Interlinked patterns in movements and otolith formation of cod (*Gadus morhua*) in the southern Baltic Sea

Dissertation

with the aim of achieving a doctoral degree

at the Faculty of Mathematics, Informatics and Natural Sciences

Department of Biology

Institute of Marine Ecosystem and Fishery Science

University of Hamburg

submitted by

Stefanie Haase

2021 in Hamburg

Dissertation evaluation commission:

Prof. Dr. Axel Temming
Institute of Marine Ecosystem and Fishery Science, University of Hamburg

Dr. Uwe Krumme
Thünen Institute of Baltic Sea Fisheries, Rostock

Date of oral defence: 09.08.2021

A mind is like a parachute. It doesn’t work if it isn’t open.

-Frank Zappa-

Contents

SUMMARY 1

ZUSAMMENFASSUNG 1

GENERAL INTRODUCTION 9

CHAPTER 1 33

Short-term tagging mortality of Baltic cod (*Gadus morhua*)

CHAPTER 2 55

Validation approaches of a geolocation framework to reconstruct movements of demersal fish equipped with data storage tags in a stratified environment

CHAPTER 3 81

Movement of cod (*Gadus morhua* L.) in the southern Baltic Sea: evidence from data storage tags

CHAPTER 4 115

Validation of otolith zone formation and otolith growth of adult wild cod (*Gadus morhua* L.) in the southern Baltic Sea through mark-recapture and tetracycline marking

GENERAL DISCUSSION 145

ACKNOWLEDGEMENTS 173

DECLARATION OF AUTHOR'S CONTRIBUTIONS 175

DECLARATION ON OATH 179

APPENDIX 181

Summary

Some of the best studied fish species with the longest monitored stock time series worldwide occur in the North Atlantic. Over the decades numerous publications added knowledge to our understanding of ecology and behaviour of these fish species and stocks. However, there are also gaps in knowledge, obvious uncertainties in ecological interactions and potential biases. These are rarely profoundly questioned unless environmental changes or inconsistent stock assessment results challenge our established points of view.

In the Baltic Sea, Atlantic cod (*Gadus morhua* L.) has been intensively surveyed and investigated for decades. Yet, our understanding of this marine species living in a semi-enclosed, brackish environment is still incomplete. For example, despite numerous otolith exchanges to improve the age reading and large scientific efforts to understand the ecology of Baltic cod, the analytical stock assessment of the Eastern Baltic cod (EBC) stock was suspended in 2014; obviously age data were uncertain and the aut- and synecological knowledge was incomplete to explain the changes occurring in this stock in distress. For instance, spreading hypoxic areas in the central Baltic basins forced EBC to contract in the southern Baltic Sea but behaviour and movements of cod in response to the consequences are poorly understood.

This dissertation aims at providing a more thorough understanding of cod ecology in the southern Baltic Sea, with a particular focus on patterns in movements of wild, free-ranging cod and validation of ring patterns in otoliths. Tagging performance, data storage tags recording depth and ambient temperature and otoliths of recaptured cod were analysed. Tagged cod had been released in the southern Baltic Sea within the scope of the international mark-recapture project TABACOD ("Tagging Baltic cod").

In **chapter 1** ("Short-term tagging mortality of Baltic cod (*Gadus morhua*)") the post-release short-term mortality of cod after tagging with T-bar tags and tetracycline was assessed. The mortality associated with catching and on-board handling was estimated to be 16% with no significant influence of the tagging process itself. While no effect of tagging month was observed, mortality rate was decreasing with increasing fish length. The low mortality rate confirmed the appropriateness of the tagging technique and potentially explained the lack of smaller recaptures.

In **chapter 2** (“Validation approaches of a geolocation framework to reconstruct movements of demersal fish equipped with data storage tags in a stratified environment“) a geolocation framework was adapted to reconstruct the movements of cod in the southern Baltic Sea tagged with temperature-depth data storage tags (DSTs). The adapted geolocation framework was tested with five validation experiments including i) simulated tracks, ii) stationary, nearshore moored DSTs, iii) temperature data from an offshore measuring station, iv) a temperature-depth probe attached to an otter board of a commercial vessel, and v) DSTs mounted on the CTD and otter boards of a survey vessel. The difference between true and modelled positions was on average between 2 and 19 km and generally better when there was a pronounced stratification of the water column and simulated individuals regularly conducted vertical movements.

In **chapter 3** (“Movement of cod (*Gadus morhua* L.) in the southern Baltic Sea: evidence from data storage tags”) the adapted and validated geolocation model was applied to the temperature-depth DSTs from 28 recaptured Baltic cod assigned to the EBC or Western Baltic cod (WBC) stock by genetics or otolith shape analysis to reconstruct daily positions. The temperature and depth profiles were supplemented with information on salinity and oxygen estimates from the regional ocean model also used for geolocation. Individual movements could be classified into three behavioural types: 1) coastal, shallow-water WBC, 2) resident EBC, and 3) migratory EBC. Unlike WBC, EBC generally occupied deeper waters, were exposed to higher salinities and regularly spent short period in hypoxic waters. While resident EBC stayed within the Bornholm Basin year-round, migratory EBC moved between spawning grounds in the Bornholm Basin during summer and coastal feeding grounds during autumn and spring. This study highlights the importance of coastal shallow-water feeding grounds, especially in autumn and spring which are underrepresented in the current bottom trawl survey. In addition, the temperature-depth profiles of all EBC revealed daily vertical movements in the water column which were triggered by twilight and partly followed the lunar cycle. Regular defaecation below the thermocline of small pelagics ingested during nightly feeding excursions above the thermocline may exacerbate hypoxia in the stagnant water bodies of the deeper basins.

In **chapter 4** (“Validation of otolith zone formation and otolith growth of adult wild cod (*Gadus morhua* L.) in the southern Baltic Sea through mark-recapture and tetracycline marking“) the chemically time-stamped otoliths of 258 T-bar tagged cod assigned to the WBC or EBC stock were analysed. WBC in the southern Baltic Sea confirmed the recent age validation result from the Belt Sea that the translucent zone is formed during summer when cod are stressed by adverse temperatures and reduced feeding. The translucent zone of EBC was also laid down during summer, but under very different conditions, i.e. during spawning coinciding with hypoxic conditions and food limitation. The faster otolith growth of WBC were in line with previous findings showing that EBC exhibit slower somatic length growth and, hence, severely reduced productivity of the EBC stock.

Through this dissertation, it was possible to interlink the environmental conditions experienced by cod tagged with data storage tags to the growth and patterns in ring formation in the otoliths of cod tagged with T-bar tags. The evaluation of movement patterns in the present centre of cod distribution thus provided new insights into the behaviour and ecology of cod inhabiting the permanently stratified brackish-water ecosystem of the southern Baltic Sea.

Zusammenfassung

Einige der am meist untersuchten Fischarten mit den längsten Bestandszeitreihen kommen im Nordatlantik vor. Im Laufe der Jahrzehnte haben zahlreiche Veröffentlichungen unser Verständnis von Ökologie und Verhalten dieser Fischarten und Bestände erweitert. Allerdings gibt es auch Wissenslücken, offensichtliche Unsicherheiten bei ökologischen Wechselwirkungen und mögliche Verzerrungen. Diese werden selten tiefgreifend in Frage gestellt, es sei denn, Umweltveränderungen oder widersprüchliche Bestandsberechnungsergebnisse stellen unsere etablierten Sichtweisen in Frage.

In der Ostsee wird der Dorsch (*Gadus morhua* L.) seit Jahrzehnten intensiv untersucht und in Surveys erfasst. Dennoch ist unser Verständnis dieser marinen Art, die hier in einem brackigen Binnenmeer lebt, immer noch unvollständig. Trotz zahlreicher Otolithen-Austausche zur Verbesserung der Altersbestimmung und großer wissenschaftlicher Anstrengung, die Ökologie des Ostseedorsches zu verstehen, wurde beispielsweise die analytische Bestandsbewertung des östlichen Ostdorschbestandes (Eastern Baltic cod, EBC) im Jahr 2014 ausgesetzt; offensichtlich waren die Altersdaten unsicher und das aut- und synökologische Wissen zu unvollständig, um die Veränderungen in diesem unter Stress stehenden Bestand zu erklären. Zum Beispiel zwangen die sich ausbreitenden hypoxischen Gebiete in den zentralen Ostseebecken den EBC Bestand dazu, sich in der südlichen Ostsee zu konzentrieren. Das Verhalten und die Bewegungen des Dorsches als Reaktion auf die Folgen sind nur unzureichend verstanden.

Diese Dissertation zielt darauf ab, ein gründlicheres Verständnis der Ökologie des Dorsches, mit besonderem Augenmerk auf die Bewegungsmuster wildlebender Tiere und die Validierung von Ringmustern in Otolithen in der südlichen Ostsee zu erlangen. Es wurde die Überlebensrate nach der Markierung bestimmt und die Informationen von Datenloggern, die die Umgebungstemperatur und Wassertiefe messen, sowie Otolithen wiedergefangener Dorsche analysiert. Die markierten Dorsche wurden in der südlichen Ostsee im Rahmen des internationalen Markierungsprojekts TABACOD ("Tagging Baltic cod") ausgesetzt.

In **Kapitel 1** ("Short-term tagging mortality of Baltic cod (Gadus morhua)") wurde die Sterblichkeit von Dorschen kurze Zeit nach der Markierung mit T-bar Tags und Tetracyclin bewertet. Die mit dem Fang und der Handhabung an Bord verbundene Sterblichkeit betrug 16%, wobei der Markierungs-Prozess selbst keinen signifikanten Einfluss aufwies. Auch der

Markierungsmonat zeigte keinen Effekt auf die Sterbklichkeit, jedoch nahm die Sterblichkeitsrate mit zunehmender Fischlänge ab. Die niedrige Sterblichkeitsrate bestätigte die Eignung der Markierungstechnik und erklärte das Fehlen kleinerer Wiederfänge.

In **Kapitel 2** ("Validation approaches of a geolocation framework to reconstruct movements of demersal fish equipped with data storage tags in a stratified environment") wurde eine Geolokalisierungs-Methode angepasst, um die Bewegungen von Dorschen, die mit Temperatur-Tiefen-Datenloggern (data storage tags, DSTs) markiert wurden, in der südlichen Ostsee zu rekonstruieren. Die angepasste Geolokalisierungs-Methode wurde mit fünf Validierungsexperimenten getestet, darunter i) simulierte Tracks, ii) stationäre, küstennah verankerte DSTs, iii) Temperaturdaten von einer Offshore-Messstation, iv) eine Temperatur-Tiefen-Sonde, die an einem Scherbrett eines kommerziellen Schiffes angebracht war, und v) DSTs, die an der CTD- und am Scherbrett eines Fischereiforschungsschiffes montiert waren. Die Differenz zwischen korrekten und modellierten Positionen lag im Durchschnitt zwischen 2 und 19 km und war im Allgemeinen besser, wenn eine ausgeprägte Schichtung der Wassersäule vorlag und die simulierten Individuen regelmäßig vertikale Bewegungen durchführten.

In **Kapitel 3** ("Movement of cod (*Gadus morhua* L.) in the southern Baltic Sea: evidence from data storage tags") wurde die angepasste und validierte Geolokalisierungs-Methode auf die Temperatur-Tiefen-Profile, die mit den Datenloggern von 28 wieder gefangenen Ostseedorschen, aufgenommen wurden, angewandt, um tägliche Positionen zu rekonstruieren. Diese Individuen wurden durch Genetik oder Otolithenformanalyse dem EBC- oder dem Westdorschbestand (Western Baltic cod, WBC) zugeordnet. Die Temperatur- und Tiefenprofile wurden mit Informationen zu Salz- und Sauerstoffgehalt aus dem regionalen Ozeanmodell ergänzt, das auch zur Geolokalisierung verwendet wurde. Die individuellen Bewegungen konnten in drei Verhaltenstypen klassifiziert werden: 1) küstennahe Flachwasser WBC, 2) stationäre EBC und 3) wandernde EBC. Im Gegensatz zu den WBC, hielten sich die EBC in tieferen Wasserschichten auf, waren höheren Salzgehalten ausgesetzt und verbrachten regelmäßig kurze Zeiträume in hypoxischen Bereich. Während die stationären EBC das ganze Jahr über im Bornholm-Becken blieben, zogen die wandernden EBC zwischen den Laichgründen im Bornholm Becken im Sommer und den küstennahen Nahrungsgründen im Herbst und Frühjahr hin und her. Diese Studie unterstreicht die Bedeutung der küstennahen Flachwasser-Futterplätze, insbesondere im Herbst und Frühjahr, die in der aktuellen

Grundschleppnetzerhebung unterrepräsentiert sind. Darüber hinaus zeigten die Temperatur-Tiefen-Profile aller EBC tägliche vertikale Bewegungen in der Wassersäule, die durch die Dämmerung ausgelöst wurden und teilweise dem Mondzyklus folgten. Die regelmäßige Defäkation von kleinen pelagischen Tieren, die bei nächtlichen Fütterungsausflügen oberhalb der Sprungschicht von den EBC aufgenommen wurden, kann die hypoxische Situation in den stehenden Gewässern der tieferen Becken verschärfen.

In **Kapitel 4** ("Validation of otolith zone formation and otolith growth of adult wild cod (*Gadus morhua* L.) in the southern Baltic Sea through mark-recapture and tetracycline marking") wurden die chemisch und mit T-bar Tags markierten Otolithen von 258 wiedergefangenen Dorschen, die dem WBC- oder EBC-Bestand zugeordnet wurden, analysiert. Die WBC in der südlichen Ostsee bestätigten das jüngste Ergebnis der Altersvalidierung aus der Beltsee, dass die durchscheinende Zone („translucent zone") im Sommer gebildet wird, wenn die Dorsche durch ungünstige Temperaturen und reduzierte Futterbedingungen gestresst sind. Die durchscheinende Zone von EBC wurde ebenfalls im Sommer angelegt, jedoch unter ganz anderen Bedingungen, nämlich während einer Zeit in der die Tiere laichen und hypoxischen Bedingungen und Nahrungslimitierung ausgesetzt sind. Das schnellere Otolithen-Wachstum der WBC stand im Einklang mit früheren Erkenntnissen, die zeigten, dass EBC ein langsameres somatisches Längenwachstum und der EBC-Bestand damit eine stark reduzierte Produktivität aufweist.

Durch diese Arbeit war es möglich, die Umweltbedingungen, denen die mit Datenloggern markierten Dorsche ausgesetzt waren, mit dem Wachstum und den Mustern in der Otolithen-Ringbildung von Dorschen, die mit T-bar Tags markiert wurden, zu verknüpfen. Die Auswertung von Bewegungsmustern im derzeitigen Verbreitungsschwerpunkt des Dorsches lieferte somit neue Erkenntnisse über das Verhalten und die Ökologie des Dorsches, der das permanent-geschichtete Brackwasser-Ökosystem der südlichen Ostsee bewohnt.

General Introduction

Movement

Organisms have three fundamental tasks: acquisition of resources, survival, and reproduction (Nentwig et al., 2017; Kiørboe et al., 2018). To fulfil these, each species follows its own strategies, many of which involve movement. Movements were classified as a major contribution to system functioning and considered one of the ten greatest “inventions of evolution” (Lane, 2010) because they contribute to the adaption of species, the characteristics of life history patterns, and, finally, to the dynamics of populations (Dingle, 2014). Movements can occur at multiple temporal and spatial scales (Koehn and Crook, 2013) and are often driven by a combination of internal drivers such as reproduction, feeding, and taking shelter, in response to external stimuli including biotic and abiotic conditions (Nathan et al., 2008; Pinti, 2020). As a result, individuals often move within so-called “home ranges”, which describes an area used by an organism containing the resources needed for survival and reproduction (Dingle, 2014).

Box 1: Movements and migrations

Because the terms “movement” and “migration” are often confused and ambiguously used in the literature, I have based this thesis on the following definitions:

Movement is the umbrella term for a range of different movement behaviours. It simply describes a change in location.

Migration is the mostly directed, often periodic, movement to a new habitat to find more favourable conditions (Dingle and Drake, 2007; Dingle, 2014). Main migrations of fish include 1) feeding migrations, 2) spawning migrations, and 3) wintering migrations.

Recent advances in animal tracking, above all the integration of high-resolution Global Positioning Systems (GPS), have significantly improved the ability to describe terrestrial animal movements (Kays et al., 2015). Recording the movements of mobile, marine organisms, however, remains challenging due to the fast absorption of microwave frequencies in GPS communication in water. Despite the constant development of new techniques, individuals can often not be directly observed. A thorough understanding of animal movements is necessary to sustainably assess and manage harvested stocks (Cadrin and Secor, 2009; Hooten et al., 2017). Further, the timing and direction of individual movements combined with knowledge on environmental conditions can give insights about niche occupation and behaviour of populations and help to optimize monitoring and sampling strategies.

Conventional tagging has been a widely used approach in fisheries management to study the movements, growth, abundance, and survival of marine fish in the wild (e.g. Hilborn, 1990; Guy et al., 1996; Pine et al., 2012; Hall, 2014). For conventional tagging, externally attached tags such as T-bar tags, usually with a unique identifier, are used to identify individual fish and match it to release and recapture information. Tagging studies are conducted under the assumption that data are unbiased and representative of the population but tag loss or post-release tagging mortality can lead to a biased sample from the marked population (Rude et al., 2011; Pine et al., 2012,). Further, it is assumed that the tags do not alter fish behavior and growth (Pine et al., 2012; Smith et al., 2017). Some fish are more sensitive to the tagging process than others, e.g. body size, maturity, sex, the tag type itself, or the tag location may have an influence (Buckmeier and Reeves, 2012). Therefore, species-specific or even stock-specific evaluations are suggested during a tagging study (Smith et al., 2017).

One major limitation of conventional tagging is that only release and recapture information are available and it is unknown what happened in between (Kohler and Turner, 2001). As a result, movements between release and recapture are often significantly underestimated (Lucas and Baras, 2000). Further, recaptures often depend on commercial and recreational fishing effort. Recapture positions are therefore highly influenced by the temporal and spatial distribution of fishing activities (Bolle et al., 2005; Pine et al., 2012). Therefore, a detailed understanding of major migrations, their timing and how they link spawning and feeding habitats is often speculative or lacking (Nielsen, 2019). Advances in bio-logging and biotelemetry help to address these issues by recording environmental data during the fish’s time at liberty. They enable scientists to acquire fisheries-independent and individual data between release and recapture (Young and Isely, 2004; Cooke et al., 2006) to couple environmental conditions to behavior across a number of relevant temporal and spatial resolutions (Åkesson, 2002; Cooke et al., 2012).

Electronic tags to study fish movements and behavior

One such example is acoustic telemetry. It is frequently used to characterize small-scale behavior patterns such as homing behavior (Robichaud and Rose, 2004), spawning site fidelity (Zemeckis et al., 2019), or to test the effect of closed or marine protected areas (Lindholm and Auster, 2003). Within a limited listening range (usually a few hundred meters), the hydrophone of the acoustic receiver records when a previously tagged fish enters the area. But since the listening range is limited, prior knowledge of migrations and daily home ranges of the species of interest is necessary for a successful study.

A bio-logging approach that is not restricted to such a fine scale are data storage tags (DSTs; also called archival tags). DSTs collect and store individual in-situ data from release to recapture. DSTs were first deployed in the 1990s and have since then been used in many studies worldwide (Hussey et al., 2015). Previous studies demonstrated the value of DSTs to analyze migrations (Hunter et al., 2006; Ådlandsvik et al., 2007; Zemeckis et al., 2017), population structures (Block et al., 2005; Galuardi and Lam, 2014), homing behavior (Svedäng et al., 2007), habitat use (Cooke et al., 2012) and mortality (Kurota et al., 2009; Eveson et al., 2012); in some cases, it was even used to inform spatially resolved stock assessment models (Taylor et al., 2011; Eveson et al., 2012; Sippel et al., 2015). DSTs record and store time-stamped measurements of sensors at intervals ranging from seconds to minutes. Built-in sensors can record environmental parameters such as ambient temperature, salinity, pressure (provides information on water depth), and light level or behavioural parameters such as acceleration or heart rate (Thorstad et al., 2014). In larger species even cameras have been mounted (Gleiss et al., 2019; Jewell et al., 2019). Tags vary by the manufacturer in the structure of the data collected, the number and types of sensors included, and the size (Nielsen, 2019). DSTs can be relatively small and thus can be used for tagging also smaller fish, but larger tags often have increased storage volume and battery capacity. These archival tags can be attached externally (there are also options that the tag is released from the fish and data are transferred via satellite, so-called “Pop-up Satellite Archival Tags”; short: PSATs), although, especially for smaller individuals, internal implantation is preferred to avoid abrasion at the tag attachment (Jadot, 2003; Cooke et al., 2012) and drag which might alter swimming performance (Lewis and Muntz, 1984; Jepsen et al., 2015). Otherwise, one of the strongest assumptions in tagging studies, namely that fish behave naturally during their time at liberty, might be impaired (Pine et al., 2012). DST studies are often limited by low recapture rates and the number of tags deployed because they are relatively costly (~300 €). Physical recapture is necessary which makes them only partly fisheries-independent (Galuardi and Lam, 2014). Extrapolating results to population level is on the small sample size of correlated measurements often limited (Cooke et al., 2012; Sequeira et al., 2019). But even though sample sizes are often lower than in conventional tagging studies, each recovered DST is very valuable and adds information on the independent behavior of wild fish (Cooke et al., 2012 and references therein).

Figure 1: Cod tagged with a yellow T-bar tag in the dorsal musculature and schematic illustration of a data storage tag. Both tags are labelled with unique numbers for identification and a telephone number to call when the fish is recaptured (© A. Schütz/ Thünen Institute).

Geolocations derived from DSTs

DST data often lack direct observation of spatial context, but geolocation models can reconstruct daily locations between release and recapture when the fish is recaptured. The principle approach of geolocation models is to compare environmental data recorded by the DST to regional ocean models and to find the most probable track. The choice of environmental parameters to consider is dependent on the heterogeneity within the chosen ecosystem. Typically, parameter are chosen that reflect a high variability of the system and the behaviour of the species of interest to achieve a high precision in reconstructed positions (Liu, 2019; Nielsen, 2019). Another prerequisite is a spatially and temporally highly resolved grid of the parameters of interest for the study area which can display daily movements of the tagged species (Nielsen, 2019). Various geolocation methods are available to estimate daily positions from DST data, including light-level, temperature, pressure (for depth or tides), and salinity.

While light-based geolocation is popular for highly migratory pelagic species and thus experiencing variation in light-level as revealed by day-length, local noon, and midnight (Sibert et al., 2003; Nielsen et al., 2006), this method is less applicable for demersal groundfish mainly inhabiting higher latitude ecosystems (Seitz et al., 2006). For tracking demersal species, which are closely related to the seafloor and inhabit an ecosystem with sufficient influence of tides and multiple amphidromic points, the phase and amplitude of the signal recorded in the pressure sensor can be compared with an oceanographic tidal model to reconstruct fish positions, the so-called tidal location method (Metcalfe and Arnold, 1997; Pedersen et al., 2008; Thygesen, 2009; Le Bris, 2014). For environments with a pronounced salinity gradient, such as the Baltic Sea where salinity decreases from West to East, salinity can be used to support geolocation (Neuenfeldt et al., 2007). But since tags that record salinity need to be attached externally to the individual, drag and wound infections might alter fish behavior, and tagged fish might not be representative of the population anymore (Pine et al., 2012). When neither light, nor tidal, nor salinity measurements can assist in a geolocation approach, depth and temperature data recorded with the DSTs can be compared to a bathymetry and temperature grid of the area of interest (Righton and Mills, 2008).

Geolocation frameworks based on Hidden Markov Models (HMMs), which are a variation of non-parametric state-space models, resulted in good track estimations, especially for near-shore demersal species tagged with depth sensors (Andersen et al., 2007; Nielsen et al., 2019). The origin of many HMMs is a tidal geolocation framework developed for North Sea cod (Pedersen et al., 2008; Thygesen, 2009). Since these geolocation frameworks are highly case-specific to the area, species, and tag-type, various modifications of this approach have been developed and applied (Thorsteinsson et al., 2012; Le Bris et al., 2013; Woillez et al., 2016; Biais et al., 2017; Liu et al., 2017; Braun et al., 2018).

When deriving tracking data from temperature-depth data of DSTs, it is highly recommended to test the geolocation framework for robustness and performance of the chosen specifications. This is needed to assess the uncertainty of the positional assignments to avoid misinterpretation of behavior and movement patterns derived from these geolocations (Braun et al., 2015; Nielsen et al., 2019). The uncertainty involved in such geolocation studies is ideally evaluated by directly comparing modeled to known positions but known positions of wild, free-ranging fish are typically rare. There are, however, a few examples where fish have been double-tagged with DSTs and acoustic transmitters to compare modeled positions to recorded positions when the fish is within the telemetry array (Liu et al., 2017). Other examples include double-tagging with pop-up satellite tags and satellite-linked location tags (Teo et al., 2004; Braun et al., 2019) but these options are only available to a limited number of cases and mostly restricted to species regularly surfacing or that are above a certain size (Arnold and Dewar, 2001; Block et al., 2005).

Other methods, which do not rely on wild fish, include simulated tracks which preferably imitate natural fish behavior (Neuenfeldt et al., 2007; Righton and Mills, 2008; Liu et al., 2017; Nielsen et al., 2019), moored measuring stations (Hunter et al., 2003; Thorsteinsson et al., 2012; Liu et al., 2017) or attaching sensors to fishing gear (Righton and Mills, 2008). When validation is conducted, most studies choose a range of different validation methods to imitate different behaviors of a fish such as stationary behavior or movements covering larger distances across different scales of space and time. The results of these studies have in common that the geolocation frameworks performed well when the ecosystem was highly heterogeneous in terms of environmental parameters (Liu et al., 2017; Nielsen et al., 2019). Although the effort to investigate model performance is often within reasonable time and financial bounds, many geolocation studies still lack this assessment (Liu, 2019).

When applying a geolocation framework to cod in the Baltic Sea both the characteristics of the hydrography and of the species have to be considered to identify the most promising approach. One goal of this dissertation is to adapt and validate an existing HMM geolocation framework and apply it for the geolocation of cod tagged with temperature-depth DSTs in the southern Baltic Sea. By reconstructing the tracks, I want to gain detailed knowledge on movement timing between spawning and feeding grounds, and the environmental conditions related to this.

The Baltic Sea

The Baltic Sea is a large, semi-enclosed, non-tidal sea characterized by strong environmental gradients (Snoeijs-Leijonmalm et al., 2017). Particularly pronounced is the horizontal and vertical gradient of salinity. It decreases from approximately 20 PSU in the West to almost freshwater (5 PSU) in the North-East and at any given basin, salinities are typically lower at the surface than those at the seafloor (Zweifel and Laamanen, 2009; Snoeijs-Leijonmalm and Andrén, 2017). While highly variable saltwater-inflows from the Kattegat transport marine water via the shallow and narrow Danish straits, the over 200 river-runoffs framing the area lead to a constant freshwater inflow, resulting in the brackish nature of the Baltic Sea (Snoeijs-Leijonmalm and Andrén, 2017). The rather shallow western Baltic Sea is highly influenced by the irregular saltwater-inflows (Mohrholz, 2018a; Mohrholz, 2018b; Naumann et al., 2020). In the southern Baltic, the Arkona Basin (about 45- 50 m water depth) and the Bornholm Basin (up to 100 m water depth) are characterized by a three-layer structure (Fig. 1). A permanent halocline near the bottom and a seasonal surface thermocline with an average thickness of 15-20 m result in a double stratification of the water column. The lack of vertical mixing of the deep-water layers leads to constant hypoxic conditions in the deep basins (Naumann et al., 2020). As a continental sea, the Baltic is heavily influenced by the surrounding landmasses and particularly harmed by nutrient inputs leading to eutrophication (Wulff et al., 1990; Snoeijs-Leijonmalm and Andrén, 2017; Reusch et al., 2018; Savchuk, 2018). Due to its challenging salinity conditions, the Baltic Sea faunal community is relatively species-poor (Mackenzie et al., 2007) and both freshwater and marine species meet their physiological limits along the salinity gradient (Zweifel and Laamanen, 2009).

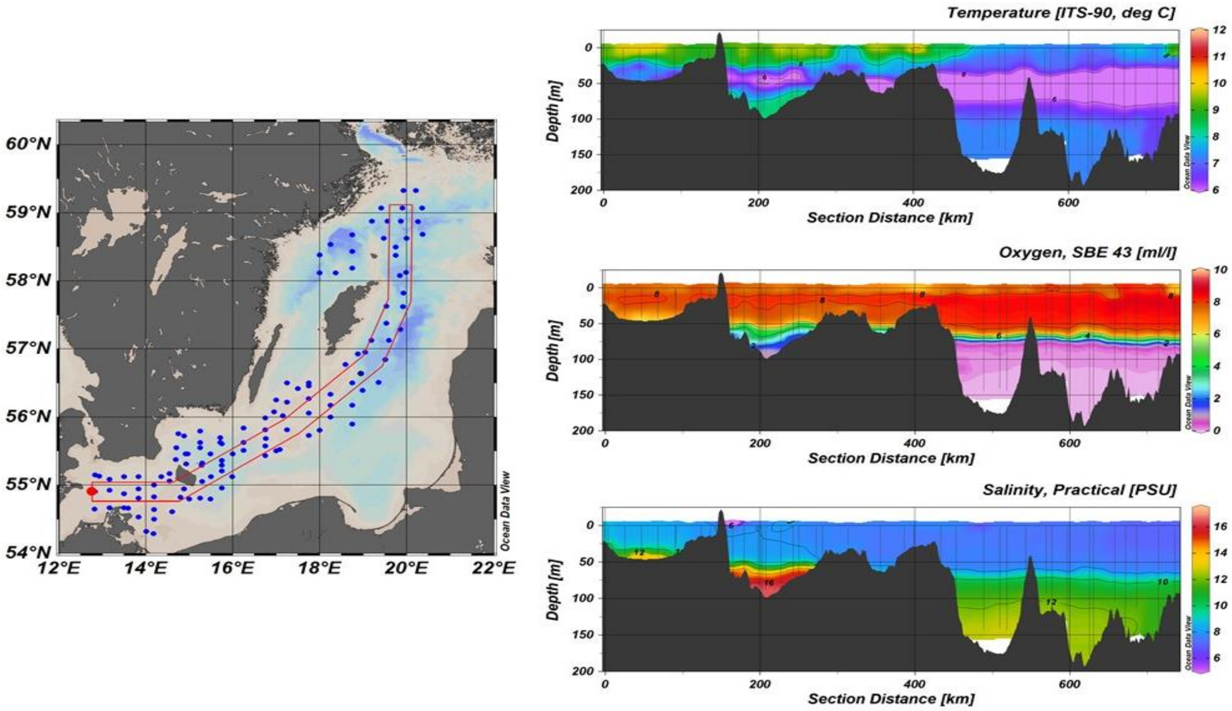

Figure 2: Hydrographic situation in the southern and eastern Baltic Sea during a research cruise in May 2020. The vertical stratification in temperature, oxygen and salinity is clearly visible. The sections are interpolated from CTD-casts which are shown as blue dots on the map in the left panel. The red lines indicate the south-west to north-east section depicted in the three cross-sections.

The two cod populations in the southern Baltic Sea

Atlantic cod (*Gadus morhua* L. 1758) is a demersal species and is found throughout the shelves and coastal areas of the North Atlantic. Most cod stocks are or were economically and ecologically valuable and have been heavily exploited for centuries (Kurlansky, 1998). Because of high fishing pressure, many stocks have collapsed since then, and some stocks are still severely threatened by exploitation. The Baltic Sea is inhabited by two genetically distinct populations of Atlantic cod. Cod in the Baltic experience conditions which are at the edge of their tolerance because temperatures are at the higher and salinity at their lower tolerance limit, respectively (Köster et al., 2005; Mackenzie et al., 2007; Cardinale and Svedäng, 2011).

The two cod stocks in the Baltic Sea are commonly referred to as “Western Baltic cod” (WBC) and “Eastern Baltic cod” (EBC). The distribution area of WBC ranges from the Belt Sea at the western border to the Öresund and the Arkona Sea (Fig. 1), often also referred to as western Baltic Sea. The EBC currently mainly inhabits the Arkona Sea and the Bornholm Basin because the hydrographic conditions on the spawning grounds further East prevent successful, regular survival of cod eggs since the mid-1980s and led to a contraction of the stock to the southern Baltic Sea (Köster et al., 2009; Eero et al., 2012). Before stock decline and hypoxic areas reduced the range, EBC was also common in the central and northern Baltic Sea up to 63 °N (Aro, 1989). The distribution ranges of both stocks overlap, and mixing is most pronounced in the Arkona Sea (Eero et al., 2014; Hemmer-Hansen et al., 2019; Weist et al., 2019). Recent studies confirmed that EBC move as far West as to the Belt Sea (Schade et al., 2019) and that WBC can be found in the Bay of Gdansk (Hüssy et al., 2020). The current knowledge of movements of cod in the southern Baltic Sea have been mainly reconstructed from several conventional tag-release studies (e.g. Berner, 1962; Thurow, 1963; Berner, 1971; Berner, 1981). These movements outside the core distribution area are likely driven by feeding migrations (Aro, 1989), spill-over events (Eero et al., 2014), or favorable hydrographic conditions (Stroganov et al., 2017). Genetic analysis revealed, however, that the stocks are genetically distinct from each other and hybridization does apparently not occur (Hemmer-Hansen et al., 2019; Weist et al., 2019). Although the two cod stocks live in partly overlapping habitats, they differ in several characteristics such as otolith shape (Schade et al., 2019; Weist et al., 2019), haemoglobin type (Sick, 1965) or behavior, noticeable, for example, in the spawning location and timing (Bleil et al., 2009). Presently, the stocks also differ significantly in productivity which is the result of a slower growth rate and higher natural mortality of EBC compared to WBC (Eero et al., 2015; McQueen et al., 2020). Also deteriorated conditions, earlier maturation, and the lack of larger individuals have been stated as indices of the distress of EBC (Eero et al., 2015). Multiple natural stressors such as increasing hypoxic conditions (Casini et al., 2016), limitation in food availability, and a shift in food composition, potentially leading to a thiamine deficiency (Engelhardt et al., 2020) and increasing infestation rates by nematodes (Behrens et al., 2014; Sokolova et al., 2018) were identified. The body condition of WBC is currently not worrying but high fishing pressure and a series of years with low recruitment led to a severe reduction in stock size and currently (commercial and recreational) catches largely depend on the 2016-year-class (ICES, 2020a).

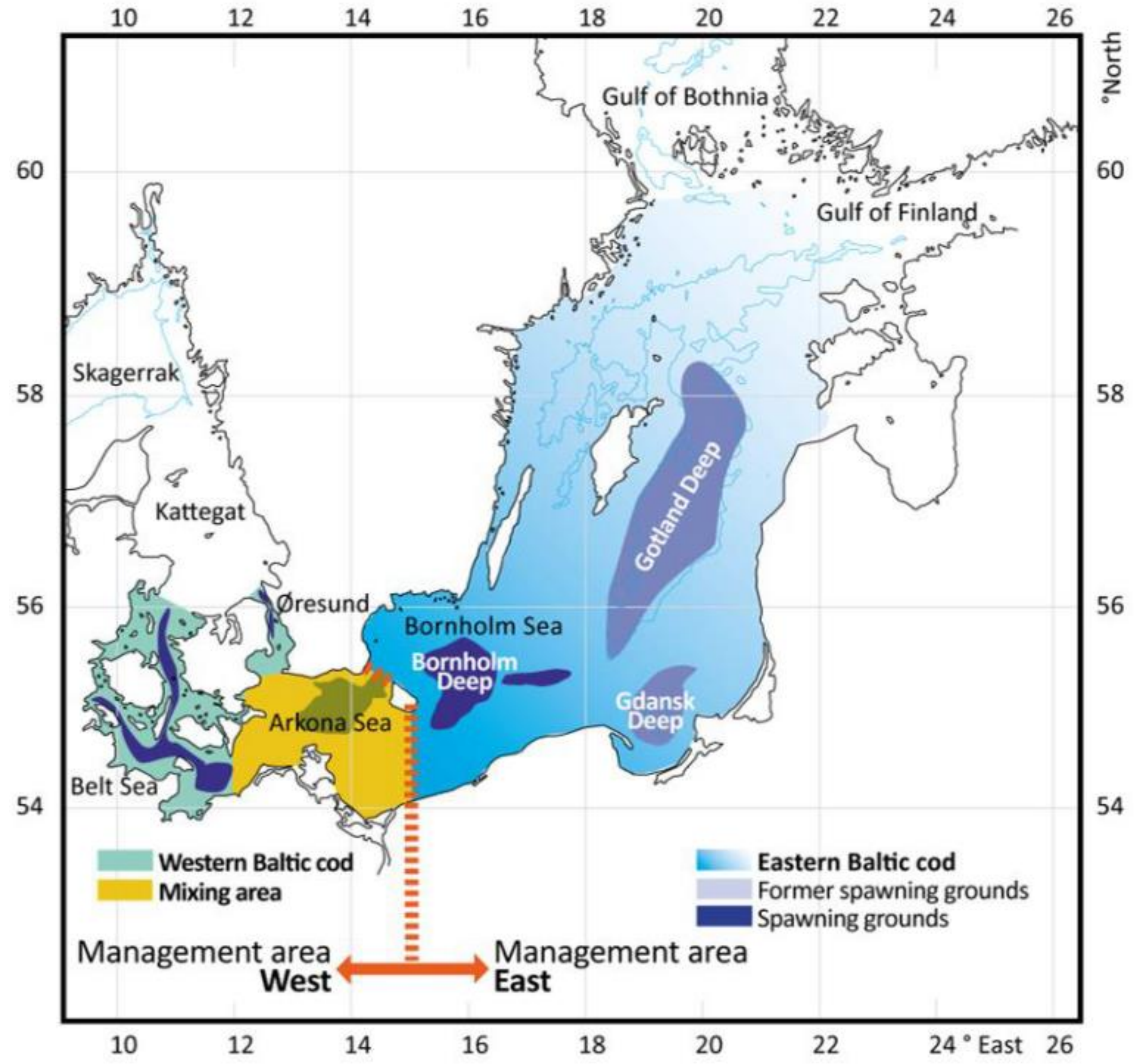

Figure 3: Schematic overview of the distribution area of Western and Eastern Baltic cod (© C. Zimmermann/ Thünen Institute).

The differences in stock status between WBC and EBC could be the result of different environmental conditions experienced by the stocks. This is on the one hand due to the result of different behaviors but also due to the different characteristics of the shallow western Baltic Sea and deeper and continuously stratified eastern Baltic Sea.

Some of these behavioral characteristics and the environmental conditions experienced are reflected in visible zones in the otoliths of fish. Otoliths are calcium carbonate structures; three pairs of otoliths can be found in the inner ear of fish and are used for balance and hearing (Campana, 1999). Because otoliths form distinct, visible zones, the sagitta, the largest otolith, is often used for age estimation (Campana, 2001). The zone formation of WBC otoliths has recently been validated for juvenile (McQueen et al., 2019; Plonus et al., 2021) and adult cod (Krumme et al., 2020) and is now routinely used in age determination. Age determination is an important component of the WBC stock assessment. Contrary, the zone formation of EBC is considered to be irregular and unrelated to season (Hüssy, 2010; Hüssy et al., 2016). Due to the low precision and accuracy of traditional age reading of EBC as revealed by age reader comparisons (ICES, 2006; ICES, 2015), the influence of age data in the current stock assessment model is reduced (ICES, 2020b). Validated otolith material does not yet exist which could shed light on the zone formation, especially in combination with the newly gained knowledge of the environmental conditions experienced by adult cod tagged with DSTs in the southern Baltic Sea.

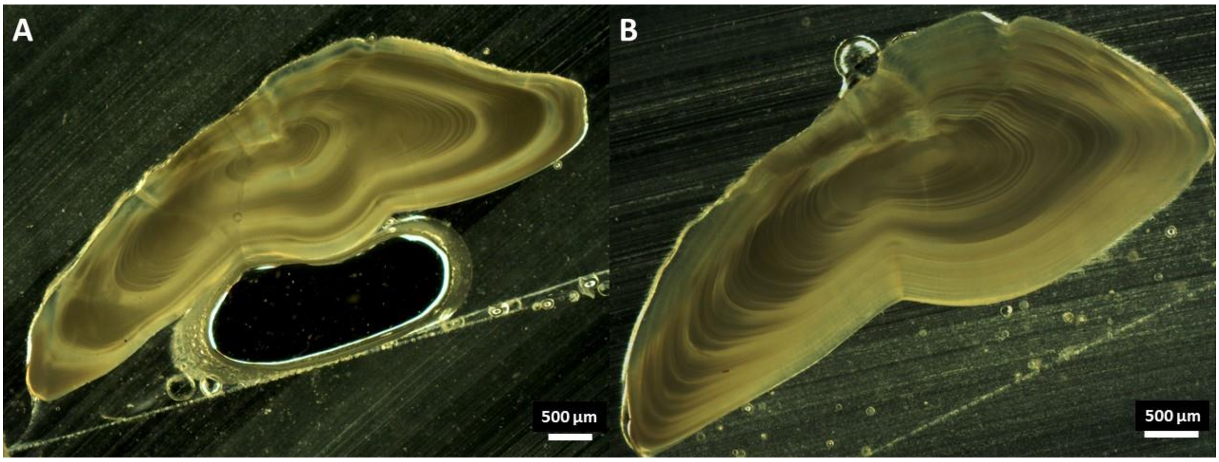

Figure 4: Cross-section of a (A) Western and (B) Eastern Baltic cod otolith. (A) This 3-year-old Western Baltic cod (sex not classified) was captured in April 2017 with a total length of 59 cm and 1800 g. (B) This female Eastern Baltic cod was captured in June 2018 with a total length of 42 cm and a weight of 809 g. The cod was at least five years old but age determination is accompanied by a high uncertainty. The photographs are taken under a transmitted light microscope so that the translucent zones appear lighter than the opaque zone. While the zone transitions appear distinct in (A), transitions are more diffuse in (B) and ageing is associated with high inaccuracies. Photos taken by B. Rotzoll (Thünen Institute).

Research objective and overview

The overall aim of this dissertation is to contribute new findings of individual behavior, movements, and otolith formation of cod to better understand cod ecology under the specific conditions currently characterizing the southern Baltic Sea. This is intended to improve our understanding of the niche separation of the two cod stocks inhabiting the southern Baltic Sea. Results from this dissertation can help to better account for biases in the surveys or reduce uncertainties in the aging of individual cod, thus ultimately contributing to a more sustainable resource management in the Baltic Sea. To achieve this, I analyse recaptured cod tagged with T-bar tags and DSTs recording temperature and depth released in the southern Baltic Sea between 2016-2019 within the scope of the international mark-recapture project TABACOD (“Tagging Baltic cod”).

During my research, I addressed four specific objectives:

> Objective 1: Estimate the short-term post-release mortality of cod associated with T-bar tagging in the southern Baltic Sea

In **Chapter 1**, I aim at quantifying the post-release mortality associated with T-bar tagging, specifically for cod tagged in the southern Baltic Sea during the TABACOD project. This is done to check if the applied technique leads to satisfactory results. A generalized linear mixed effect model is used to test the effect of tagging on the short-term mortality and if this varies with fish length and month.

> Objective 2: Adapt and test a geolocation framework to reconstruct the movements of cod tagged with temperature-depth DSTs in the southern Baltic Sea and assess the uncertainty associated with the adapted framework

In **Chapter 2**, I introduce a geolocation method which is a direct modification of the geolocation model HMMoce, primarily developed for highly migratory pelagic species (Braun et al., 2018). The adapted HMMoce includes modification in the likelihood calculations to account for the demersal behavior of cod in the southern Baltic Sea. The adapted geolocation framework was subsequently validated, and the uncertainty assessed using five methods, including 1) artificially constructed tracks, 2) near-shore mooring experiments with DSTs, 3) mooring experiments with an offshore measurement station, 4) temperature-depth sensors attached to the otter boards of a commercial trawler, and 5) DSTs attached to the otter boards or the CTD of a research vessel.

> Objective 3: Reconstruct the vertical and horizontal movements of cod assigned to EBC or WBC tagged with temperature-depth DSTs in the southern Baltic Sea and assess seasonal patterns and differences between individuals

In **Chapter 3**, I apply the adapted and validated geolocation framework from Chapter 2 on DST profiles of 28 recaptured DST-tagged WBC and EBC assigned by genetics and otolith shape analysis from the southern Baltic Sea. The movement behavior, supplemented with further information e.g. on ambient temperatures recorded by the DSTs, salinity and oxygen values derived from a regional ocean model, and vertical movements, is analyzed to provide a first thorough understanding of the diversity in the patterns of movements of cod in the southern Baltic Sea. This will give first insights into the two cod populations in a mixing area.

> Objective 4: Assess the zone formation and increment patterns in otoliths of adult EBC and WBC from the southern Baltic Sea

Finally, in **Chapter 4**, I analyse the formation and growth of cod otoliths in the southern Baltic Sea by using chemically marked otoliths of wild recaptures. This chapter contrasts the formation and growth rate of otoliths from the WBC and EBC stock to which they were assigned by genetics or otolith shape analysis. Since this study provides the first validated set of chemically marked otoliths recaptured from wild EBC, it is an essential baseline to critically re-assess the uncertainties linked to the age determination of EBC otoliths. The results hopefully advance the international age reading routines used to determine the age of EBC and built the foundation for further studies. Finally, I link these patterns to the gained knowledge of movements and environmental conditions described in Chapter 3.

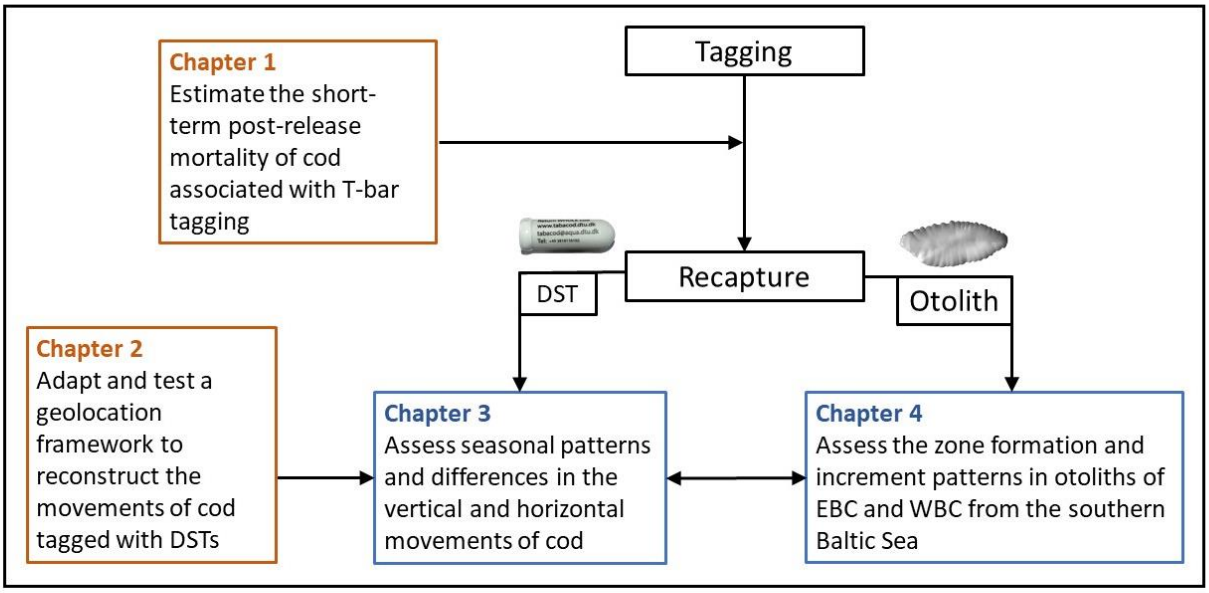

Figure 5: Conceptual diagram of my thesis objectives and the chapter that address them. The thesis consists of methodological (orange) and applied (blue) chapters. Arrows indicate how methods or information developed in one chapter are used in other chapters (DST © A. Schütz/ Thünen Institute, otolith © B. Rotzoll/ Thünen Institute).

References

Ådlandsvik B, Huse G, Michalsen K (2007) Introducing a method for extracting horizontal migration patterns from data storage tags. Hydrobiologia 582: 187–197

Åkesson S (2002) Tracking fish movements in the ocean. Trends Ecol Evol 17: 56–57

Andersen KH, Nielsen A, Thygesen UH, Hinrichsen H-H, Neuenfeldt S (2007) Using the particle filter to geolocate Atlantic cod (Gadus morhua) in the Baltic Sea, with special emphasis on determining uncertainty. Can J Fish Aquat Sci 64: 618–627

Arnold G, Dewar H (2001) Electronic Tags in Marine Fisheries Research: A 30-Year Perspective. *In* JR Sibert, JL Nielsen, eds, Electron. Tagging Track. Mar. Fish. Proc. Symp. Tagging Track. Mar. Fish Electron. Devices Febr. 7–11 2000 East-West Cent. Univ. Hawaii. Springer Netherlands, Dordrecht, pp 7–64

Aro E (1989) Review of fish migration patterns in the Baltic Sea. Rapp P-V Reun Cons Int Explor Mer 190: 72–96

Behrens JW, Seth H, Axelsson M, Buchmann K (2014) The parasitic copepod Lernaeocera branchialis negatively affects cardiorespiratory function in Gadus morhua: response to parasite infection by gadus morhua. J Fish Biol 84: 1599–1606

Berner M (1962) Die Ostseedorscherträge 1960/61 und die Fangaussichten 1962. Dtsch Fisch Zeitg 9: 126–131

Berner M (1971) Ergebnisse der Dorschmarkierungen des Jahres 1968 in der Bornholm- und Arkonasee. Fisch.-Forsch. 9, 15–20.

Berner M (1981) Dislocation parameters of tagging experiments on cod in the Baltic (subdivision 22-25) from 1959 to 1975. ICES CM 1981 J15 26 pp.

Biais G, Coupeau Y, Séret B, Calmettes B, Lopez R, Hetherington S, Righton D (2017) Return migration patterns of porbeagle shark (Lamna nasus) in the Northeast Atlantic: implications for stock range and structure. ICES J Mar Sci 74: 1268–1276

Bleil M, Oeberst R, Urrutia P (2009) Seasonal maturity development of Baltic cod in different spawning areas: importance of the Arkona Sea for the summer spawning stock. J Appl Ichthyol 25: 10–17

Block BA, Teo SLH, Walli A, Boustany A, Stokesbury MJW, Farwell CJ, Weng KC, Dewar H, Williams TD (2005) Electronic tagging and population structure of Atlantic bluefin tuna. Nature 434: 1121–1127

Bolle LJ, Hunter E, Rijnsdorp AD, Pastoors MA, Metcalfe JD, Reynolds JD (2005) Do tagging experiments tell the truth? Using electronic tags to evaluate conventional tagging data. ICES Journal of Marine Science, Volume 62, Issue 2, 2005, Pages 236–246, https://doi.org/10.1016/j.icesjms.2004.11.010

Braun CD, Galuardi B, Thorrold SR (2018) HMMoce : An R package for improved geolocation of archival-tagged fishes using a hidden Markov method. Methods Ecol Evol 9: 1212–1220

Braun CD, Gaube P, Afonso P, Fontes J, Skomal GB, Thorrold SR (2019) Assimilating electronic tagging, oceanographic modelling, and fisheries data to estimate movements and connectivity of swordfish in the North Atlantic. ICES J Mar Sci. doi: 10.1093/icesjms/fsz106

Braun CD, Skomal GB, Thorrold SR, Berumen ML (2015) Movements of the reef manta ray (Manta alfredi) in the Red Sea using satellite and acoustic telemetry. Mar Biol 162: 2351–2362

Buckmeier DL, Reeves KS (2012) Retention of Passive Integrated Transponder, T-Bar Anchor, and Coded Wire Tags in Lepisosteids. North Am J Fish Manag 32: 573–576

Cadrin SX, Secor DH (2009) Accounting for Spatial Population Structure in Stock Assessment: Past, Present, and Future. *In* RJ Beamish, BJ Rothschild, eds, Future Fish. Sci. N. Am. Springer Netherlands, Dordrecht, pp 405–426

Campana SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J Fish Biol 59: 197–242

Campana SE (1999) Chemistry and composition of fish otoliths: pathways, mechanisms and applications. Mar Ecol Prog Ser 188: 263–297

Cardinale M, Svedäng H (2011) The beauty of simplicity in science: Baltic cod stock improves rapidly in a ‘cod hostile’ ecosystem state. Mar Ecol Prog Ser 425: 297–301

Casini M, Käll F, Hansson MV, Plikshs M, Baranova T, Karlsson OM, Lundström K, Neuenfeldt S, ardmark AG, Hjelm J (2016) Hypoxic areas, density-dependence and food limitation drive the body condition of a heavily exploited marine fish predator. R Soc Open Sci. doi: 10.6084/m9.figshare.c.3518970.v1

Cooke SJ, Hinch S, Lucas MC, Lutcavage M (2012) Biotelemetry and biologging. Fish. Tech. 3rd Ed Zale Parrish T Sutton Eds. American Fisheries Society, Bethesda, Maryland, pp 819–881

Cooke SJ, Hinch SG, Crossin GT, Patterson DA, English KK, Healey MC, Shrimpton JM, Van Der Kraak G, Farrell AP (2006) Mechanistic basis of individual mortality in Pacific salmon during spawning migrations. Ecology 87: 1575–1586

Dingle, H., and Drake, V. A. 2007. What Is Migration? BioScience, 57: 113–121.

Dingle H (2014) Migration: The Biology of Life on the Move, 2. Edition. Oxford University Press, Oxford ; New York

Eero M, Hemmer-Hansen J, Hüssy K (2014) Implications of stock recovery for a neighbouring management unit: experience from the Baltic cod. ICES J Mar Sci 71: 1458–1466

Eero M, Hjelm J, Behrens J, Buchmann K, Cardinale M, Casini M, Gasyukov P, Holmgren N, Horbowy J, Hüssy K, et al (2015) Eastern Baltic cod in distress: biological changes and challenges for stock assessment. ICES J Mar Sci 72: 2180–2186

Eero M, Vinther M, Haslob H, Huwer B, Casini M, Storr-Paulsen M, Köster FW (2012) Spatial management of marine resources can enhance the recovery of predators and avoid local depletion of forage fish. Conserv Lett 5: 486–492

Engelhardt J, Frisell O, Gustavsson H, Hansson T, Sjöberg R, Collier TK, Balk L (2020) Severe thiamine deficiency in eastern Baltic cod (Gadus morhua). PLOS ONE 15: e0227201

Eveson JP, Basson M, Hobday AJ (2012) Using electronic tag data to improve mortality and movement estimates in a tag-based spatial fisheries assessment model. Can J Fish Aquat Sci 69: 869–883

Galuardi B, Lam CH (Tim) (2014) Telemetry Analysis of Highly Migratory Species. Stock Identif. Methods. Elsevier, pp 447–476

Gleiss AC, Schallert RJ, Dale JJ, Wilson SG, Block BA (2019) Direct measurement of swimming and diving kinematics of giant Atlantic bluefin tuna (Thunnus thynnus). R Soc Open Sci 6: 190203

Guy CS, Blankenship HL, Nielsen LA (1996) Tagging and marking. Pages 353–383 in B. R. Murphy and D. W. Willis, editors. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Hall DA (2014) Chapter Sixteen - Conventional and Radio Frequency Identification (RFID) Tags. *In* SX Cadrin, LA Kerr, S Mariani, eds, Stock Identif. Methods Second Ed. Academic Press, San Diego, pp 365–395

Hemmer-Hansen J, Hüssy K, Baktoft H, Huwer B, Bekkevold D, Haslob H, Herrmann J, Hinrichsen H, Krumme U, Mosegaard H, et al (2019) Genetic analyses reveal complex dynamics within a marine fish management area. Evol Appl 12: 830–844

Hilborn R (1990) Determination of Fish Movement Patterns from Tag Recoveries using Maximum Likelihood Estimators. Can J Fish Aquat Sci 47: 635–643

Hooten MB, Johnson DS, Morales JM, McClintock BT, eds (2017) Animal movement: statistical models for telemetry data. CRC Press/Taylor & Francis Group, Boca Raton

Hunter E, Aldridge JN, Metcalfe JD, Arnold GP (2003) Geolocation of free-ranging fish on the European continental shelf as determined from environmental variables: I. Tidal location method. Mar Biol 142: 601–609

Hunter E, Berry F, Buckley AA, Stewart C, Metcalfe JD (2006) Seasonal migration of thornback rays and implications for closure management. J Appl Ecol 43: 710–720

Hussey NE, Kessel ST, Aarestrup K, Cooke SJ, Cowley PD, Fisk AT, Harcourt RG, Holland KN, Iverson SJ, Kocik JF, et al (2015) Aquatic animal telemetry: A panoramic window into the underwater world. Science 348: 1255642–1255642

Hüssy K (2010) Why is age determination of Baltic cod (Gadus morhua) so difficult? ICES J Mar Sci 67: 1198–1205

Hüssy K, Casini M, Haase S, Hilvarsson A, Horbowy J, Krüger-Johnsen M, Krumme U, Limburg KE, McQueen K, Mion M, et al (2020) Tagging Baltic Cod – TABACOD. Eastern Baltic cod: Solving the ageing and stock assessment problems with combined state-of-the-art tagging methods. 64

Hüssy K, Radtke K, Plikshs M, Oeberst R, Baranova T, Krumme U, Sjöberg R, Walther Y, Mosegaard H (2016) Challenging ICES age estimation protocols: lessons learned from the eastern Baltic cod stock. ICES J Mar Sci 73: 2138–2149

ICES (2020a) Cod (Gadus morhua) in subdivisions 22?24, western Baltic stock (western Baltic Sea). doi: 10.17895/ICES.ADVICE.5942

ICES (2006) Report of the Study Group on Ageing Issues of Baltic Cod (SGABC), 16–19 May 2006, Gdynia, Poland. ICES CM 2006/ BCC:08. 45 pp.

ICES (2015) Report of the Benchmark Workshop on Baltic Cod stocks (WKBALTCOD), 2-6 March 2015, Rostock, Germany. ICES CM 2015/ACOM:35. ICES Sci Rep 172 pp.

ICES (2020b) Baltic Fisheries Assessment Working Group (WGBFAS). ICES Scientific Reports. 2:45. 643 pp. http://doi.org/10.17895/ices.pub.6024.

Jadot C (2003) Comparison of two tagging techniques for Sarpa salpa: external attachment and intraperitoneal implantation. Oceanol Acta 26: 497–501

Jepsen N, Thorstad EB, Havn T, Lucas MC (2015) The use of external electronic tags on fish: an evaluation of tag retention and tagging effects. Anim Biotelemetry 3: 49

Jewell OJD, Gleiss AC, Jorgensen SJ, Andrzejaczek S, Moxley JH, Beatty SJ, Wikelski M, Block BA, Chapple TK (2019) Cryptic habitat use of white sharks in kelp forest revealed by animal-borne video. Biol Lett 15: 20190085

Kays R, Crofoot MC, Jetz W, Wikelski M (2015) Terrestrial animal tracking as an eye on life and planet. Science. doi: 10.1126/science.aaa2478

Kiørboe T, Visser A, Andersen KH (2018) A trait-based approach to ocean ecology. ICES J Mar Sci 75: 1849–1863

Koehn JD, Crook DA (2013) Movement and Migration, In, Ecology of Australian Freshwater Fishes, Humphries, P and Walker, K. (eds), pp 105-129, CSIRO Publishing, Victoria, Australia.

Kohler NE, Turner PA (2001) Shark Tagging: A Review Of Conventional Methods and Studies. Environ Biol Fishes 60: 191–224

Köster F, Vinther M, MacKenzie B, Eero M, Plikshs M (2009) Environmental effects on recruitment and implications for biological reference points of Eastern Baltic cod (Gadus morhua). J Northwest Atl Fish Sci 41: 205–220

Köster FW, Möllmann C, Hinrichsen H-H, Wieland K, Tomkiewicz J, Kraus G, Voss R, Makarchouk A, MacKenzie BR, St. John MA, et al (2005) Baltic cod recruitment – the impact of climate variability on key processes. ICES J Mar Sci 62: 1408–1425

Krumme U, Stötera S, McQueen K, Pahlke E (2020) Age validation of age 0-3 wild cod Gadus morhua in the western Baltic Sea through mark-recapture and tetracycline marking of otoliths. Mar Ecol Prog Ser 645: 141–158

Kurlansky M (1998) Cod: A Biography of the Fish that Changed the World, Reprint Edition. Penguin Books, New York

Kurota HK, McAllister MKMK, Lawson GLLL, Nogueira JINI, Teo SLHTLH, Block BABA (2009) A sequential Bayesian methodology to estimate movement and exploitation rates using electronic and conventional tag data: application to Atlantic bluefin tuna (Thunnus thynnus). Can J Fish Aquat Sci. doi: 10.1139/F08-197

Lane N (2010) Life Ascending: The Ten Great Inventions of Evolution, Reprint Edition. W W NORTON & CO, New York

Le Bris A (2014) Mechanisms and consequences of variation in the migratory behaviour of Atlantic cod (Gadus morhua L.) in the northern Gulf of St. Lawrence. doctoral. Memorial University of Newfoundland

Le Bris A, Fréchet A, Galbraith PS, Wroblewski JS (2013) Evidence for alternative migratory behaviours in the northern Gulf of St Lawrence population of Atlantic cod (Gadus morhua L.). ICES J Mar Sci 70: 793–804

Lewis AE, Muntz WRA (1984) The effects of external ultrasonic tagging on the swimming performance of rainbow trout, Salmo gairdneri Richardson. J Fish Biol 25: 577–585

Lindholm J, Auster P (2003) Site Utilization by Atlantic Cod (*Gadus morhua*) in Off-Shore Gravel Habitat as Determined by Acoustic Telemetry: Implications for the Design of Marine Protected Areas. Mar Technol Soc J 37: 27–34

Liu C (2019) Geolocation Methods for Demersal Fish Species Using Archival Tagging Data. The School for Marine Science and Technology, Department of Fisheries Oceanography

Liu C, Cowles GW, Zemeckis DR, Cadrin SX, Dean MJ (2017) Validation of a hidden Markov model for the geolocation of Atlantic cod. Can J Fish Aquat Sci 74: 1862–1877

Lucas MC, Baras E (2000) Methods for studying spatial behaviour of freshwater fishes in the natural environment. Fish Fish 1: 283–316

Mackenzie BR, Gislason H, Möllmann C, Köster FW (2007) Impact of 21st century climate change on the Baltic Sea fish community and fisheries. Glob Change Biol 13: 1348–1367

McQueen K, Casini M, Dolk B, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Mion M, Mohr T, Radtke K, et al (2020) Regional and stock-specific differences in contemporary growth of Baltic cod revealed through tag-recapture data. ICES J Mar Sci fsaa104

McQueen K, Hrabowski J, Krumme U (2019) Age validation of juvenile cod in the Western Baltic Sea. ICES J Mar Sci 76: 430–441

Metcalfe JD, Arnold GP (1997) Tracking fish with electronic tags. Nature 387: 665–666

Mohrholz V (2018a) Major Baltic Inflow Statistics – Revised. Front Mar Sci 5: 384

Mohrholz V (2018b) Baltic saline barotropic inflows (SBI) 1887 - 2018. doi: 10.12754/data-2018-0004

Nathan R, Getz WM, Revilla E, Holyoak M, Kadmon R, Saltz D, Smouse PE (2008) A movement ecology paradigm for unifying organismal movement research. Proc Natl Acad Sci 105: 19052–19059

Naumann M, Gräwe U, Mohrholz V, Kuss J, Kanwischer M, Feistel S, Hand I, Waniek JJ, Schulz-Bull DE (2020) Hydrographic-hydrochemical assessment of the Baltic Sea 2019. Meereswissenschaftliche Berichte No 114 2020 - Mar Sci Rep No 114 2020 13,1 MB, 97 pages

Nentwig W, Bacher S, Brandl R (2017) Ökologie kompakt, 4., korrigierte Auflage. Springer Spektrum, Berlin

Neuenfeldt S, Hinrichsen H-H, Nielsen A, Andersen KH (2007) Reconstructing migrations of individual cod (Gadus morhua L.) in the Baltic Sea by using electronic data storage tags. Fish Oceanogr 16: 526–535

Nielsen A, Bigelow KA, Musyl MK, Sibert JR (2006) Improving light-based geolocation by including sea surface temperature. Fish Oceanogr 15: 314–325

Nielsen J, Mueter, F.J., Adkison, M.D. (2019) Effect of study area bathymetric heterogeneity on parameterization and performance of a depth-based geolocation model for demersal fishes.

Nielsen JK (2019) Multi-scale movement of demersal fishes in Alaska. Thesis

Pedersen MW, Righton D, Thygesen UH, Andersen KH, Madsen H (2008) Geolocation of North Sea cod (Gadus morhua) using hidden Markov models and behavioural switching. Can J Fish Aquat Sci 65: 2367–2377

Pine WE, Hightower JE, Coggins LG, Lauretta MV, Pollock KH (2012) Design and Analysis of Tagging Studies. Zale AV Parrish DL Sutton TM Eds Fish. Tech., 3rd ed. American Fisheries Society, Bethesda, MD, pp 521–572

Pinti JPA (2020) Vertical Migration: Structure and function of pelagic ecosystems.

Plonus R, McQueen K, Günther C, Funk S, Temming A, Krumme U (2021) Is analysis of otolith microstructure a valid method for investigating early life history of Western Baltic cod? Mar Biol 168: 30

Reusch TBH, Dierking J, Andersson HC, Bonsdorff E, Carstensen J, Casini M, Czajkowski M, Hasler B, Hinsby K, Hyytiäinen K, et al (2018) The Baltic Sea as a time machine for the future coastal ocean. Sci Adv 4: eaar8195

Righton D, Mills CM (2008) Reconstructing the movements of free-ranging demersal fish in the North Sea: a data-matching and simulation method. Mar Biol 153: 507–521

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

Rude NP, Whitledge GW, Phelps QE, Hirst S (2011) Long-Term PIT and T-Bar Anchor Tag Retention Rates in Adult Muskellunge. North Am J Fish Manag 31: 515–519

Savchuk OP (2018) Large-Scale Nutrient Dynamics in the Baltic Sea, 1970–2016. Front Mar Sci. doi: 10.3389/fmars.2018.00095

Schade FM, Weist P, Krumme U (2019) Evaluation of four stock discrimination methods to assign individuals from mixed-stock fisheries using genetically validated baseline samples. Mar Ecol Prog Ser 627: 125–139

Seitz AC, Norcross BL, Wilson D, Nielsen JL (2006) Evaluating light-based geolocation for estimating demersal fish movements in high latitudes. Fish Bull 104: 571578

Sequeira AMM, Heupel MR, Lea M -A., Eguíluz VM, Duarte CM, Meekan MG, Thums M, Calich HJ, Carmichael RH, Costa DP, et al (2019) The importance of sample size in marine megafauna tagging studies. Ecol Appl. doi: 10.1002/eap.1947

Sibert JR, Musyl MK, Brill RW (2003) Horizontal movements of bigeye tuna (Thunnus obesus) near Hawaii determined by Kalman filter analysis of archival tagging data. Fish Oceanogr 12: 141–151

Sick K (1965) Haemoglobin Polymorphism of Cod in the Baltic and the Danish Belt Sea. Hereditas 54: 19–48

Sippel T, Paige Eveson J, Galuardi B, Lam C, Hoyle S, Maunder M, Kleiber P, Carvalho F, Tsontos V, Teo SLH, et al (2015) Using movement data from electronic tags in fisheries stock assessment: A review of models, technology and experimental design. Fish Res 163: 152–160

Smith NJ, McCall PL, Sutton TM (2017) Effects of different tagging protocols on survival, growth, and tag retention in juvenile least cisco Coregonus sardinella. Fish Res C: 68–72

Snoeijs-Leijonmalm P, Andrén E (2017) Why is the Baltic Sea so special to live in? Springer, pp 23–84

Snoeijs-Leijonmalm P, Schubert H, Radziejewska T, eds (2017) Biological Oceanography of the Baltic Sea. doi: 10.1007/978-94-007-0668-2

Sokolova M, Buchmann K, Huwer B, Kania P, Krumme U, Galatius A, Hemmer-Hansen J, Behrens J (2018) Spatial patterns in infection of cod Gadus morhua with the seal-associated liver worm Contracaecum osculatum from the Skagerrak to the central Baltic Sea. Mar Ecol Prog Ser 606: 105–118

Stroganov AN, Bleil M, Oeberst R, Semenova AV, Winkler H (2017) First evidence of spawning of eastern Baltic cod (Gadus morhua callarias) in the Belt Sea, the main spawning area of western Baltic cod (Gadus morhua L.). J Appl Ichthyol 34: 527–534

Svedäng H, Righton D, Jonsson P (2007) Migratory behaviour of Atlantic cod Gadus morhua: natal homing is the prime stock-separating mechanism. Mar Ecol Prog Ser 345: 1–12

Taylor NG, McAllister MK, Lawson GL, Carruthers T, Block BA (2011) Atlantic Bluefin Tuna: A Novel Multistock Spatial Model for Assessing Population Biomass. PLOS ONE 6: e27693

Teo S, Boustany A, Blackwell S, Walli A, Weng K, Block B (2004) Validation of geolocation estimates based on light level and sea surface temperature from electronic tags. Mar Ecol Prog Ser 283: 81–98

Thorstad EB, Rikardsen AH, Alp A, Okland F (2014) The Use of Electronic Tags in Fish Research – An Overview of Fish Telemetry Methods. Turk J Fish Aquat Sci. doi: 10.4194/1303-2712-v13_5_13

Thorsteinsson V, Pálsson Ó, Tómasson G, Jónsdóttir I, Pampoulie C (2012) Consistency in the behaviour types of the Atlantic cod: repeatability, timing of migration and geo-location. Mar Ecol Prog Ser 462: 251–260

Thurow F (1963) Dorschmarkierungen in der Westlichen Ostsee. Informationen Aus Fisch. Inf. Fish. Res. 10, 12–17.

Thygesen UH (2009) Geolocating fish using Hidden Markov Models and Data Storage Tags. Rev Methods Technol Fish Biol Fish 277–293

Weist P, Schade FM, Damerau M, Barth JMI, Dierking J, André C, Petereit C, Reusch T, Jentoft S, Hanel R, et al (2019) Assessing SNP-markers to study population mixing and ecological adaptation in Baltic cod. PLOS ONE 14: e0218127

Woillez M, Fablet R, Ngo T-T, Lalire M, Lazure P, de Pontual H (2016) A HMM-based model to geolocate pelagic fish from high-resolution individual temperature and depth histories: European sea bass as a case study. Ecol Model 321: 10–22

Wulff F, Stigebrandt A, Rahm L (1990) Nutrient Dynamics of the Baltic Sea. Ambio 19: 126–133

Young SP, Isely JJ (2004) Temporal and spatial estimates of adult striped bass mortality from telemetry and transmitter return data. North Am J Fish Manag 24: 8

Zemeckis DR, Dean MJ, DeAngelis AI, Van Parijs SM, Hoffman WS, Baumgartner MF, Hatch LT, Cadrin SX, McGuire CH (2019) Identifying the distribution of Atlantic cod spawning using multiple fixed and glider-mounted acoustic technologies. ICES J Mar Sci 76: 1610–1625

Zemeckis DR, Liu C, Cowles GW, Dean MJ, Hoffman WS, Martins D, Cadrin SX (2017) Seasonal movements and connectivity of an Atlantic cod (Gadus morhua) spawning component in the western Gulf of Maine. ICES J Mar Sci 74: 1780–1796

Zweifel U, Laamanen M (2009) Biodiversity in the Baltic Sea – An integrated thematic assessment on biodiversity and nature conservation in the Baltic Sea. undefined, /paper/Biodiversity-in-the-Baltic-Sea-%E2%80%93-An-integrated-on-Zweifel-Laamanen/f0f79b50ae98ed407fdef9a26d59c308b71fd6e8

Chapter 1

Short-term tagging mortality of Baltic cod (*Gadus morhua*)

Stefanie Haase[1,*], Kate McQueen[1,*], Monica Mion[2], Magnus Andersson[2], Annelie Hilvarsson[2], Hans Jakob Olesen[3], Anders Svenson[2], Michele Casini[2,4], Karin Hüssy[3], Krzysztof Radtke[5], Uwe Krumme[1]

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

[2]Department of Aquatic Resources, Institute of Marine Research, Swedish University of Agricultural Sciences, Turistgatan 5, 45330 Lysekil, Sweden.

[3]National Institute of Aquatic Resources, Technical University of Denmark, Kemitorvet, 2800 Kgs. Lyngby, Denmark.

[4]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Selmi 3, Bologna, Italy.

[5]National Marine Fisheries Research Institute, Kołłątaja 1, 81-332 Gdynia, Poland.

[*]Equal authorship

This chapter has already been published in Fisheries Research:

Haase S, McQueen K, Mion M, Andersson M, Hilvarsson A, Olesen HJ, Svenson A, Casini M, Hüssy K, Radtke K, Krumme U (2021) Short-term tagging mortality of Baltic cod (*Gadus morhua*). Fish Res 234:105804, DOI:10.1016/j.fishres.2020.105804

Abstract

Tagging-induced mortality experiments are an important component of mark-recapture studies, as they can be used to assess the appropriateness of the tagging methodology, and to improve the reliability of estimates of recapture rates used for calculations of mortality rates and population size. Here, short-term tagging mortality of Baltic cod was estimated through containment experiments in the southern Baltic Sea. Experimental cod were selected from trawl catches, and approximately half were tagged externally with T-bar tags and received an intraperitoneal injection of tetracycline-hydrochloride. The rest of the experimental cod formed the control group, and received neither tag nor injection. The tagged and control cod were mixed evenly within submersible cages, and held for 5-8 days. The experiments were conducted in different regions and during different months by different tagging teams. Overall mortality rate was 16% (n = 324), with the mortality rate of the tagged group 19%, and the mortality rate of the control group 13%. A general linear mixed model was fit to assess the effect of tagging, month, experiment duration, fish length and tagging site (i.e. the combined effect of region and tagging team) on mortality. Tagging had no effect on mortality, indicating that mortality can be attributed mainly to the capture and handling procedure. There was a significantly negative relationship between fish length (range: 20-55 cm) and mortality. Mortality did not differ between the months tested, but there was a significant effect of tagging site on mortality. Tagging-related mortality should be accounted for in analyses of data from mark-recapture studies of Baltic cod, and some variability in mortality between tagging sites can be expected.

Keywords: mark-recapture, T-bar, tetracycline-hydrochloride, Baltic Sea cod, post-release survival

Introduction

Mark-recapture studies can be an effective method of gaining information on the population size, total, natural and fishing mortality rates (Pine et al., 2003), individual growth rates (Fabens, 1965) and movement patterns (Hilborn, 1990) of fish within a stock. Short-term tagging mortality experiments are an important component of an effective mark-recapture study involving conventional tagging, to estimate the proportion of fish that die soon after release due to direct effects of the tagging process (e.g. stress of capture, handling and tagging) (Brattey and Cadigan, 2004). Given the ethical considerations associated with field research on live fish (Bennett et al., 2016), short-term mortality experiments can be used to ensure that the tagging method has minimal influence on the survival of the fish, and to determine the optimum gear type and season of tagging (Brattey and Cadigan, 2004). Estimation of short-term tagging mortality rates is also key to avoiding bias in estimates of population size and mortality rate calculated from recapture rate (Brownie and Robson, 1983).

Between 2016-2019, >25000 cod (*Gadus morhua*) in the southern Baltic Sea were tagged and released as part of the large-scale, international tagging project “TAgging BAltic COD” (TABACOD, Hüssy et al., 2020). Deteriorating body condition, decreasing relative abundance of large fish, and diminished spatial distribution range observed in the Eastern Baltic cod stock in recent years is a fisheries management concern (Eero et al., 2015). A lack of reliable age data has hindered the estimation of growth and mortality rates, which contributed to the suspension of the age-based analytical stock assessment in 2014 (Eero et al., 2015). The aims of the TABACOD tagging project were to gain new information on the growth, otolith formation and movements of the Eastern Baltic cod stock, and to estimate fishing and natural mortality rates independently from the stock assessment. The cod for the TABACOD tagging project were mainly caught by bottom trawl, injected with tetracycline-hydrochloride (an antibiotic which induces a permanent mark in fish otoliths) and tagged with external T-bar anchor tags (Hüssy et al., 2020).

Several studies have previously estimated short-term mortality rates of tagged Atlantic cod, for example in Newfoundland (Brattey and Cadigan, 2004) and the western Baltic Sea (Kock, 1975a, Stötera et al., 2019; Weltersbach and Strehlow, 2013). A variety of capture methods have been used in these studies, which can significantly influence the survival probability of cod (Weltersbach and Strehlow, 2013). Tagging procedure also varied between studies, with

Brattey and Cadigan (2004) and Weltersbach and Strehlow (2013) applying T-bar anchor tags, Kock (1975a) applying spaghetti and Carlin tags, and Stötera et al. (2019) using both T-bar anchor tags and intraperitoneal injection of tetracycline-hydrochloride and/or strontium chloride. The reported short-term mortality estimates also ranged widely, even within the Baltic Sea, from 0 % (Kock, 1975a) to 25.7 % (Weltersbach and Strehlow, 2013), probably due to the diversity of the capture and tagging methods used. Given the variability in estimates from previous studies, and with the potential for several variables (e.g. tagging procedure, capture gear, depth and temperature) to influence the mortality rates, it was considered valuable to conduct further short-term mortality experiments under the conditions specific to the recent tagging study of Eastern Baltic cod.

The aim of this study was to estimate the short-term mortality rates of cod trawled in the southern Baltic Sea and tagged with T-bar anchor tags and intraperitoneal injection of tetracycline-hydrochloride. Additionally, the influence of fish length, month of capture, experiment duration and the cumulative effects of capture region and tagging team specific procedural differences were investigated as possible factors contributing to the variability in short-term mortality rates.

Materials and Methods

Experimental design and data collection

The study design was adapted from containment studies (Pollock and Pine, 2007), similar to those conducted to estimate the short-term catch-and-release mortality associated with recreational angling for Baltic cod (Weltersbach and Strehlow, 2013) and short-term tagging mortality of Atlantic cod (Brattey and Cadigan, 2004). Cod were captured, handled, and tagged using the same methods as in the international tagging project “TABACOD” (Hüssy et al., 2020). The experiments were carried out by trained scientists and technicians coordinated by three different experimental coordinators, on-board three separate research vessels, thus forming three “tagging teams” (A, B and C). This approach was taken to reflect some of the variability in capture location, procedure and handling on-board of different vessels, which is unavoidable in an international tagging study.

Cod were caught in the Arkona and Bornholm Basin regions of the southern Baltic Sea (ICES subdivisions 24 and 25, REGULATION EC 218/2009), with bottom trawls (OTB TV3-520, OTB 300/60) of short duration (5-30 minutes). CTD casts performed close to the catch locations shortly before or after the trawls, for all experiments except experiment 7, provided information on the water temperature of the entire water column. Temperatures at surface and fishing depth are presented in Table 1. Immediately after catch, cod were transferred to a tank on board which was supplied with an inflow of fresh, surface seawater. Individuals without external signs of injury or illness (e.g. abrasions, bleeding, and barotrauma) were randomly selected from the catch and measured and weighed. The implementation of this selection procedure ensures that the estimates of mortality from these experiments reflect the mortality of cod selected for inclusion in the tagging project, but cannot provide insight into the general mortality rates of Baltic cod caught by trawl. Where possible, fish representing the full range of length classes available from the catch were selected. Total fish lengths included in the experiment ranged from 20 cm to 55 cm (Fig. 1). The length range of cod available from the catches is fairly typical for the size-truncated EBC stock, where catches in recent years have been dominated by fish <45 cm (ICES, 2020).

Cod selected for the tagged group were tagged with a T-bar anchor tag (Hallprint TBA) at the base of the first dorsal fin. They were then laid on their dorsal side and a solution of 10 mg/ml tetracycline was administered, using a syringe that was inserted at a shallow angle into the body cavity, approximately at the end of the pelvic fin tips. They received 100 mg tetracycline-hydrochloride per kg wet mass of cod (Stötera et al., 2019). Tagged cod were returned to a tank on board to check for immediate recovery from the tagging procedure, before they were transferred to the experimental cages. Individuals for the control group were handled in the same way, but received neither an injection nor a tag and thus had no individual mark. Handling of individual fish generally took 1-2 minutes, and time spent on-board between catch and transfer to the cages lasted about 1 hour.

Experimental cod were placed in cages, which differed between the tagging teams. Team A used cages with dimensions 150 cm x 100 cm x 120 cm. Team B used cages with dimensions 120 cm x 80 cm x 100 cm. Team C used a round cage with diameter 150 cm and height 55 cm, and a square cage with dimensions 130 cm x 130 cm x 52 cm. Depending on the size of the individuals and the cage, 3-16 (mean = 7) cod were placed in each cage, with cod placed in cages with individuals of a similar size, to reduce the risk of cannibalism. Approximately equal numbers of control and treatment fish were placed in each cage. The cages were then lowered to the seafloor, at a depth similar to the capture depth. The duration of each experiment was 5-8 days (Table 1), dependent on weather conditions and practical constraints of research cruises.

In total, 415 cod caught between April and November 2017 were used for 9 containment experiments. A small subsample of cod (n = 24) were additionally surgically implanted with data storage tags (DSTs), as part of a qualitative investigation into the short-term survival and healing from this surgery. Another small subsample (n = 28) was treated for barotrauma by venting the gas-bladder with a hypodermic needle. These two groups were excluded from subsequent analysis as the sample sizes were too small for a quantitative analysis on the effects of these treatments on survival probability across the range of experimental conditions. Additionally, 23 cod escaped from the cages or were in cages that were lost and 16 cod were excluded because there was evidence that the cage was attacked by seals, which resulted in higher mortality. This resulted in 324 cod being included in this analysis (197, 80 and 47 handled by Tagging Team A, B and C, respectively). The tagging group and the control group included 168 and 156 individuals, respectively (Table 1).

Table 1: Overview of experimental set up, and overall mortality rate of cod for each experiment, sorted by date. Sample sizes and survival rates refer to cod included in the analysis. Water temperatures were measured with a CTD cast shortly before or after trawls.

Experiment Number	Deployment date	Tagging team	Number of cages	Fishing depth (m)	Surface water temperature (°C)	Water temperature at fishing depth (°C)	Number of tagged cod	Mean lengths (range) of tagged cod (cm)	Number of control cod	Mean lengths (range) of control cod (cm)	Experiment duration (days)	Mortality rate of tagged cod	Mortality rate of control cod
1	20.04.2017	A	11	21	6.6	6.0	24	40 (21-49)	27	37 (21-50)	7	0.17	0.07
2	11.05.2017	A	8	20	7.6	7.1	23	34 (20-49)	24	36 (25-47)	7	0.17	0.17
3	23.05.2017	B	4	43	10.5	6.0	24	32 (29-44)	24	32 (27-43)	5	0.13	0.0
4	01.06.2017	A	4	21	12.9	12.0	10	40 (29-49)	4	43 (38-47)	8	0.10	0.0
5	09.06.2017	A	4	34	13.4	11.8	12	36 (27-48)	9	35 (27-52)	6	0.17	0.22
6	15.06.2017	A	4	23-38	15.5	14.0	13	20 (26-35)	12	29 (25-35)	7	0.54	0.42
7	20.09.2017	C	5	53-60	-	-	26	32 (20-49)	21	33 (22-55)	5	0.27	0.29
8	07.11.2017	B	3	44	9.6	8.0	16	31 (26-43)	16	31 (27-36)	6	0.0	0.0
9	15.11.2017	A	4	20-23	9.4	9.4	20	39 (30-44)	19	38 (31-43)	5	0.2	0.05
Sum	20.4.-15.11.2017		42	20-60	6.6-15.5	6.0-14.0	168	35 (20-49)	156	34 (21-55)	5-8	0.19	0.13

The experiments were carried out during different months, with cod caught with bottom trawls from regions with different depths (Table 1). Team A trawled cod from depths ranging between 20-38 m (mean depth = 23.2 m) on the southern slopes of the Arkona Basin and deployed the experimental cages at a depth of 20m off the northern coast of the island of Rügen. Team B trawled cod from depths ranging between 43-44 m (mean depth = 43.4 m), and Team C trawled cod from depths ranging between 53-60 m (mean depth = 56.7 m). Team B and C both trawled and deployed their experimental cages at depths similar to capture on the northern and western slopes of the Bornholm Basin. The fishing areas and depths were within the regions commonly fished to collect cod for the TABACOD tagging project in the relatively shallow Arkona and Bornholm Basin regions of the southern Baltic (see Hüssy et al., 2020 for a map of TABACOD tagging locations).

The average condition factor of experimental cod ($Fulton's\ K = \frac{weight\ (g)}{length\ (cm)^3} * 100$) varied only slightly between sites, with the average (± standard deviation) condition of cod of Team A 0.97 ± 0.14, of Team B 0.84 ± 0.11, and of Team C 0.94 ± 0.13. The condition of 10 cod of Tagging Team A could not be estimated, as weight measurements were not recorded.

Data analysis

Two-sample Kolmogorov-Smirnov test was used to investigate differences in length frequency distributions of the control and treatment group. Chi-squared tests were conducted to check whether the proportion of experimental cod in the tagged and control group differed significantly between tagging teams and tagging months. Mortality (number died/sample size) of tagged and control cod was calculated separately, and for all experimental cod combined.

A generalized linear mixed effect model (GLMER) with a random intercept a_i was used to analyse the fixed effect of the categorical variables month (*month*) and tagging treatment (*treatment*: tagged/control), and the continuous variables total fish length (*TL*) and experiment duration (*ED*) on the survival of individual *j*. As condition factor could not be calculated for every experimental cod, this variable was not included in the model, to avoid reduction of the sample size. Due to the binary response variable, we chose a model with logit-link function. Site *i* was included as a random effect, being a combination of fishing depth, tagging team, gear and vessel. This variable was included as a random effect, as we wanted to account for potential unavoidable variability introduced by conducting the experiments across different tagging sites and teams, but quantifying these differences was not part of our research question.

The full model was:

$$Y_{ij} \sim Bin(1, p_{ij})$$

$$logit(p_{ij}) = \alpha + \beta_1 x\ month_{ij} + \beta_2\ x\ TL_{ij} + \beta_3\ x\ ED_{ij} + \beta_4\ x\ treatment_{ij} + a_i$$

Y_{ij} is 0 if individual *j* at site *i* died and 1 otherwise. We assumed that the random intercept a_i is normally distributed with mean 0 and variance σ_a^2. The parameters α and β are the intercept and slopes of the fixed effects, respectively. Stepwise backwards elimination of the predicting variables based on Akaike information criterion (AIC, Akaike (1973)) was used for model selection. Models with lower AIC are considered to have a better fit, with a ΔAIC > 2 considered to signify a significant difference between model fits. For models with ΔAIC < 2, the model with fewer predicting variables was selected as the most appropriate (Burnham and Anderson, 2002). Fitted values and predictor variables versus residuals were visually checked for dependence and obvious non-linear patterns.

All statistical analyses were performed in R version 3.5.1 (R Development Core Team, 2008) and the lme4 package (Bates et al., 2012).

Results

There were no differences between the proportions of experimental cod distributed between the two treatments (tagged vs. control) within the three tagging teams (chi-square = 0.34, df = 2, p = 0.85, Table S1) or the five tagging months (chi-square = 1.96, df = 4, p = 0.74, Table S2). The length distributions of the control and tagged groups were not significantly different (Kolmogorov-Smirnov test: D = 0.09, p = 0.53), with the mean length for the tagged fish (35±7 cm) slightly larger than the control group (34±7 cm, Figure 1).

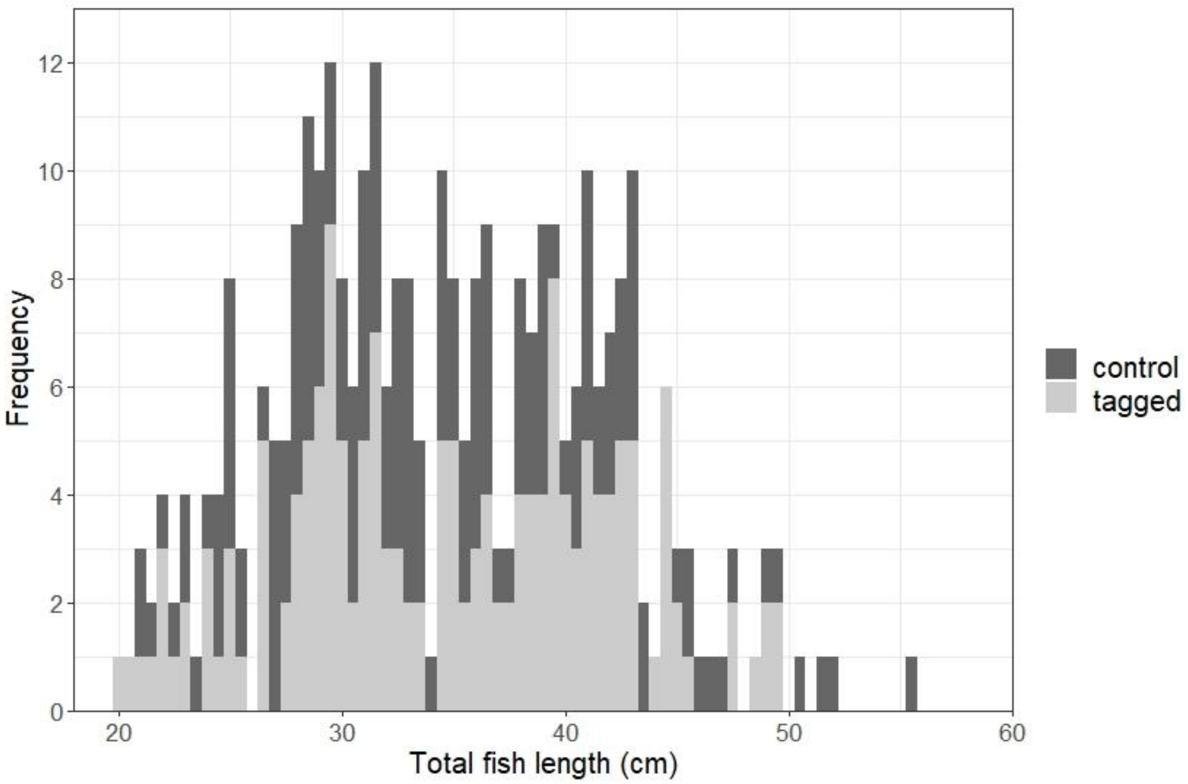

Figure 1: Cumulative length distribution of cod from the control group (black) and tagged with T-bars and injected with tetracycline-hydrochloride (grey).

The proportion of cod that died from the control and tagged groups combined was 0.16 (s.e. ± 0.02). Mortality rate of the control group was 0.13 (s.e. ± 0.03) and of the tagged group 0.19 (s.e. ± 0.03). Two (0.08) of the DST-tagged cod and 9 (0.32) of the cod treated for barotrauma died during the experiment.

Factors influencing mortality

The full model estimated β_1, the effect of month, as 0.06 (95% CI: -1.12-1.24) for May, 0.70 (95% CI: -0.38-1.77) for June, 0.88 (95% CI: -1.41-3.16) for September, and -0.45 (95% CI: -2.02-1.12) for November relative to April, respectively. β_2, the effect of total fish length, was estimated as -0.09 (95% CI: -0.14-0.04), β_3, the effect of experiment duration, as -0.16 (95% CI: -0.73-0.42), β_4, the effect of treatment, as 0.50 (95% CI: -0.15-1.15) for a tagged fish relative to an untagged fish, and α, the intercept as 1.49 (95% CI: -2.89-5.86). According to the binomial GLMER with tagging site as a random effect, including fish length as a fixed effect, provided the best fit to the data ($AIC_{\text{full model}}$ = 268.06; $AIC_{\text{final model}}$ = 263.49, Table 2). None of the other variables (treatment, month, experiment duration) significantly improved model fit. The probability of being dead at the end of the experiments decreased with increasing fish length (Figure 2), with the predicted probability of mortality decreasing from 0.35 (95% CI: 0.42-0.14) for a 20 cm cod, to 0.02 (95% CI: 0.05-0.0) for a 55 cm cod. The estimated among-site variance σ_a^2 was 0.91 indicating rather large variation in mortality between sites.

Table 2: AIC values, degrees of freedom (df) and deviance used to select the best-fitting GLMER. The final model is marked in bold.

Fixed effects included	df	AIC	deviance
month, treatment, length, experiment duration	9	268.1	250.1
month, treatment, length	8	266.3	250.3
treatment, length	4	262.8	254.8
length	**3**	**263.5**	**257.5**
intercept only	2	281.9	277.9

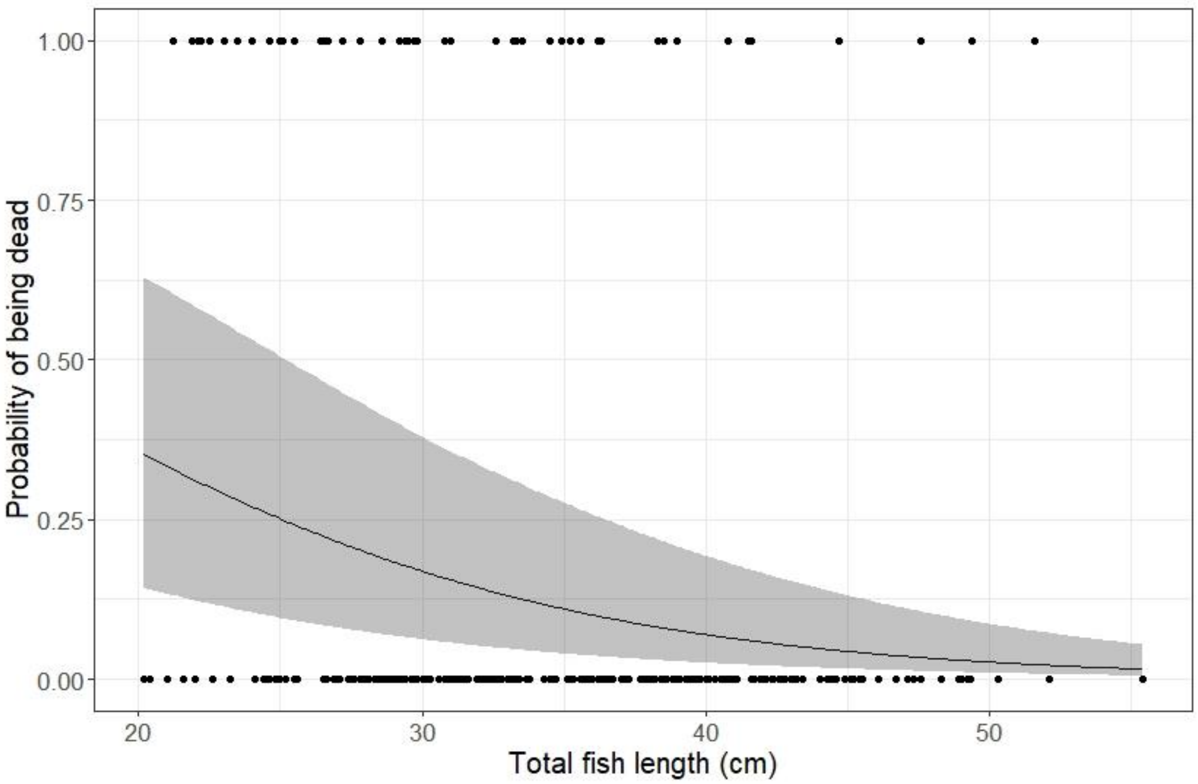

Figure 2: Mortality rate of experimental cod relative to total fish length (grey area indicating the 95% confidence intervals, black circles represent individual fish).

Discussion

Tagging with T-bar tags and injection with tetracycline-hydrochloride had no effect on mortality of Baltic cod selected from trawls as suitable for inclusion in a tagging study. However, the overall mortality of cod in these experiments, which can likely be attributed to the catch and handling procedure, was non-negligible and should be considered during analysis of data from the tagging project. The short-term (5-8 days) mortality rates of the tagged group of cod (excluding control fish) was 19.0%, which is between the reported short-term (10 days) mortality rates of tagged cod caught by pots (14.5%) and angling (25.7%) in a previous study on mortality rates of tagged Western Baltic cod (Weltersbach and Strehlow, 2013). Mortality rates of cod in the tagged group of the present study were higher than the short-term (1, 40 or 47 days) mortality rates reported for cod from the western Baltic Sea, captured in pound nets, and tagged with T-bar tags and the same concentrations of tetracycline as used here (4.0%, Stötera et al., 2019, and 5.0%, Krumme et al., 2020), and higher than the short-term (15-27 days) mortality of cod caught by trawl or gillnet from the western Baltic Sea in autumn and tagged with Carlin or spaghetti tags, which was negligible (Kock, 1975a). Overall short-term (5-10 days) mortality of tagged cod captured by various gears (hand-lines, Japanese cod traps, otter trawl, line trawls) off the coast of Newfoundland was also lower (11.8%, Brattey and Cadigan, 2004) than mortality rates estimated in this study. This variability in short-term tagging mortality rates between studies highlights the importance of estimating mortality rates that correspond to the project-specific tagging and handling procedure, gear type and area.

In the present study, there was no significant difference between the short-term mortality rates of tagged and control cod. These results agree with those of Brattey and Cadigan (2004), who found no difference in mortality rates between a control group of untagged cod and cod tagged with T-bar tags. The results differ slightly to those of Stötera et al. (2019), where injection with tetracycline-hydrochloride had a positive effect on survival rates of tagged cod, relative to a control group injected with a saline solution. In Stötera et al. (2019), experimental cod were reported to have visible injuries caused by cormorant (*Phalacrocorax carbo*) attacks and net abrasions from time spent in the cod-end of pound nets, and it was postulated that tetracycline may have effectively suppressed bacterial infections in injured cod. In the present study, cod had no external injuries and our control group received neither a tag nor an injection. This lack of previous injuries, additional handling and puncture wounds may counteract the relative advantage the treatment group gained through receiving a dose of antibiotics. Our findings indicate that the capture and handling procedures were responsible for the short-term mortality observed, implying that the additional stress of the tagging and injection procedures is negligible in terms of survival of tagged Baltic cod.

Although tagging had no effect on the mortality of Baltic cod, the best fitting GLMER indicated that the probability of mortality was not constant across all experimental fish. It is difficult to unambiguously explain the variation in mortality rates of experimental cod, due to the pre-selection of cod for the experiments. In tagging studies, only fish without visible external damage should be selected for tagging (Brattey and Cadigan, 2004), and the selection of apparently undamaged, healthy-looking fish for our experiments therefore allowed us to assess the mortality of cod included in the tagging study. However, the selection process may have introduced some bias, and this should be considered during interpretation of the results.

Fish length had a significantly negative effect on short-term mortality of experimental cod (tagged and control), within the length range used for our study (20-55 cm). This finding is in contrast to the results of previous experiments of short-term mortality of tagged cod, which found no relationship between cod length and mortality rates (Brattey and Cadigan, 2004). However, the length range of experimental cod in the Brattey and Cadigan (2004) study was 41-115 cm, which therefore does not include smaller fish which may be particularly vulnerable to the catch and handling procedures.

Although not directly comparable with the survival of cod selected for our mortality experiments, some previous studies indicate that mortality from trawling can be related to fish size in certain gadoid species.

Experiments on the survival of escaped haddock (*Melanogrammus aeglefinus*) (range: 12-61 cm) and whiting (*Merlangius merlangus*) (range: 17-35 cm) from trawl gears have indicated that mortality is higher for smaller individuals (Sangster et al., 1996, Ingólfsson et al., 2007). Poorer swimming ability causing exhaustion-related stress and injury during trawling has been proposed as a potential mechanism explaining the inverse relationship between size and mortality of haddock escaping from towed fishing gear (Ingólfsson et al., 2007). However, in the same study, the mortality of cod (range: 22-94 cm) and saithe (*Pollachius virens*) (range: 26-68 cm) that escaped from trawls was negligible (Ingólfsson et al., 2007). Stressors associated with trawling may have contributed to the higher mortality of smaller cod in our short-term tagging mortality experiment, but further research would be required to confirm this finding.

In the present study, there was significant variation in mortality rates between tagging sites. The tagging site variable combines the effects of region, including depth of capture, and tagging team, which represents research vessel specific equipment, personnel and procedures. These potentially interacting effects can unfortunately not be disentangled in this study. It has been demonstrated previously that depth of capture can influence survival of cod captured by hook and line (Ferter et al., 2015; Milliken et al., 2009). Barotrauma related injuries from rapid decompression increase with increasing capture depths, though high barotrauma recovery and survival rates for cod able to descend back to depth after a capture event have been reported (Ferter et al., 2015). Variability in the skill level and experience of the individuals carrying out the tagging in the different teams is also likely to contribute to variation in tagging mortality (Dicken et al., 2009; Hoyle et al., 2015). Additional sources of variability which may have influenced the mortality rates between experiments, but which were not addressed in our study, include trawl duration and catch size. Targeted experiments would need to be carried out to address the effects of these additional variables on cod survival. Although we cannot assess the relative importance of all these effects on mortality of the experimental cod, including these sources of variability more realistically reflects the conditions associated with the large-scale, international tagging study.

Our study did not reveal a significant influence of month of capture on mortality rates. This is contrary to previous short-term tagging mortality experiments which demonstrated that mortality was highest for cod kept in enclosures during summer or autumn, when water temperatures in the enclosures were warmer or fluctuated more (Brattey and Cadigan, 2004). A previous cod tagging study in the western Baltic Sea reported higher mortality rates in summer, which were attributed to the stress associated with bringing cod from below the thermocline to the surface (Kock, 1975a), and recapture rates of tagged Baltic cod have previously been reported to be lowest for cod tagged during summer (Netzel 1976). Higher mortality rates of cod captured by longline during the summer have also been reported, presumably due to the rapid changes in temperatures experienced while being hauled from cold bottom waters to surface waters that can be up to 10°C warmer (Milliken et al., 2009).

The lack of a significant influence of month on mortality of experimental cod in our study could be related to the observation that most experimental fish apparently would not have experienced large changes in temperature during catch, regardless of month. The Baltic Sea is stratified, with the seasonal thermocline beginning to form in May, and breaking down again in autumn (Snoeijs-Leijonmalm and Andrén, 2017). In 2017, the seasonal thermocline was at depths between 20-30m (Naumann et al., 2018). The fishing depths of Team A were therefore close to the thermocline depth, which is reflected in the similarities between temperatures recorded at the surface and at fishing depth for experiments carried out by this team (Table 1). The largest differences in temperature during Team A's experiments were recorded in June, though temperatures at fishing depth were still only 0.9-1.6°C colder than at the surface (Table 1). The temperature differences between surface and fishing depth were larger during the experiments of Team B, which were conducted at greater depths. In May, while the thermocline was present, the temperature at the surface was 3.5°C warmer than at fishing depth (experiment 3, Table 1), whereas in November, the temperature difference between surface and fishing depth was only 1.6°C (experiment 8, Table 1). Although no temperature data is available for experiment 7, it is expected that the seasonal thermocline would still be present in September, and as Team C fished at depths deeper than the thermocline, experimental cod would have crossed the thermocline during catch. Although not significant, the lowest survival rates recorded in this study were for cod captured in June and September (Table 1), during which experimental fish experienced the warmest temperatures or were likely hauled across the thermocline during catch.

The summer period also coincides with spawning time of Eastern Baltic cod (Köster et al., 2017; Wieland et al., 2000). Spawning-related exhaustion or physiological stress may also contribute to reduced survival of Baltic cod captured during this time, as was observed in experiments focusing on the short-term survival of trawled Western Baltic cod in on-board tanks (Kock, 1975b). As we released surviving cod at the end of the experiment, we were however unable to determine the sex and maturity of all experimental cod, and can therefore only speculate that this may have influenced survival. Nevertheless, given the current understanding of cod physiology, carrying out tagging studies in winter months, i.e. quarter 4 and quarter 1, when temperatures and solar radiation are low, the water column is more mixed, and eastern Baltic cod are not spawning, should increase the likelihood of survival.

In conclusion, these experiments indicate that the capture of Baltic cod by trawl and tagging-associated handling result in short-term mortality that should be accounted for in estimates of tag-recapture rates. Although mortality rates were higher for smaller individuals, it is generally important to cover a wide size-range in tagging studies, so that results are representative of as much of the stock as possible. Furthermore, although the inclusion of several tagging teams and sites complicated the interpretation of results, experiments including such sources of variation produce results which more realistically reflect the variable mortality associated with a large, international tagging study that is conducted by different tagging teams in different regions.

Acknowledgements

This study was funded by BalticSea2020 (http://balticsea2020.org) through the project "*Tagging Baltic Cod*" (TABACOD). Thanks go to all staff involved in conducting the experiments. We would like to thank Axel Temming for valuable feedback on the manuscript. We thank the two anonymous reviewers for their constructive comments. The experiments were conducted under the animal test permissions AZ 7221.3.1-029/15 and 016-15-0201-0092.

References

Akaike, H. (1973) Information theory as an extension of the maximum likelihood principle. Pages 267–281*in*B. N. Petrov and F. Csaki, editors. Second International Symposium on Information Theory. Akademiai Kiado, Budapest, Hungary.

Bates, D.M., Maechler, M, and Bolker, B.(2012) Lme4: Linear mixed-effect models using the S4 classes. R package version 0.999999-0.

Bennett, R.H., Ellender, B.R., Mäkinen, T., Miya, T., Pattrick, P., Wasserman, R.J., Woodford, D.J., Weyl, O.L.F. (2016) Ethical considerations for field research on fishes. Koedoe 58, 15 pp. https://doi.org/10.4102/koedoe.v58i1.1353

Brattey, J., Cadigan, N. (2004) Estimation of short-term tagging mortality of adult Atlantic cod (Gadus morhua). Fish. Res. 66, 223–233. https://doi.org/10.1016/S0165-7836(03)00203-0

Brownie, C., Robson, D.S. (1983) Estimation of Time-Specific Survival Rates from Tag-Resighting Samples: A Generalization of the Jolly-Seber Model. Biometrics 39, 437. https://doi.org/10.2307/2531015

Burnham, K. P. and Anderson D.R.(2002) Model selection and multimodel inference: a practical information-theoretic approach. Second edition. Springer-Verlag, New York, New York, USA.

Claireaux, G., Webber, D.M., Kerr, S.R., Boutilier, R.G. (1995) Physiology and behaviour of free-swimming Atlantic cod (*Gadus morhua*) facing fluctuating temperature conditions. J. Exp. Biol. 198, 49–60.

Davis, M.W., Ryer, C.H. (2003). Understanding Fish Bycatch Discard and Escapee Mortality. AFSC Quarterly Report, Seattle, 9 pp.

Dicken, M.L., Booth, A.J., Smale, M.J. (2009) Factors affecting recapture rates of raggedtooth sharks *Carcharias taurus* tagged off the east coast of South Africa. Afr. J. Mar. Sci. 31, 365–372. https://doi.org/10.2989/AJMS.2009.31.3.9.997

Eero, M., Hjelm, J., Behrens, J., Buchmann, K., Cardinale, M., Casini, M., Gasyukov, P., Holmgren, N., Horbowy, J., Hüssy, K., Kirkegaard, E., Kornilovs, G., Krumme, U., Köster, F.W., Oeberst, R., Plikshs, M., Radtke, K., Raid, T., Schmidt, J., Tomczak, M.T., Vinther, M., Zimmermann, C., Storr-Paulsen, M. (2015) Eastern Baltic cod in distress: biological changes and challenges for stock assessment. ICES J. Mar. Sci. J. Cons. 72, 2180–2186. https://doi.org/10.1093/icesjms/fsv109

Fabens, A.J. (1965) Properties and fitting of the von Bertalanffy growth curve. Growth 29, 265–289.

Ferter, K., Weltersbach, M.S., Humborstad, O.-B., Fjelldal, P.G., Sambraus, F., Strehlow, H.V., Vølstad, J.H. (2015) Dive to survive: effects of capture depth on barotrauma and post-release survival of Atlantic cod (*Gadus morhua*) in recreational fisheries. ICES J. Mar. Sci. J. Cons. 72, 2467–2481. https://doi.org/10.1093/icesjms/fsv102

Hemmer-Hansen, J., Hüssy, K., Baktoft, H., Huwer, B., Bekkevold, D., Haslob, H., Herrmann, J.-P., Hinrichsen, H.-H., Krumme, U., Mosegaard, H., Nielsen, E.E., Reusch, T.B.H., Storr-Paulsen, M., Velasco, A., von Dewitz, B., Dierking, J., Eero, M. (2019) Genetic analyses reveal complex dynamics within a marine fish management area. Evol. Appl. https://doi.org/10.1111/eva.12760

Hüssy, K., Casini, M., Haase, S., Hilvarsson, A., Horbowy, J., Krüger-Johnsen, M., Krumme, U., Limburg, K., McQueen, K., Mion, M., Olesen, H.J., Radtke, K. (2020) Tagging Baltic Cod – TABACOD**.** Eastern Baltic cod: Solving the ageing and stock assessment problems with combined state-of-the-art tagging methods. DTU Aqua Report no. 368-2020. National Institute of Aquatic Resources, Technical University of Denmark. 64 pp

Hüssy, K., Hinrichsen, H.-H., Eero, M., Mosegaard, H., Hemmer-Hansen, J., Lehmann, A., Lundgaard, L.S. (2016) Spatio-temporal trends in stock mixing of eastern and western Baltic cod in the Arkona Basin and the implications for recruitment. ICES J. Mar. Sci. J. Cons. 73, 293–303. https://doi.org/10.1093/icesjms/fsv227

Hilborn, R. (1990) Determination of Fish Movement Patterns from Tag Recoveries using Maximum Likelihood Estimators. Can. J. Fish. Aquat. Sci. 47, 635–643. https://doi.org/10.1139/f90-071

Hoyle, S.D., Leroy, B.M., Nicol, S.J., Hampton, W.J. (2015) Covariates of release mortality and tag loss in large-scale tuna tagging experiments. Fish. Res. 163, 106–118. https://doi.org/10.1016/j.fishres.2014.02.023

ICES (2020) Baltic Fisheries Assessment Working Group (WGBFAS). ICES Scientific Reports. 2:45. 643 pp. http://doi.org/10.17895/ices.pub.6024Ingólfsson, Ó.A., Soldal, A.V., Huse, I., Breen, M., 2007. Escape mortality of cod, saithe, and haddock in a Barents Sea trawl fishery. ICES J. Mar. Sci. 64, 1836–1844. https://doi.org/10.1093/icesjms/fsm150

Kock, K.-H. (1975a) Über die Haltung von Dorschen (*Gadus morhua* L.) in Netz-käfigen. Arch. Fisch Wiss. 26, 35-48.

Kock, K.-H. (1975b) Untersuchungen über die markierungsbedingte Sterblichkeit und Markenverluste am Dorsch (*Gadus morhua* L.) der westlichen Ostsee. Berichte der Deutschen Wissenschaftlichen Kommission für Meeresforschung, 35:189-206.

Köster, F. W., Huwer, B., Hinrichsen, Hans-H., Neumann, V., Makarchouk, A., Eero, M., Dewitz, B. V., Hűssy, K., Tomkiewicz, J., Margonski, P., Temming, A., Hermann, Jens-P., Oesterwind, D., Dierking, J., Kotterba, P., Plikshs, M. (2017) Eastern Baltic cod recruitment revisited - dynamics and impacting factors. ICES J. Mar. Sci., 74(1), 3–19. doi:10.1093/icesjms/fsw172

Krumme, U., Stötera, S., McQueen, K., Pahlke, E. (2020) Age validation of age 0-3 wild cod (*Gadus morhua*) in the western Baltic Sea through mark-recapture ad tetracycline marking of otoliths. Mar. Ecol. Prog. Ser. https://doi.org/10.3354/meps13380.

Milliken, H.O., Farrington, M., Rudolph, T., Sanderson, M. (2009) Survival of Discarded Sublegal Atlantic Cod in the Northwest Atlantic Demersal Longline Fishery. North Am. J. Fish. Manag. 29, 985–995. https://doi.org/10.1577/M08-008.1

Naumann, M., Umlauf, L., Mohrholz, V., Kuss, J., Siegel, H., Waniek, J. J., Schilz-Bull, D. E. (2018) Hydrographic-hydrochemical assessment of the Baltic Sea 2017. Meereswiss. Ber., Warnemünde, 107. doi:10.12754/msr-2018-0107

Netzel, J (1976) Migrations of the Baltic cod and an attempt to evaluate the dynamics of the population based on tagging experiments in 1954-1963. (in Polish) Studia i Materiały. Seria B Nr 37. Gdynia 1976. 100 pp.

Pine, W.E., Pollock, K.H., Hightower, J.E., Kwak, T.J., Rice, J.A. (2003) A Review of Tagging Methods for Estimating Fish Population Size and Components of Mortality. Fisheries 28, 10–23. https://doi.org/10.1577/1548-8446(2003)28[10:AROTMF]2.0.CO;2

Pollock, K.H., Pine, W.E. (2007) The design and analysis of field studies to estimate catch-and-release mortality. Fish. Manag. Ecol. 14, 123–130. https://doi.org/10.1111/j.1365-2400.2007.00532.x

R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

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

Sangster, G. I., Lehmann, K., Breen, M. (1996) Commercial fishing experiments to assess the survival of haddock and whiting after escape from four sizes of diamond mesh cod-ends. Fish. Res. 25, 323-345.

Snoeijs-Leijonmalm, P., Andrén, E. (2017) Why is the Baltic Sea so special to live in?, in: Snoeijs-Leijonmalm, P., Schubert, H., Radziejewska, T. (Eds.), Biological Oceanography of the Baltic Sea. Springer Netherlands, Dordrecht, pp. 23–84. https://doi.org/10.1007/978-94-007-0668-2_2

Stötera, S., Degen-Smyrek, A.K., Krumme, U., Stepputtis, D., Bauer, R., Limmer, B., Hammer, C. (2019) Marking otoliths of Baltic cod (*Gadus morhua* Linnaeus, 1758) with tetracycline and strontium chloride. J. Appl. Ichthyol. 35, 427–435. https://doi.org/10.1111/jai.13829

Weltersbach, M. S., Strehlow, H.V. (2013) Dead or alive - estimating post-release mortality of Atlantic cod in the recreational fishery. ICES J. Mar. Sci. 70, 864–872. https://doi.org/10.1093/icesjms/fst038

Weist, P., Schade, F.M., Damerau, M., Barth, J.M.I., Dierking, J., André, C., Petereit, C., Reusch, T., Jentoft, S., Hanel, R., Krumme, U. (2019) Assessing SNP-markers to study population mixing and ecological adaptation in Baltic cod. PLOS ONE 14, e0218127. https://doi.org/10.1371/journal.pone.0218127

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

Wilde, G.R. (2002) Estimation of catch-and-release fishing mortality and its sampling variance, in: Regional Experiences for Global Solutions. Presented at the Proceedings for the 3rd World Recreational Fishing Conference, Darwin, NT, Australia, pp.

Chapter 2

Validation approaches of a geolocation framework to reconstruct movements of demersal fish equipped with data storage tags in a stratified environment

Stefanie Haase[1], Uwe Krumme[1], Ulf Gräwe[2], Camrin D. Braun[3,4], Axel Temming[5]

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

[2]Department of Physical Oceanography and Instrumentation, Leibniz Institute for Baltic Sea Research Warnemünde, 18119 Rostock, Germany

[3]School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat St, Seattle, WA 98105 USA

[4]Biology Department, Woods Hole Oceanographic Institution, 266 Woods Hole Rd, Woods Hole, MA 02543 USA

[5]Institute of Marine Ecosystem and Fisheries Science, Centre for Earth System Research and Sustainability (CEN), University of Hamburg, Große Elbstraße 133, 22767 Hamburg, Germany

This chapter has already been published in Fisheries Research:

Haase S, Krumme U, Gräwe U, Braun CD, Temming A (2021) Validation approaches of a geolocation framework to reconstruct movements of demersal fish equipped with data storage tags in a stratified environment. Fish Res 237:105884, DOI:10.1016/j.fishres.2021.105884

Abstract

Geolocation models are essential tools to derive detailed information on the movement patterns of demersal fish equipped with data storage tags recording temperature and depth. However, modelled horizontal positions are accompanied by an often unknown error which needs to be assessed to support the appropriate interpretation. We conducted five validation experiments in the southern Baltic Sea comprising a) simulated tracks, b) stationary, nearshore moored tags, c) temperature data from an automated offshore measuring station, d) a temperature-depth probe mounted to an otter board of a commercial vessel and e) tags mounted to the conductivity-temperature-depth probe (CTD) and the otter board of a survey vessel to obtain temperature-depth data from known locations. Using depth-temperature data from each experiment, we calculated modelled positions using an adapted R-package *HMMoce* model framework. These experiments allowed us to compare true and modelled positions to quantify model performance and assess its potential efficacy for future geolocation studies on tagged cod. Correctly modelled start and end positions were a good indicator for all validation methods; modelled and true tracks were generally similar with differences in daily positions varying on average between 2 and 19 km. The performance of the geolocation model was improved when temperature-depth profiles indicated heterogeneity in the oceanographic environment, particularly in stratified water columns. The validation methods confirmed that the modified *HMMoce* framework could be used as a geolocation tool for Eastern Baltic cod when an uncertainty of less than 20 km from the true position is acceptable. Since all presented methods are cost-effective, transparent, open source and reproducible, they can be routinely applied to understand expected model uncertainties and thus make more robust inference about movements of tagged fish.

Keywords: Geolocation uncertainty, cod movement, HMMoce, Hidden Markov model, validation

Introduction

Tracking of aquatic animals has provided unique insights into their behavior and distribution and has fundamentally altered our understanding of the structure and function of global aquatic ecosystems (e.g. Hussey et al. 2015). Data storage tags (DSTs) are among the wide range of technologies available to track movements of marine species. DSTs deliver time-stamped measurements of environmental parameters experienced by the tagged individual (e.g. ambient temperature, salinity, pressure, light level), and, more recently, individual parameters (e.g. acceleration, heart rate); however, these data often lack spatial context. Geolocation tools are regularly used to infer movements of tagged individuals by comparing spatially resolved oceanographic data from, for example, 3-D ocean models with in-situ environmental data recorded by the tags (Arnold and Dewar, 2001; Skomal et al., 2009; Thorstad et al., 2014; Le Bris et al., 2018) while accounting for characteristics of the individual fish movement, such as distribution and range of swimming speeds.

Historically, many geolocation tools have been developed for particular areas, species and tag types, resulting in several case-specific solutions. Most recent geolocation model frameworks have been based on non-parametric state-space models, such as the Hidden Markov Model (HMM) (Nielsen et al., 2019). Several applications of HMMs have demonstrated these approaches perform well for near-shore demersal species tagged with depth sensors (Andersen et al., 2007; Nielsen et al., 2019), including the baseline framework for many current HMMs that was originally developed for the geolocation of North Sea cod (*Gadus morhua*) using tidal information (Pedersen et al., 2008; Thygesen, 2009). This has since been applied to geolocate Atlantic cod (e.g. Thorsteinsson et al. (2012); Le Bris et al. (2013a); Liu et al. (2017)) and has been adapted to a variety of other systems, mainly by adjusting the likelihood estimation to better account for the characteristics of different tags, species and areas (Le Bris et al., 2013b; Woillez et al., 2016; Biais et al., 2017; Braun et al., 2018a). Many of the original applications of the Pedersen et al. (2008) model framework were case-specific and written for use in Matlab, likely restricting wider adoption of these methods. However, Braun et al. (2018) generalised the core functionality to facilitate applications beyond specific species and regions and translated the base functionality into the open-source R language (R Core Team, 2017) as the R-package *HMMoce* (https://github.com/camrinbraun/HMMoce), thus widening the range of potential users and making it readily adaptable to other applications.

To apply a geolocation model to a new area, species and or tag type, it is essential to estimate the performance, classify the associated uncertainty and test for the robustness of the desired model framework and specifications (Nielsen et al., 2019). Otherwise, movements and behavior derived through sub-optimal geolocation techniques might be error-prone (Braun et al., 2015) and could lead to misinterpretation. Validation of geolocation methods is usually conducted by comparing modelled and known tracks. While data on known movements of tagged individuals is typically rare, some surface-oriented, epipelagic species have been effectively double-tagged with archival tags (e.g. DSTs, pop-up satellite archival transmitting tags) and satellite-linked location tags (e.g. salmon sharks (Teo et al., 2004); blue sharks (Braun et al., 2019a)). These double-tagging efforts can provide independent, known tracks for validation of geolocation techniques. Similarly, some studies have successfully double-tagged demersal species with a DST and an acoustic tag to supplement geolocation likelihoods with additional known positions from an acoustic receiver array (Liu et al., 2017). However, both double-tagging with satellite and acoustic tags are only applicable to a limited number of cases and are relatively costly (Arnold and Dewar, 2001; Block et al., 2005).

Other studies have used simulated tracks, generating potential natural behavior of the species of interest, for model validation. These simulated tracks can then be matched with oceanographic parameters which are used to subsequently reconstruct the tracks with a geolocation model (Neuenfeldt et al., 2007; Righton and Mills, 2008; Liu et al., 2017; Nielsen et al., 2019). This simple but effective method can emulate the present knowledge of fish behavior and helps to develop a better understanding of processes within the geolocation model (Jonsen et al., 2013) and the choice of input parameters. However, geolocation models are rarely tested in their ability to reconstruct stationary behavior, for example, by applying these methods to stationary, moored DSTs or temperature values recorded at an automated measuring station (Hunter et al., 2003; Thorsteinsson et al., 2012; Liu et al., 2017). Furthermore, testing the model’s ability to detect mobile behavior by attaching sensors to fishing gear to reconstruct the vessel’s track has, to our knowledge, only been presented in Righton and Mills (2008). These approaches have usually only covered small areas compared to the stock distribution area and simulated rather local behavior. Preferably, a combination of different validation methods across a spectrum of space and timescales should be conducted to account for different types of movement behavior (ideally for the target species), but these validation methods are not routinely used before applying a geolocation model to a new study species or area.

Previous validation studies found that a higher horizontal or vertical spatial heterogeneity in environmental parameters, and thus larger variations in water depth and temperature measurements of the DST, can lead to improved accuracy of the geolocation (Liu et al., 2017; Nielsen et al., 2019). DSTs implanted in cod in the southern Baltic Sea between 2016 and 2019 indicated high heterogeneity in the recorded temperature and depth data as tagged individuals made regular daily vertical movements across several meters of a stratified water column (Hüssy et al., 2020). Thus, the combination of dynamic oceanography and animal behavior can be used to further constrain the geolocation problem and should thus be leveraged to improve reconstructed tracks (e.g. Braun et al., 2018b).

Here, we adapt the existing *HMMoce* model framework to better handle geolocation of DSTs deployed on demersal species and quantify the accuracy of the adapted model to assess its utility for future studies geolocating tagged cod in the southern Baltic Sea. In particular, we tested the sensitivity of the model results to the vertical resolution of the regional ocean model used to calculate likelihoods, as well as swimming speed parameters, using five experiments: (1) artificial tracks, (2) stationary DSTs moored inshore, (3) an automated offshore measuring station, (4) temperature-depth probes attached to an otter board of a commercial fishing vessel, and (5) DSTs attached to the CTD probe and the otter board of a fisheries research vessel. We compared known positions to modelled outcomes from the adapted *HMMoce* model to assess the uncertainty of the geolocation model across a wide range of possible fish behavior and movement types.

Material & Methods

Oceanography and cod behavior in the southern Baltic Sea

The bathymetry of the temperate, non-tidal southern Baltic Sea is characterized by shallow nearshore areas and deeper offshore basins which provide a high degree of heterogeneity in bathymetry (Fig. 1A). In each basin there are weak horizontal gradients in water temperature (i.e. within the same depth layer). A three-layer structure characterizes the water column in the Arkona and Bornholm Basin. This double stratification is composed of a permanent near-bottom halocline and a seasonal surface thermocline with an average thickness of 15-20 metres. However, during full thermal stratification, strong winds or storm events can mix the water column down to the halocline.

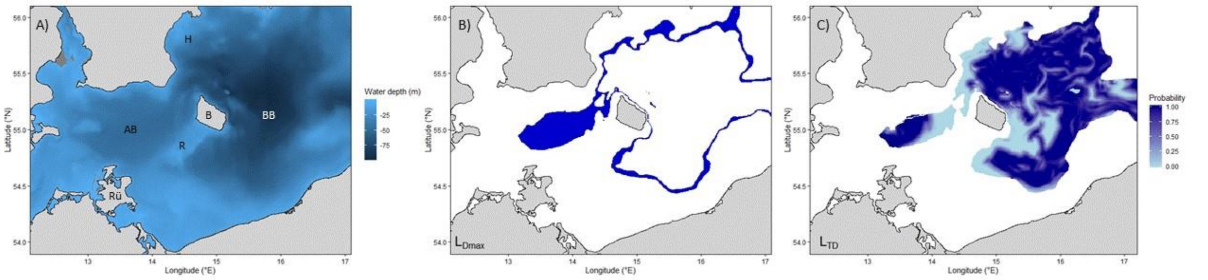

Figure 1: A) Bathymetry map of the southern Baltic Sea with locations mentioned in this study (Rü: Rügen Island, AB: Arkona Basin, R: Rönnebank, H: Hanö Bight, B: Bornholm, BB: Bornholm Basin). B) Illustration of a daily maximum-depth likelihood (L_{Dmax}) and C) a temperature-depth likelihood (L_{TD}) profile. White areas in B) and C) indicate areas not fitting the depth restrictions and are thus masked from the likelihood at that time step.

Cod in the southern Baltic Sea are primarily demersal but are known to display daily vertical movements across the thermocline up to 20 m upwards during sunset and downwards around sunrise, presumably to prey on clupeids (Schaber et al., 2012; Hüssy et al. 2020). Furthermore, Baltic cod concentrate in the deeper, more saline and often hypoxic basins for spawning in summer (Nielsen et al., 2013) to ensure buoyancy of the pelagic eggs (Nissling et al., 1994; Nissling and Westin, 1991) and use the shallower waters and basin slopes for feeding otherwise. To validate the reconstruction of cod movements in the southern Baltic Sea for a future study, we mimicked the tag deployment with temperature-depth DSTs of the manufacturer star-oddi (DST milli-TD (resolution: 0.032 °C and 0.03 m, accuracy: ±0.1°C and ±0.6 m, temperature response time: 12 sec.), and DST micro-TD (resolution: 0.032 °C and 0.12 m, accuracy: ±0.2°C and ±0.6 m, temperature response time: 8 sec.)) which were customized to record temperature and depth data once per minute.

Geolocation method

To reconstruct the movements based on temperature-depth data, we used R3.6.2 (R Core Team, 2017) and the R-based *HMMoce* package version 1.0.0 (Braun et al., 2018a). *HMMoce* constructs the most probable track according to the data measured by the archival tag using outputs from a regional oceanographic model (ROM), among other possible likelihoods (e.g. sea surface temperature). To determine possible positions, daily likelihood distributions were estimated, which are the derivatives of depth and temperature data recorded by the DST and the corresponding values from the ROM. A likelihood close to 1 suggests a high overlap between the temperature-depth profile recorded by the DST and the vertical profile at a given position. We assembled the estimated likelihoods into a Hidden Markov Model, which computes the posterior probability distributions. Based on this, the most likely position of each individual is estimated for each day. Finally, we provided a start and end location in all model runs. The most probable track was calculated as the mean of the daily distributions. Detailed information about *HMMoce* is given in Braun et al. (2018a) and the associated GitHub page (https://github.com/camrinbraun/HMMoce).

HMMoce was developed primarily for geolocating pelagic and highly migratory species (e.g. basking sharks, Braun et al. (2018b); swordfish, Braun et al. (2019b) thresher sharks, Arostegui et al. (2020)). Thus, several adjustments were necessary to maximize applicability to demersal species.

First, we adapted the likelihood framework to account for the demersal behaviour of cod (following Nielsen et al. (2019)) and the specific bathymetry of the southern Baltic Sea. This involved adding a maximum-depth likelihood (hereafter called L_{Dmax}) to account for 1) the heterogeneous bathymetry of the southern Baltic Sea with shallower areas at the coast and deeper areas in the basins, and 2) the demersal behaviour of Baltic cod. L_{Dmax} was estimated by filtering the bathymetry map of the Baltic Sea ROM for areas, which were within ±5 m of the maximum daily depth recorded by the DST assuming that cod approach the seafloor to within 5 m at least once a day. L_{Dmax} was thus a binary likelihood (composed of zeros and ones) indicating whether or not the maximum bottom depth was within ±5 m of the maximum daily reported depth (Fig. 1B).

Second, we modified the original temperature-depth-likelihood (hereafter called L_{TD}) functionality to better account for the temperature stratification of the southern Baltic Sea and regular daily vertical movements of cod. It was calculated by comparing the temperature at a certain depth recorded by the DST with the temperature at the same depth of the Baltic ROM at a predefined vertical resolution (2 m, 3 m or 5 m). To consider the daily vertical movements of cod, each fish needed to be recorded in at least three different depth layers within a day (24 hours starting at 00:00 hs), resulting in a minimum required vertical movement of 6 m, 9 m or 15 m, respectively, depending on the resolution chosen. Respective individual likelihoods of each depth level were then multiplied to get one temperature-depth-likelihood profile per day with probability values between 0 and 1 for each grid cell (Fig. 1C). For days where fish did not cross at least three vertical layers, they were expected to stay close to the bottom within 6 m, 9 m or 15 m. The bathymetry map was then filtered as in the maximum-depth-likelihood method L_{Dmax} described above, only that this time the maximum bottom depth was within ±6 m, ±9 m or ±15 m of the maximum daily depth recorded by the DST. The daily likelihood output of L_{TD} could thus be a probability distribution consisting of continuous values between 0 and 1 when the fish crossed three or more layers, or of binary values when not.

Further, smaller changes in the code were necessary to account for the data-structure of the star-oddi tags (*HMMoce* was originally designed for tags manufactured by Wildilfe Computers), and the data structure of the Baltic Sea ROM. The R-scripts of the adapted *HMMoce* are permanently archived at https://github.com/StefanieHaase/Adapted-HMMoce for others to use and adapt.

Regional ocean model

To generate the temperature forcing fields for the temperature-depth likelihood calculations, we applied the ocean model of Gräwe et al. (2015), covering the entire southern Baltic Sea. The numerical model has a grid resolution of 1/180° in latitude (~600 m) and 1/110° in longitude (~600 m). In the vertical, 42 terrain-following, adaptive levels were employed. We set the storage time interval to one day. Error measurements of the ocean model showed that the near bottom temperature bias ranged between -0.1°C and 0.1°C, which is well below the natural variability (as measured by the standard deviation) of 4.5°C (Gräwe et al., 2015). Seifert & Kayser (1995) quantified the average sampling error of the bathymetry as ±0.5 m and ±1.5 m for the Arkona and Bornholm Basin, respectively, by comparing various data sources (regular soundings (Reissmann (1999), and soundings during various research cruises). For the geolocation experiments, we interpolated the ocean model output to z-levels with a vertical grid spacing of 2 m, 3 m and 5 m to test the effect of vertical resolution on model performance. We limited the tracking experiments to the area 12-17.3 °E and 53.8-56.2 °N, which includes the Arkona and Bornholm Basin (Fig. 1A).

Validation experiments

To assess the performance of the adapted *HMMoce*, we compiled five dataset types including temperature, depth and known positions during 24-hour cycles covering periods of 12-365 days. Temperature and depth data as well as the first and last known position of the datasets were used as input parameters for *HMMoce*, thus reflecting the standard input data from DSTs. Additionally, maximum swimming speed (m/s) was set as an input parameter. It limits the distance an individual can move within a day. It was set according to the constructed maximum speed (m/s) which results from the distance between positions of two consecutive days as artificially constructed in the different validation experiments. We tested the model's ability to reconstruct the positions of the datasets for maximum swimming speeds between 0.1 and 1m/s and vertical resolutions of 2, 3 and 5 m. After the model run, the most probable track was then compared to the true daily locations. Uncertainties of predicted daily locations were not considered. We qualitatively assessed whether the modelled track depicted the general trend and main features of the true track and whether the release and recapture position were estimated within 0.5 km to the true positions. Modelled start and end location could differ from the given start and end location when the convolution of the movement kernel and likelihood does not allow the true position as a possible solution because the highest likelihood of the previous and subsequent day exceeded the distance that could be covered according to maximum swimming speed. Additionally, the great-circle distance between each true and modelled daily position was calculated (Hijmans, 2019) to quantify the overall track error. Shorter distances between true and modelled daily position indicate better model performance (horizontal accuracy). The minimum, median, mean, maximum and standard deviation were calculated from the daily distances between true and modelled positions per track. The distances between true and modelled tracks were visually checked for trends over time. This qualitative and quantitative assessment informs about the horizontal accuracy of the model and about the effect of the chosen parametrization (maximum swimming speed and vertical resolution). We prepared all maps with the R-packages *ggplot* (Wickham, 2016) and *ggPlotBathymetry* (Lipka, 2020) using the bathymetry values from the Baltic ROM.

1) *Simulated tracks:*

The first experiment tested the model's ability to reconstruct simulated tracks covering a large part of the stock's distribution area in the southern Baltic Sea. We created three simulated tracks to emulate a movement from three different release locations: close to Rügen (German waters, GER), Hanö Bight (Swedish waters, SWE) and close to Bornholm (Danish waters, DK). The simulated cod released in GER and SWE swam to the Bornholm Basin (spawning ground) and back towards the respective release site (directed, random movement of between 0 and 20 km a day from the position of the previous day in the direction of the Bornholm Basin or release site, respectively). Both trajectories were characterized by a stationary period at the release site and in the Bornholm Basin (undirected, random movement of between 0 and 7 km per day from the position of the previous day; Fig. 2d-f). The simulated cod released in DK performed an undirected, random movement throughout the experiment. The resulting horizontal distances between successive days of the artificial tracks varied between 0 km and 19.4 km (average: 3.5 km), 0.03 km and 12.9 km (average: 3 km) and 0.1 km and 6.4 km (average: 3.4 km) for the German, Swedish and Danish release position, respectively. This resulted in constructed maximum speeds of 0.2 m/s, 0.2 m/s and 0.1 m/s, respectively. The trajectories covered one year to account for an annual cycle of the changes in oceanography in the study area. Bottom depth was extracted from the Baltic ROM for each simulated daily position. To emulate vertical movements, we added additional depths (a random number between 0 and 10), which were up to 20 m shallower than the bottom depth given the range of natural vertical movements displayed by Baltic cod (Hüssy et al., 2020; Schaber et al., 2012, Haase unpublished data). The water temperature was extracted from the ROM for each depth, position and day. A random uniform error of ±0.2 °C and ±0.6 m was added to the temperature and depth values, respectively, to account for the measurement accuracy of the DST. The three resulting tracks with their corresponding temperature-depth profiles are shown in Fig. 2.

2) *Moored DSTs*:

The second dataset contained temperature-depth data recorded with moored DSTs. This experiment aimed at testing the model's ability to correctly reconstruct a fixed position, i.e. stationary behavior, in shallow, nearshore waters. Water temperature and depth data were recorded from three DSTs which were moored at a stationary rig (deployment: 54.593° N, 13.574° E, heave: 54.593° N, 13.581° E, Fig. S1). The DSTs sampled data for 18 days (20.06.2019 - 08.07.2019; date format: dd.mm.yyyy) at ~2.5 m, ~9 m and ~18 m water depth. GPS-positions between deployment and heaving of the moored DSTs differed by 433 m, either due to drift or inaccurate GPS-positioning. Deployment and heave positions were taken as the release and recapture positions, respectively. Since the deepest DST had an offset of 2 m, it was corrected for zero offset.

3) *Automated offshore measuring station:*

Water temperature at eight depth strata (2, 5, 7, 16, 25, 33, 40, and 43 m) were obtained from the automated measuring station (MARNET) in the Arkona Basin (54.883 °N, 13.866 °E) in an hourly resolution to test the model's ability to reconstruct a fixed position in deeper, offshore areas. The data period covered the same 18 days as the moored DSTs (20.06.2019 - 08.07.2019). The dataset contained some unrealistically low water temperatures so that all temperature measurements below 7.5 °C were removed. The position of the measuring station was used as release and recapture position.

4) Temperature-depth probes attached to the otter board of a commercial trawler:

The fourth dataset was sampled by a temperature-depth probe (NKE STPS 300, temperature accuracy <0.05 °C, resolution 0.001 °C at 10 °C, response time <0.5 s at 63 %, depth accuracy 90 cm, resolution 7.2 cm) which was attached to the otter board of a commercial fishing vessel during a 12-day fishing trip to the Bornholm Basin (29.4.2016-11.5.2016). The fishing ground covered a longitudinal spread of 49.4 km and a latitudinal spread of 57.5 km. The probe logged water temperature and depth during fishing hauls. GPS-positions were recorded at the bridge and matched with temperature and depth recordings. On days where no fishing hauls were conducted and thus no temperature-depth data recorded, stationary behavior was created by repeating the maximum depth of the previous day (necessary on 2.5.2016 and 8.5.2016). The maximum distance between the positions of the commercial trawler on successive days was 56.6 km, corresponding to a constructed maximum speed of 0.65 m/s and the maximum swimming speed used as input parameter was adapted accordingly. The Baltic ROM output suggested that bottom temperatures, as well as temperatures modelled at 20 m and 40 m depth, showed little variation in this area throughout the year (Fig. S12-S14). This experiment tested the model's ability to reconstruct fast movements on the spawning ground in the Bornholm Basin.

5) DSTs attached to the CTD and otter board of a scientific survey vessel:

The fifth dataset was sampled by two DSTs, one attached to the CTD-probe and the other to the otter board of a research vessel during a routine 15-day trawl survey (08.11.2019-23.11.2019) in the Baltic Sea. While the DST attached at the CTD was slowly lowered in the water column, remained a short period at the bottom before again being lifted to the surface, the DST attached to the otter board was characterized by a short period in the water column and a prolonged period at the bottom. Only the 13 days during which the vessel was within the model domain were selected (11.03.2019-23.3.2019). The area covered by the research vessel had a longitudinal spread of 164.8 km and a latitudinal spread of 122.1 km. The crew performed four to five hauls per day, with a CTD cast covering the whole water column on each station. Positions were recorded at the bridge and matched to temperature and depth recordings. On days where no fishing hauls and CTD stations were conducted due to bad weather, stationary behavior was created by repeating the maximum depth of the previous day (necessary on 15.3.2019, 16.3.2019 and 19.3.2019). The maximum distance between the positions of the research vessel on successive days was 93.4 km, corresponding to a constructed maximum speed of 1.1 m/s. Maximum swimming speed was adapted accordingly so that the model's ability to reconstruct swift movements within coastal areas and offshore areas was tested for maximum swimming speeds of up to 3 m/s.

Results

Geolocation of simulated tracks

The general trend of the simulated tracks could be depicted correctly with the adapted *HMMoce* (Fig. 2d-f). Release and recapture position were modelled correctly when the maximum swimming speed was chosen equal to or above the constructed maximum speeds, otherwise the gaussian-shaped movement kernel prioritized smaller movements that the extent of movement required. Even choosing the maximum swimming speed multiple times higher than the modelled track led to the correctly constructed release and recapture positions in all three tracks (Figs. 2 & 3). Generally, computer-running time increased with increasing maximum swimming speed because the number of grid cells used in the convolution (movement kernels) increases with increasing allowable diffusion. Depending on maximum swimming speed and vertical resolution, the mean distance between modelled and true daily position varied between 8.3 km (DK track, vertical resolution 2 m, maximum swimming speed 0.1 m/s) and 34.9 km (SWE track, vertical resolution 5 m, maximum swimming speed 0.1 m/s), respectively (Fig. 3). The performance criteria mean and median difference of true and modelled locations ranked model performance of different resolutions and maximum swimming speeds similar (Figs. 3 & S2-S4). The lowest mean distance between true and modelled track was achieved at a vertical resolution of 3, 2 and 2 m and maximum swimming speeds of 0.5, 0.3 and 0.1 m/s for the GER (10.7 km (±6.7)), SWE (11.6 km (±9.9)) and DK (8.3 km (±6.3)) track, respectively, indicating that the most suitable vertical resolution and maximum swimming speed differed with fish behavior.

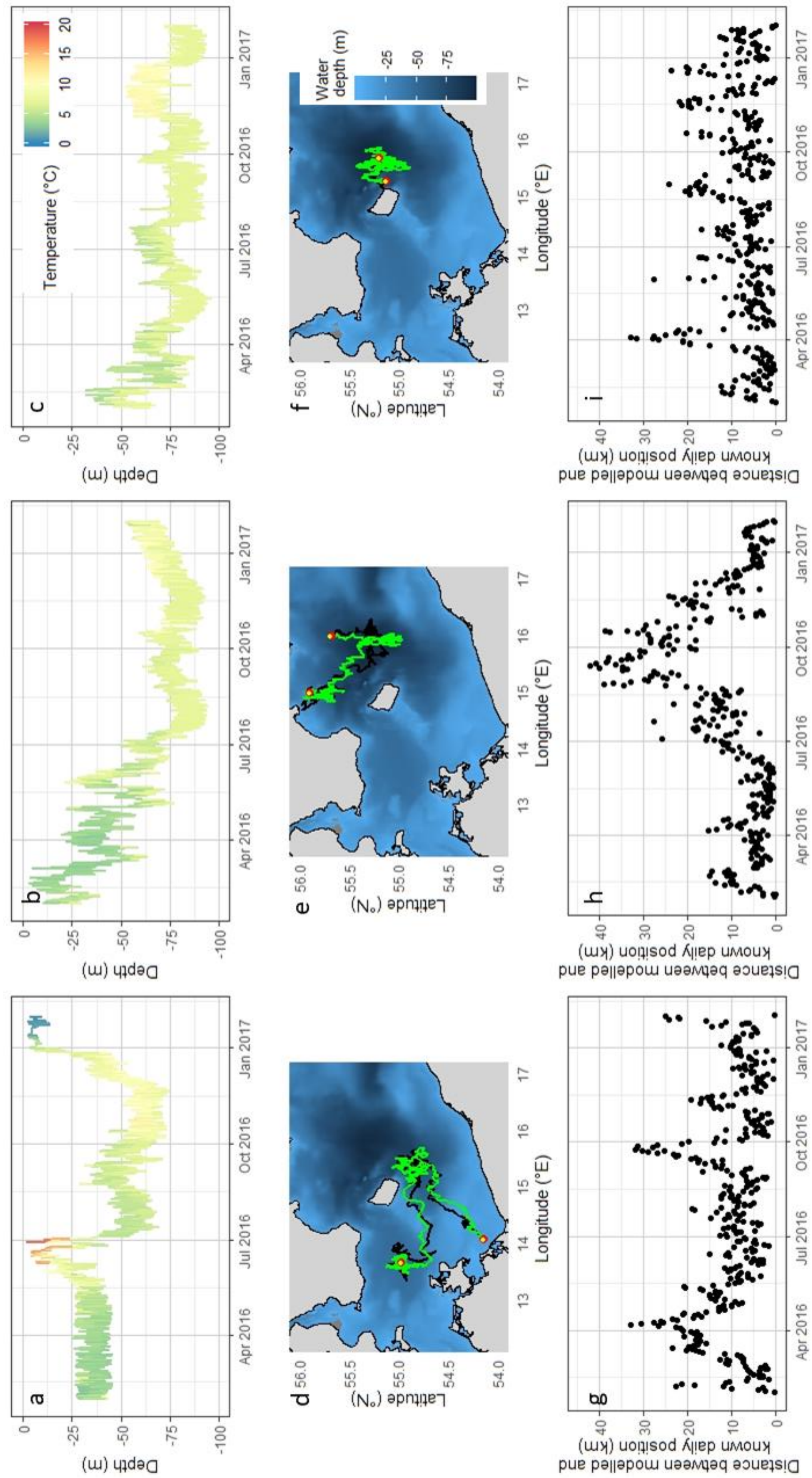

Figure 2: a-c Temperature-depth profiles of simulated trajectories. d-f: True (green) and reconstructed (black) track with release positions (yellow circles: true release and recapture position, red circles: modelled release and recapture positions) track on a bathymetry map of the southern Baltic Sea. g-i: Distance between modelled and true daily positions. a, d, g: GER track, b, e, h: SWE track, c, f, I: DK track.

Distances between true and modelled daily positions generally increased during stationary periods at the release positions and in the Bornholm Basin for the GER and DK track; however, distances did not generally increase over time, and season did not appear to influence model performance (Fig. 2g-i). Horizontal accuracy was highest for the DK track likely because the maximum daily depth restricted possible positions to the deep Bornholm Basin (Figs. 2 & 3).

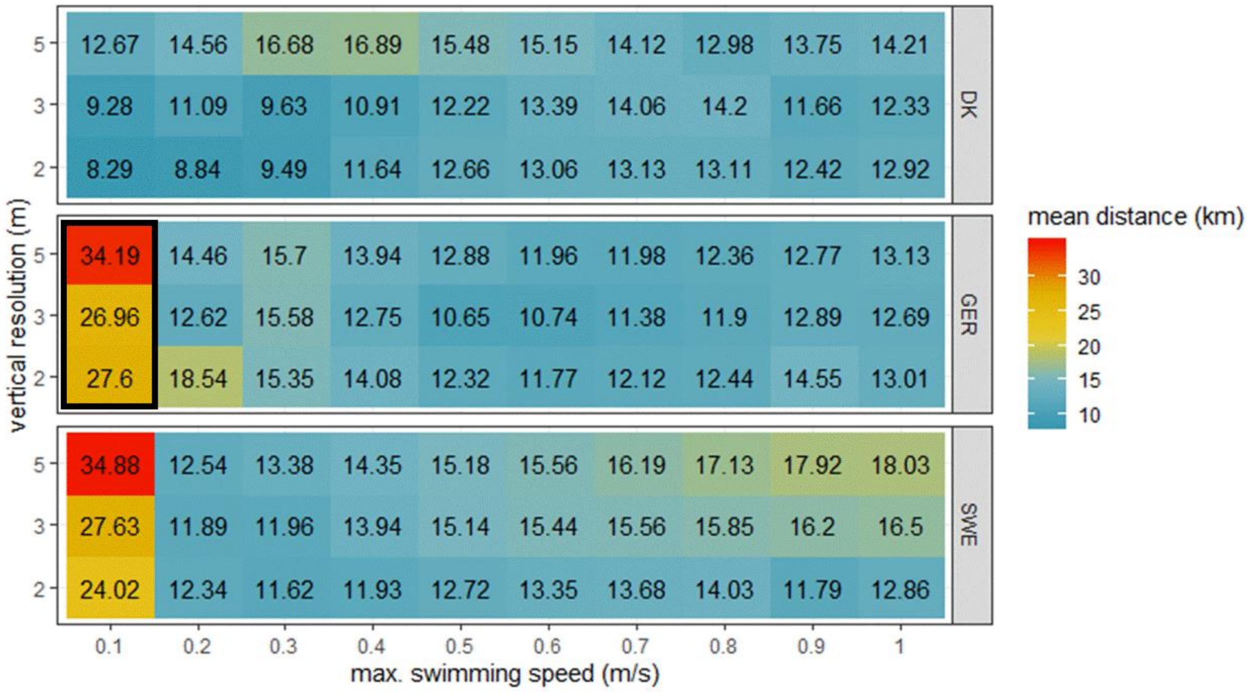

Figure 3: Mean daily difference between true and reconstructed position for the Danish, German and Swedish release positions. The black square indicates that start and end-position were not modelled correctly for this combination of vertical resolution and swimming speed; these tracks would have been discarded in a future geolocation study. Median, minimum and maximum distances between true and modelled daily positions are presented in the supplementary material Figs. S2-S4.

Geolocation of the stationary method

DST-recordings varied by 5.9 °C (13.2-19.2 °C) and 2.9 m (1.2-4.0 m) beneath the surface, 8.6 °C (10.9-19.5 °C) and 3.0 m (7.7-10.7 m) for the middle and 10.1 °C (8.8-19.9 °C) and 2.0 m (17.0-18.9 m) for the deepest position (Fig. S5). Stationary behavior could be depicted at all vertical resolutions; however, accuracy and precision decreased with increasing maximum swimming speed (Figs. S6-S9). Start position could not be reproduced correctly for a resolution of 2 m and maximum swimming speed of 0.1 m/s. The modelled location differed from the true location by 1.9 km (±2.0) for a vertical resolution of 3 m and a maximum swimming speed of 0.1 m/s (Fig. 4a).

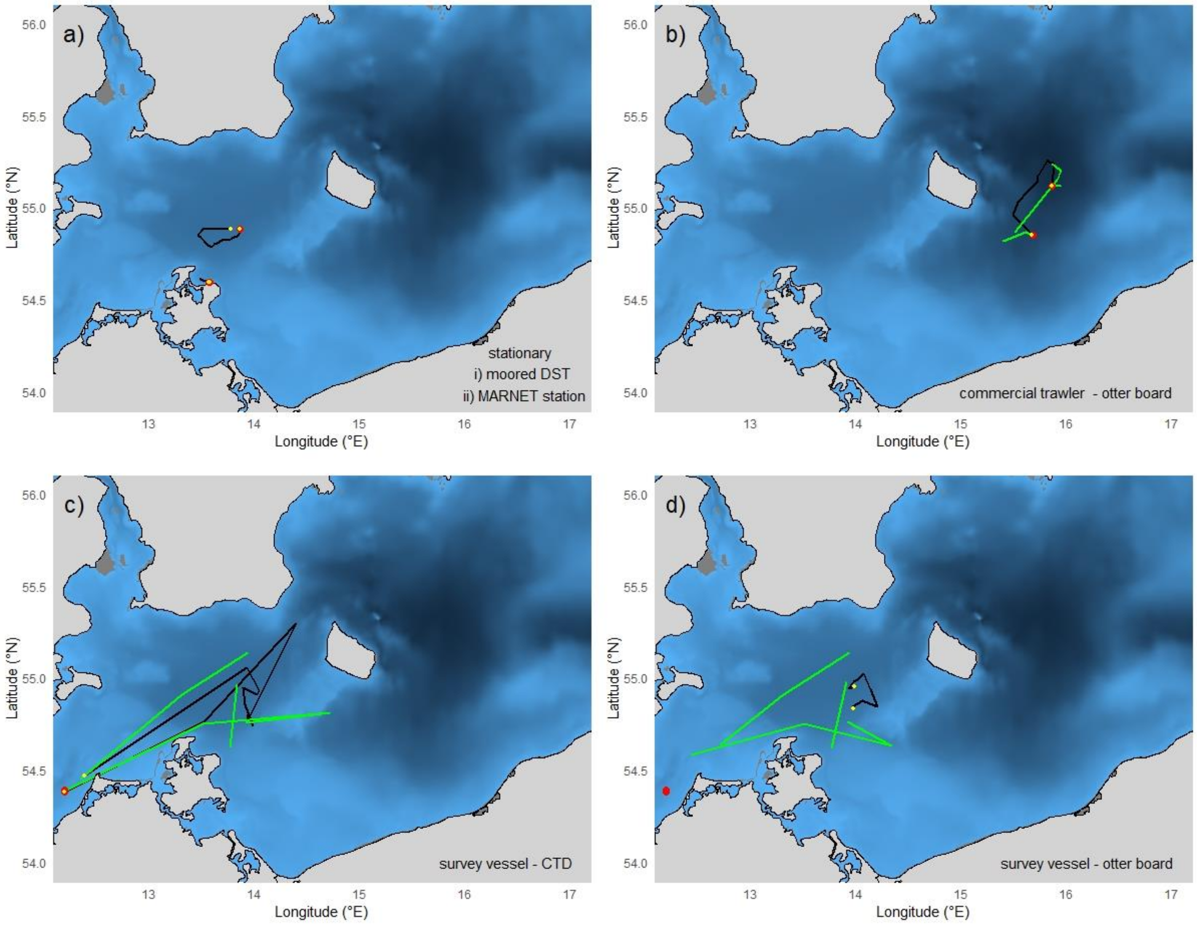

Figure 4: True (green) and reconstructed (black) track with known start and end position (red dot) and reconstructed start and end position (yellow dot) for the a) stationary method (i) moored DST: vertical resolution 3 m, maximum swimming speed 0.1 m/s, ii) MARNET-station: vertical resolution 3 m, maximum swimming speed 0.1 m/s), the green track is hidden below the red dot since it was stationary, b) temperature-depth probe attached to the otter board of a commercial vessel (vertical resolution 2 m, maximum swimming speed 0.2 m/s), c) DST attached to the CTD of a survey vessel (vertical resolution 2 m, maximum swimming speed 2.1 m/s) and d) DST attached to the otter board of a survey vessel (vertical resolution 5 m, maximum swimming speed 0.4 m/s). Fragmented appearance in b), c) and d): We displayed the true position which was closest to the modelled position. For days without fishing hauls or CTD casts no true position is displayed. Daily distances between true and modelled positions can be found in Fig. S20.

Temperatures recorded from the MARNET-station ranged between 8.3 °C at 43 m and 18.2 °C at 2 m water depth (Fig. S10), indicating strong stratification in the water column, and exhibited a pronounced thermocline at ~16 m. However, a mixing event occurred at the end of the experiment on the 03.07.2019; it was less distinct in the ROM and led to differences between measurements and ROM at the position of the MARNET-station of up to 7 °C (Fig. S11). The position of the MARNET-station could be modelled with a difference between modelled and true position of 13.0 km (±9.1) for a vertical resolution of 3 m and a maximum swimming speed of 0.1 m/s (Fig. 4a). For a vertical resolution of 3 m, the track was modelled precisely and accurate up to a maximum swimming speed of 0.8 m/s. Start and end positions were not modelled correctly for a vertical resolution of 5 m although all daily positions were modelled within the Arkona Basin. Distances between true and modelled positions for a vertical resolution of 2 m was generally large, but daily positions were within the Arkona Basin at maximum swimming speeds between 0.4 and 0.8 m/s (Figs. S6-S9).

Temperature-depth probes attached to the otter board of a commercial trawler

The probe attached to the otter board recorded a temperature range of 8.2 °C (4.2 °C-12.4 °C) and depths ranging from 0.2 m to 93.4 m during fishing hauls. The general direction of the vessel's track could be reconstructed with the adapted *HMMoce* with some uncertainty when swimming speed was set to 0.2 m/s or faster for all vertical resolutions tested (Figs. 4b, S6-S9, S15). The track of the vessel could be reconstructed with an average daily accuracy of 10.1 km (±8.1) and a maximum deviation of 25.8 km (vertical resolution 2 m, maximum swimming speed 0.2 m/s).

DSTs attached to the CTD and otter board of a scientific survey vessel

At a maximum swimming speed of 1.7 m/s or higher with a vertical resolution of 2 m or 1.5 m/s or higher with a vertical resolution of 3 m, the start and end positions were modelled correctly when using the data sampled with the DST attached to the CTD probe. Start and end positions could not be modelled correctly within the tested range of maximum swimming speeds when using a vertical resolution of 5 m. For a vertical resolution of 2 m and a maximum swimming speed of 2.1 m/s, differences between true and modelled daily positions varied between 0.2 km and 57.8 km with an average of 18.8 km (±19.5) (Fig. 4c). The general trend was depicted with some restrictions, i.e. one station was modelled north of the Rönnebank although the vessel was south of Rönnebank.

Neither pattern nor release and recapture position could be modelled correctly for the DST attached to the otter board. Only the relatively stationary period in the Arkona Basin with a water depth of around 45 m was depicted. Differences between daily modelled and true position differed between 10.2 and 103.3 km (average 39.3 km (±31.9) at a vertical resolution of 5 m and a maximum swimming speed of 0.4 m/s with large differences at the beginning and end of the track (Fig. 4d, Figs. S16-S19).

Discussion

To put data from archival tags such as DTSs into spatio-temporal context, geolocation tools must be adapted to and tested in new environments and for new applications. We have described five validation approaches which imitated the stationary and mobile behavior of cod in the southern Baltic Sea tagged with temperature-depth DSTs. Both simulated behaviors could be reconstructed with the adapted R-based geolocation model *HMMoce*. The uncertainty associated with the estimation of daily positions was, on average, <20 km between true and modelled daily position for both stationary and mobile behavior. This is within the purported daily range of 20 km of Baltic cod (Neuenfeldt et al., 2007). Contrary to Atlantic cod on the north-east Newfoundland Shelf, Baltic Sea cod likely do not need to cover daily distances of up to 59 km as observed with echosounders (Rose et al., 1995) since feeding and spawning grounds are relatively close to each other. This assessment of the expected horizontal error can aid interpretation of geolocated movements and behavior for DST-tagged demersal species in the wild, such as Baltic cod. The horizontal accuracy of these methods, despite the diversity of validation methods and the spatio-temporal variability of experiments used to generate reconstructed tracks (e.g. ranging from a specific mooring location for multiple days to the whole study area for an annual cycle (Tab. 1)), suggests this approach can be readily applied in this new study system for this species.

Table 2: Comparison of the different validation approaches used in this study.

Approach	Area covered	Time covered	Costs	Trend of track modelled correctly	Average distance between true and modelled daily positions*	Test case
Artificial tracks	large	long	low	yes	8-12	fish behavior during an annual cycle
Moored DSTs	small	short	medium	no	2	stationary behavior in shallow, nearshore water
MARNET-station	small	short	low	yes	13	stationary behavior in offshore, deeper water
Probe at otter board-commercial	medium	short	medium	yes	10	mobile behavior in the Bornholm Basin
DST at CTD/ otter board-survey	large	short	medium	yes/no	19/ 39	fast mobile behavior in coastal areas and the Arkona Basin

*rounded to full km

In general, the variety of model validation methods used in this study improved the understanding of the model performance under different vertical resolutions and maximum swimming speeds. For example, the validation showed that, in this scenario, the model was insensitive to a wide range of maximum swimming speeds (Fig. S15). We also tested model sensitivity to vertical resolution. While a lower vertical resolution reduced computer-running time, it could lead to fewer days in which the likelihood L_{TD} could be estimated because the depth recorded by the DST of tagged individual did not cover a vertical range of more than 6 m, 9 m and 15 m (for a vertical resolution of 2 m, 3 m and 5 m, respectively; Table S1). When the necessary vertical range threshold was not recorded by the DST, daily L_{TD} was filtered based on the area within 6 m, 9 m, or 15 m to the maximum recorded swimming depth that day. Hence, with these assumptions, a lower vertical resolution resulted in a larger available likelihood area for that day. Finally, although not directly modelled or included in the sensitivity analyses, the results suggest that a stronger stratification of the water column and regular fish movements across a dynamic thermal regime may lead to higher horizontal accuracy of the modelled daily positions when the speed of those vertical movements does not exceed the temperature response time of the DST.

Comparison of validation approaches

The five validation methods differed both in spatial and temporal extent but generally resulted in average daily accuracies between 8.3 km and 18.8 km when start and end position were reconstructed correctly within 0.5 km to the true positions and 39.3 km otherwise (Tab. 1). Tracks where start and end positions were not modelled within this range would not be used for further analysis so that the accuracy of the geolocation model tested lies within the upper range of what hydrodynamic geolocation methods normally achieve (accuracy between 5-50 km (Evans and Arnold, 2008)). However, comparing the accuracies of validation methods between studies is limited: accuracies are a combination of a) the suitability of different validation methods (i.e. considering species behavior) and b) the model performance in different seasons and areas (i.e. the accompanied heterogeneity of recorded data) (Evans and Arnold, 2008). Consequently, the estimates from this validation study can only be used as a guideline for other studies and model accuracy needs to be estimated separately for each area, species and tag type.

The three artificially-constructed tracks and the resulting temperature-depth profiles were simulated based on the accuracy of the DST. Since the simulated tracks are not fully independent from the ROM, they did not include potential bias or discrepancies that could arise between temperature-depth measurements of the DST in the field and the associated predictions of the ROM. The simulated movements covered large parts of the distribution area of Eastern Baltic cod and comprised a full year of oceanographic data, including a range of water column stratifications. The geolocation model reconstructed the simulated tracks with an average mean difference between true and modelled position of 8.3 km (±6.3) and 11.6 km (±9.9), with lower precision during stationary periods such as in the Arkona Basin or Bornholm Basin. This difference is slightly larger than the error of 2.9 km (± 4.7) observed in the validation of a simulated track of cod in the Baltic Sea (Neuenfeldt et al., 2007). However, that study used salinity as a third parameter which may lead to more constraints in the geolocation model and thus these approaches cannot be directly compared. Salinity, in particular, is a unique feature in the Baltic Sea since it decreases from West to East but external tags, which are necessary to record salinity, likely lead to abnormal behavior of smaller fish (Jepsen et al., 2015) and were therefore not considered in this study. Advantages of using simulated tracks are that fish behavior based on current knowledge can be tested to improve our understanding of model performance under different conditions covering large parts of the fish's distribution area and long time periods with different degrees of stratification. Furthermore, simulation experiments are inexpensive and thus can be readily used to estimate the horizontal accuracy of geolocation models prior to extensive, field-based tagging studies.

All other methods in this study included temperature-depth data sampled independently from the ROM, an automated measuring station in the Arkona Basin, or temperature-depth probes. Temperature-depth data, such as from CTDs or moored observing stations, are often readily available in a target study region. As such, these data can be used to conduct inexpensive, preliminary validation studies prior to DST deployment (Tab. 1). The moored DSTs and the automated measuring station in this study mimicked stationary behavior of cod over a period of three weeks. While cod are unlikely to occupy the same habitat for multiple weeks, the geolocation model achieved high precision and accuracy with a difference between the true and modelled position of 1.9 km (±2.0) for the moored DST and 13.0 km (±9.1) for the MARNET-station, respectively. Other studies using moored DSTs had estimated differences between true and modelled positions of 11 km (Liu et al., 2017) and 19 km (Thorsteinsson et al., 2012). These studies included tidal information and could therefore also benefit from a third recorded parameter. The higher horizontal accuracy of the moored DSTs indicates that the geolocation model performs better when the bathymetry of the area is characterized by higher contrast such as in the heterogeneous nearshore areas (moored DSTs) in contrast to the rather homogenous offshore area in the Arkona Basin (MARNET-station, Figs. 1a & 4a). Higher heterogeneity in bathymetry (Nielsen et al., 2019) and strong temperature gradients in dynamic oceanographic regimes (Braun et al., 2018b) can also lead to better geolocation model. Despite these differences, both stationary approaches used here confirmed that stationary behavior can be modelled precisely using the adapted *HMMoce* model framework. The stationary method can further be tested during different seasons to quantify the potential impact of water column stratification on model performance. Additionally, temporary moorings of DSTs at several locations (e.g. inshore vs. deep basins) could yield better accuracy estimates across a range of different habitats (Hunter et al., 2003; Liu et al., 2017).

Attaching temperature-depth probes and DSTs to otter boards and the CTD of research and commercial vessels proved relatively straightforward and has the advantage that the vessels fish within the habitat of the distribution area of the fish. Those methods were, however, limited by vessel time and characterized by smaller areas and shorter periods covered. Further, vertical and horizontal movements as constructed with the DSTs and probes attached to fishing gears may have exceeded realistic swimming speed of cod. The temperature-depth probes attached to the commercial vessel in this study had higher accuracy and much faster response time compared to the DST. This resulted in a lower mean difference between true and modelled position (10.1 km (±8.1)) compared to the DST attached to the otter board of the research vessel for which the track could not be reconstructed successfully (mean difference between true and modelled daily position 39.3 km (±31.9)). The otter board passes through the water column faster than the response time of the DST so that temperature data are matched with wrong depth information especially close to the thermocline. In contrast, when using the temperature-depth data sampled by the DST attached to the CTD (which was moved slower through the water column) the track could be reconstructed successfully with an average distance between true and modelled track of 18.8 km (±19.5). The geolocation of fish can thus be impeded if they regularly cross the thermocline within short time periods and exceed the response time of the DST's temperature sensor (12 s).

Implications for testing the adapted HMMoce for cod in the southern Baltic

The difference between true and modelled daily positions varied on average between 2 and 20 km when start and end position were modelled correctly. When reconstructing the tracks of wild tagged fish, a horizontal error of on average 10-20 km has to be included in the interpretation of the tracks of Baltic cod estimated with the modified *HMMoce* geolocation model. Stationary periods in homogenous water bodies, i.e. with less variation in temperature and depth for prolonged periods, such as in the deeper basins of the Arkona and Bornholm Basin, tend to be accompanied by higher uncertainties in horizontal position. In the Baltic cod case, this could lead to higher uncertainties during stationary periods in deeper water, e.g. for spawning (Köster et al., 2017; Nielsen et al., 2013). However, our results suggest that movements between core areas used, for example, for spawning and feeding, and depth-related shifts in habitat used should be appropriately characterized with this model framework in this region. We demonstrate that different validation methods can be inexpensive and readily applied to inform the interpretation of geolocation model outputs for field studies on tagged fish. In particular, these methods can be used to quantify the expected uncertainty in horizontal geolocation specifically for each species, area and tag type. Ultimately, these sensitivity analyses and validation experiments can help create more robust estimates of movements of demersal fishes that cannot otherwise be directly observed.

Acknowledgements

We thank Ina Hennings and Anne Georgi (OF) for deploying and heaving the stationary DST buoy. We are also grateful to Kay Briesewitz, captain of SAS111 “Christina Bettina”, and Andreas Hermann (OF) for deploying the temperature-depth probe at the otter board and the BSH for providing the data from the MARNET-station. We also thank the captain of the FRV “Solea” and the cruise leader Andrés Velasco for attaching DSTs at the CTD and the otter board and Lena von Nordheim for revising an early draft of this manuscript. S. Haase was partly funded by TABACOD, a Baltic2020 funded project. The compute power to integrate the numerical ocean model was provided by the North-German Supercomputing Alliance (HLRN). We thank the two anonymous reviewers for their constructive comments.

References

Andersen, K.H., Nielsen, A., Thygesen, U.H., Hinrichsen, H.-H., Neuenfeldt, S. (2007) Using the particle filter to geolocate Atlantic cod (Gadus morhua) in the Baltic Sea, with special emphasis on determining uncertainty. Can. J. Fish. Aquat. Sci. 64, 618–627. https://doi.org/10.1139/f07-037

Arnold, G., Dewar, H. (2001) Electronic Tags in Marine Fisheries Research: A 30-Year Perspective, in: Sibert, J.R., Nielsen, J.L. (Eds.), Electronic Tagging and Tracking in Marine Fisheries: Proceedings of the Symposium on Tagging and Tracking Marine Fish with Electronic Devices, February 7–11, 2000, East-West Center, University of Hawaii, Reviews: Methods and Technologies in Fish Biology and Fisheries. Springer Netherlands, Dordrecht, pp. 7–64. https://doi.org/10.1007/978-94-017-1402-0_2

Arostegui, M., Gaube, P., Berumen, M., DiGiulian, A., Jones, B., Røstad, A., Braun, C. (2020) Vertical movements of a pelagic thresher shark (Alopias pelagicus): insights into the species' physiological limitations and trophic ecology in the Red Sea. Endanger. Species Res. https://doi.org/10.3354/esr01079

Biais, G., Coupeau, Y., Séret, B., Calmettes, B., Lopez, R., Hetherington, S., Righton, D. (2017) Return migration patterns of porbeagle shark (Lamna nasus) in the Northeast Atlantic: implications for stock range and structure. ICES J. Mar. Sci. 74, 1268–1276. https://doi.org/10.1093/icesjms/fsw233

Block, B.A., Teo, S.L.H., Walli, A., Boustany, A., Stokesbury, M.J.W., Farwell, C.J., Weng, K.C., Dewar, H., Williams, T.D. (2005) Electronic tagging and population structure of Atlantic bluefin tuna. Nature 434, 1121–1127. https://doi.org/10.1038/nature03463

Braun, C.D., Galuardi, B., Thorrold, S.R. (2018a) HMMoce: An R package for improved geolocation of archival-tagged fishes using a hidden Markov method. Methods Ecol. Evol. 9, 1212–1220. https://doi.org/10.1111/2041-210X.12959

Braun, C.D., Skomal, G.B., Thorrold, S.R. (2018b) Integrating Archival Tag Data and a High-Resolution Oceanographic Model to Estimate Basking Shark (Cetorhinus maximus) Movements in the Western Atlantic, in: Front. Mar. Sci. https://doi.org/10.3389/fmars.2018.00025

Braun, C.D., Gaube, P., Sinclair-Taylor, T.H., Skomal, G.B., Thorrold, S.R. (2019a) Mesoscale eddies release pelagic sharks from thermal constraints to foraging in the ocean twilight zone. Proc. Natl. Acad. Sci. 116, 17187–17192. https://doi.org/10.1073/pnas.1903067116

Braun CD, Gaube P, Afonso P, Fontes J, Skomal GB, Thorrold SR. (2019b) Assimilating electronic tagging, oceanographic modelling, and fisheries data to estimate movements and connectivity of swordfish in the North Atlantic. ICES J Mar Sci. doi: 10.1093/icesjms/fsz106

Braun, C.D., Skomal, G.B., Thorrold, S.R., Berumen, M.L. (2015) Movements of the reef manta ray (Manta alfredi) in the Red Sea using satellite and acoustic telemetry. Mar. Biol. 162, 2351–2362. https://doi.org/10.1007/s00227-015-2760-3

Evans, K.J., Arnold, G. (2008) Report on a geolocation methods workshop convened by the SCOR Panel on New Technologies for Observing Marine Life, 5-6 October 2007, San Sebastían, Spain. CSIRO Marine and Atmospheric Research, Hobart, Tas.

Gräwe, U., Naumann, M., Mohrholz, V., Burchard, H. (2015) Anatomizing one of the largest saltwater inflows into the Baltic Sea in December 2014: ANATOMIZING A MAJOR BALTIC SEA INFLOW. J. Geophys. Res. Oceans 120, 7676–7697. https://doi.org/10.1002/2015JC011269

Hijmans, R.J. (2019) geosphere: Spherical Trigonometry. R package version 1.5-10. https://CRAN.R-project.org/package=geosphere.

Hunter, E., Aldridge, J.N., Metcalfe, J.D., Arnold, G.P. (2003) Geolocation of free-ranging fish on the European continental shelf as determined from environmental variables: I. Tidal location method. Mar. Biol. 142, 601–609. https://doi.org/10.1007/s00227-002-0984-5

Hüssy, K., Casini, M., Haase, S., Hilvarsson, A., Horbowy, J., Krüger-Johnsen, M., Krumme, U., Limburg, K.E., McQueen, K., Mion, M., Olesen, H.J., Radtke, K. (2020) Tagging Baltic Cod – TABACOD. Eastern Baltic cod: Solving the ageing and stock assessment problems with combined state-of-the-art tagging methods, DTU Aqua Report no. 368-2020. National Institute of Aquatic Resources, Technical University of Denmark.

Hussey, N.E., Kessel, S.T., Aarestrup, K., Cooke, S.J., Cowley, P.D., Fisk, A.T., Harcourt, R.G., Holland, K.N., Iverson, S.J., Kocik, J.F., Mills Flemming, J.E., Whoriskey, F.G. (2015) Aquatic animal telemetry: A panoramic window into the underwater world. Science 348: 1255642–1255642

Jepsen, N., Thorstad, E.B., Havn, T., Lucas, M.C. (2015) The use of external electronic tags on fish: an evaluation of tag retention and tagging effects. Anim. Biotelemetry 3, 49. https://doi.org/10.1186/s40317-015-0086-z

Jonsen, I.D., Basson, M., Bestley, S., Bravington, M.V., Patterson, T.A., Pedersen, M.W., Thomson, R., Thygesen, U.H., Wotherspoon, S.J. (2013) State-space models for bio-loggers: A methodological road map. Deep Sea Res. Part II Top. Stud. Oceanogr. 88–89, 34–46. https://doi.org/10.1016/j.dsr2.2012.07.008

Köster, F.W., Huwer, B., Hinrichsen, H.-H., Neumann, V., Makarchouk, A., Eero, M., Dewitz, B.V., Hüssy, K., Tomkiewicz, J., Margonski, P., Temming, A., Hermann, J.-P., Oesterwind, D., Dierking, J., Kotterba, P., Plikshs, M. (2017) Eastern Baltic cod recruitment revisited—dynamics and impacting factors. ICES J. Mar. Sci. 74, 3–19. https://doi.org/10.1093/icesjms/fsw172

Le Bris, A., Fisher, J.A.D., Murphy, H.M., Galbraith, P.S., Castonguay, M., Loher, T., Robert, D. (2018) Migration patterns and putative spawning habitats of Atlantic halibut (Hippoglossus hippoglossus) in the Gulf of St. Lawrence revealed by geolocation of pop-up satellite archival tags. ICES J. Mar. Sci. 75, 135–147. https://doi.org/10.1093/icesjms/fsx098

Le Bris, A., Fréchet, A., Galbraith, P.S., Wroblewski, J.S. (2013a) Evidence for alternative migratory behaviours in the northern Gulf of St Lawrence population of Atlantic cod (Gadus morhua L.). ICES J. Mar. Sci. 70, 793–804. https://doi.org/10.1093/icesjms/fst068

Le Bris, A., Fréchet, A., Wroblewski, J.S. (2013b) Supplementing electronic tagging with conventional tagging to redesign fishery closed areas. Fish. Res. 148, 106–116. https://doi.org/10.1016/j.fishres.2013.08.013

Lipka, M. (2020) markolipka/ggPlotBathy.

Liu, C., Cowles, G.W., Zemeckis, D.R., Cadrin, S.X., Dean, M.J. (2017) Validation of a hidden Markov model for the geolocation of Atlantic cod. Can. J. Fish. Aquat. Sci. 74, 1862–1877. https://doi.org/10.1139/cjfas-2016-0376

Neuenfeldt, S., Hinrichsen, H.-H., Nielsen, A., Andersen, K.H. (2007) Reconstructing migrations of individual cod (Gadus morhua L.) in the Baltic Sea by using electronic data storage tags. Fish. Oceanogr. 16, 526–535. https://doi.org/10.1111/j.1365-2419.2007.00458.x

Nielsen, B., Hüssy, K., Neuenfeldt, S., Tomkiewicz, J., Behrens, J., Andersen, K. (2013) Individual behaviour of Baltic cod Gadus morhua in relation to sex and reproductive state. Aquat. Biol. 18, 197–207. https://doi.org/10.3354/ab00505

Nielsen, J., Mueter, F.J., Adkison, M.D. (2019) Effect of study area bathymetric heterogeneity on parameterization and performance of a depth-based geolocation model for demersal fishes.

Nissling, A., Kryvi, H., Vallin, L. (1994) Variation in egg buoyancy of Baltic cod Gadus morhua and its implications for egg survival in prevailing conditions in the Baltic Sea. https://doi.org/10.3354/meps110067

Nissling, A., Westin, L. (1991) Egg buoyancy of Baltic cod (Gadus morhua) and its implications for cod stock fluctuations in the Baltic. Mar. Biol. 111, 33–35. https://doi.org/10.1007/BF01986342

Pedersen, M.W., Righton, D., Thygesen, U.H., Andersen, K.H., Madsen, H. (2008) Geolocation of North Sea cod (Gadus morhua) using hidden Markov models and behavioural switching. Can. J. Fish. Aquat. Sci. 65, 2367–2377. https://doi.org/10.1139/F08-144

R Core Team (2017) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Reissmann, J. H. (1999). Bathymetry of four deep Baltic basins. Deutsche Hydrographische Zeitschrift, 51(4), 489–497. https://doi.org/10.1007/BF02764169

Righton, D., Mills, C.M. (2008) Reconstructing the movements of free-ranging demersal fish in the North Sea: a data-matching and simulation method. Mar. Biol. 153, 507–521. https://doi.org/10.1007/s00227-007-0818-6

Rose, G.A., deYoung, B., Colbourne, E.B. (1995) Cod (Gadus morhua L.) migration speeds and transport relative to currents on the north-east Newfoundland Shelf. ICES J. Mar. Sci. 52, 903–913. https://doi.org/10.1006/jmsc.1995.0087

Schaber, M., Hinrichsen, H.-H., Gröger, J. (2012) Seasonal changes in vertical distribution patterns of cod (Gadus morhua) in the Bornholm Basin, central Baltic Sea. Fish. Oceanogr. 21, 33–43. https://doi.org/10.1111/j.1365-2419.2011.00607.x

Seifert, T., B. Kayser (1995) "A high resolution spherical grid topography of the Baltic Sea", Meeresw. Berichte (Marine Science Reports), No. 9, 72-88.

Skomal, G.B., Zeeman, S.I., Chisholm, J.H., Summers, E.L., Walsh, H.J., McMahon, K.W., Thorrold, S.R. (2009) Transequatorial Migrations by Basking Sharks in the Western Atlantic Ocean. Curr. Biol. 19, 1019–1022. https://doi.org/10.1016/j.cub.2009.04.019

Teo, S., Boustany, A., Blackwell, S., Walli, A., Weng, K., Block, B. (2004) Validation of geolocation estimates based on light level and sea surface temperature from electronic tags. Mar. Ecol. Prog. Ser. 283, 81–98. https://doi.org/10.3354/meps283081

Thorstad, E.B., Rikardsen, A.H., Alp, A., Okland, F. (2014) The Use of Electronic Tags in Fish Research – An Overview of Fish Telemetry Methods. Turk. J. Fish. Aquat. Sci. 13. https://doi.org/10.4194/1303-2712-v13_5_13

Thorsteinsson, V., Pálsson, Ó., Tómasson, G., Jónsdóttir, I., Pampoulie, C. (2012) Consistency in the behaviour types of the Atlantic cod: repeatability, timing of migration and geo-location. Mar. Ecol. Prog. Ser. 462, 251–260. https://doi.org/10.3354/meps09852

Thygesen, U.H. (2009) Geolocating fish using Hidden Markov Models and Data Storage Tags. Rev. Methods Technol. Fish Biol. Fish. 277–293. https://doi.org/10.1007/978-1-4020-9640-2_17

Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York.

Woillez, M., Fablet, R., Ngo, T.-T., Lalire, M., Lazure, P., de Pontual, H. (2016) A HMM-based model to geolocate pelagic fish from high-resolution individual temperature and depth histories: European sea bass as a case study. Ecol. Model. 321, 10–22. https://doi.org/10.1016/j.ecolmodel.2015.10.02

Chapter 3

Movement of cod (*Gadus morhua* L.) in the southern Baltic Sea: evidence from data storage tags

Stefanie Haase[1], Ulf Gräwe[2], Camrin D. Braun[3,4], Axel Temming[5], Kate McQueen[1,6], Monica Mion[7], Franziska Maria Schade[1], Annelie Hilvarsson[7], Hans Jakob Olesen[8], Michele Casini[7,9], Karin Hüssy[8], Krzysztof Radtke[10], Uwe Krumme[1]

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

[2]Department of Physical Oceanography, Leibniz Institute for Baltic Sea Research Warnemünde, 18119 Rostock, Germany

[3]School of Aquatic and Fishery Sciences, University of Washington, 1122 NE Boat St, Seattle, WA 98105 USA

[4]Biology Department, Woods Hole Oceanographic Institution, 266 Woods Hole Rd, Woods Hole, MA 02543 USA

[5]Institute of Marine Ecosystem and Fisheries Science, Centre for Earth System Research and Sustainability (CEN), University of Hamburg, Große Elbstraße 133, 22767 Hamburg, Germany

[6]Institute of Marine Research, P.O. Box 1870 Nordnes, 5817 Bergen, Norway

[7]Department of Aquatic Resources, Institute of Marine Research, Swedish University of Agricultural Sciences, Turistgatan 5, 45330 Lysekil, Sweden

[8]Technical University of Denmark, National Institute of Aquatic Resources, Kemitorvet, 2800 Kgs. Lyngby, Denmark

[9]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Selmi 3, 40126 Bologna, Italy

[10]National Marine Fisheries Research Institute, Ul. Kołłątaja 1, 81-332 Gdynia, Poland.

Abstract

The three-dimensionality of marine ecosystems enables species for distribution in horizontal and vertical directions and can lead to intraspecific behavioural differences in stratified environments in habitats of close proximity. Knowledge about movements of individual fish linked to environmental conditions helps fisheries scientists to interpret the variation in seasonal stock structure and distribution of a population. We analysed the temperature and depth profiles of 28 individual cod tagged with data storage tags in the southern Baltic Sea and reconstructed their movements throughout their time at liberty with a geolocation framework adapted to cod in the southern Baltic Sea. Based on depth occupation and estimated daily positions, we matched salinity and oxygen conditions obtained from a regional ocean model. Either genetics or otolith shape analysis were used to assign individuals to the Western Baltic (WBC) or Eastern Baltic cod (EBC) stock. The data on EBC demonstrated a high variation in horizontal and vertical movement patterns ranging from residence limited to the Bornholm Basin throughout the year to migration between coastal feeding grounds (November-April) and deeper spawning grounds (May-October). In contrast, WBC stayed in shallower, coastal, normoxic areas year-round and were exposed to greater seasonal fluctuations in water temperature. Depth time-series data revealed daily vertical movements especially of EBC, triggered by twilight, some EBC individuals showed vertical movements related to the lunar cycle. Our data suggest a significant vertical separation of WBC and EBC in the southern Baltic Sea which results in different conditions of temperature, depth, salinity and oxygen experienced and results in different biological conditions of the two stocks.

Keywords: Baltic cod, migratory behaviour, electronic tagging, geolocation model

Introduction

Habitat transitions in longitude and latitude are known to cause intraspecific differentiation in marine environments (Piacenza et al., 2015; Bowen et al., 2016; Gaither et al., 2018). The influence of vertical habitat transition on species diversification is less understood but depth and strong environmental gradients are known to separate closely related species and lead to changes in functionality and size (Jennings et al., 2013; Mindel et al., 2016; Gaither et al., 2018). Studies on vertical separation are mainly restricted to the deep sea where depth gradients are large (e.g. *Coryphaenoides rupestris* (Gaither et al., 2018)) or tidal coasts where environmental zonation occurs on scales of meters (e.g. *Littorina saxatalis* (Rolán-Alvarez, 2007)), *Bellapiscis medius* and *B. lesleyae* (Hilton et al., 2008)) and Whiting in the North Sea are known to have specific feeding strategies depending on their habitat preference (Pedersen, 2005). Understanding the underlying mechanisms can be achieved by observing the species temporal space use and the interaction with environmental conditions with the help of timing and direction of individual fish movements (Griffiths et al., 2018). The occurrence of different biological niches can thus lead to the co-existence of genetically distinct individuals of the same species, each adapted to a respective niche (Goudarzi et al., 2019).

Atlantic cod (*Gadus morhua*) experience a wide range of physical and biological factors and occupies a wide range of ecological niches throughout the coastal and continental shelf regions in the North Atlantic (Brander, 1994). Depth-specific niche occupation and behavioural patterns of co-existing cod populations were observed in several areas of the North Atlantic and are key to sustain a wide range of biotic and abiotic ecosystem properties (reviewed in Robichaud and Rose (2004)). The Baltic Sea is an extreme environment for cod because salinity and temperature are at the lower and higher tolerance limit, respectively (Mackenzie et al., 2007). While living at the eastern boundary of the geographic range, Baltic cod populations are also genetically distinct to other Atlantic cod populations (Nielsen et al., 2003; O’Leary et al., 2007; Kijewska et al., 2011). Although both stocks are morphological very similar, they differ, among others, in growth rate (McQueen et al., 2020), otolith shape (Hüssy et al., 2016; Schade et al., 2019), number of vertebrae (reviewed in Bagge et al., 1994), spawning season (Bleil et al., 2009) and haemoglobin type (Sick, 1965; Andersen et al., 2009). The two cod stocks, that are genetically different, inhabit the southern Baltic Sea (Hemmer-Hansen et al., 2019; Weist et al., 2019) and are managed as two separate management units, namely “Western” (WBC, ICES Subdivision (SD) 22-24) and “Eastern”

(EBC, SD 25-32) Baltic cod. The region of the Arkona Basin (SD 24) is a mixing area between the stocks (Hemmer-Hansen et al., 2019; Weist et al., 2019). Both stocks used to be of high commercial value but recent negative stock developments resulted in a bycatch only quota for the EBC stock in 2020 and 2021 (ICES, 2020a) and severe reductions in quota for the WBC stock (ICES, 2020b). The reasons for this differ between the stocks: while WBC stock size decreased due to high fishing pressure and poor recruitment in recent years (ICES, 2020b), EBC suffer from reduced condition, reduced growth rate and increased natural mortality due to a variety of stressors such as high levels of liver infestation with *Contraceacum osculatum*, poor nutritional conditions and thiamine deficiency (Eero et al., 2015; Horbowy, 2016; Sokolova et al., 2018; ICES, 2019; Engelhardt et al., 2020; ICES, 2020c; Mion et al., 2020).

A comparison of growth rates of conventionally tagged individuals from both stocks revealed that both area and stock affiliation contribute to the very low growth rate and productivity of EBC (McQueen et al., 2020) but reasons are yet not fully understood. In fact, even though conventional tag-recapture studies are useful for e.g. estimating growth rates or the extent of the distribution area (Latour, 2005), the great disadvantage is that movements between release and recapture cannot be reconstructed and it is unsure which conditions the individuals experience during their time at liberty (Kohler and Turner, 2001).

While scientific trawl and hydroacoustic surveys can give an indication about the seasonal distribution of fish stocks, they only provide snapshots of a situation and the behaviour and ecology of individuals often remains unclear. Bio-logging devices such as data storage tags (DSTs) have therefore become valuable tools to collect basic information on the natural history of wild, free-ranging fish (Lucas and Baras, 2000; Cooke et al., 2011) under the assumption that tagged individuals are a subsample of the whole stock and behave like untagged fish after release (Pine et al., 2012). Although common trade-offs with DSTs are the sample size, the duration of battery life and the type and resolution of information collected (Hussey et al., 2015; Andrzejaczek et al., 2019; Andrzejaczek et al., 2020), DSTs have become popular to estimate fish movements and their interaction with the surrounding environment (Neuenfeldt et al., 2007; Costa et al., 2012; Hussey et al., 2015; Hays et al., 2016; Griffiths et al., 2018). However, in most cases daily positions cannot be obtained directly from archival tags and geolocation frameworks are needed to reconstruct positions between release and recapture (e.g. Pedersen et al., 2008; Thygesen, 2009; Neat et al., 2014; Braun et al., 2018).

In addition to horizontal movements, vertical movements can be directly observed and can give an indication about feeding strategies and the response to different environmental conditions (Hobson et al., 2007; Nielsen et al., 2013). Often, also directly recorded temperature values inform about the range of ambient temperatures a certain fish species can tolerate. Free-ranging fish can give a better reference of the temperature niche used than experiments in captivity (Righton et al., 2010). Therefore, studies involving DSTs of free-ranging fishes have greatly contributed to enhance our understanding of habitat use (Metcalfe et al., 2008; Galuardi and Lutcavage, 2012), migration patterns (Hunter et al., 2006; Metcalfe et al., 2008; Zemeckis et al., 2017), and population structure (Hunter et al., 2004; Block et al., 2005; Galuardi and Lutcavage, 2012).

Past mark-recapture studies of cod in the southern Baltic Sea (SD 24-26) revealed that individuals are sedentary within their specific basins (reviewed in Robichaud and Rose (2004)). Other studies describe movement patterns between shallower feeding grounds to the deep basins for spawning (Aro, 1989; Wieland et al., 2000; Neuenfeldt et al., 2007), but the timing and behaviour on the feeding and spawning grounds remains mostly unclear (Nielsen et al., 2013) and a separation of EBC and WBC did not occur in these studies.

To understand behaviour under the recent environmental conditions and with the possibility to allocate individuals to one of the two stocks, we analysed 28 individual cod tagged with temperature-DSTs in the southern Baltic Sea between 2016 and 2018. The temperature-depth recordings were used in a geolocation framework (Haase et al., 2021; uncertainty of daily positions: on average below 20 km) to reconstruct likely horizontal movements as well as salinity and oxygen experience of individual cod. Subsequently, all individuals were assigned to the EBC and WBC stock using genetics or otolith shape analysis. We examined temporal and spatial patterns in movements of the recaptured cod in response to changes in environmental parameters.

Materials and methods

Study area

The Baltic Sea is a semi-enclosed, non-tidal, brackish-water area characterized by a series of deep basins separated by shallower sills. In the basins of the southern Baltic, the water column is characterized by a strong thermohaline stratification which leads to stagnation of the deep-water and occurrence of hypoxic and anoxic areas (Møller and Hansen, 1994; Naumann et al., 2020) but weak horizontal gradients in water temperature exist within each basin. In contrast, the water column in the shallow, near-shore areas is regularly mixed during autumn and winter storms and hypoxic areas at the bottom might only occur in late summer (Conley et al., 2011; Carstensen and Conley, 2019; LLUR, 2020).

Data storage tags and tagging procedure

In Danish, German and Swedish waters (SD 24-25), 1260 cod were tagged with DSTs between 2016 and 2019. Cod were mostly caught with bottom trawls (trawl duration 5-30 min) from research and commercial vessels. A smaller proportion was caught with angling, pound nets and fish traps.

Cod which did not show any sign of skin damage or barotrauma were selected and transferred into a water bath containing MS-222 3-aminobenzoate methanesulfonate (Sigma-Aldrich, dose 5 g/50 l) for sedation. Cod were monitored closely, and internal DSTs were surgically implanted after cod did no longer response to grabbing the tail. Cod were measured (in mm) and weighted (in g). Depending on cod weight (to not exceed 3% of bodyweight), either a milli-DST or micro-DST (Star-Oddi Iceland), weighting 7 g and 1.9 g in water and capable of storing 1,398,100 and 87,906 measurements, respectively, was implanted. The DSTs measured temperature (resolution: 0.032 °C, accuracy: +/-0.1°C (milli), +/-0.2°C (micro)) and pressure which was transformed into depth (resolution: 0.03 m (milli), 0.12 (micro), accuracy: +/-0.6 m (milli), +/- 0.6 m (micro)). Depending on the time interval to be covered, tags were programmed to record pressure and temperature every 1, 3 or 30 minutes.

Tetracycline-hydrochloride (TET) was internally injected in the body cavity which was used for analysis on otoliths in Haase et al. (in prep.). Cod were tagged externally with two T-bar anchor tags. After recapture, cod were assigned genetically (Hemmer-Hansen et al., 2019) or by shape analysis of the otolith (Schade et al., 2019) to the WBC or EBC stock. The fish length of returned frozen cod were transformed using a shrinkage key developed for cod in the southern Baltic Sea (McQueen et al., 2019b). Sex and maturity were estimated following the Manual for the Baltic International Trawl Surveys (ICES, 2014). The condition factor was calculated for each fish (Fulton's K = weight (g)/ length (cm)^3 * 100). Liver infestation was quantified by counting the total number of liver worms visible on the surface and assigning the number to the infestation category (Tab. 1).

Estimation of geographic positions

We adapted the geolocation model HMMoce (Braun et al., 2018) to geolocate cod in the southern Baltic Sea (Haase et al., 2021) using a regional ocean model (ROM, Gräwe et al., 2015). The R-script of the adapted HMMoce is permanently stored at https://github.com/StefanieHaase/Adapted-HMMoce. This approach combines likelihoods derived from tag-based depth and temperature data to reconstruct daily positions of cod tagged with DSTs. Likelihoods are combined in a gridded hidden Markov model framework that computes posterior probability distributions to estimate the most likely position of the animal at each time point (for model details see Braun et al. 2018).

This framework was primarily adapted to account for the demersal behaviour of cod and the specific stratification regime of the southern Baltic Sea. Subsequent validation experiments found that the adapted model was able to consistently reconstruct tracks to within 20 km of known locations and often exhibited higher accuracy depending on stratification, heterogenous bathymetry and the number of vertical movements conducted by the tagged individual (see Haase et al. (2021) for details of the validation experiments). The model performance was further tested in this study by comparing temperature data recorded by the DST and modelled temperature from the Baltic ROM.

Data analysis

For each individual we calculated the straight-line distance between subsequent daily positions as well as the overall displacement distance between release and recapture using the great-circle method (Hijmans, 2019). The resulting tracks were separately analysed for WBC and EBC. Further, EBC were visually classified into two behavioural types: individuals which stayed within or in close proximity to the Bornholm Basin throughout their time at liberty (resident EBC) and individuals moving between the Bornholm Basin during spawning and their coastal feeding grounds (migratory EBC). For individuals with days-at-liberty (DAL) less than a complete year, the behavioural mode was classified based on the available track and the covered months. Individual U11227 was classified as EBC by shape analysis, however, visual inspection of the depth profile and track showed similar behaviour to the other WBCs and U11227 was therefore subsequently analysed as WBC.

Based on daily position and depth, salinity and oxygen values (in mol/kg) were matched from the same Baltic ROM. Oxygen was transformed to ml/l, assuming 1 l of water weighting 1.025 kg. Temperature and depth recordings and matched salinity and oxygen values were then summarised for each individual by calculating daily means (±sd, averaged over the depths occupied), minimum and maximum values. Mean (±sd), minimum and maximum monthly temperature, depth, salinity and oxygen values were further summarised per behaviour type. To account for possible short-term post-treatment effects, we removed the first six days after release (van der Kooij et al., 2007). Additionally, we excluded the day of recapture, to avoid extraordinary depth and temperature changes caused by the recapture process (Hobson et al., 2007).

The distributional area of each individual was estimated with the kernel density estimation (KDE) of the R package adehabitatHR (Calenge, 2006). We used the 50% and 90% volume density contours to estimate the core area and home range of each individual, respectively (Worton, 1989; Seaman and Powell, 1996). These estimates were then compared between the three distinguished behavioural modes.

A generalized additive mixed model (GAMM) was used to investigate the temporal depth use of cod in the southern Baltic Sea. Behavioural type was used as categorial variable (coastal WBC, migratory EBC and resident EBC). We considered time of day (hour of day), day of the year (DOY) and fraction of moon illumination (0-1) as continuous variables. The daily fractions of lunar illumination was extracted from the R package ‘suncalc’ (Thieurmel and Elmarhraoui, 2019) and arcsin transformed prior to the statistical analysis (Zar, 1996; Braun et al., 2014). Individual fish ID was included as a random effect.

GAMMS were constructed with a normally distributed error structure using maximum likelihood estimates with the R package ‘mgcv’. A cyclic smoother was applied to day of the year to account for the circularity of this variable (Andrzejaczek et al., 2020). The model was corrected for autocorrelation in the time series. Model comparison was conducted with the Akaike’s information Criterion (AIC). Models with lower AIC are considered to have better fit; ΔAIC>2 indicates a significant improvement.

Statistical analyses were carried out in R 3.4.3 (R Core Team, 2017).

Results

From a total of 1260 DST-tagged cod (mean length at release: 355 mm, range 148-952 mm), 43 tags (3.4%) were returned (mean length at release: 397 mm, range: 269-585 mm) and 32 tags exceeded 30 days at liberty. The track of four individuals could not be reconstructed with the adapted HMMoce because release and recapture position were not reproduced correctly. The tracks of 28 DST tagged cod were reconstructed (Tab. 2) resulting in 5336 daily positions with corresponding records of depth and temperature. The direct distance between release and recapture was on average 28.8% of the track revealed by the daily positions (Tab. 1). Visual inspection of the temperature error plots (Figs. 1 & S1) suggest that the adapted HMMoce performed well in most cases but exceptions are B1817 and U11093. After excluding the first day to remove the influence of release (the tag adopted the air temperature before tagging), temperature recorded by the DST and modelled by the ROM varied on average by 0.22 ± 1.25°C.

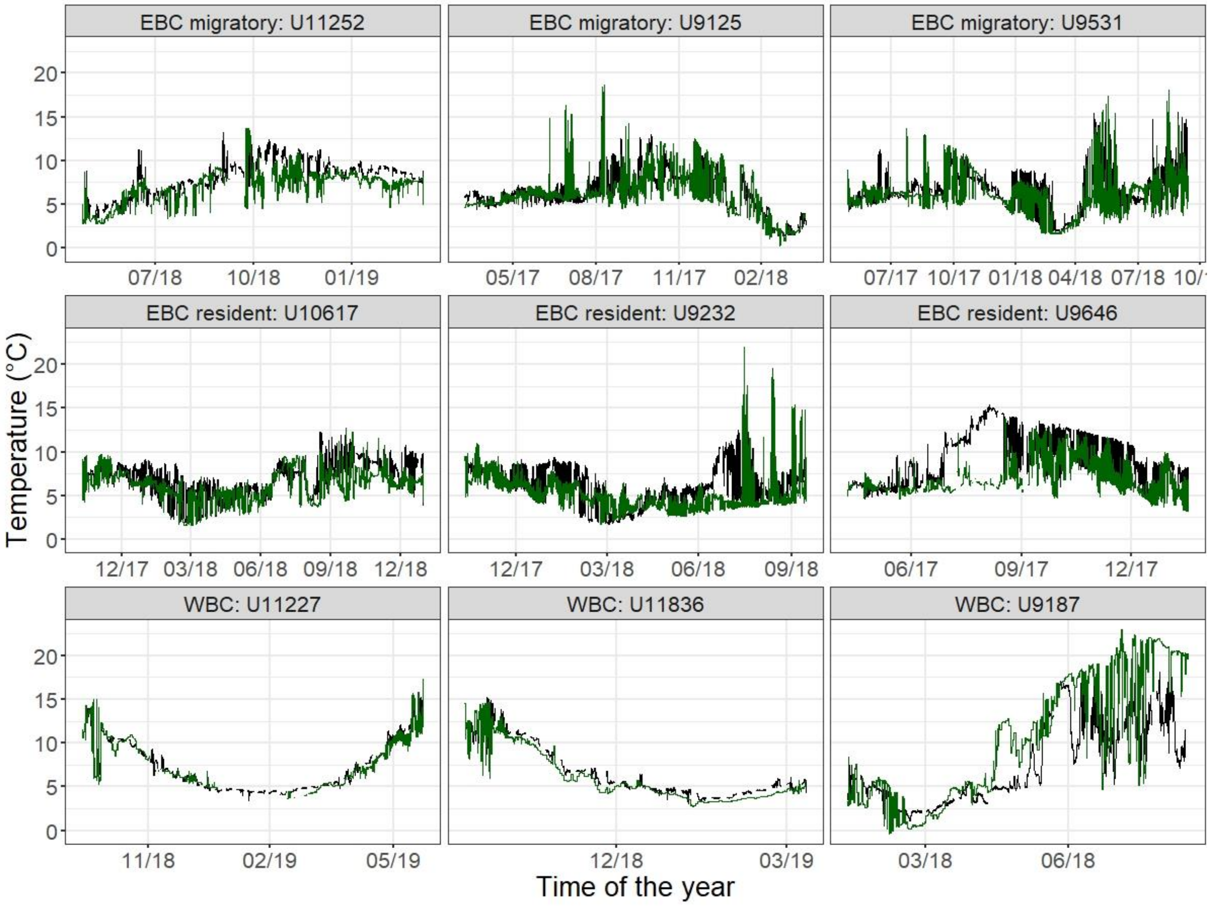

Figure 1: Temperature profile as recorded by nine DSTs (black line) and estimated by the geolocation model (green line). ID U11227 was classified as Eastern Baltic cod by shape analysis but the temperature and depth profile suggested to reassign this individual to the Western Baltic cod stock. The temperature profiles of all DSTs can be found in S1.

Differences in behavioural modes

The reconstructed tracks of the recaptured cod in the southern Baltic Sea showed a high degree of individual and seasonal variability in horizontal movement patterns (Fig. 2), depth occupation (Figs. 3, 4, S2), ambient temperature (Figs. 1, 3, S1), associated oxygen (Fig. 3, 5, S3) and salinity conditions (Figs. 3, 6, S4). Five individuals were assigned to the WBC stock (Tab. 1). Seven of the 24 EBC remained resident to the Bornholm Basin, while 16 performed movements between the Bornholm Basin and coastal feeding grounds (Fig. 2). Since not all individuals were at liberty for a complete year, time of the year was considered when classifying the behavioural type. The three behavioural modes differed in the extend of the home range and core area with resident EBC having smaller home ranges and core areas than migratory EBC and WBC (Tabs. 1, 2, Fig. S5).

WBC (n = 5) primarily occupied shallower (mean = 15.1 ± 6.3 m, min = 0 m, max = 49.6 m) and warmer (mean = 7.3 ± 3.5 °C, min = 1.1 °C, max = 18.1 °C), more coastal habitats in the southern Baltic Sea throughout their time at liberty (Fig. 2, Tab. 2). The temperature profile of WBC showed a strong seasonal signal with coldest temperatures in March and warmest temperatures between July and October (Figs. 1, 3 & S1). Four WBC used the shallow waters of the Rönnebank and around the island of Bornholm, one inhabited the shallow waters of the German and Polish coast (Fig. 2). Daily distances travelled varied between 0.7 km and 4.7 km with an average of 2.2 ± 1.5 km (Tab. 1). On average, WBC inhabited waters with a salinity of 7.7 ± 0.6 and did not experience hypoxia during their time at liberty (mean = 7.7 ± 0.8 ml/l, min = 3.8 ml/l, max = 9.4 ml/l). WBC had the highest condition of the three behavioural groups (1.0 ± 0.1) and liver worm infestation was only detected in the individual staying close to the island of Bornholm throughout the time at liberty (U11227, Tab. 1). One fish was recaptured during the spawning process in late January (U11203), another was classified as spent in early April (U11836) and likely used the stationary period in February and March to spawn (Fig. 4). Both WBC used the southern rim of the Arkona Basin at the Rönnebank for spawning.

Resident EBC (n = 7) inhabit the deeper Bornholm Basin or adjacent areas throughout the year (Fig. 2) and travelled on average distances of 2.2 ± 1.4 km a day. Seasonal temperature differences were also visible for resident EBC but the extend was smaller compared to WBC (Fig. 3). During vertical movements, resident EBC occupied water depth between 5.8 m and 78.0 m corresponding to temperatures between 1.7°C and 15.3°C (Tab. 2). The largest depth and temperature range recorded within a day was 62.1 m and 9.6°C, respectively. Since resident EBC stayed within the Bornholm Basin year-round, they were exposed to hypoxic and anoxic condition throughout the year which resulted in the lowest mean oxygen content (6.6 ± 1.6 ml/l, Tab. 2). Resident EBC showed the highest degree of liver infestation of the three behavioural groups, and only one highly mobile cod (ID 10617) did not show any infestation. Resident EBC had on average a condition factor of 0.8 ± 0.1 and apart from one individual which was not classified, the rest of the individuals had a maturity stage of spent or resting and did not show any sign of skipped spawning.

Migratory EBC (n = 16) performed seasonal movements between shallower, coastal feeding areas, close to Rügen and the Hanö Bight (residence on the feeding grounds: November-April), and the deeper Bornholm Basin during spawning (residence on the spawning ground: May-November, Figs. 2, S2). Arrival on the spawning ground (May-June) was visually detected by abrupt descents in the water column to depth below 50 m and coincided with the arrival in the Bornholm Basin as revealed by horizontal geolocation. Residence on the spawning ground varied slightly between individuals (Figs. 4, S2). Daily horizontal movements were on average the highest among the three behavioural groups (mean = 2.6 ± 1.1 km, Tab. 2). The DSTs of migratory EBC recorded depth and temperatures between 0 m and 86.9 m and 0.6°C and 17.0°C with daily ranges of 58.4 m and 12.5°C (Tab. 2). Temperatures were on average lower during the first quarter but the seasonal pattern was less distinct than in WBC (Fig. 3). On the spawning ground, migratory EBC regularly moved between hypoxic and non-hypoxic waters (Figs. 5, S3). Migratory EBC experienced on average the highest salinity values 9.8 (±2.0), min = 6.6, max = 19.2). The condition factor of migratory EBC (0.9 ± 0.1) was on average slightly higher compared to resident EBC but lower than WBC. Two individuals (U9144, U9105) were recaptured in the Bornholm Basin during spawning in August and July, several individuals were recaptured between February and June while preparing for spawning (Tab. 1). U9023 was recaptured preparing for spawning although that individual already arrived on the spawning ground 22 days before recapture. Individual U91206 might have skipped spawning because the individual was recaptured in June in resting stage. Four migratory EBC depicted distinct movements to and off the spawning ground and stayed between 124 (U9008) and 184 days (U9127) deeper than 50 m.

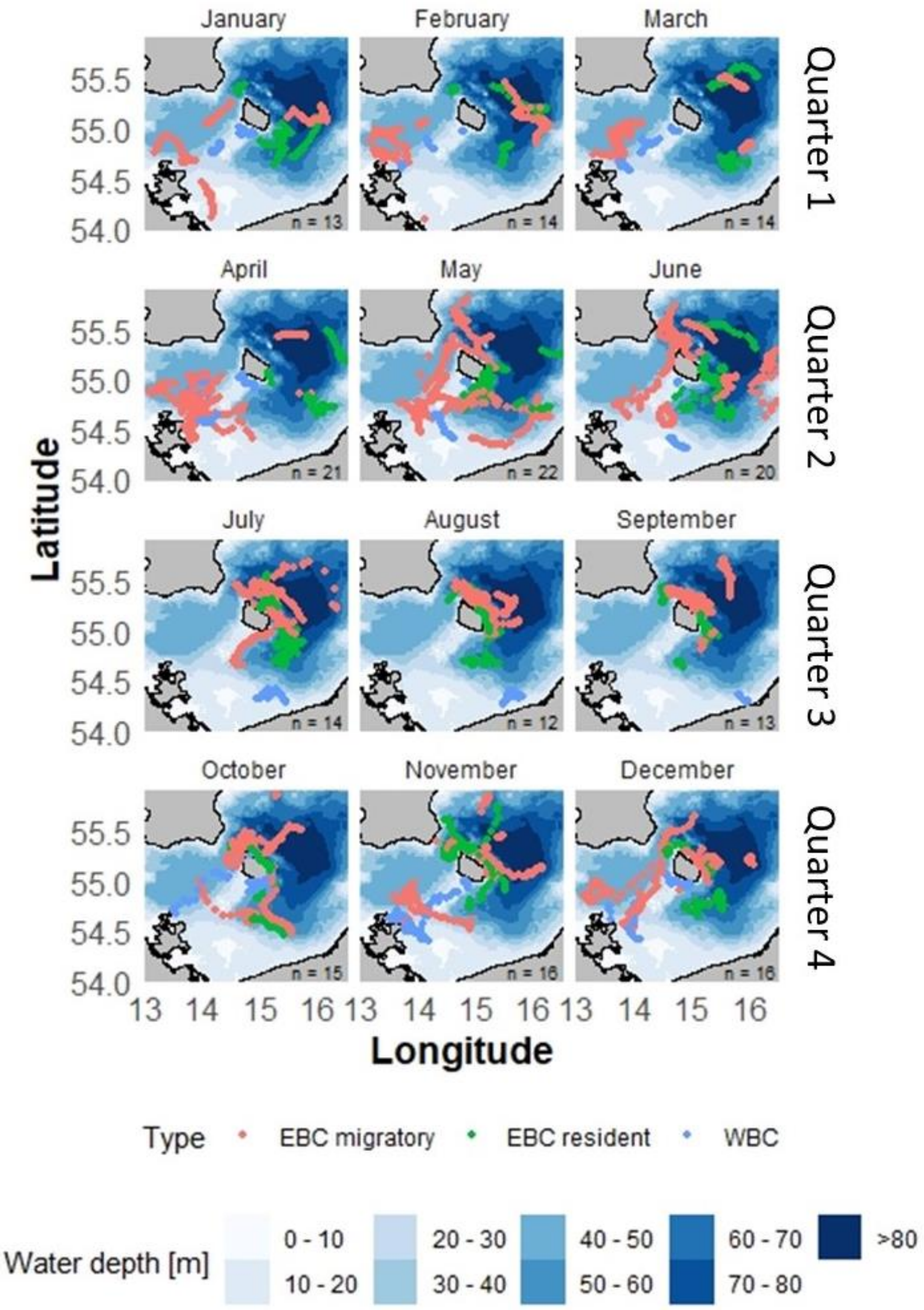

Figure 2: Daily most probable positions of individual cod tagged with DSTs separated into month. Cod were allocated to the western (blue) and eastern (red, green) stock. Further, visually inspections of the geolocated tracks for EBC revealed two distinct behavioural modes: cod resident to the Bornholm Basin (green) and cod migratory between feeding grounds and known spawning area (red). The uncertainty for each daily position is less than20 km (for comparison: the distance between Rönne and Nexö on the island of Bornholm is 29 km).

Table 1: Details of individual cod tagged with DSTs and spatial statistics summarizing movements. DAL: days at liberty. Maturity stages: 1: virgin, 2: maturing, 3: spawning, 4: spent, 5: resting, 6: abnormal. Daily length growth was calculated as the difference in fish length between release and recapture divided by the DAL. Liver infection: Macroscopic quantification of liver worms on the surface of the liver (scale: 0: 0 worms, 1: 1-10 worms, 2: 11-20 worms, 3: >20 worms). Direct distance: distance between reported release and recapture position. "-" indicates missing information. The recapture position of U10479 is unknown but was estimated based on the temperature-depth profile of the last days.

Behavioural type	ID	Release date	DAL	Sex	Maturity	Liver infection	Condition factor	Fish length at release (mm)	Estimated daily length growth (mm/day)	Direct distance between release and recapture (km)	Total distance during DAL (km)	Average daily distance (km) (±sd)	Home range (km^2)	Core area (km^2)
Coastal WBC	U11836^{*,1}	10.10.2018	181	F	4	0	0.78	497	0.08	36.4	542	3.0 (4.4)	4562	1063
	B2055	10.10.2018	55	F	2	0	0.89	443	0.15	106.3	258.6	4.7 (2.9)	10048	3620
	U11203^{1}	11.10.2018	106	F	3	0	1.05	407	0.25	75.2	149.5	1.4 (4.2)	432	120
	U11227^{*,2}	10.10.2018	253	F	-	2	1.14	468	0.17	6	177.9	0.7 (1.2)	189	42
	U9187*	07.02.2018	218	F	5	-	1.00	316	0.15	152.3	281.8	1.3 (1.3)	9357	3367
Resident EBC	U9646*	06.05.2017	285	M	5	1	0.86	355	0.11	46.8	360.3	1.3 (1.0)	2085	693
	B1980	06.05.2017	135	F	4	3	0.74	471	0.12	24.5	712	5.3 (3.9)	3345	1046
	B1960	06.05.2017	39	F	d	1	0.82	398	0.23	12.8	34.1	0.9 (0.5)	182	61
	U10573	27.04.2018	231	M	5	3	0.93	408	0.07	45.6	208.6	0.9 (1.1)	1023	291
	U10617*	07.11.2017	447	M	5	0	0.93	269	0.08	121.8	1019.7	2.3 (2.0)	13145	4401
	U11093	27.04.2018	50	F	4	3	0.85	418	-0.04	58	99.7	2.0 (1.7)	1142	264
	U9232*	09.11.2017	337	F	5	2	0.70	400	0.18	141.4	864	2.6 (2.6)	5060	1476
Migratory EBC	U9125*	07.04.2017	375	M	1	0	1.29	328	0.08	15.3	1254.9	3.3 (2.9)	15343	4940
	U11252^{*,1}	22.05.2018	319	M	2	1	0.94	329	0.11	108.3	538.4	1.7 (1.8)	7422	2485
	U9127^{1}	07.04.2017	278	M	2	0	0.76	319	0.06	34.6	1461.7	5.3 (5.2)	20125	5101
	U9008	29.03.2017	278	M	5	1	0.75	385	0.1	75.5	867.7	3.1 (3.4)	11167	2901
	U10989	22.03.2018	172	-	-	-	-	375	0.04	95.3	363.2	2.1 (1.8)	7430	2592
	U9105	07.04.2017	115	M	3	0	0.78	370	0.24	127.5	218.4	1.9 (2.1)	8779	3026
	U9023	01.02.2017	123	F	2	1	0.88	364	0.08	85.9	390.5	3.2 (2.4)	9213	2425
	U10479	26.09.2017	92	M	5	0	0.62	388	0.1	-	276.7	3.0 (3.0)	7962	2432
	U9106	06.04.2017	75	M	5	0	1.11	386	0.43	45.1	164.3	2.2 (2.9)	771	209
	U10731	08.02.2018	60	M	2	2	0.81	398	0.07	36.5	230.2	3.8 (3.0)	2400	626
	B1845	17.11.2017	77	M	2	1	0.66	487	0.1	61	143.8	1.9 (1.4)	1669	494
	U9531*	25.05.2017	546	F	6	2	0.71	345	0.14	36	1171.5	2.1 (3.5)	6215	2084
	U9144	08.02.2018	156	M	3	2	0.84	432	0.14	229.6	450.3	2.9 (2.5)	30764	9614
	B1815	06.04.2017	58	M	2	3	1.04	404	0.33	28	56.1	1.0 (0.8)	410	102
	B1817^{3}	07.04.2017	52	-	-	-	-	399	-	129.4	151	2.0 (1.9)	11148	4054
	U8882	24.10.2016	195	M	2	1	0.94	338	0.11	109.6	235.8	1.2 (2.0)	7158	1767

[1] stock was assigned by shape analysis

[2] cod was classified retrospectively as EBC by shape analysis but depth profile and track suggested that this is a WBC

[3] no fish was returned so no stock assignment was possible; assignment was suggested by the track

* the profiles of these cod are shown in Figs. 1, 4,5,6, all other profiles are shown in the supplementary material

Table 2: Summary of the depth and temperature experience for each behavioural type as recorded with the DSTs. Salinity and oxygen values are extracted, based on daily position and depth, from the Baltic ROM. Mean values were calculated from daily means for each individual. Home range and core area are rounded to full km².

		Behavioural type		
		WBC (n=5)	migratory EBC (n=16)	resident EBC (n=7)
	No. days with measurements	540	2852	1738
Depth (m)	mean (±sd)	15.1 (±6.3)	42.5 (±16.1)	48.2 (±11.0)
	range (min-max)	0-49.6	0-86.9	5.8-78.0
	max. range within a day	37.2	58.4	62.1
Temperature (°C)	mean (±sd)	7.3 (±3.5)	6.8 (±2.2)	7.2 (±2.3)
	range (min-max)	1.1-18.1	0.6-17.0	1.7-15.3
	max. range within a day	9	12.5	9.6
Salinity (PSU)	mean (±sd)	7.7 (±0.6)	9.8 (±2.0)	9.6 (±1.4)
	range (min-max)	6.7-16.3	6.6-19.2	7.0-14.2
	max. range within a day	8.9	11.5	6.8
Oxygen (ml/l)	mean (±sd)	7.7 (±0.8)	6.7 (±1.9)	6.5 (±1.7)
	range (min-max)	3.8-9.4	0-9.4	0-9.1
	max. range within a day	3.8	8.5	8.8
Home range (km²)	mean(±sd)	4323 (±4043)	10594 (±6862)	55848.06(±4903)
	range (min-max)	189 -10048	409.78-30764	182.38-13145
Core area (km²)	mean(±sd)	1415 (±1477)	3187.04 (±2032)	1890.32 (±1667)
	range (min-max)	48-3620	101.97-9614	61.10-4401

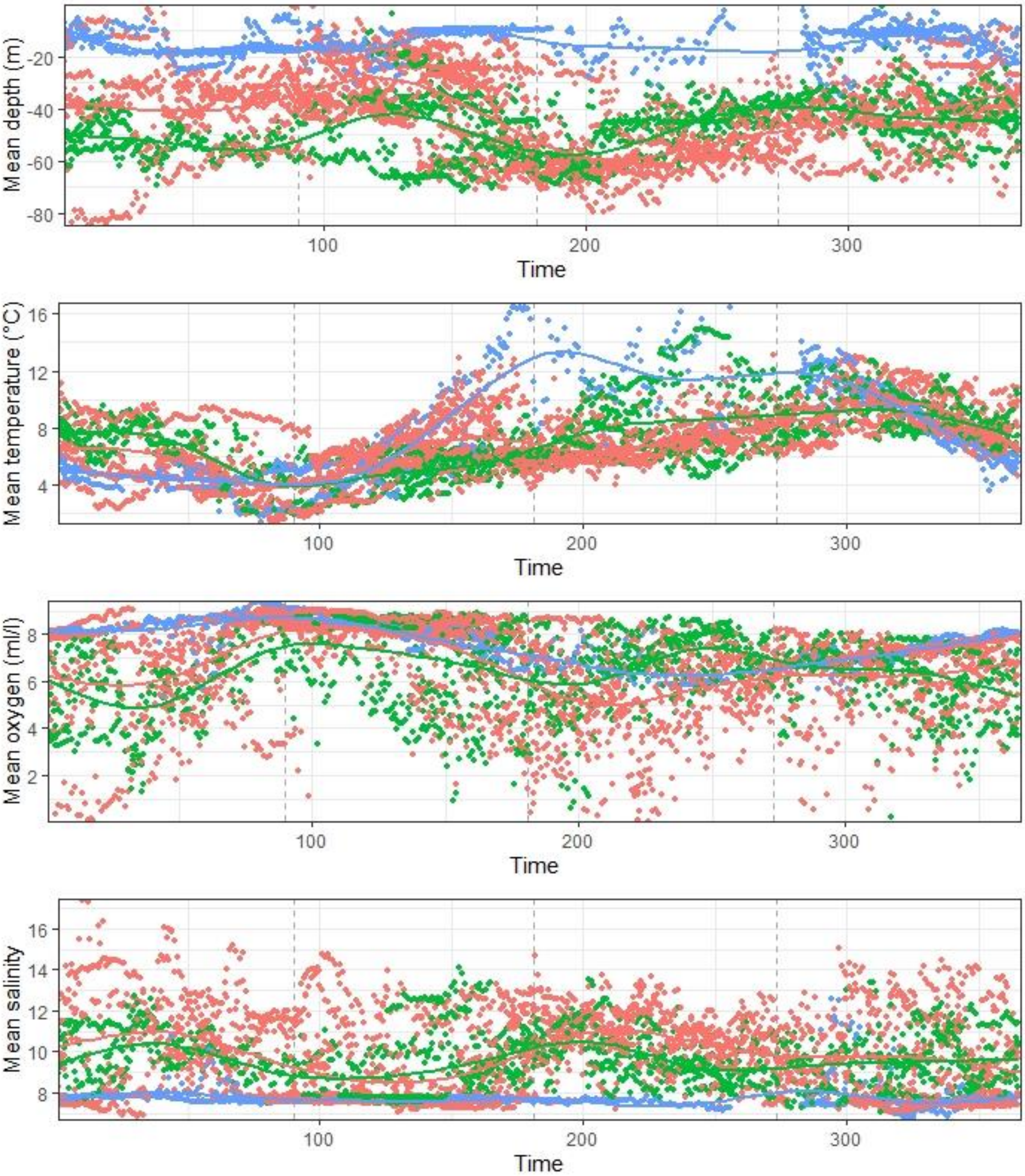

Figure 3: Mean depth, temperature, oxygen and salinity values per individual and day (n = 5 data-storage-tag (DST) profiles of WBC, blue; n = 7 DST profiles of resident EBC, green; n = 16 DST profiles of migratory EBC, red). Single dots mark the mean of the recorded values per individual and day. A LOESS smoother with grey-shaded area as the 95% confidence interval shows the overall trend of the data points. Vertical dashed lines mark the transition between quarters (31.3., 30.6., 30.9.).

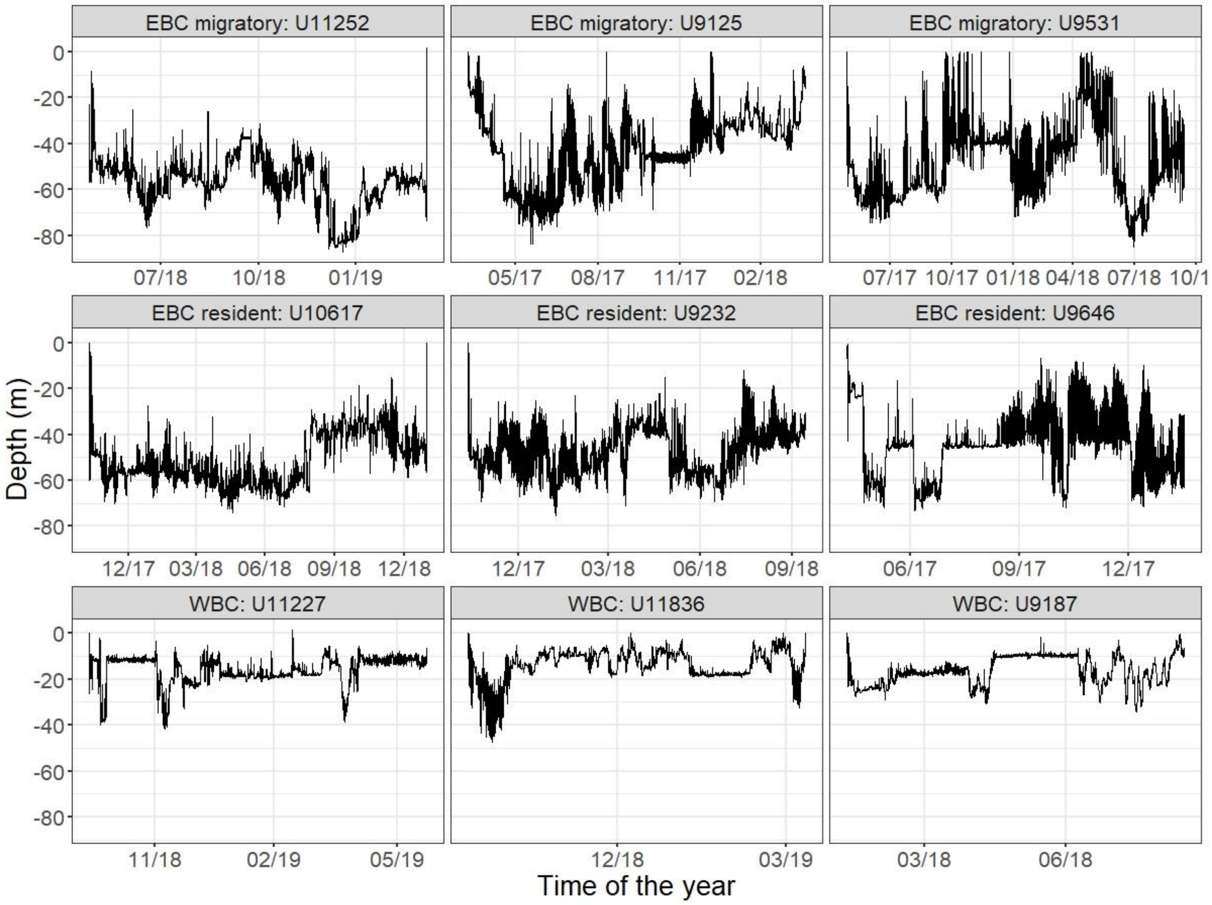

Figure 4: Depth as recorded by nine DSTs (date format: mm/yy). The depth profiles of all DSTs can be found in S2.

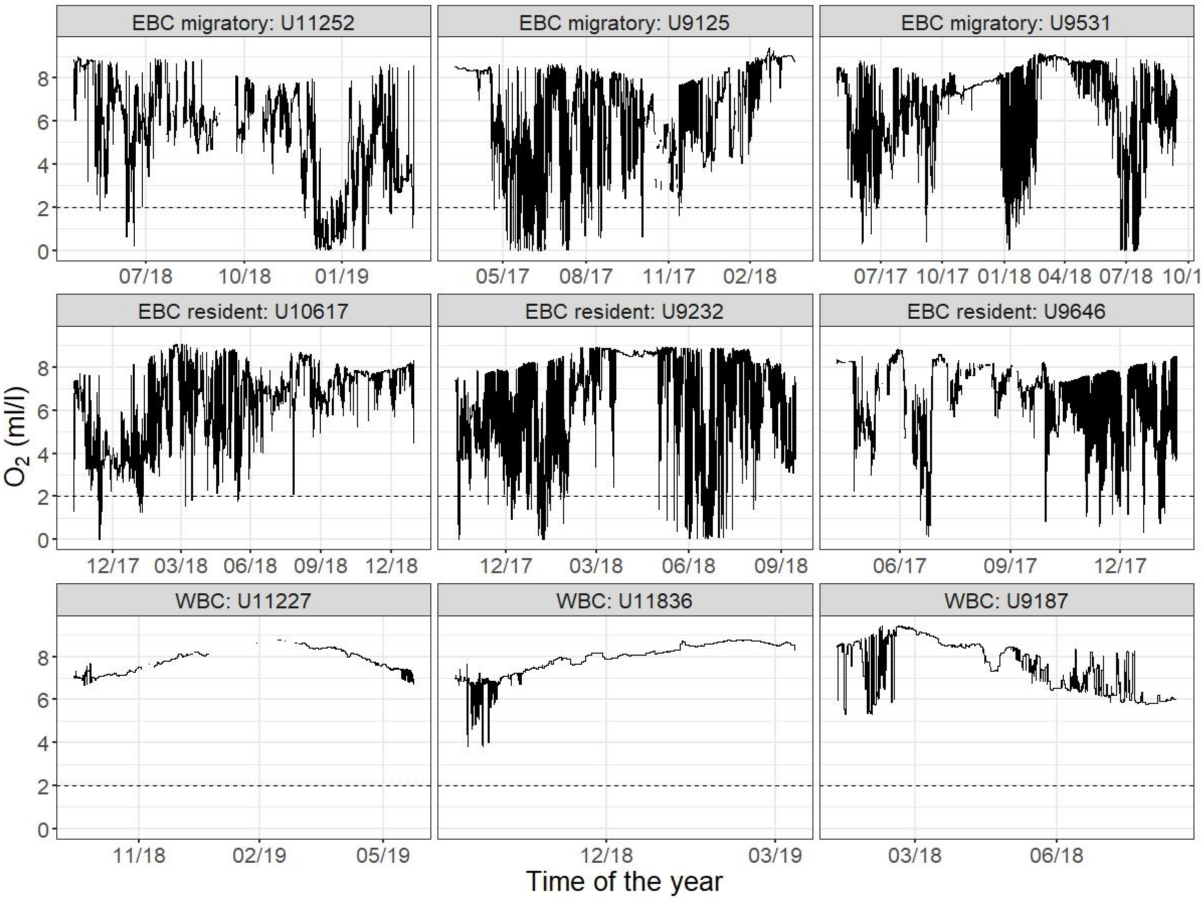

Figure 5: Oxygen content extracted from the Baltic regional ocean model based on the daily position and depth of nine selected cod (date format: mm/yy). The dashed line at 2 ml/l indicates the transition to hypoxic conditions. The oxygen profiles of all cod can be found in S3.

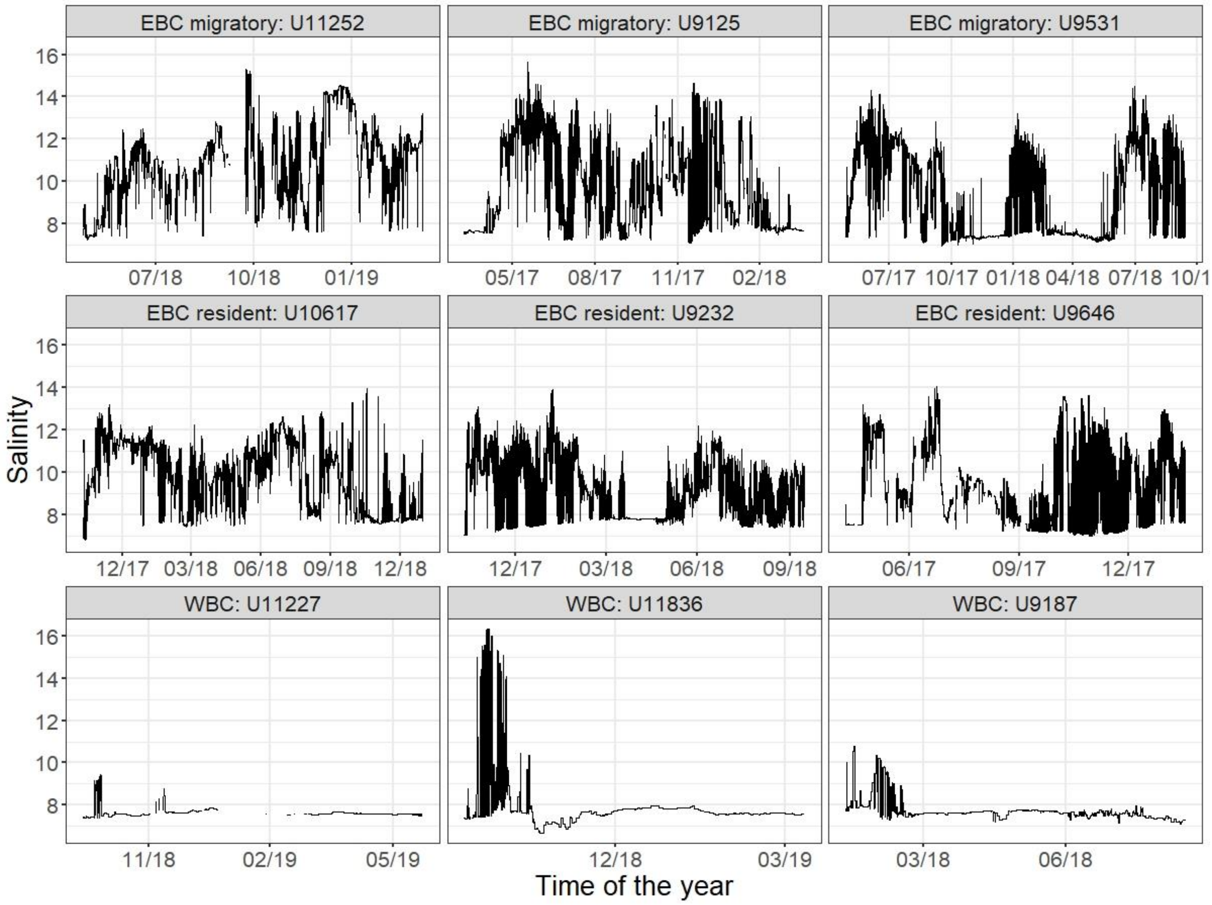

Figure 6: Salinity extracted from the Baltic regional ocean model based on the daily position and depth of nine selected cod (date format: mm/yy). The salinity profiles of all cod can be found in S4.

Vertical movements of cod in the southern Baltic Sea

The final GAMM chosen through model selection included 108874 hourly records of depth and included behavioural type, DOY and hour of the day (Fig. 7). The final model could explain 46.2% of the variation present in the underlying data. As already recognizable in Tab. 2, resident EBC inhabited significantly deeper depth than migratory and WBC (Fig. 7A). The GAMM also reconstructed the seasonal movements between shallow coastal and deeper waters of the Bornholm Basin in the third quarter (Fig. 7B). Cod occupied deeper waters during day-time than night-time (Fig. 7C). Daily vertical movements were accompanied by large variations in temperature, salinity and oxygen (Figs. 1, 5 & 6).

Visual inspection of the depth time series revealed regular vertical movements of EBC triggered by twilight, irrespective of the month (Fig. S6). With the onset of sunset, cod ascent in the water column while they returned to deeper water at sunrise. Only two of the five WBC showed such a pattern and the extend is much smaller due to the general shallower depth distribution. Although the lunar illumination was not significant in the final model, nine individuals showed a clear signal related to moon illumination with larger vertical movements during new moon and very restricted vertical movements during full moon (Fig. 8).

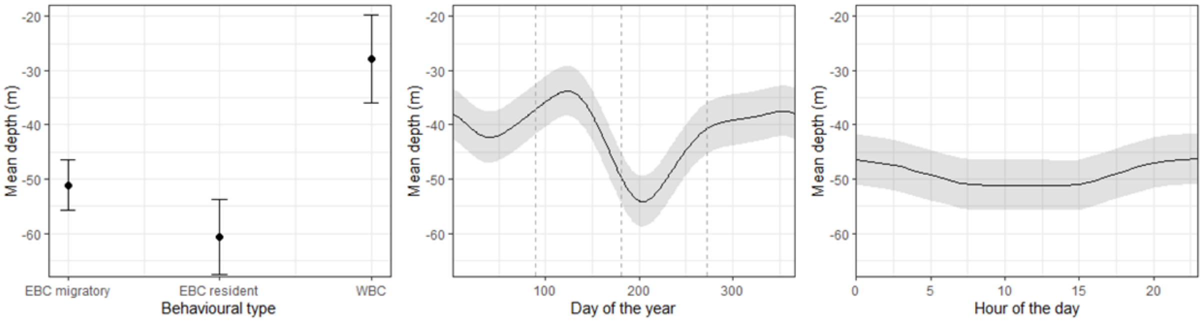

Figure 7: Average depth plots derived from the top-ranked generalized additive mixed model indicating the significant effects of (A) behavioural type, (B) day of the year, and (C) hour of the day of individual tagged cod in the southern Baltic Sea between 2016 and 2019. Error bars in (A) and shaded areas in (B,C): 95% confidence interval. Dashed line in (B) indicates transitions between quarters. Note the different scales on the y-axes.

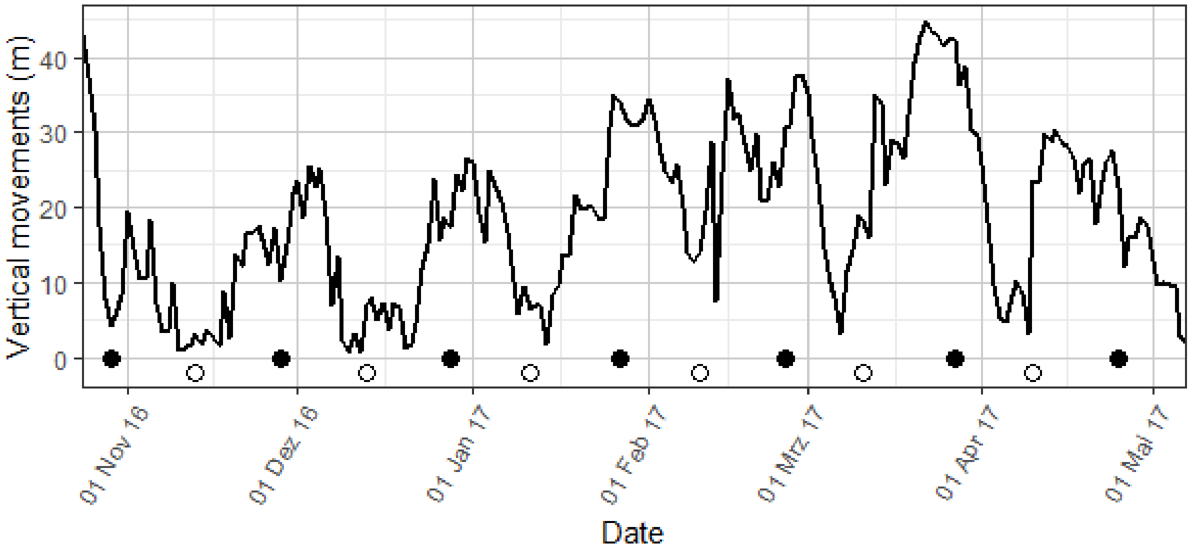

Figure 8: Extend of vertical movements within a day of the cod with ID U8882.Circle indicate moon illumination (full circle: new moon, empty circle: full moon).

Discussion

The southern Baltic Sea is characterized by strong thermohaline stratification, but movements of cod were not directly linked to this yet. The comprehension of individual cod movements in this area was limited and mostly relied on conventional tagging, survey data (Aro, 1989; Schaber et al., 2012) and one study were the movement of cod was reconstructed with temperature-depth-salinity DSTs (Neuenfeldt et al., 2007). In this study, the temperature and depth profiles of cod tagged with DSTs enabled us to reconstruct the horizontal movements of 28 cod and revealed diverse movements of individual fish which could not have been revealed otherwise. Based on daily positions and depth, salinity and oxygen values were matched. This is the first time that DSTs of recaptured cod were assigned to the WBC or EBC stock. Although all cod displayed individual variation in horizontal and vertical movements, WBC used shallower, more coastal and well-oxygenated waters throughout the course of the year. Further, EBC showed two distinct behavioural strategies: while resident EBC stayed within the Bornholm Basin year-round, migratory EBC moved between the spawning ground in the Bornholm Basin and coastal feeding grounds. Both EBC behavioural types experienced hypoxia during their time at liberty. Vertical movements depicted significant seasonal and daily patterns and some individuals showed movements which were correlated with moon illumination.

Seasonality in movements

A new set of recaptures tagged with temperature-depth DSTs and a geolocation framework adapted to cod in the southern Baltic Sea with an uncertainty of less than 20 km enabled us to analyse seasonal horizontal movements of individual cod. The recaptured DSTs depicted variable patterns in residency and migration. WBC stayed in shallow, coastal waters throughout the year and experienced therefore a strong seasonal signal in temperature (Fig. 1). This seasonal variation in ambient temperature was already detected in WBC in the western Baltic Sea (Funk et al., 2020) and is a major driver for the clear patterns in otolith zone formation and highly precise age reading of WBC (McQueen et al., 2019a; Krumme et al., 2020). Contrary, the seasonal temperature pattern of EBC is less distinct because EBC inhabit the cooler waters below the thermocline during summer time which coincides with the spawning time. While resident EBC stay in the Bornholm Basin year-round, migratory EBC move to shallower feeding ground where mixing with the WBC stock occurs. During spawning, migratory and resident EBC mixed on the spawning ground. Nevertheless, both types do not experience large seasonal variation in temperature and signals are overlaid by the large daily vertical movements during which the thermocline was often crossed (Fig. 1). Contrary to previous tagging studies (Robichaud and Rose, 2004; Neuenfeldt et al., 2007) which stated that cod in the southern Baltic Sea demonstrate sedentary behaviour to within the basins, our results showed that at least a subset of the EBC does perform seasonal movements between spawning and feeding grounds. Due to the limited number of days at liberty, it remains unclear if individuals stick to one movement strategy throughout their lifetime or switch between them under certain conditions.

Different movement strategies will influence the physiological rates such as feeding and metabolic rates of individuals from the same stock. The migratory EBC likely move to the coastal feeding grounds to follow the pre-spawning aggregations of herring off the coast of Rügen from November till May. Contrary to resident EBC, the condition factor is slightly higher and liver infestation lower indicating, despite the limited sample size, that the richer food source in coastal areas (P. Hornetz, unpublished data) benefits the overall fish conditions of migratory EBC. Resident EBC likely prey on the food supply in the area near the spawning ground which is likely dominated by clupeids because hypoxic conditions in deeper waters avoid the occurrence of benthic prey (Casini et al., 2016; Neuenfeldt et al., 2020). WBC displayed the highest condition factor and might benefit from extended access to benthic food and seasonal occurrence of clupeids (Funk et al. 2020; P. Hornetz, unpublished data). This difference in movement strategy and depth use likely also led to the increased growth rate of WBC compared to EBC even in the same area as reported in conventional tagging studies (McQueen et al., 2020).

The horizontal movement patterns coupled with the depth profile of four EBC showing distinct movements to and from the Bornholm Basin suggest that EBC stayed on the spawning ground significantly longer than the spawning period of 4-8 weeks as observed in laboratory experiments (Vallin and Nissling, 2000). Cod staying up to three month in the deeper Bornholm Basin have been observed before (Nielsen et al., 2013) but the individuals of this study stayed up to 6 month before they returned to shallower waters. This variability between studies is in line with the inter-annual variation in spawning season related to the occurring environmental conditions reported in Hinrichsen et al. (2007). It remains unclear why individuals stay in the hypoxic Bornholm Basin longer than necessary for reproduction, as it is often characterized by anoxic conditions. However, oxygen values in this study suggest that cod stay above the hypoxic area and only conduct occasionally dives into deeper waters (but compare Brander, 2020). Food limitation leads in turn to reduced energy intake (Neuenfeldt et al., 2020).

Vertical habitat transition

The DSTs revealed a clear vertical separation between the WBC and EBC stock. Vertical segregation depicts different ecological niches and indicating why coexistence of these two cod stocks can occur. While WBC did not occupy waters deeper than 50 m, both migratory and resident EBC regularly ascended above the thermocline during night, likely to follow clupeid prey during their daily vertical migrations (Nilsson et al., 2003; Schaber et al., 2012; Andersen et al., 2017; Casini et al., 2019). The vertical movements of EBC were directly associated with the onset of dusk and dawn. The stomach contents of EBC showed a high proportion of pelagic clupeids (Andersen et al., 2017). When cod perform vertical movements to prey e.g. on clupeids such as herring and sprat in shallower waters and subsequently return to deeper waters where the prey is excreted or respirated, this can lead to a transport of nutrients in the form of faeces to deeper areas where anoxic condition avoid decomposition (Ducklow et al., 2001; Hansen and Visser, 2016; Pinti and Visser, 2018). Due to the high stagnation of water masses in deeper areas, the nutrients deposit in the basins and amplify anoxic conditions in these regions.

Contrary, the extend of vertical movements of WBC was less pronounced and could be the result of different feeding strategies. Stomach sample data showed that WBC mainly feed on benthic invertebrates and demersal fish and thus their food search is characterized by demersal dwelling while searching for prey (Funk, 2020). Only the WBC U11836 and B2055 showed daily vertical movements in October and October-November, respectively, when they both were occupying waters between Bornholm and Rügen, likely feeding on herring and sprat which perform daily vertical migrations (Schaber et al., 2012, Kulke et al., 2018).

The vertical and partially horizontal habitat segregation likely promotes the co-existence of both genetically distinct cod stocks in close proximity to each other. Both cod stocks in the Baltic have developed several adaptions to the specific habitat and behaviour such as otolith shape (Hüssy et al., 2016; Schade et al., 2019), number of vertebrae (reviewed in Bagge et al., 1994), spawning season (Bleil et al., 2009) and haemoglobin type (Sick, 1965; Andersen et al., 2009) and are further reviewed in Schade et al. (submitted). Vertical segregation of cod populations have been observed in other areas (e.g. in the Northeast Atlantic (Nordeide et al., 2011) or Icelandic waters (Bardarson et al., 2017)) demonstrating different manifestations of life history parameters. Thus, vertical niche formation enables the occurrence of several distinct cod populations which differ in life history strategies. In the Baltic Sea case, the EBC stock developed two different life history strategies to deal with the extreme condition characterizing this area.

Conclusion

The DSTs of 28 recaptured cod assigned to either WBC or EBC in the southern Baltic Sea recording temperature and depth enabled the successful reconstruction of movements between release and recapture. Based on daily positions and occupied depth, salinity and oxygen values were matched and represent a unique dataset of environmental conditions cod experienced during their time at liberty. Based on their behaviour, EBC were split into resident EBC and migratory EBC. This study confirmed the coexistence of WBC and EBC in the southern Baltic Sea and especially from autumn till spring intermixing between WBC and migratory EBC exists in shallow coastal areas. WBC constantly occupied shallower depths than EBC and thus occupied different depth niches. The vertical segmentation of habitats is likely the reason for the coexistence of the two stocks each adapted to different habitats characterized by different environmental conditions. The different movement strategies of cod in the southern Baltic Sea likely lead to different vulnerabilities to commercial fishing and the scientific surveys and might lead to a survey bias because shallow water habitats <20 m in the SDs 25-26 and <10 m in SD 24 are not covered and thus under-represented in the active trawl surveys. This is especially important for WBC and seasonally also for migratory EBC which used rather shallow waters in quarter 1 and 4 when the Bottom International Trawl Survey (BITS), an ICES organized survey to estimate the abundance of demersal species, is conducted. This suggests that a significant part of the cod stocks use areas outside the main survey area. This habitat use and distribution might bias the survey indices when the proportion of cod in these area is not stable between e.g. sex, age groups, season or year. This short-coming of the BITS survey was recently also identified for cod in the western Baltic Sea (Funk et al., 2020). These new insights in stock-specific movements of cod in the southern

Baltic Sea provide important insights into how to improve scientific monitoring of these stocks.

Acknowledgements

We thank the technical staff from the National Marine Fisheries Institute (NMFRI) in Poland, the Technical University of Denmark (DTU-Aqua), the Swedish University of Agriculture Sciences (SLU) and the Thünen Institute of Baltic Sea Fisheries (OF) involved in the tagging, collection and processing of samples used in this study and all fishers and anglers returning tagged cod. We thank Jakob Hemmer-Hansen und DTU-Aqua for providing the genetic assignment. The tagging was conducted under the following animal test permissions: OF: TABACOD T-bar tagging: AZ 7221.3.1-029/15; TABACOD DST tagging: AZ 7221.3.1-007/18; DTU TABACOD T-bar and DST tagging: 016-15-0201-00929, NMFRI TABACOD T-bar tagging: Permission no 19/2016, dated 28.06.2016, SLU TABACOD T-bar and DST tagging: Dnr 5.8.18-14823/2018. This study was funded by BalticSea2020 (http://balticsea2020.org) through the project “TABACOD” (Tagging Baltic cod).

References

Andersen N, Lundgren B, Neuenfeldt S, Beyer J (2017) Diel vertical interactions between Atlantic cod Gadus morhua and sprat Sprattus sprattus in a stratified water column. Mar Ecol Prog Ser 583: 195–209

Andersen Ø, Wetten OF, De Rosa MC, Andre C, Carelli Alinovi C, Colafranceschi M, Brix O, Colosimo A (2009) Haemoglobin polymorphisms affect the oxygen-binding properties in Atlantic cod populations. Proc R Soc B Biol Sci 276: 833–841

Andrzejaczek S, Chapple TK, Curnick DJ, Carlisle AB, Castleton M, Jacoby DMP, Peel LR, Schallert RJ, Tickler DM, Block BA (2020) Individual variation in residency and regional movements of reef manta rays Mobula alfredi in a large marine protected area. Mar Ecol Prog Ser 639: 137–153

Andrzejaczek S, Gleiss AC, Pattiaratchi CB, Meekan MG (2019) Patterns and drivers of vertical movements of the large fishes of the epipelagic. Rev Fish Biol Fish 29: 335–354

Aro E (1989) Review of fish migration patterns in the Baltic Sea. Rapp P-V Reun Cons Int Explor Mer 190: 72–96

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

Bardarson H, McAdam BJ, Thorsteinsson V, Hjorleifsson E, Marteinsdottir G (2017) Otolith shape differences between ecotypes of Icelandic cod (Gadus morhua) with known migratory behaviour inferred from data storage tags. Can J Fish Aquat Sci. doi: 10.1139/cjfas-2016-0307

Bleil M, Oeberst R, Urrutia P (2009) Seasonal maturity development of Baltic cod in different spawning areas: importance of the Arkona Sea for the summer spawning stock. J Appl Ichthyol 25: 10–17

Block BA, Teo SLH, Walli A, Boustany A, Stokesbury MJW, Farwell CJ, Weng KC, Dewar H, Williams TD (2005) Electronic tagging and population structure of Atlantic bluefin tuna. Nature 434: 1121–1127

Bowen BW, Gaither MR, DiBattista JD, Iacchei M, Andrews KR, Grant WS, Toonen RJ, Briggs JC (2016) Comparative phylogeography of the ocean planet. Proc Natl Acad Sci 113: 7962–7969

Brander K (2020) Reduced growth in Baltic Sea cod may be due to mild hypoxia. ICES J Mar Sci 77: 2003–2005

Brander KM (1994) Patterns of distribution, spawning, and growth in North Atlantic cod: the utility of inter-regional comparisons. ICES Mar Sci Symp Cph Den Int Counc Explor Sea 1991 406–413

Braun CD, Galuardi B, Thorrold SR (2018) HMMoce : An R package for improved geolocation of archival-tagged fishes using a hidden Markov method. Methods Ecol Evol 9: 1212–1220

Braun CD, Skomal GB, Thorrold SR, Berumen ML (2014) Diving Behavior of the Reef Manta Ray Links Coral Reefs with Adjacent Deep Pelagic Habitats. PLOS ONE 9: e88170

Calenge C (2006) The package adehabitat for the R software: tool for the analysis of space and habitat use by animals. Ecol Model 197: 1035

Carstensen J, Conley DJ (2019) Baltic Sea Hypoxia Takes Many Shapes and Sizes. Limnol Oceanogr Bull 28: 125–129

Casini M, Käll F, Hansson MV, Plikshs M, Baranova T, Karlsson OM, Lundström K, Neuenfeldt S, ardmark AG, Hjelm J (2016) Hypoxic areas, density-dependence and food limitation drive the body condition of a heavily exploited marine fish predator. R Soc Open Sci. doi: 10.6084/m9.figshare.c.3518970.v1

Casini M, Tian H, Hansson M, Grygiel W, Strods G, Statkus R, Sepp E, Grohsler T, Orio A, Larson N (2019) Spatio-temporal dynamics and behavioural ecology of a “demersal” fish population as detected using research survey pelagic trawl catches: the Eastern Baltic Sea cod (Gadus morhua). 10

Conley DJ, Carstensen J, Aigars J, Axe P, Bonsdorff E, Eremina T, Haahti B-M, Humborg C, Jonsson P, Kotta J, et al (2011) Hypoxia Is Increasing in the Coastal Zone of the Baltic Sea. Environ Sci Technol 45: 6777–6783

Cooke SJ, Woodley CM, Brad Eppard M, Brown RS, Nielsen JL (2011) Advancing the surgical implantation of electronic tags in fish: a gap analysis and research agenda based on a review of trends in intracoelomic tagging effects studies. Rev Fish Biol Fish 21: 127–151

Costa DP, Breed GA, Robinson PW (2012) New Insights into Pelagic Migrations: Implications for Ecology and Conservation. Annu Rev Ecol Evol Syst 43: 73–96

Ducklow H, Steinberg D, Buesseler K (2001) Upper Ocean Carbon Export and the Biological Pump. Oceanography 14: 50–58

Eero M, Hjelm J, Behrens J, Buchmann K, Cardinale M, Casini M, Gasyukov P, Holmgren N, Horbowy J, Hüssy K, et al (2015) Eastern Baltic cod in distress: biological changes and challenges for stock assessment. ICES J Mar Sci 72: 2180–2186

Engelhardt J, Frisell O, Gustavsson H, Hansson T, Sjöberg R, Collier TK, Balk L (2020) Severe thiamine deficiency in eastern Baltic cod (Gadus morhua). PLOS ONE 15: e0227201

Funk S (2020) Spatio-temporal distribution, food intake and growth of cod (Gadus morhua L.) in the Western Baltic Sea. Räumlich-zeitliche Verteilung, Nahrungsaufnahme und Wachstum des Dorsches (Gadus morhua L.) in der Westlichen Ostsee

Funk S, Krumme U, Temming A, Möllmann C (2020) Gillnet fishers' knowledge reveals seasonality in depth and habitat use of cod (Gadus morhua) in the Western Baltic Sea. ICES J Mar Sci fsaa071

Gaither MR, Gkafas GA, de Jong M, Sarigol F, Neat F, Regnier T, Moore D, Gröcke DR, Hall N, Liu X, et al (2018) Genomics of habitat choice and adaptive evolution in a deep-sea fish. Nat Ecol Evol 2: 680–687

Galuardi B, Lutcavage M (2012) Dispersal Routes and Habitat Utilization of Juvenile Atlantic Bluefin Tuna, Thunnus thynnus, Tracked with Mini PSAT and Archival Tags. PLoS ONE 7: e37829

Goudarzi F, Hemami M-R, Rancilhac L, Malekian M, Fakheran S, Elmer KR, Steinfartz S (2019) Geographic separation and genetic differentiation of populations are not coupled with niche differentiation in threatened Kaiser's spotted newt (Neurergus kaiseri). Sci Rep 9: 1–12

Gräwe U, Naumann M, Mohrholz V, Burchard H (2015) Anatomizing one of the largest saltwater inflows into the Baltic Sea in December 2014: ANATOMIZING A MAJOR BALTIC SEA INFLOW. J Geophys Res Oceans 120: 7676–7697

Griffiths CA, Patterson TA, Blanchard JL, Righton DA, Wright SR, Pitchford JW, Blackwell PG (2018) Scaling marine fish movement behavior from individuals to populations. Ecol Evol 8: 7031–7043

Haase S, Krumme U, Gräwe U, Braun CD, Temming A (2021) Validation approaches of a geolocation framework to reconstruct movements of demersal fish equipped with data storage tags in a stratified environment. Fish Res 237: 105884

Haase S, McQueen K, Temming A, Schade FM, Hüssy K, Hilvarsson A, Casini M, Mion M, Radtke K, Krumme U (in prep.) Validation of otolith zone formation and otolith growth of adult wild cod (Gadus morhua L.) in the southern Baltic Sea through mark-recapture and tetracycline marking

Hansen AN, Visser AW (2016) Carbon export by vertically migrating zooplankton: an optimal behavior model. Limnol Oceanogr 61: 701–710

Hays GC, Ferreira LC, Sequeira AMM, Meekan MG, Duarte CM, Bailey H, Bailleul F, Bowen WD, Caley MJ, Costa DP, et al (2016) Key Questions in Marine Megafauna Movement Ecology. Trends Ecol Evol 31: 463–475

Hemmer-Hansen J, Hüssy K, Baktoft H, Huwer B, Bekkevold D, Haslob H, Herrmann J, Hinrichsen H, Krumme U, Mosegaard H, et al (2019) Genetic analyses reveal complex dynamics within a marine fish management area. Evol Appl 12: 830–844

Hijmans RJ (2019) geosphere: Spherical Trigonometry. R package version 1.5-10. https://CRAN.R-project.org/package=geosphere.

Hilton Z, Wellenreuther M, Clements KD (2008) Physiology underpins habitat partitioning in a sympatric sister-species pair of intertidal fishes. Funct Ecol 22: 1108–1117

Hinrichsen H-H, Voss R, Wieland K, Koster F, Andersen K, Margonski P (2007) Spatial and temporal heterogeneity of the cod spawning environment in the Bornholm Basin, Baltic Sea. Mar Ecol-Prog Ser 345: 245–254

Hobson V, Righton D, Metcalfe J, Hays G (2007) Vertical movements of North Sea cod. Mar Ecol Prog Ser 347: 101–110

Horbowy J (2016) Effects of varying natural mortality and selectivity on the assessment of eastern Baltic cod (Gadus morhua Linnaeus, 1758) stock. J Appl Ichthyol 32: 1032–1040

Hunter E, Berry F, Buckley AA, Stewart C, Metcalfe JD (2006) Seasonal migration of thornback rays and implications for closure management. J Appl Ecol 43: 710–720

Hunter E, Metcalfe JD, Arnold GP, Reynolds JD (2004) Impacts of migratory behaviour on population structure in North Sea plaice. J Anim Ecol 73: 377–385

Hussey NE, Kessel ST, Aarestrup K, Cooke SJ, Cowley PD, Fisk AT, Harcourt RG, Holland KN, Iverson SJ, Kocik JF, et al (2015) Aquatic animal telemetry: A panoramic window into the underwater world. Science 348: 1255642–1255642

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

ICES (2020a) Cod (Gadus morhua) in subdivisions 24-32, eastern Baltic stock (eastern Baltic Sea). doi: 10.17895/ICES.ADVICE.5943

ICES (2020b) Cod (Gadus morhua) in subdivisions 22?24, western Baltic stock (western Baltic Sea). doi: 10.17895/ICES.ADVICE.5942

ICES (2019) ICES WGBFAS REPORT 2019. 661

ICES (2020c) Baltic Fisheries Assessment Working Group. doi: 10.17895/ICES.PUB.6024

ICES (2014) Manual for the Baltic International Trawl Surveys (BITS). Series of ICES Survey Protocols SISP 7 - BITS. 71

Jennings RM, Etter RJ, Ficarra L (2013) Population Differentiation and Species Formation in the Deep Sea: The Potential Role of Environmental Gradients and Depth. PLOS ONE 8: e77594

Kijewska A, Więcaszek B, Kijewski T (2011) Analysis of Population and Taxonomical Structure of Atlantic Cod, <I>Gadus Morhua</I> (Actinopterygii: Gadiformes: Gadidae) from the Baltic Sea with Use of Microsatellite DNA. Acta Ichthyol Piscat 41: 307–314

Kohler NE, Turner PA (2001) Shark Tagging: A Review Of Conventional Methods and Studies. Environ Biol Fishes 60: 191–224 van der Kooij J, Righton D, Strand E, Michalsen K, Thorsteinsson V, Svedäng H, Neat FC, Neuenfeldt S (2007) Life under pressure: insights from electronic data-storage tags into cod swimbladder function. ICES J Mar Sci 64: 1293–1301

Krumme U, Stötera S, McQueen K, Pahlke E (2020) Age validation of age 0-3 wild cod Gadus morhua in the western Baltic Sea through mark-recapture and tetracycline marking of otoliths. Mar Ecol Prog Ser 645: 141–158

Kulke R, Bödewadt V, Hänselmann K, Herrmann JP, Temming A (2018) Ignoring the vertical dimension: biased view on feeding dynamics of vertically migrating sprat (Sprattus sprattus). ICES Journal of Marine Science, Volume 75, Issue 7, December 2018, Pages 2450–2462, https://doi.org/10.1093/icesjms/fsy136

Latour R (2005) Tagging methods and associated data analysis. Manag. Tech. Elasmobranch Fish.

LLUR (2020) Sauerstoffmangel im bodennahen Wasser der westlichen Ostsee.

Lucas MC, Baras E (2000) Methods for studying spatial behaviour of freshwater fishes in the natural environment. Fish Fish 1: 283–316

Mackenzie BR, Gislason H, Möllmann C, Köster FW (2007) Impact of 21st century climate change on the Baltic Sea fish community and fisheries. Glob Change Biol 13: 1348–1367

McQueen K, Casini M, Dolk B, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Mion M, Mohr T, Radtke K, et al (2020) Regional and stock-specific differences in contemporary growth of Baltic cod revealed through tag-recapture data. ICES J Mar Sci fsaa104

McQueen K, Hrabowski J, Krumme U (2019a) Age validation of juvenile cod in the Western Baltic Sea. ICES J Mar Sci 76: 430–441

McQueen K, Mion M, Hilvarsson A, Casini M, Olesen HJ, Hüssy K, Radtke K, Krumme U (2019b) Effects of freezing on length and mass measurements of Atlantic cod *Gadus morhua* in the Baltic Sea. J Fish Biol 95: 1486–1495

Metcalfe JD, Righton DA, Hunter E, Eastwood P (2008) Migration and habitat choice in marine fish. *In* C Magnhagen, VA Braithwaite, E Forsgren, BG Kapoor, eds, Fish Behav. Science Publishers Inc, Enfield, pp 187–233

Mindel BL, Neat FC, Trueman CN, Webb TJ, Blanchard JL (2016) Functional, size and taxonomic diversity of fish along a depth gradient in the deep sea. PeerJ 4: e2387

Mion M, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Krüger-Johnsen M, Krumme U, McQueen K, Plikshs M, Radtke K, et al (2020) Multidecadal changes in fish growth rates estimated from tagging data: A case study from the Eastern Baltic cod (Gadus morhua, Gadidae). Fish Fish. doi: https://doi.org/10.1111/faf.12527

Møller JS, Hansen IS (1994) Hydrographic processes and changes in the Baltic Sea. 18

Naumann M, Gräwe U, Mohrholz V, Kuss J, Kanwischer M, Feistel S, Hand I, Waniek JJ, Schulz-Bull DE (2020) Hydrographic-hydrochemical assessment of the Baltic Sea 2019. Meereswissenschaftliche Berichte No 114 2020 - Mar Sci Rep No 114 2020 13,1 MB, 97 pages

Neat FC, Bendall V, Berx B, Wright PJ, Cuaig MÓ, Townhill B, Schön P-J, Lee J, Righton D (2014) Movement of Atlantic cod around the British Isles: implications for finer scale stock management. J Appl Ecol 51: 1564–1574

Neuenfeldt S, Bartolino V, Orio A, Andersen KH, Andersen NG, Niiranen S, Bergström U, Ustups D, Kulatska N, Casini M (2020) Feeding and growth of Atlantic cod (Gadus morhua L.) in the eastern Baltic Sea under environmental change. ICES J Mar Sci 77: 624–632

Neuenfeldt S, Hinrichsen H-H, Nielsen A, Andersen KH (2007) Reconstructing migrations of individual cod (Gadus morhua L.) in the Baltic Sea by using electronic data storage tags. Fish Oceanogr 16: 526–535

Nielsen B, Hüssy K, Neuenfeldt S, Tomkiewicz J, Behrens J, Andersen K (2013) Individual behaviour of Baltic cod Gadus morhua in relation to sex and reproductive state. Aquat Biol 18: 197–207

Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D, Grønkjær P (2003) Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. Mol Ecol 12: 1497–1508

Nilsson LAF, Thygesen UH, Lundgren B, Nielsen BF, Nielsen JR, Beyer JE (2003) Vertical migration and dispersion of sprat (Sprattus sprattus) and herring (Clupea harengus) schools at dusk in the Baltic Sea. Aquat Living Resour 16: 317–324

Nordeide JT, Johansen SD, Jørgensen TE, Karlsen BO, Moum T (2011) Population connectivity among migratory and stationary cod Gadus morhua in the Northeast Atlantic—A review of 80 years of study. Mar Ecol Prog Ser 435: 269–283

O’Leary DB, Coughlan J, Dillane E, McCarthy TV, Cross TF (2007) Microsatellite variation in cod Gadus morhua throughout its geographic range. J Fish Biol 70: 310–335

Pedersen J (2005) Diet comparison between pelagic and demersal whiting in the North Sea. J Fish Biol 55: 1096–1113

Pedersen MW, Righton D, Thygesen UH, Andersen KH, Madsen H (2008) Geolocation of North Sea cod (Gadus morhua) using hidden Markov models and behavioural switching. Can J Fish Aquat Sci 65: 2367–2377

Piacenza SE, Barner AK, Benkwitt CE, Boersma KS, Cerny-Chipman EB, Ingeman KE, Kindinger TL, Lee JD, Lindsley AJ, Reimer JN, et al (2015) Patterns and Variation in Benthic Biodiversity in a Large Marine Ecosystem. PLOS ONE 10: e0135135

Pine WE, Hightower JE, Coggins LG, Lauretta MV, Pollock KH (2012) Design and Analysis of Tagging Studies. Zale AV Parrish DL Sutton TM Eds Fish. Tech., 3rd ed. American Fisheries Society, Bethesda, MD, pp 521–572

Pinti J, Visser AW (2018) Predator-Prey Games in Multiple Habitats Reveal Mixed Strategies in Diel Vertical Migration. Am Nat 193: E65–E77

R Core Team (2017) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Righton DA, Andersen KH, Neat F, Thorsteinsson V, Steingrund P, Svedäng H, Michalsen K, Hinrichsen HH, Bendall V, Neuenfeldt S, et al (2010) Thermal niche of Atlantic cod Gadus morhua: limits, tolerance and optima. Mar Ecol Prog Ser 420: 1–13

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

Rolán-Alvarez E (2007) Sympatric speciation as a by-product of ecological adaptation in the Galician Littorina saxatilis hybrid zone. J Molluscan Stud 73: 1–10

Schaber M, Hinrichsen H-H, Gröger J (2012) Seasonal changes in vertical distribution patterns of cod (Gadus morhua) in the Bornholm Basin, central Baltic Sea. Fish Oceanogr 21: 33–43

Schade FM, Weist P, Krumme U (2019) Evaluation of four stock discrimination methods to assign individuals from mixed-stock fisheries using genetically validated baseline samples. Mar Ecol Prog Ser 627: 125–139

Seaman DE, Powell RA (1996) An Evaluation of the Accuracy of Kernel Density Estimators for Home Range Analysis. Ecology 77: 2075–2085

Sick K (1965) Haemoglobin Polymorphism of Cod in the Baltic and the Danish Belt Sea. Hereditas 54: 19–48

Sokolova M, Buchmann K, Huwer B, Kania P, Krumme U, Galatius A, Hemmer-Hansen J, Behrens J (2018) Spatial patterns in infection of cod Gadus morhua with the seal-associated liver worm Contracaecum osculatum from the Skagerrak to the central Baltic Sea. Mar Ecol Prog Ser 606: 105–118

Thieurmel B, Elmarhraoui A (2019) suncalc: Compute Sun Position, Sunlight Phases, Moon Position and Lunar Phase.

Thygesen UH (2009) Geolocating fish using Hidden Markov Models and Data Storage Tags. Rev Methods Technol Fish Biol Fish 277–293

Vallin L, Nissling A (2000) Maternal effects on egg size and egg buoyancy of Baltic cod, Gadus morhua: Implications for stock structure effects on recruitment. Fish Res 49: 21–37

Weist P, Schade FM, Damerau M, Barth JMI, Dierking J, André C, Petereit C, Reusch T, Jentoft S, Hanel R, et al (2019) Assessing SNP-markers to study population mixing and ecological adaptation in Baltic cod. PLOS ONE 14: e0218127

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

Worton BJ (1989) Kernel Methods for Estimating the Utilization Distribution in Home-Range Studies. Ecology 70: 164–168

Zar JH (1996) Biostatistical Analysis, 3rd edition. Prentice Hall College Div

Zemeckis DR, Liu C, Cowles GW, Dean MJ, Hoffman WS, Martins D, Cadrin SX (2017) Seasonal movements and connectivity of an Atlantic cod (Gadus morhua) spawning component in the western Gulf of Maine. ICES J Mar Sci 74: 1780–1796

Chapter 4

Validation of otolith zone formation and otolith growth of adult wild cod (*Gadus morhua* L.) in the southern Baltic Sea through mark-recapture and tetracycline marking

Stefanie Haase[1,*], Kate McQueen[1,2], Axel Temming[3], Franziska M Schade[1], Karin Hüssy[4], Annelie Hilvarsson[5], Michele Casini[5,6], Monica Mion[5], Krzysztof Radtke[7], Uwe Krumme[1]

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

[2]Institute of Marine Research, P.O. Box 1870 Nordnes, 5817 Bergen, Norway

[3]Institute of Marine Ecosystem and Fisheries Science, Centre for Earth System Research and Sustainability (CEN), University of Hamburg, Große Elbstraße 133, 22767 Hamburg, Germany

[4] Technical University of Denmark, National Institute of Aquatic Resources, Kemitorvet, 2800 Kgs. Lyngby, Denmark

[5] Department of Aquatic Resources, Institute of Marine Research, Swedish University of Agricultural Sciences, Turistgatan 5, 45330 Lysekil, Sweden

[6] Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Selmi 3, 40126 Bologna, Italy

[7]National Marine Fisheries Research Institute, Ul. Kołłątaja 1, 81-332 Gdynia, Poland.

Abstract

For decades, unvalidated patterns in otolith zone formation and otolith growth of Eastern Baltic cod (EBC) have introduced uncertainties into age-based data from this stock, severely hampering our understanding of changes in stock status and productivity. Otoliths of 258 recaptures of wild cod, which were released during an international mark-recapture study in the southern Baltic Sea after being tagged externally and internally with tetracycline-hydrochloride to induce a permanent, fluorescent time-stamp on the otolith, were analysed. Recaptured fish were assigned to either the EBC or Western Baltic cod (WBC) stock. Identification of zone types formed both at the time of release and recapture showed that the translucent zone of adult EBC was formed between April and October when fish usually spawn below the halocline. This period is characterized by reduced feeding and exposure to hypoxic conditions. The opaque zone is formed during winter and spring, which coincides with the major feeding time. Otolith growth followed a linear relationship with somatic length growth. The daily otolith growth rate was on average significantly higher for WBC (2.3±0.6 µm) compared to EBC (1.4±0.6 µm) from the same area, reflecting the currently lower somatic growth rate of EBC. Concurrently, for a given fish length, EBC had larger otoliths than WBC, suggesting that slower growing individuals have larger otolith than faster growing individuals at a given fish length. This study validates the periodicity and timing of annulus formation and otolith growth in adult EBC, which is the first step to return to a reliable age determination.

Keywords: chemical marking, tagging, translucent summer ring, Baltic cod, periodicity, annulus formation

Introduction

Otoliths are calcified structures in the inner ear of teleosts and consist of opaque and translucent zones (Secor et al., 1995; Campana and Thorrold, 2001). Fish otoliths display seasonal alternations between opaque and translucent zones which can be used for age determination (Campana, 2001; Campana and Thorrold, 2001; Fablet et al., 2011), necessary to inform stock assessment models through age-length distributions or individual growth rates. The validation of first increment formation and of the seasonality of otolith zones is essential (Campana, 2001) but many fish stocks are lacking this validation (Beamish and McFarlane, 1983; ICES, 2020a).

Many ecologically and economically important gadoid stocks suffer from uncertainties in age reading (ICES, 2013). While European hake (*Merluccius merluccius)* is a prominent example of a stock with age reading problems from a fully marine environment (de Pontual et al., 2006; Bertignac and de Pontual, 2007), Eastern Baltic cod (EBC; *Gadus morhua*) is a notorious example from a brackish-water ecosystem. For decades, exchanges and workshops have tried to decrease the uncertainties in the age reading of otoliths from EBC (Hüssy et al. 2016). The zone formation of EBC otoliths was believed to be unpredictable and unrelated to season, with indistinct annuli (Hüssy et al., 2016; Svedäng et al., 2020), and with notable inter-annual variation and geographic differences (Berner, 1968). Baranova et al. (2011) argued that it is almost impossible to estimate the age of EBC otoliths without knowing the maturity stage of the individual. However, analyses of temporal changes at the outer edge of EBC otoliths from the 1950s-1990s suggested that adult EBC formed the translucent zone (TZ) from January to June (during the spawning time in those times), while juvenile EBC formed their first TZ in June the year after hatching (Tokareva, 1963; Baranova et al., 2011). Since the mid-1990s, the EBC spawning time has shifted towards summer, followed by a post-spawning and feeding period from autumn until March of the following year, which was also assumed to result in a shift in the formation of the TZ towards summertime (Wieland et al., 2000; Baranova et al., 2011).

The low precision and accuracy of traditional age determination of EBC (ICES, 2006; Hüssy, 2010; ICES, 2014; Hüssy et al., 2016) has mainly been driven by the lack of validated otolith material. Since growth and mortality rates of EBC could not be estimated reliably due to the uncertainties around the international age data, the analytical age-based stock assessment was rejected in 2014 (Eero et al., 2015). After a series of benchmark workshops (ICES, 2015; ICES, 2019a), it was subsequently decided to use the stock assessment model "Stock Synthesis" to reduce the influence of uncertain age data (Methot and Wetzel, 2013; ICES, 2019b).

For the neighbouring, genetically-distinct, Western Baltic cod (WBC) stock, which overlaps in distribution area with EBC in the Arkona Sea (Hemmer-Hansen et al., 2019; Weist et al., 2019), there have also been uncertainties in age reading (ICES, 1994; ICES, 2000; ICES, 2005), but annulus formation has recently been validated for both juveniles (through edge type analysis (McQueen et al., 2019) and daily increment counts (Plonus et al., 2021)) and young adults (through a mark-recapture study (Krumme et al., 2020)). Age data are routinely used in the WBC stock assessment (ICES, 2020b). In contrast to previous assumptions that the TZ of WBC is formed during winter, the validation showed that the TZ of WBC aged 0-3 is actually formed during summertime, when metabolic needs are apparently not met (McQueen et al., 2019; Krumme et al., 2020).

In addition to providing validation of the timing and frequency of otolith zone formation, chemically marked otolith material from tag-recapture studies is useful to gain reliable estimates of otolith growth. Various factors can affect the growth rate and changes of opacity in otoliths (Irgens et al., 2020). This involves both the fish's exposure to seasonal variations in environmental factors such as water temperature, salinity, oxygen, pH and feeding conditions (Romanek and Gauldie, 1996; Fablet et al., 2011; Bignami et al., 2013; Maneja et al., 2013) and physiological factors such as metabolism and reproductive activity (Høie et al., 2008; Svedäng et al., 2020). Otolith growth is related to fish growth and hence, to stock productivity. It is often assumed that otolith size follows a direct linear relationship with fish size so that it can be used for back-calculations of fish lengths at earlier ages, for example to assess growth rates (Lea, 1910). In reality, this relationship is seldom constant over longer time periods because somatic growth can cease under unfavourable environmental conditions, or under food limitation, while otolith growth may continue (Mosegaard et al., 1988; Hüssy and Mosegaard, 2004; Høie et al., 2008). Otolith growth is thus better related to the fish's metabolic activity which, however, also depends on environmental conditions and food intake (Mosegaard et al., 1988; Hüssy and Mosegaard, 2004; Fablet et al., 2011; Svedäng et al., 2020). As a result, somatic growth and otolith growth of a fish could be potentially decoupled, i.e. for a given fish length, the otolith of a faster-growing fish could be smaller compared to the otolith of a slower-growing older fish (Svedäng et al., 2020 and references therein), first identified what became known as the "growth effect" by Secor and Dean (1989).

In this study, we want to use the knowledge gained from the successful validation of the zone formation of age 0-3 WBC (Krumme et al., 2020) to improve our understanding of growth and opacity patterns in EBC otoliths. Chemical and external marking of wild fish has proven to be a successful method because the intraperitoneal injection of tetracycline-hydrochloride (TET) marks the timing of release on the otolith and is visible under UV light when the fish is recaptured (Campana, 2001; Stötera et al., 2018). Although such studies are expected to be costly and restricted in sample size and area covered (Beamish and McFarlane, 1983), they can give direct observations from wild fish and are not affected by the conditions usually restricting laboratory experiments.

In the international mark-recapture project "TABACOD" (Tagging Baltic cod), wild cod from the southern Baltic Sea were marked with TET and released. Recaptures were assigned to either the EBC or WBC stock using genetics or otolith shape analysis, and the sectioned otoliths were used to assess the seasonal patterns of zone formation. Furthermore, we compared otolith growth and size between the WBC and EBC stock and linked this to somatic growth. This is the first time that chemically marked otoliths of EBC are used for the validation of visible patterns in ring formation of these notoriously difficult-to-read otoliths, allowing for stock-specific differentiation in otolith formation and growth in the southern Baltic Sea.

Methods

Oceanography and behaviour of cod in the southern Baltic Sea

The Baltic Sea is a semi-enclosed, non-tidal, brackish-water ecosystem characterized by a decrease in salinity from near marine in the west to almost freshwater in the northeast, with a series of deep basins separated by shallower sills. In the basins of the southern Baltic (Fig. 1), the water column is characterized by a strong thermohaline stratification which leads to stagnation of the deep-water and regular occurrence of hypoxic areas (Møller and Hansen, 1994; Naumann et al., 2020).

Temperature-depth profiles of cod tagged with data storage tags in 2016-2019 through the TABACOD project suggest that contemporary EBC use shallower waters from autumn to spring and display a deep-water-use period during the spawning time in summer (Hüssy et al., 2020). While WBC experience a clear seasonal temperature oscillation with a maximum during summertime, EBC undergo a flat seasonal temperature cycle with slightly higher temperatures in autumn and winter and lower temperatures in spring (Hüssy et al., 2020a, Haase S unpublished data)).

Tagging of cod

A total of 25,352 cod were tagged between March 2016 and May 2019 in the southern Baltic Sea within the TABACOD project (Figs. S1, S2). The tagging procedure is described in detail in Hüssy et al. (2020). In brief, live cod were caught in German, Danish, Swedish and Polish national waters (Fig. 1). Cod were mostly caught with bottom trawl hauls (trawl duration 5-30 min) from research and commercial vessels. A smaller proportion of cod was caught by angling or with pound nets and fish traps.

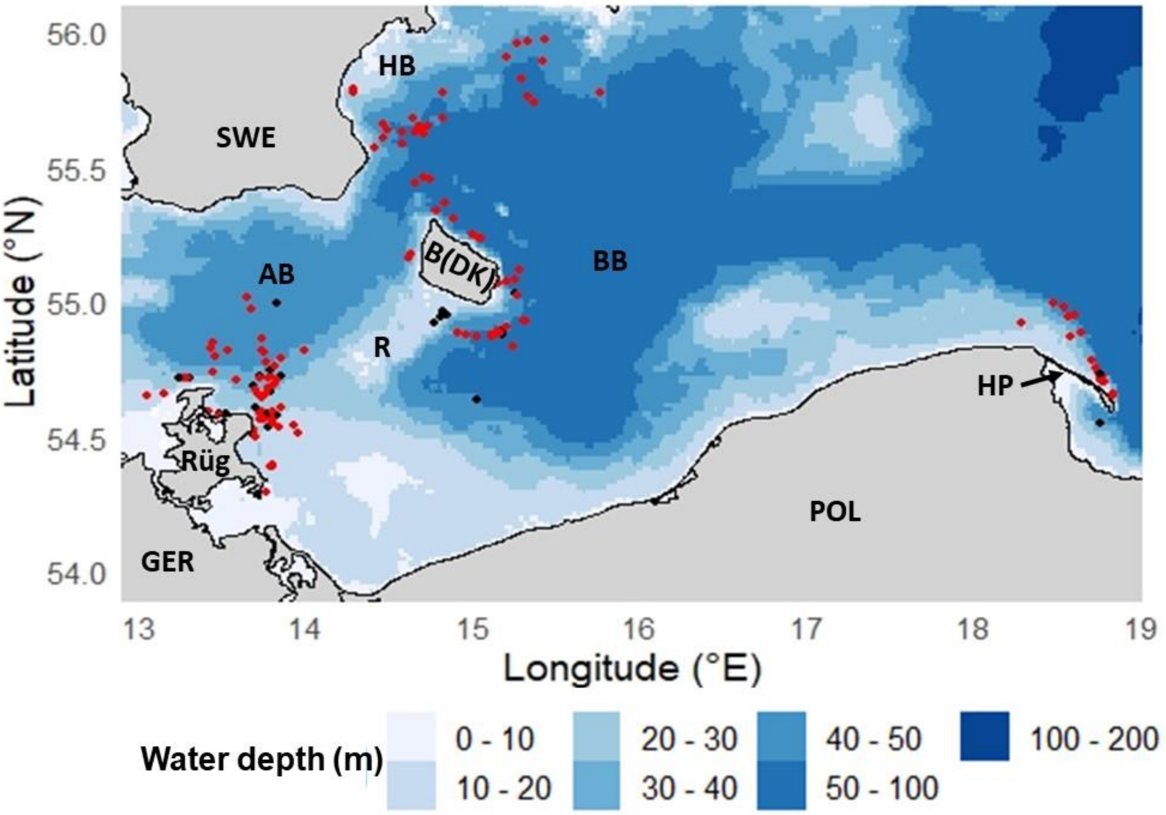

Figure 1: Release position of recaptured cod in the southern Baltic Sea for which otoliths were available for analysis. Individuals were assigned to the EBC (red) or WBC (black) stock by genetics or otolith shape analysis. Locations mentioned in this study are marked (GER: Germany, Rüg: Rügen Island, SWE: Sweden, AB: Arkona Basin, R: Rönnebank, HB: Hanö Bight, B: Bornholm, DK: Denmark, BB: Bornholm Basin, POL: Poland, HP: Hel Peninsula)

The total length (in mm) and the wet mass (in g) were measured for each fish before tagging procedures. Cod were then chemically marked by injecting TET intraperitoneally following the procedure described in Stötera et al. (2018). 93.7 % of all released cod were TET marked. The injected volume of the chemical compound was proportional to individual wet mass (100 mg TET per kg wet mass of cod). TET is embedded into the otolith within approximately 24 hours (Panfili et al., 2002, Campana and Thorrold, 2001), 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 T-bar anchor tag (type TBF-1, Hallprint) for individual identification upon recapture. All fish were released immediately or returned to the water tank and released together at the end of the day. Within TABACOD, the overall mortality associated with tagging events was 16% without significant effects of the tagging procedure itself (Haase et al., 2021).

Recaptured cod

Recaptured cod were returned by commercial and recreational fishers together with information on recapture date and time, location and gear type. By March 2020, 383 recaptured cod had been reported, resulting in a return rate of 1.5%. Total fresh or frozen length (to the nearest mm), fresh or frozen weight (to the nearest g) and sex were recorded. Measurements of length and weight of frozen fish were transformed to fresh length and weight with the shrinkage key developed for cod in the southern Baltic Sea (McQueen et al 2019b). The sagittal otoliths were removed and stored dry and dark to prevent fading of the fluorescent mark (Krumme and Bingel, 2016) until further processing. Recaptures were genetically assigned to the WBC or EBC stock based on tissue samples following the procedure described in (Hemmer-Hansen et al., 2019). Individuals that could not be assigned genetically (14.8%) were assigned to their respective stock using otolith shape analysis. For more details on the method refer to (Schade et al., 2019).

Otolith preparation followed the procedure described in Krumme et al. (2020). Each otolith section was first photographed under transmitted light (to distinguish between TZ and opaque zones (OZ), normally used for age reading) and then under fluorescent light with an Olympus U-MNV filter cube (barrier filter: 455 nm, excitation filter: 400-410 nm, dichroic mirror: 455 nm) to visualize the fluorescent TET mark. The TET mark was visible in 80.2% of the recaptured otoliths.

Validation of zone formation and otolith growth

The zone formation of each sectioned otolith was classified by overlaying the photo taken under fluorescent light and the one taken under transmitted light using Microsoft PowerPoint to make both the TZ and OZ as well as the TET mark visible (Fig. 2, Fig. S3).

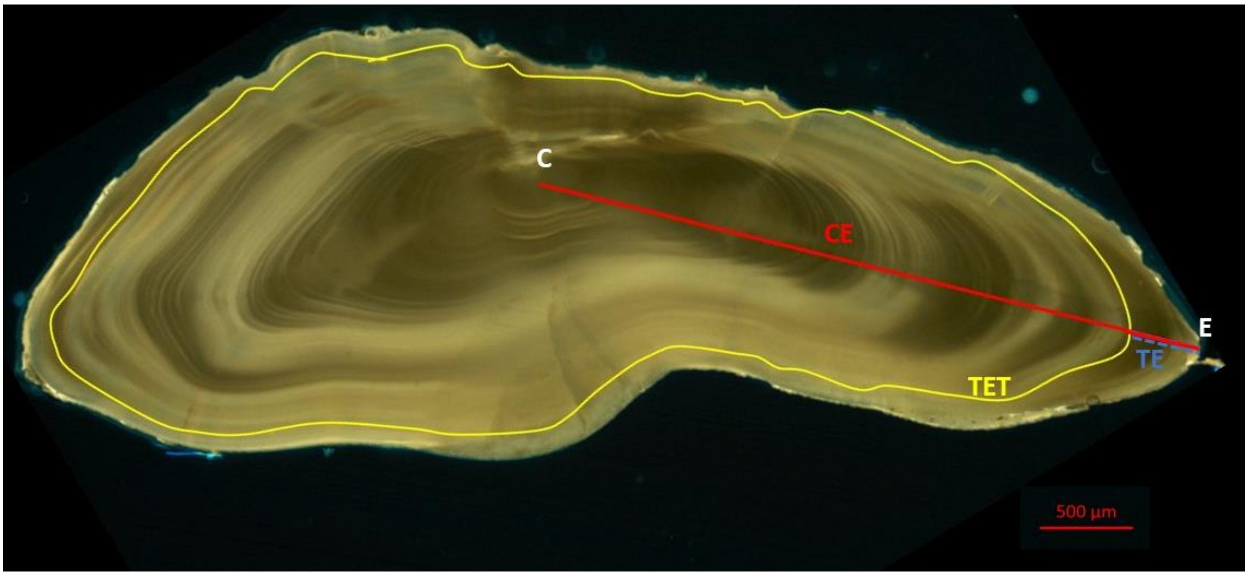

Figure 2: Photograph of an otolith taken under transmitted light overlaid with the tetracycline mark (TET) observed under fluorescent light. C – otolith core, E – otolith edge, CE – distance core to edge at recapture, TE – distance TET mark (=release) to edge (=recapture). Cod was released on 15.11.2016 (390 mm) and recaptured after 410 days at liberty on 30.12.2017 (421 mm). The zone type at release and recapture were classified as opaque and translucent, respectively. Three research assistants measured the distances TE and CE as 368 (±43) µm and 3753(±24) µm, respectively.

For each otolith, the zone type at the edge and at the TET mark, when visible, was classified as either opaque or translucent by one researcher, using a photograph taken under transmitted light. The zone category at the TET mark was only recorded when there was sufficient growth between mark and edge (> 40 µm of otolith growth). In addition, the number of zone transitions between OZs and TZs from the TET mark to recapture was counted following Krumme et al. (2020). The seasonality of zone formation was assessed by examining the monthly distribution of both zone types at release (TET mark) and recapture (edge). In some cases, zone type at the edge could not be classified because of reflections at the edge or because the zone type was not clear. Zone type at the TET mark could not be classified when (i) the TET mark was too close to the edge, (ii) a TET mark was missing or (iii) the zone type was uncertain. The number of zone changes between release and recapture gives an indication about the frequency of zone changes.

Largest distances (in µm) between TET mark and edge (TE) and between core and edge (CE, Fig. 2) were measured with the software Zen 2 (blue edition). These measurements represented otolith growth during the time at liberty (TE) and a measure for otolith size (CE).

The distance TE was only measured when the TET was clearly separated from the edge (i.e. > 40 µm of otolith growth). All measurements were separately conducted by three research assistants, resulting in three independent measurements for each otolith. Measurements between research assistants varied on average by 36 and 76 µm for the distance TET mark and edge, and distance core and edge, respectively. The mean distance for each otolith and measurement were calculated from the measurements by the three research assistants. Average daily otolith growth (DOG, in µm) was calculated by dividing the otolith growth after release (i.e. from the TET mark) by the days at liberty (DAL). Several sources of error can be associated with the distance measurements. These involve (i) an imprecise cut through the core, (ii) a poor quality of the photograph impeding the distinction of the edge type, or (iii) a blurred TET mark and reflections of the outer edge. Further, (iv) since individual measuring axes were used, differences in the chosen angle could lead to differences in distance measurements.

Statistical analysis

We applied four ANOVA models to test the following objectives:

1. Does otolith growth (TE) follow a linear relationship with fish growth ($\text{growth}_{\text{somatic}}$; difference between length at release and length at recapture (mm))? Does it differ between stocks (WBC vs. EBC)?

   $\text{TE} \sim \text{growth}_{\text{somatic}} * \text{stock}$

2. Does otolith growth increase linearly with the days at liberty (DAL)?

   $\text{TE} \sim \text{DAL} * \text{stock}$

3. Does daily otolith growth (DOG) depend on fish length (TL; total length at recapture (mm))? Does it differ between stocks?

   $\text{DOG} \sim \text{TL} * \text{stock}$

4. Does the size of the otolith (CE), as measured from the core to the edge, depend on fish length? Does stock affiliation and sex (female vs. male) have an influence on this?

   $\text{CE} \sim \text{TL} * \text{stock} * \text{sex}$

Continuous variables included TE, $growth_{somatic}$, DAL, DOG, and TL . Stock and sex were used as categorial variables. Initial checking revealed that sex might only be significant in model 4, therefore it was not included in the other models since it reduces the sample size by 54 individuals which had not been assigned a sex. Insignificant terms were dropped successively using F-tests until all terms were significant. The criterion for statistical significance was $p<0.05$. Model selection was conducted by stepwise backwards elimination of the predicting variables based on the Akaike information criterion (AIC, Akaike, 1974). Models with lower AIC are considered to have a better fit, with $\Delta AIC>2$ indicating a significant improvement. The model with less predicting variables was then selected as the most appropriate (Burnham and Anderson, 2002). Assumptions of homogeneity of the residuals and normality of the variables were verified by visual inspection. All analyses were conducted in R 4.0.3 (R Core Team, 2020).

Results

Overview of recaptures

Otoliths from 262 recaptured cod were available. Four of those could not be assigned to either the WBC or EBC stock and were excluded from the analysis. 85.7 % and 14.3 % of the individuals were assigned to the EBC or WBC stock, respectively (Tab. 1). Eight individuals were recaptured after one day. The longest period after which a cod was recaptured was 868 days; average time at liberty was 210 days (Fig. S4). Release and recapture lengths of recaptured individuals ranged between 240 mm and 541 mm (average length of EBC: 386 ± 51 mm and average length of WBC: 389 ± 72 mm) and 253 mm and 585 mm (average length of EBC: 420 ± 48 mm and average length of WBC: 425 ± 73 mm), respectively (Figs. S1 & S2). At recapture, WBC covered ages between 1 and 4 (Tab. S1).

Table 1: Number of returned marked cod by country and stock assigned by genetics or otolith shape analysis (EBC: Eastern Baltic cod stock, WBC: Western Baltic cod stock). Number in brackets indicates number of individuals without visible TET-mark.

	Stock		
Tagging location	EBC	WBC	Total
Southern Arkona Sea	107 (17)	23 (1)	130 (18)
around Bornholm	50 (6)	8 (0)	58 (6)
Hanö Bight	27 (3)	0 (0)	27 (3)
off Hel Pensinsula	37 (17)	6 (6)	43 (23)
Total	221 (43)	37 (7)	258 (50)

Validation of zone formation

Zone categories at release (TET mark) and recapture (edge) could be classified for 179 and 247 individuals out of 258 available, respectively (Fig. S7). TheThe number of classifications of the zone type at the TET mark was particularly low for individuals with DAL less than 30 days.

The proportion of OZs was higher between November and May for EBC at the TET mark, while the TZ dominated from July until October (Fig. 3, refer to Fig. 4 and Tab. 2 for examples). Sample size of TET mark classifications in July (n=2) and December (n=3) was low due to low number of releases in these months (Fig. S1). Despite the low number of WBC recaptures, a clear seasonal pattern was discernible: the OZ dominated in November and from January to April while there was a higher proportion of TZ in May, July and October.

The temporal pattern of the edge type classification was similar to the patterns of opacity at the TET mark but the proportion of TZ was higher in the former (Fig. 3). Generally, classification at the TET mark had a lower level of uncertainty because the zone type could be assigned more easily. The classification at the edge was more uncertain due to uncompleted zone formation and reflections of the edge (Fig. S5).

The majority of the recaptured individuals formed one TZ and one OZ per year (Fig. 4(1)), resulting in 0-2 zone changes if DAL<365 and 2-4 zone changes if DAL>364 and <730. 94.3% of the EBC had 0 (n=42), 1 (n=59) or 2 (n=15) zone changes within the first year after tagging, while 0.03% (n=4) and 0.02% (n=3) had 3 and 4 changes, respectively (Fig. 4(2)). 66.7% of the EBC recaptured within the second year at liberty had 2 (n=11), 3 (n=11) or 4 (n=6) zone changes, while 23.8% (n=10) displayed only 1 zone change and 0.07% (n=3) and 0.02% (n=1) had 5 and 6 zone changes, respectively (Fig. 4(3)). One EBC with 868 DAL displayed 10 zone changes (Fig. 4 (4)). 93.1 % of the WBC displayed 0 (n=14), 1 (n=12) or 2 (n=1) zone changes in the first year at liberty, while 6.9% (n=2) had 4 zone changes. Only one WBC had DAL>1 year and displayed 3 zone changes. In total, 86.8% of the EBC and 93.1% of the WBC supported the hypothesis that one TZ and two OZs are formed per year.

Hence, a calendar year in EBC otoliths consists of three parts: an OZ between January and May, a TZ until October and again an OZ, although some individuals still display a TZ until January. The zone changes for individuals which were at liberty for more than one year, however, suggest that irregularities occur. The few WBC otoliths analysed formed an OZ from January until May, followed by a TZ in summer and again an OZ from November onwards.

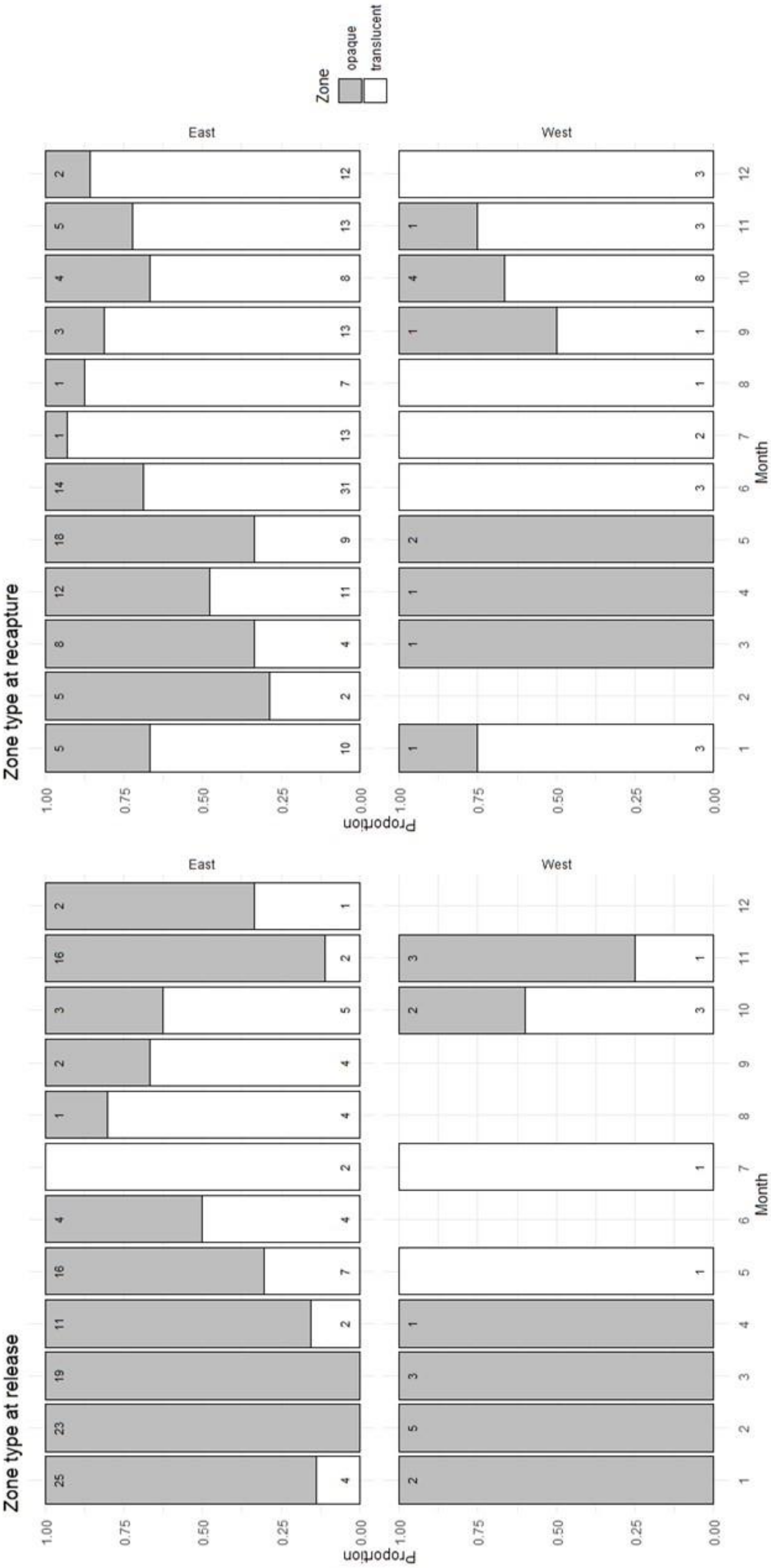

Figure 3: Proportions of zone types at release (left) and recapture (right) per month separated by assigned stocks. Numbers within the bars indicate the sample size.

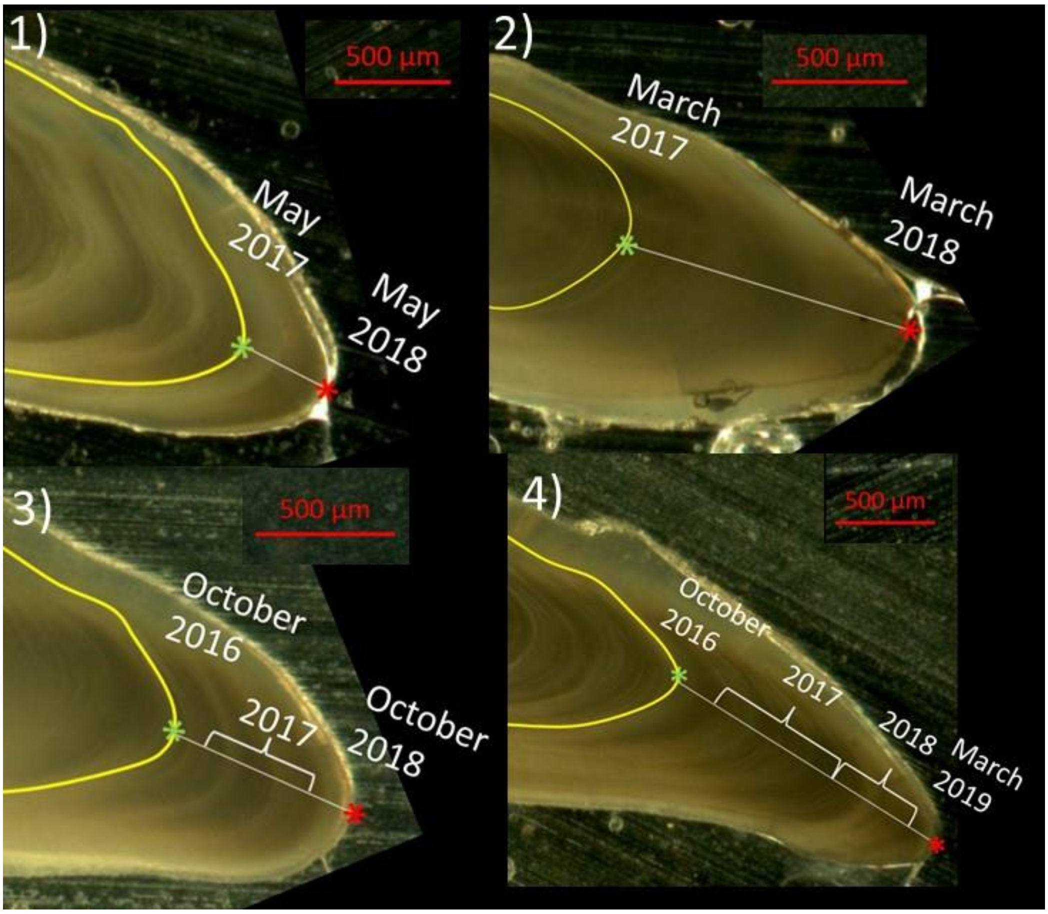

Figure 4: Zoom into the cross section of otoliths from Eastern Baltic cod at liberty for 364 days (1) and (2) and more than a year (3) and (4). The tetracycline-mark (yellow line) as marked on the image taken under the fluorescent light is overlaid onto the image of the otolith viewed under transmitted light. Release (green star with month and year) and recapture (red star with month and year) events are marked. The curly brackets in (3) and (4) indicate the approximation of one year of otolith growth with the corresponding year. Further information can be found in Table 2. Note that (2), (3) and (4) are examples of the 15.2% where more than two zone transitions per year occurred and double rings were visible. Photographs of the complete otoliths can be found in Figure S6.

Table 2: Release and recapture information from the four fish presented in Fig. 4. Date format: dd.mm.yyy. Zone types are classified as opaque (O) and translucent (T) at the tetracycline-mark (release) and at the edge (recapture). Zone transitions: number of changes between translucent and opaque zones between release and recapture. Otolith length at recapture: Mean measured distance between core and edge. Otolith growth: Mean measured distance between tetracycline-mark and edge. Otolith length at release: Difference between Otolith growth and otolith length at recapture. All individuals were assigned as Eastern Baltic cod by genetics (ID 1 and 3) or otolith shape analysis (ID 2 and 4).

	Release				Time at liberty		Recapture						
Otolith ID	Date	Zone	Fish-length (mm)	Otolith length (µm)	Days	Zone transitions	Date	Zone	Fish-length (mm)	Otolith length (µm)	Otolith growth (µm)	Sex	Maturity
1	27.05.2017	O	371	3949	364	2	26.05.2018	O	447	4357	408	F	spent
2	25.03.2017	O	249	2001	364	4	24.03.2018	O	446	3072	1071	-	-
3	26.10.2016	T	278	2580	712	6	08.10.2018	T	346	3273	693	M	abnormal
4	19.10.2016	T	277	2930	868	10	06.03.2019	T	456	4456	1526	F	maturing

Stock differences in otolith growth

The distance between the TET mark and edge were measured for 214 individuals. The largest otolith growth after release was 1523 µm in 868 DAL, for an individual belonging to the EBC stock (Fig. 4 (1)).

After tagging, otolith growth (TE, the largest distances between TET mark and edge) increased linearly with fish growth (F=1192, df=3, $p<0.05$, Fig. 5) with no significant difference between WBC and EBC ($AIC_{full\ model}=2600.1$, $AIC_{final\ model}=2597.3$, Fig. S7). On average, otolith growth increased 6 (±0.17) µm for each mm of somatic growth between release and recapture.

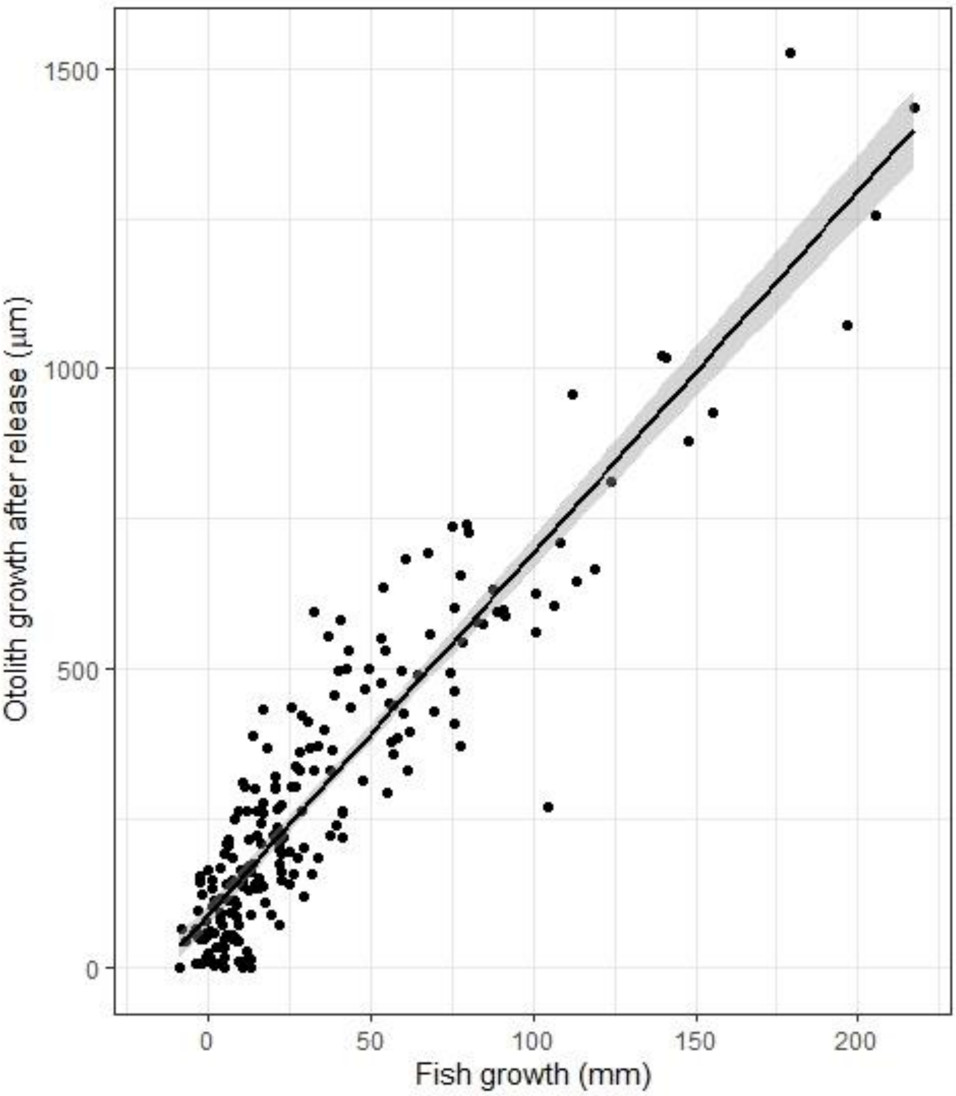

Figure 5: Otolith growth vs. fish growth after release with a linear model fitted with 95%-confidence intervals to the data ($R^2 = 0.85$, n=213) for both stocks combined.). Otolith growth vs. fish growth separately for stock can be found in Fig. S7. Negative fish growth could occur due to measurement errors and shrinkage when DAL was short.

The width of the otolith growth after the TET mark increased with increasing DAL (Fig. 6). The residuals were not distributed homogeneously but visual inspection showed that with increasing DAL, otolith growth increased faster for WBC than for EBC. EBC individuals that seemed to be outliers due to their fast otolith growth were consistently tagged in German or Polish national waters (Fig. S8). For cod with DAL>30, average daily otolith growth (DOG) was significantly faster for WBC (2.3 ±0.6 µm day^{-1}) compared to EBC (1.4 ± 0.6 µm day^{-1}, Fig. S6, F=45.2, df=1, $p<0.05$, Fig. S9). Fish length did not significantly influence daily otolith growth ($AIC_{full\ model}$=308.3, $AIC_{final\ model}$=305.6).

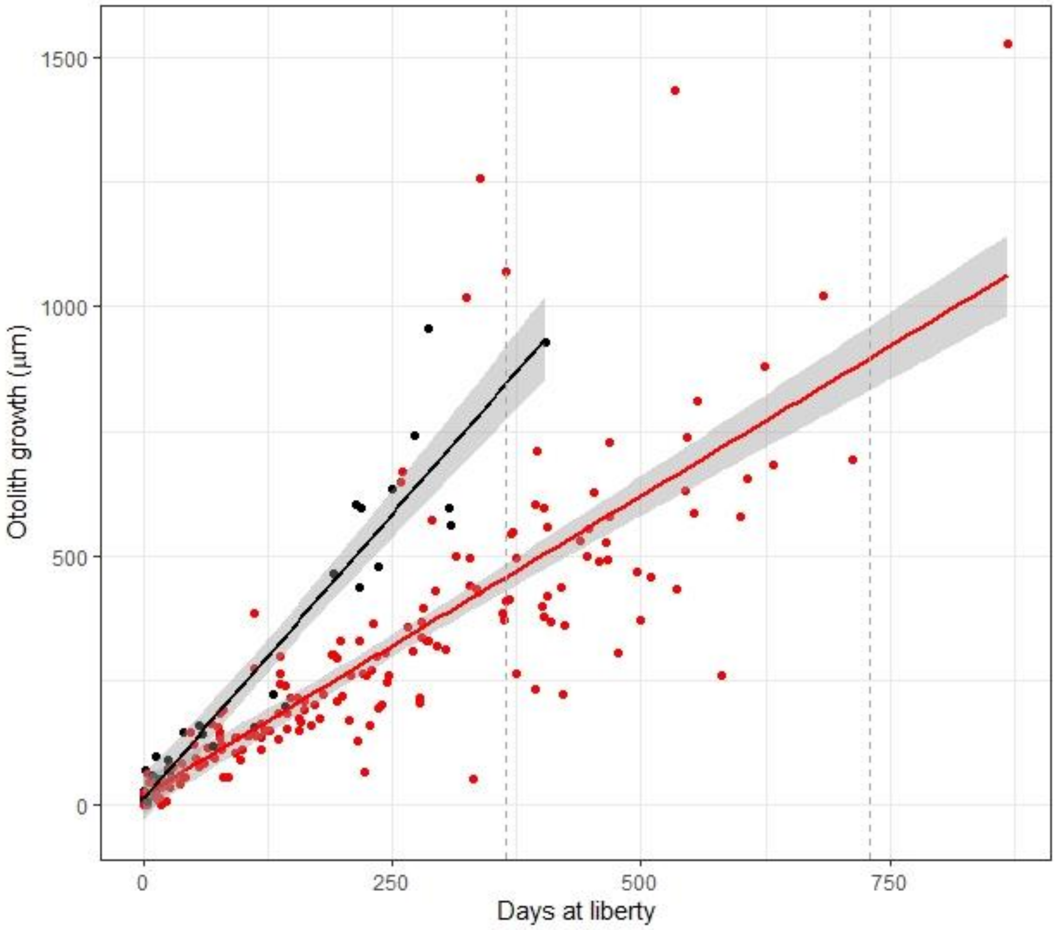

Figure 6: Otolith growth after release vs. days at liberty separately for Western Baltic cod (WBC, black dots) and Eastern Baltic cod (EBC, red dots); linear model fitted with 95%-confidence intervals ($R^2 = 0.71$, n=213).

Stock differences in otolith size

There was a significant positive relationship between CE and recapture length. CE was significantly greater for EBC compared to WBC and for males compared to females, with no interaction at the same fish length (Figure 7, Tab. 3, F=52.92, df=5, p<0.001, n=204, $AIC_{full\ model}$=2963.92, $AIC_{final\ model}$=2962.83), suggesting that EBC and male individuals have larger otoliths at the same fish length. It has to be considered that the number of WBC samples which were assigned a sex and included in this analysis is rather small (n=24) with only 12 individuals per sex.

Table 3: Parameter estimates and standard error (SE) for ANOVA exploring the effect of stock and recapture length on the distance core-edge (F=113.8, df=2, p<0.001). Parameter estimates significantly different from 0 at the 5% level (p<0.05) are marked with a "*".

Term	Parameter estimate	SE	t-value	p-value
Intercept (EBC)	1229.97	200.68	6.13	<0.001*
$TL_{recapture}$	5.87	0.46	12.78	<0.001*
WBC	-445.95	73.94	-6.03	<0.001*
male	146.85	48.51	3.03	0.003*

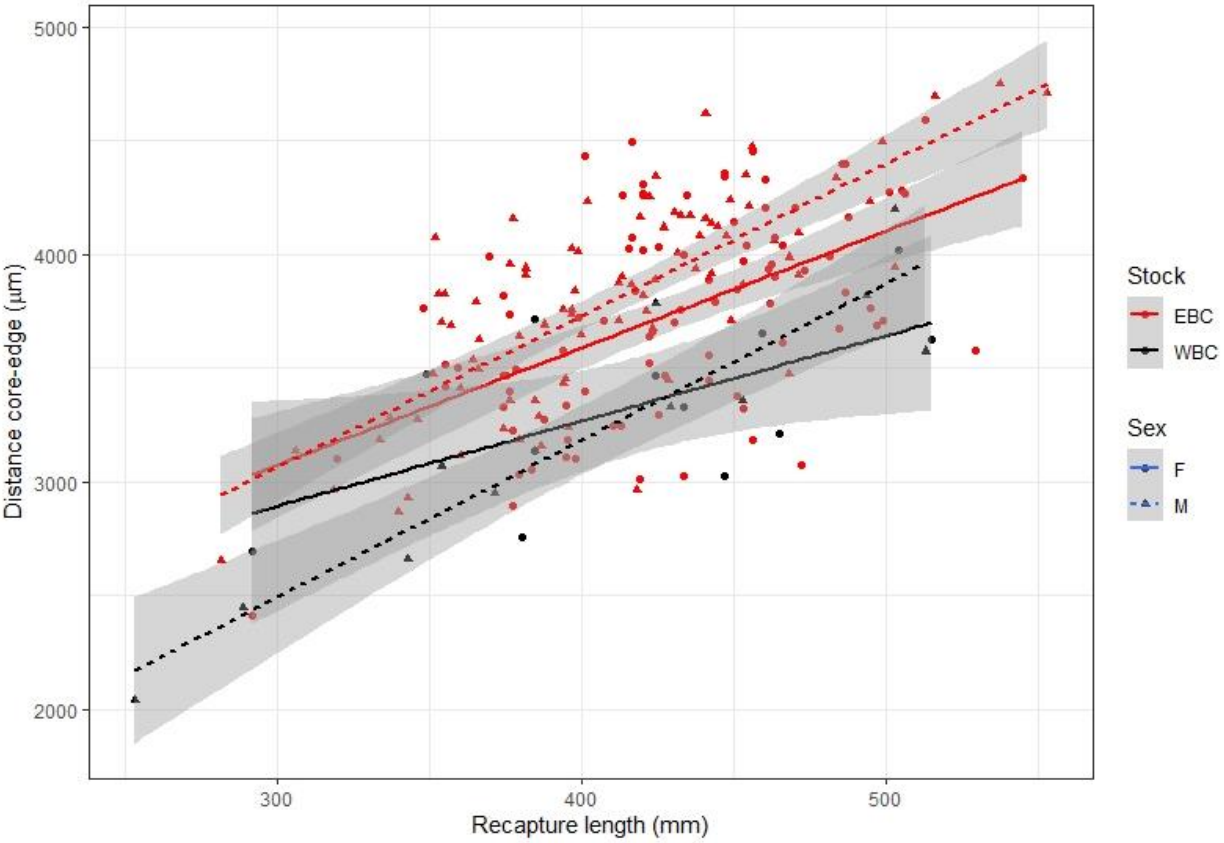

Figure 7: Distance core-edge, a measure for otolith size, over recapture length, separately for stock (Eastern Baltic cod (EBC): red dots; Western Baltic cod (WBC): black dots, n=256); a linear model is fitted to the data with 95%-confidence intervals.

Discussion

After decades of uncertainty in the interpretation of ring patterns, this study is the first to validate otolith zone formation of wild adult EBC using TET-marked recaptures. Recently, the zone formation of juvenile and young adult WBC was validated (Krumme et al., 2020), making EBC the second demersal fish stock from the Baltic Sea where chemically marked otoliths have been used to validate ring formation patterns. The present analysis revealed that contrary to previous assumptions, zone formation of EBC does show a visible seasonal signal within the otolith, namely that the TZ of EBC is deposited between April and October, predominantly during the summer months. Furthermore, the time-stamped otoliths provided the first validated otolith growth measurements which revealed that contemporary WBC otoliths grew faster than EBC, while EBC otoliths were generally larger than otolith of WBC at the same fish length. This is in line with the slow body growth of EBC estimated from mark-recapture data (McQueen et al., 2020; Mion et al., 2020). Moreover, sex-specific differences in otolith growth were detected with male EBC having larger otoliths.

Seasonal pattern in zone formation

Zone formation of EBC was assumed to be unpredictable and unrelated to season (Hüssy, 2010; Hüssy et al., 2016). However, the chemically marked otoliths of adult (25-59 cm) EBC from this study showed a clear pattern of TZ formation between April and October at the TET mark. There were few individuals which formed their TZ until January, indicating either a prolonged period of TZ formation or a second, separate TZ formation in December-January (Fig. 5). Zone formation at the edge was less interpretable than at the TET mark. Classification at the outer edge is often ambiguous (see examples of WBC in Krumme et al. 2020). In EBC, incomplete zones, slow otolith growth, and reflections at the edge of the slices may have contributed to the uncertainties in past attempts using edge type analysis (Baranova et al., 2011 and references therein).

The wide range of months in which the TZ is formed is in line with the results of edge type analysis of Baranova et al. (2011). The extended period of TZ formation coincides with the presently prolonged spawning period of EBC, ranging from May until September (Köster et al., 2017). During spawning, which in recent years mainly takes place in the Bornholm Basin, EBC use areas below the halocline where salinity is higher, hypoxic conditions prevail and food uptake is reduced (Eero et al., 2015; Casini et al., 2016, Hornetz P unpublished data).

This likely results in a physiologically stressful period in which metabolic needs are not met. Temperature is less likely to play an important role in the formation of the TZ, because temperature differences experienced by EBC during the course of the year, as observed in DST data, are minor compared to WBC (Haase S. unpublished data). There is not such a distinct increase in ambient temperatures experienced by EBC during summer when the fish spawn in deeper, colder areas below the halocline (Wieland et al., 2000). An increase in ambient temperature in EBC only occurs when they move to shallower grounds in late autumn (Hüssy et al., 2020a; Haase S unpublished data). The OZ of adult EBC is mainly formed post-spawning in winter and spring. This is also a period of more intense feeding (Baranova et al., 2011; Hornetz P, unpublished data), even though somatic growth of EBC shows negligible seasonal variation (McQueen et al., 2020; Mion et al., 2020).

It is noteworthy that the TZ of both EBC (this study) and WBC (Krumme et al. 2020) is formed during summer. However, different combinations of factors are involved (Tab. 4), which may lead to similar metabolic states resulting in TZ formation. Unlike EBC, WBC spawn mainly during the first quarter of the year (Hüssy, 2011) and experience increased water temperatures in summer. Recent evidence from WBC in the relatively shallow Belt Sea (ICES subdivision 22) revealed that the fish conduct seasonal shifts in vertical distribution (Funk et al. 2020). In summer, WBC concentrate on the basin slopes to “over-summer”, thus avoiding high water temperatures in shallower waters (Freitas et al. 2015, 2016) and hypoxic areas building up in the deeper basin (Naumann et al., 2020). This period is associated with reduced feeding (Funk et al., 2020a) and reduced growth of WBC (McQueen et al. 2019), suggesting that the metabolic needs of WBC are not met and a TZ is formed, both in juveniles and young adults (McQueen et al., 2019; Krumme et al., 2020; Plonus et al. 2021). The fact that WBC from the Arkona, Bornholm and Gdansk basin also form the TZ during summer strongly suggests that WBC display in these areas the same seasonal changes in depth use as observed in the Belt Sea. This is further supported by recent findings from otolith shape analyses (Schade et al., under review in CJAFS) and patterns in depth use of DST recaptures (Haase S, unpublished data) showing that WBC usually use shallower waters than EBC. Consequently, WBC generally experience stronger seasonal temperature fluctuations than EBC. Contrarily, WBC and EBC also share common stressors during the period of TZ formation, such as the decreases in food intake, increase in ambient salinity and decrease in oxygen while concentrating in spawning aggregations (EBC) or concentrating in over-summering groups (WBC) (Tab. 4).

Table 4: Comparison of stressors affecting the formation of a translucent zones during summer in Eastern Baltic cod (EBC) and Western Baltic cod (WBC) otoliths (Y = yes, N = no). References can be found in the text.

		Stock	
	Stressor in summer	WBC	EBC
Behaviour	Spawning	N[1]	Y[2]
	Decrease in food intake	Y[4]	Y[5, 6]
	Aggregation into large groups	Y[1]	Y[2, 6]
Environmental condition	Increase in salinity	Y[7]	Y[7]
	Decrease in oxygen	Y[1]	Y[6]
	Increase in temperature	Y[1]	N[8]

[1] Funk et al. (2020b)
[2] Wieland et al. (2000)
[3] Köster et al. (2017)
[4] Funk et al. (2020a)
[5] Hornetz P, unpubl. data
[6] Casini et al. (2016)
[7] Nissling and Westin (1997)
[8] Hüssy et al. (2020)

The environmental factors influencing the formation of TZ and OZ are difficult to disentangle in wild fish (Fablet et al., 2011 and references therein), and only a few ambient conditions can be recorded simultaneously in individuals tagged with data-storage tags in mark-recapture studies. Controlled experiments conducted with Atlantic cod revealed that high temperatures and changes in metabolic activity expressed for example through food limitation, lead to the formation of a TZ in otoliths (Dannevig, 1956; Høie et al., 2008; Neat et al., 2008; Fablet et al., 2011; Stanley et al., 2015). Further research linking evidence from controlled experiments and environmental data recorded by tagged wild fish is needed to disentagle the relative role of different processes influencing zone formation in Baltic cod otoliths.

Irregularities in otolith zone formation

Most EBC and WBC had up to two zone changes within a year, indicating that one TZ (April-October) and two OZs (January-March and November-December) were formed per calendar year, and thus pairs of zones can be counted to estimate age. There were, however, 7.2% of the EBC forming more and 6.0% less than one TZ bordered by two OZs per year (see Fig. 4(2)-4(4)). 6.9 % of the WBC had more than two zone changes within a year. This suggests that there is not necessarily one period of favourable conditions followed by one with unfavourable conditions every year, but deviations can occur. Double rings indicate either the return of unfavourable conditions or the temporary occurrence of alleviating conditions. On the other hand, a continuous OZ or TZ during a year indicates that the individual experienced either favourable or unfavourable conditions for time periods which exceed a year. The proportion of irregular otoliths (13.2%) is not minor and may impede the age reading of EBC when chemically-marked recaptures from continuous tagging efforts are not available.

Differences in otolith growth between mixing cod stocks

The on average 61% higher daily otolith growth rate of WBC compared to EBC indicates better fish growth conditions for WBC in the same area. Otolith growth followed a strict linear relationship with fish growth, with no differences between the two stocks (Fig. 7). This emphasizes that in Baltic cod body growth is reflected in otolith growth. The difference in otolith growth between the two stocks in the southern Baltic is lower than the difference in somatic growth, as somatic growth of cod from the western Baltic Sea has been reported to be roughly twice as fast as cod in the eastern Baltic Sea (McQueen et al., 2020). Considering only cod tagged within the same region, the stock-specific differences in somatic fish growth are lower than the regional differences, but with an average difference of 41% still statistically significant (McQueen et al., 2020). Due to the limited number of recaptured WBC (likely reflecting the decreasing abundance of WBC towards the east) and overlapping distributions of WBC and EBC in the area, it was impossible to statistically assess whether or not differences are due to a genetic or to a spatial effect, but the difference in otolith growth rate is another clear signal for the reduced productivity of EBC compared to WBC (McQueen et al., 2020). Grouping by tagging country, however, revealed that otoliths of recaptured individuals tagged in Polish and German national waters showed faster otolith growth rates compared to individuals tagged in Danish and Swedish national waters (Fig. S7). This indicates an area-effect which is likely related to the exposure to different environmental conditions, such as an increase in benthic food availability and decrease in hypoxic areas closer to the coast (Casini et al., 2016; Naumann et al., 2020).

Differences in otolith size between mixing cod stocks

Whereas the daily otolith growth during the individual's time at liberty was faster for WBC, the distance between core and edge was larger for EBC at the same fish length (Fig. 7). This indicates that the otoliths of EBC grew faster or for a longer time period before the individual reached length classes covered in this tagging study (i.e. smaller than 25 cm). Following our present understanding, this is the most likely explanation, but further samples of juvenile otoliths could bring clarity.

An alternative explanation is that somatic growth and otolith growth are decoupled, resulting in continuous otolith growth while somatic growth is reduced (Mosegaard et al., 1988; Hüssy and Mosegaard, 2004; Svedäng et al., 2020). However, at least during the duration of this experiment there is no indication of decoupled somatic and otolith growth as reflected by the strictly linear relationship which did not differ between EBC and WBC (Figs. 5 & S9). Evidence for decoupling might require longer time series. A general restriction of this analysis is the low sample size of WBC.

Additionally, the distance between core and edge was larger for female than male cod. At least for EBC, where the difference was slightly larger than for WBC, this suggests that otoliths of males grew slower (Fig. 7). A potential explanation are the extended stays (earlier arrival and later leave) on the spawning grounds (Nielsen et al., 2013), so that males are longer exposed to unfavourable conditions currently occurring in the Bornholm Basin (Eero et al., 2015; Casini et al., 2016; Köster et al., 2017). This could potentially also be a first indication for reduced somatic growth.

Cod smaller than 25 cm total length were not tagged in this study, so that we still lack chemically-marked otoliths from juvenile EBC to validate the formation of the first TZ. If the age of the first visible increment formation is still unclear in EBC, age data should not be included in the current stock assessment, even though this study validates the patterns in ring formation of adult EBC. Monthly length-frequency distributions of wild juvenile EBC are needed (see McQueen et al. 2019 for the WBC as example), ideally in addition to recaptures of chemically-marked juveniles. This is, however, challenging in EBC because young-of-year are usually too small for external tagging and the mortality associated with trawling Baltic cod for tagging process increases with decreasing size (Haase et al., 2021)

Conclusion

Chemical marking combined with tagging provides a time stamp in the otolith which enables the analysis of zone formation and otolith growth between release and recapture. This method was already successfully used to validate that WBC form the TZ during summer (Krumme et al., 2020). In adult EBC, also unlike previous assumptions, TZ also occurred predominantly in summer during the extended spawning period where metabolic needs are apparently not met. However, validation of the age at first first visible increment formation in juvenile EBC is still needed before age data of EBC can be reliably estimated. Since otolith growth follows a linear pattern with fish growth irrespective of stock affiliation, the slower daily otolith growth of EBC confirmed the reduced length growth rate of adult EBC (McQueen et al., 2020). The generally larger otolith size of EBC compared to WBC at a given fish length indicate that EBC are older at the same fish length, which provides further evidence for the reduced growth rate and stock productivity of EBC. The smaller otolith size of males compared to females further suggest a sex-specific difference in somatic growth of cod in the southern Baltic Sea presenting a serious matter which requires further research.

Acknowledgements

We thank the technical staff from the National Marine Fisheries Institute (NMFRI) in Poland, the Technical University of Denmark (DTU-Aqua), the Swedish University of Agriculture Sciences (SLU) and the Thünen Institute of Baltic Sea Fisheries (OF) involved in the tagging, collection and processing of samples used in this study and all fishers and anglers returning tagged cod. We thank Jakob Hemmer-Hansen und DTU-Aqua for providing the genetic assignment. Thanks go to the research assistents Fanny Morel, Thies Rinner and Marius Hoppe for measuring and categorizing the otoliths. The tagging was conducted under the following animal test permissions: OF: TABACOD T-bar tagging: AZ 7221.3.1-029/15; TABACOD DST tagging: AZ 7221.3.1-007/18; DTU: TABACOD T-bar and DST tagging: 016-15-0201-00929, NMFRI: TABACOD T-bar tagging: Permission no 19/2016, dated 28.06.2016, SLU: TABACOD T-bar and DST tagging: Dnr 5.8.18-14823/2018.

This study was funded by BalticSea2020 (http://balticsea2020.org) through the project “TABACOD” (Tagging Baltic cod).

References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Autom Control 19: 716–723

Baranova T, Müller-Karulis B, Šics I, Plikshs M (2011) Changes in the annual life cycle of eastern Baltic cod during 1950-2010. ICES CM 2011R10

Beamish RJ, McFarlane GA (1983) The Forgotten Requirement for Age Validation in Fisheries Biology. Trans Am Fish Soc 112: 735–743

Berner M (1968) Einige orientierende Untersuchungen an den Otolithen des Dorsches (Gadus morhua L.) aus verschiedenen Regionen der Ostsee. Fischerei Forschungs Wissenschaftliche Schriftenreihe: 119–129

Bertignac M, de Pontual H (2007) Consequences of bias in age estimation on assessment of the northern stock of European hake (Merluccius merluccius) and on management advice. ICES J Mar Sci 64: 981–988

Bignami S, Enochs IC, Manzello DP, Sponaugle S, Cowen RK (2013) Ocean acidification alters the otoliths of a pantropical fish species with implications for sensory function. Proc Natl Acad Sci 110: 7366–7370

Burnham KP, Anderson DR (2002) Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach, 2nd ed. doi: 10.1007/b97636

Campana SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J Fish Biol 59: 197–242

Campana SE, Thorrold SR (2001) Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? Can J Fish Aquat Sci 58: 30–38

Casini M, Käll F, Hansson M, Plikshs M, Baranova T, Karlsson O, Lundström K, Neuenfeldt S, Gårdmark A, Hjelm J (2016) Hypoxic areas, density-dependence and food limitation drive the body condition of a heavily exploited marine fish predator. R Soc Open Sci 3: 160416

Dannevig E (1956) The influence of temperature on the formation of zones in scales and otoliths of young cod. Fiskdir Skr Ser Hav 9: 1–16

Eero M, Hjelm J, Behrens J, Buchmann K, Cardinale M, Casini M, Gasyukov P, Holmgren N, Horbowy J, Hüssy K, et al (2015) Eastern Baltic cod in distress: biological changes and challenges for stock assessment. ICES J Mar Sci 72: 2180–2186

Fablet R, Pecquerie L, De Pontual H, Høie H, Millner R, Mosegaard H, Kooijman S (2011) Shedding Light on Fish Otolith Biomineralization Using a Bioenergetic Approach. PloS One 6: e27055

Funk S, Frelat R, Möllmann C, Temming A, Krumme U (2020a) The forgotten feeding ground: patterns in seasonal and depth-specific food intake of adult cod Gadus morhua in the western Baltic Sea. J Fish Biol. doi: https://doi.org/10.1111/jfb.14615

Funk S, Krumme U, Temming A, Möllmann C (2020b) Gillnet fishers' knowledge reveals seasonality in depth and habitat use of cod (Gadus morhua) in the Western Baltic Sea. ICES J Mar Sci fsaa071

Haase S, McQueen K, Mion M, Andersson M, Hilvarsson A, Olesen HJ, Svenson A, Casini M, Hüssy K, Radtke K, et al (2021) Short-term tagging mortality of Baltic cod (Gadus morhua). Fish Res 234: 105804

Hemmer-Hansen J, Hüssy K, Baktoft H, Huwer B, Bekkevold D, Haslob H, Herrmann J, Hinrichsen H, Krumme U, Mosegaard H, et al (2019) Genetic analyses reveal complex dynamics within a marine fish management area. Evol Appl 12: 830–844

Høie H, Folkvord A, Mosegaard H, Li L, Clausen LAW, Norberg B, Geffen AJ (2008) Restricted fish feeding reduces cod otolith opacity. J Appl Ichthyol 24: 138–143

Hüssy K (2010) Why is age determination of Baltic cod (Gadus morhua) so difficult? Ices J Mar Sci - ICES J MAR SCI 67: 1198–1205

Hüssy K (2011) Review of western Baltic cod (Gadus morhua) recruitment dynamics. ICES J Mar Sci 68: 1459–1471

Hüssy K, Casini M, Haase S, Hilvarsson A, Horbowy J, Krüger-Johnsen M, Krumme U, Limburg KE, McQueen K, Mion M, et al (2020) Tagging Baltic Cod – TABACOD. Eastern Baltic cod: Solving the ageing and stock assessment problems with combined state-of-the-art tagging methods. 64

Hüssy K, Mosegaard H (2004) Atlantic cod (*Gadus morhua*) growth and otolith accretion characteristics modelled in a bioenergetics context. Can J Fish Aquat Sci 61: 1021–1031

Hüssy K, Radtke K, Plikshs M, Oeberst R, Baranova T, Krumme U, Sjöberg R, Walther Y, Mosegaard H (2016) Challenging ICES age estimation protocols: lessons learned from the eastern Baltic cod stock. ICES J Mar Sci 73: 2138–2149

ICES (2020a) Working Group on Biological Parameters (WGBIOP). doi: 10.17895/ICES.PUB.7651

ICES (2013) Report of the Workshop on Age Validation Studies of Gadoids (WKAVSG), 6-10 May 2013, IMEDEA, Mallorca. ICES CM 2013/ACOM:50. 33 pp.

ICES (2006) Report of the Study Group on Ageing Issues of Baltic Cod (SGABC), 16–19 May 2006, Gdynia, Poland. ICES CM 2006/ BCC:08. 45 pp.

ICES (2014) Report of the Workshop on Scoping for Integrated Baltic Cod Assessment (WKSIBCA). 1-3 October 2014, Gydnia, Poland. ICES CM 2014/ACOM:62. 51 pp.

ICES. 2014. Report of the Baltic Fisheries Assessment Working Group (WGBFAS), 3- 10 April 2014, ICES HQ, Copenhagen, Denmark. ICES CM 2014/ACOM:10. 919 pp.

ICES (2015) Report of the Benchmark Workshop on Baltic Cod stocks (WKBALTCOD), 2-6 March 2015, Rostock, Germany. ICES CM 2015/ACOM:35. ICES Sci Rep 172 pp.

ICES (2019a) Benchmark Workshop on Baltic Cod stocks (WKBALTCOD2). ICES Sci Rep 1: 310 pp.

ICES (2019b) Cod (Gadus morhua) in subdivisions 24-32, eastern Baltic stock (eastern Baltic Sea). doi: 10.17895/ices.advice.4747

ICES (1994) Report of the workshop on Baltic cod age reading, 6−10 June 1994, Gdynia, Poland. ICES CM 1994/J:5.

ICES (2000) Study group on Baltic cod age reading, 27−31 March 2000, Karlskrona, Sweden. ICES CM 2000/H: 01.

ICES (2005) Report of the study group on ageing issues of Baltic cod (SGABC), 17−20 May 2005, Klaipeda, Lithua- nia. ICES CM 2006/ACFM: 02.

ICES (2020b) Baltic Fisheries Assessment Working Group (WGBFAS). ICES Scientific Reports. 2:45. 643 pp. http://doi.org/10.17895/ices.pub.6024.

Irgens C, Folkvord A, Otterå H, Kjesbu OS (2020) Otolith growth and zone formation during first maturity and spawning of Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 77: 113–123

Köster FW, Huwer B, Hinrichsen H-H, Neumann V, Makarchouk A, Eero M, Dewitz BV, Hüssy K, Tomkiewicz J, Margonski P, et al (2017) Eastern Baltic cod recruitment revisited—dynamics and impacting factors. ICES J Mar Sci 74: 3–19

Krumme U, Bingel F (2016) Tetracycline marks visible in Baltic cod Gadus morhua otoliths stored for 40 years. J Fish Biol 89: 2189–2194

Krumme U, Stötera S, McQueen K, Pahlke E (2020) Age validation of age 0-3 wild cod Gadus morhua in the western Baltic Sea through mark-recapture and tetracycline marking of otoliths. Mar Ecol Prog Ser 645: 141–158

Lea E (1910) On the methods used in the Herring-investigations. Publ Circonst Cons perm int Explor Mer 14–22

Maneja R, Frommel A, Geffen A, Folkvord A, Piatkowski U, Chang M, Clemmesen C (2013) Effects of ocean acidification on the calcification of otoliths of larval Atlantic cod Gadus morhua. Mar Ecol Prog Ser 477: 251–258

McQueen K, Casini M, Dolk B, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Mion M, Mohr T, Radtke K, et al (2020) Regional and stock-specific differences in contemporary growth of Baltic cod revealed through tag-recapture data. ICES J Mar Sci fsaa104

McQueen K, Hrabowski J, Krumme U (2019) Age validation of juvenile cod in the Western Baltic Sea. ICES J Mar Sci 76: 430–441

Methot RD, Wetzel CR (2013) Stock synthesis: A biological and statistical framework for fish stock assessment and fishery management. Fish Res 142: 86–99

Mion M, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Krüger-Johnsen M, Krumme U, McQueen K, Plikshs M, Radtke K, et al (2020) Multidecadal changes in fish growth rates estimated from tagging data: A case study from the Eastern Baltic cod (Gadus morhua, Gadidae). Fish Fish. doi: https://doi.org/10.1111/faf.12527

Møller JS, Hansen IS (1994) Hydrographic processes and changes in the Baltic Sea. 18

Mosegaard H, Svedäng H, Taberman K (1988) Uncoupling of Somatic and Otolith Growth Rates in Arctic Char (*Salvelinus alpinus)* as an Effect of Differences in Temperature Response. Can J Fish Aquat Sci 45: 1514–1524

Naumann M, Gräwe U, Mohrholz V, Kuss J, Kanwischer M, Feistel S, Hand I, Waniek JJ, Schulz-Bull DE (2020) Hydrographic-hydrochemical assessment of the Baltic Sea 2019. Meereswissenschaftliche Berichte No 114 2020 - Mar Sci Rep No 114 2020 13,1 MB, 97 pages

Neat FC, Wright PJ, Fryer RJ (2008) Temperature effects on otolith pattern formation in Atlantic cod Gadus morhua. J Fish Biol 73: 2527–2541

Nielsen B, Hüssy K, Neuenfeldt S, Tomkiewicz J, Behrens J, Andersen K (2013) Individual behaviour of Baltic cod Gadus morhua in relation to sex and reproductive state. Aquat Biol 18: 197–207

Nissling A, Westin L (1997) Salinity requirements for successful spawning of Baltic and Belt Sea cod and the potential for cod stock interactions in the Baltic Sea. Mar Ecol Prog Ser 152: 261–271

Panfili J, De Pontual H, Troadec H, Wright P (2002) Manual of Fish Sclerochronology. doi: 10.1643/OT-03-266

Pannella, G. (1974). Otolith growth patterns: An aid in age determination in temperate and tropical fishes, p. 28-39. In T.B. Bagenal (ed.), The Ageing of Fish. Unwin Brothers, Old Woking, England.

Plonus R, McQueen K, Günther C, Funk S, Temming A, Krumme U (2021) Is analysis of otolith microstructure a valid method for investigating early life history of Western Baltic cod? Mar Biol 168: 30

de Pontual H, Groison AL, Piñeiro C, Bertignac M (2006) Evidence of underestimation of European hake growth in the Bay of Biscay, and its relationship with bias in the agreed method of age estimation. ICES J Mar Sci 63: 1674–1681

R Core Team (2020) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

Romanek CS, Gauldie RW (1996) A predictive model of otolith growth in fish based on the chemistry of the endolymph. Comp Biochem Physiol A Physiol 114: 71–79

Schade FM, Weist P, Krumme U (2019) Evaluation of four stock discrimination methods to assign individuals from mixed-stock fisheries using genetically validated baseline samples. Mar Ecol Prog Ser 627: 125–139

Secor DH, Dean JM (1989) Somatic Growth Effects on the Otolith–Fish Size Relationship in Young Pond-reared Striped Bass, Morone saxatilis. Can J Fish Aquat Sci. doi: 10.1139/f89-015

Secor DH, Dean JM, Campana SE, Miller AB, Belle W. (1995) Recent developments in fish otolith research. Published for the Belle W. Baruch Institute for Marine Biology and Coastal Research by the University of South Carolina Press, Columbia, S.C. Baruch Institute for Marine Biology and Coastal Research, International Symposium on Fish Otoliths: Research and Application, eds

Stanley RRE, Bradbury IR, DiBacco C, Snelgrove PVR, Thorrold SR, Killen SS (2015) Environmentally mediated trends in otolith composition of juvenile Atlantic cod (Gadus morhua). ICES J Mar Sci 72: 2350–2363

Stötera S, Degen-Smyrek A, Krumme U, Stepputtis D, Bauer R, Limmer B, Hammer C (2018) Marking otoliths of Baltic cod (Gadus morhua Linnaeus, 1758) with tetracycline and strontium chloride. J Appl Ichthyol 35: 427–435

Svedäng H, Thunell V, Pålsson A, Wikström SA, Whitehouse MJ (2020) Compensatory Feeding in Eastern Baltic Cod (Gadus morhua): Recent Shifts in Otolith Growth and Nitrogen Content Suggest Unprecedented Metabolic Changes. Front Mar Sci. doi: 10.3389/fmars.2020.00565

Tokareva GL (1963) A method of age determination and growth pe- culiarities of Baltic Sea cod. Trudy ATLANTNIRO: 179–191

Weist P, Schade FM, Damerau M, Barth JMI, Dierking J, André C, Petereit C, Reusch T, Jentoft S, Hanel R, et al (2019) Assessing SNP-markers to study population mixing and ecological adaptation in Baltic cod. PLOS ONE 14: e0218127

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

General Discussion

This thesis evolves around two main topics, namely (1) the movement of individual cod in the southern Baltic Sea and (2) the zone formation and growth of otoliths of cod from the same area. The seasonal movements and distribution patterns of individuals and the associated environmental conditions can thus be linked to patterns in otolith formation. Knowledge on the movement patterns and behavior of individual fish is important to better understand the connection between individual behaviour and link these to the dynamics in a stock (e.g. distribution, habitat use, exposure to different environmental conditions (Sutherland, 1997; Henderson et al., 2014)). Information on the individual movement behaviour relative to ambient environmental conditions is particularly valuable input for modelling bioenergetics of individuals or inferring to stock dynamics. Further, fish otoliths are commonly used for age determination and can be an indicator for the growth rate of a stock; both fish age and otolith growth are measures for the productivity and status of a fish stock and inaccurate aging due to the lack of validated otolith material might lead to a biased picture of the stock status.

This dissertation aimed at resolving some of these issues for Western Baltic cod (WBC) and Eastern Baltic cod (EBC) of the southern Baltic Sea by using data of the international tagging project “TABACOD” (Tagging Baltic cod; Hüssy et al. (2020a)) which took place between 2016 and 2019 and used both T-bar tags and archival DSTs recording temperature and depth.

Summary of key contributions

To ensure that during a tagging project a representative sample is obtained, the post-release short-term mortality after tagging with T-bar tags and tetracycline was evaluated (Objective 1, Chapter 1). This methodological check revealed that the mortality directly related to the tagging procedure in the TABACOD project was relatively low (16%) and that the tagging process itself did not have an influence on the short-term mortality of cod. However mortality related to the trawling and handling onboard was generally decreasing with increasing fish length. Besides the truncated length distribution of EBC available for tagging, this could have been one reason for the low sample size of recaptures of fish released at smaller sizes. Although the post-release mortality of DST-tagged cod was not statistically tested due to low sample size, DSTs were unlikely to have a negative impact on the post-release survival of tagged cod because the recapture rate of DSTs was generally higher (3.4%) than for T-bar tags (1.5%).

Another methodical requirement included adapting a geolocation framework to the characteristics of cod inhabiting the southern Baltic Sea tagged with temperature and depth DSTs (Objective 2, Chapter 2). Using the adapted geolocation framework, daily positions of cod could be estimated with an accuracy of less than 20 km as tested with five different validation experiments including i) simulated tracks, ii) stationary, nearshore moored tags, iii) temperature data from an offshore measuring station, iv) a temperature-depth probe attached to an otter board of a commercial vessel, and v) DSTs mounted on the CTD and otter boards of a survey vessel. Uncertainty of the geolocation framework was lower when there was a pronounced stratification of the water column and individuals regularly conducted vertical movements.

After assessing the post-release tagging mortality and the performance of the adapted geolocation framework, the adapted geolocation framework was applied to 28 temperature-depth profiles recorded by DSTs of cod which were at liberty for at least 39 days (Objective 3, Chapter 3). Unlike all previous tagging studies in the southern Baltic, recaptured cod were assigned to either the EBC or WBC stock using genetics or otolith shape analysis. Individual horizontal movements showed a high degree of variability but could be classified into three behavioural types: 1) coastal, shallow-water WBC, 2) resident EBC and 3) migratory EBC. Further, based on depth use and estimated daily positions, salinity and oxygen values from a Baltic regional ocean model were matched, to derive fluctuations of two additional key environmental parameters experienced during the time at liberty. Due to the extended use of deeper water layers, EBC experienced higher salinities than WBC and conducted short excursions into hypoxic conditions. The shallower movements of WBC highlight the importance of shallow water habitats in the southern Baltic Sea. The recorded temperature-depth profiles revealed daily vertical movements in the water column of EBC and partly of WBC which were trigged by twilight and in some cases by the lunar cycle.

The findings of Chapter 4 reveal seasonal patterns in otolith zone formation and stock-specific otolith growth of cod tagged with T-bar tags and assigned to EBC or WBC by genetics or otolith shape analysis (Objective 4). These findings show that the translucent zone of EBC is formed during summer and early autumn and is not, as previously assumed, unrelated to season. This chapter gave evidence that WBC in SD 24 and 25 also form their translucent zone in summer as already validated for cod from the Belt Sea (SD 22). Differences in growth increments of EBC and WBC otoliths were in line with the somatic growth rates of these stocks in the southern Baltic. The seasonal patterns in otolith formation, i.e. formation of the translucent zone during summer and autumn, coincided with the seasonal behaviour patterns revealed in Chapter 3, i.e. use of the Bornholm basin area below the halocline to spawn.

Altogether, evaluation of the seasonal movements and environmental conditions experienced and the seasonal zone formation of otoliths of the two cod stocks in the Baltic Sea provide new insights into the ecology and behaviour of cod inhabiting this unique brackish ecosystem.

Movement of cod in the southern Baltic Sea

On a regional scale, present knowledge on distribution of fish stocks in the Baltic Sea is mainly based on conventional tagging studies (Aro, 1989), on catches from surveys and the commercial fishery and analysis of acoustic data (Schaber et al., 2012; Andersen et al., 2017; Casini et al., 2019). The major focus of these studies was cod, likely due to its once high economic value, but studies on the distribution of prey species also exist, such as on herring and sprat (Andersen et al., 2017) or species partly using the same food sources as cod, such as flounder (Haase et al., 2020; Orio et al., 2020; but see Reßing (2020) who found no noticeable diet overlap of flounder with cod in SD 24-25).

Although cod in the Baltic Sea can be considered a relatively well-studied fish species, knowledge about individual movements are mainly based on one study where individuals were externally tagged with temperature-depth-salinity DSTs (Neuenfeldt et al., 2007). This study was, however, limited in sample size (n=10), temporal coverage (days at liberty ranged between 54 and 295 but nine of the cod were at liberty for four month or less) and spatial extent (individuals remained mostly within the Bornholm Basin, while one individual was recaptured in the Western Baltic Sea at 11.1 °E; Neuenfeldt et al., 2007). It remains unclear if the individuals from that study were WBC or EBC because stock-assignment was not conducted.

The DST-tagged cod from Chapter 3 were assigned to stock by genetics or shape analysis. Although limited in sample size (n=5), all WBC stayed in close proximity to the coast or to shallow oceanographic features at the Rönnebank and did not descent into waters below 50 m.

They stayed above the permanent halocline in normoxic waters. Contrary, EBC stayed predominately below the halocline and, thus, displayed a vertical separation from WBC. The depth profiles and estimated movements of WBC and EBC were so distinct that one individual which was assigned to the EBC by shape analysis was retrospectively classified as WBC according to its depth profile. The resident EBC stayed within the Bornholm Basin year-round and did not conduct seasonal movements to further-away feeding grounds. The behaviour of these resident EBC were similar to the behaviour described in Robichaud and Rose (2004) and Neuenfeldt et al. (2007). Contrary, migratory EBC conducted migrations between the Bornholm Basin (spawning ground) and feeding grounds close to the Island of Rügen and the Hanö Bight. These migratory individuals stayed on the spawning ground between May and November and on the feeding grounds between December and April. Thus, the DSTs revealed seasonal movements of a subsample of the tagged individuals between spawning and feeding grounds, but some individuals also stayed close to the spawning ground year-round. Cod in the southern Baltic Sea showed a diversity of different life strategies likely enabling the co-existence of the two stocks (Schade et al., under review). A review of several T-bar tagging studies (Robichaud and Rose (2004) described the movement patterns of cod in the Arkona and Bornholm basin as “sedentary” to the respective basin and within 40 – 200 km. The results from Chapter 3 give evidence that movement patterns observed from T-bar tagging alone likely underestimate the complexity in movements, especially when cod were recaptured approximately one year after release (Righton et al., 2007) when they are likely to use a similar area as the year before.

Although T-bar tags are not useful to reconstruct individual tracks, the larger amount of release and recapture positions (Fig. 1) can supplement the knowledge about movements gained from the DSTs. The distribution of released and recaptured cod tagged with T-bars clearly showed that WBC predominantly use the shallow, coastal areas (but two WBC were recaptured at the slopes of the Bornholm Basin at 50 and 56 m water depth). These recapture overview (Fig. 1) further shows that the DST recaptures did not represent the whole distribution area of the WBC and EBC stock. WBC were recaptured as far East as the Gdansk Bay (SD 26) and EBC as far West as the island of Fehmarn and in the Øresund (Fig. 1).

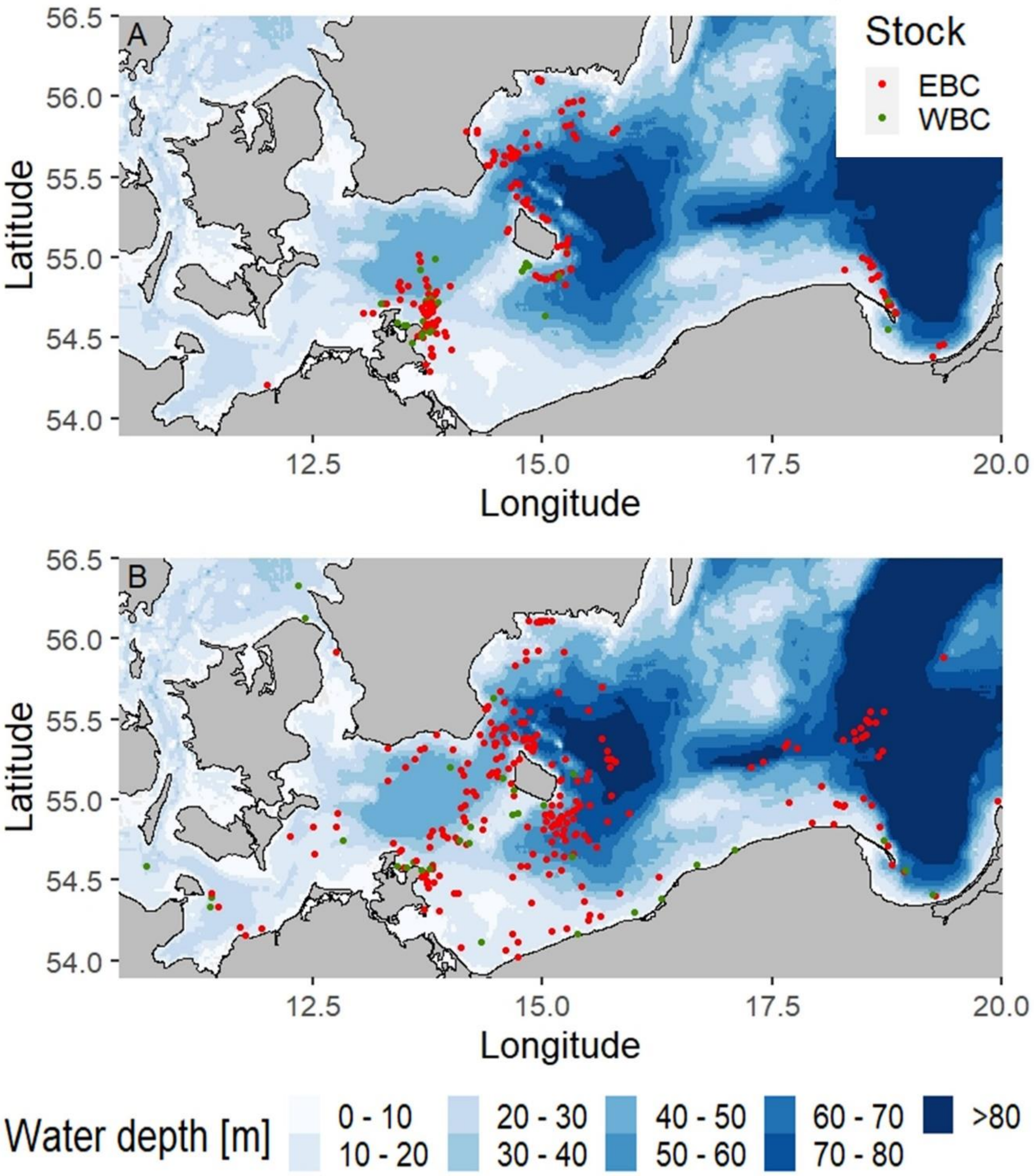

Figure 1: Release (A) and recapture (B) positions of all recaptured cod tagged with T-bar tags from the TABACOD project which were assigned to the Eastern Baltic (EBC, red) or Western Baltic (WBC, green) cod stock assigned by genetics or shape analysis. No cod released in the Gdansk Bay were tagged with data storage tags.

The DST profiles not only revealed horizontal movements, but also interesting insights into vertical movements of individual cod. During dawn, mostly EBC ascended in the water column, and descended again to the sea floor at dusk. This behaviour likely occurs when cod follow the daily vertical movements of clupeids (Schaber et al., 2012; Andersen et al., 2017) which dominate the stomach contents of cod sampled from greater water depths (Hornetz, 2020). These vertical movements, which were at least periodically observed in all EBC, lead to large variations in ambient water temperature in short time-intervals because the fish crossed the thermocline (Chapter 3, Righton et al. (2010)). These vertical movements are likely to foster a vertical transport of nutrients from the upper layer into below the halocline because cod feed on clupeids in the upper water column, which in turn consume the largest share of their daily ratio also in the upper layers and during vertical movements (Kulke et al., 2018). The decomposition of faeces is then likely realized in deeper water strata (compare e.g. Hansen and Visser, 2016; Pinti and Visser, 2018). The isolation of water masses in the basins hinders the return of nutrients into the upper layer so that nutrients, regularly transferred by piscivorous foraging excursions of cod, are deposited in the basins which can further amplify anoxic conditions in these stagnating areas. Hence, EBC, which can no longer rely on benthic food items in the deeper basins because hypoxia and anoxia resulted in large-scale loss of epibenthos (Casini et al., 2016), would further deteriorate the oxygen conditions of the stagnant water bodies by regular excretion of faeces at daytime derived from clupeids consumed in the upper, oxygenated layer at night. Since all EBC conducted, at least periodically, vertical movements a significant amount of faeces is transported into the deeper areas and further research should be conducted to quantify this process.

Further, the depth profiles of some EBC individuals revealed vertical movements which were synchronised with the lunar cycle. During full moon, some cod conducted less pronounced vertical movements than during new moon (Chapter 3). In the non-tidal Baltic Sea, during full moon, light can penetrate deeper into the water column and enables cod in coastal or slope areas to visually forage on benthos, while they might rather feed on clupeids during nights with less illumination. Alternatively, benthos abundance or availability may change with the lunar cycle during certain periods of the years and cod are likely to respond to such fluctuations in the prey field. Another possible hypothesis is that the vertical movements of clupeids are also influenced by moon light. Lunar illumination is a well-known driver in the behaviourof small pelagics and plankton, especially to synchronise spawning activity (Takemura et al., 2004; Hernández-León, 2008; Last et al., 2016) and has also been shown to influence the spawning behaviour of Atlantic cod in Icelandic waters (Grabowski et al., 2015). This is the first time that behaviour related to the lunar cycle was recognized for some cod in the Baltic Sea and understanding the mechanisms behind it need further research.

At the bottom of the southern Baltic Sea, the average annual temperature is reported to be 4-6 °C but significant variation occurs in the shallow-water areas (Snoeijs-Leijonmalm and Andrén, 2017, Fig. S12 in the supplementary material of Chapter 2). The ambient temperatures measured with the DSTs suggest that WBC, occupying shallower waters throughout the year, experience larger seasonal variations than EBC. These clear and strong seasonal variations in water temperature were recently described for cod in the relatively shallow Belt Sea where cod do not have access to deeper waters with colder temperatures during summer (Funk, 2020). Interestingly, Chapter 3 indicated that WBC in the southern Baltic Sea where deeper, colder waters can be accessed, also stay shallower and mostly above the thermocline and withstand the variation in ambient temperature.

The average ambient temperatures of WBC and EBC reported in Chapter 3, are at the medium level of the average temperatures experienced by other Atlantic cod stocks and depicted similar temperatures ranges as the stocks at the Faroe island and in the Barents Sea as measured with DSTs (Righton et al., 2010). Although temperature was highlighted to be an important predictor of the growth rates of Atlantic cod (Brander, 1995), growth rates of WBC, and especially EBC, are lower than expected under the ambient temperatures experienced (McQueen, 2019). Other factors leading to the reduced growth rate of EBC which was especially pronounced in recent years (Eero et al., 2015; Hüssy et al., 2018, Mion et al., 2021) is likely a combination of several factors including decreased condition (Eero et al., 2012; Casini et al., 2016) driven by use of hypoxic areas (Limburg and Casini, 2018; Brander, 2020), hypoxia-related changes in food availability and composition (Eero et al., 2012; Rojbek et al., 2014, P. Hornetz, pers. communication), which are linked to thiamine deficiency (Engelhardt et al., 2020), and increased infestation rates of cod livers with the juvenile stages of the nematode *Contracaecum osculatum* (Behrens et al., 2014; Sokolova et al., 2018).

The increase in permanent hypoxic areas since the mid-1980s led to a shrinkage of suitable spawning sites for EBC and to a contraction of the stock to the southern Baltic Sea (Eero et al., 2012; Casini et al., 2016; Köster et al., 2017). Also in the western Baltic Sea, hypoxic zones occur in shallow, coastal waters periodically in late summer (LLUR, 2020). Hypoxic conditions in the southern Baltic adversely influence cod because the availability of benthic food, especially food for smaller cod, is reduced (Neuenfeldt et al., 2020) and hypoxic conditions reduce the rate of digestions and thus food consumption (Chabot and Dutil, 1999;

Brander, 2020). Although oxygen depletion was visible in the estimated oxygen profiles of WBC over summer, none of the individuals visited hypoxic zones with values <2 ml/l. Contrary, both resident and migratory EBC experienced hypoxic conditions when visiting the deeper Bornholm Basin but dives into hypoxic areas were rather short and individuals did not endure chronical hypoxic conditions (Fig. 2) which are known to decrease growth (Chabot and Dutil, 1999). These shorter dives into hypoxic conditions therefore likely do not play a major role in reduced digestion rates and are thus not directly related to the reduced condition of EBC. Hypoxic or even anoxic conditions might, however, have a much larger and adverse effect on the less mobile, benthic prey species of cod (Modig and Ólafsson, 1998; Carstensen et al., 2014) and on successful spawning of cod (Köster et al., 2017).

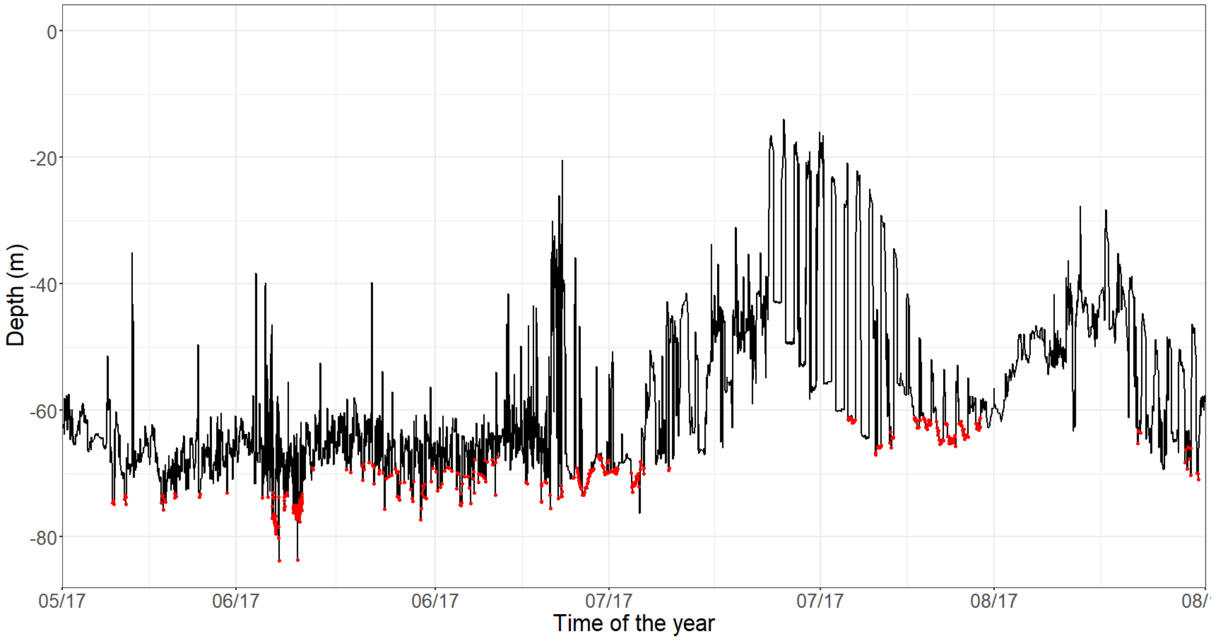

Figure 2: Section of a depth profile of an Eastern Baltic cod classified as migratory between coastal feeding grounds and the Bornholm Basin for spawning (ID U9125, date format: mm/yy). Red dots mark periods which the individual spent in hypoxic conditions (O_2 value < 2ml/l) as extracted from the Baltic regional model based on water depth and estimated position. The male cod was released on 07.04.2017 and recaptured on 19.04.2018.

Otolith formation of cod in the southern Baltic Sea

Temperature and feeding condition, which in turn is influenced by several other factors such as food availability and quality as well as oxygen content of the surrounding water, are known to affect the opacity of fish otoliths (Fablet et al., 2011). Seasonal changes in otolith opacity are commonly used for age determination but validation of these patterns is lacking in many fish stocks (Beamish and McFarlane, 1983; Campana, 2001). Although the changes between translucent and opaque zones in WBC otoliths are often described as very clear, the formation of the translucent zone during summer was in SD 22 only recently validated for both juvenile (McQueen et al., 2019; Plonus et al., 2021) and adult WBC up to the age of three (Krumme et al., 2020). WBC became the first Baltic demersal fish stock where zone formation was validated. Chapter 4 extended this validation to SD 24 and 25 and showed that the translucent zones of WBC otoliths are also formed in this region during summer. Despite the successful validation, comparisons between age readers suggest that age determination can still improve in precision (Haase and Krumme, 2020a; Haase and Krumme, 2020b; Davies, 2021) but the validation studies formed a common baseline for improving the data input for the WBC stock assessment and a recent otolith exchange could correct some mis-aged otoliths sampled during the Baltic Internation Trawl Survey (BITS) between 2017 and 2019 in the ICES database DATRAS (Davies, 2021). These studies demonstrate that age validation significantly improves the data input to the stock assessment and further improves our understanding of the stock’s ecology (McQueen, 2019).

The age reading of EBC otoliths was always considered more challenging and classified as unreliable and unrelated to seasons (Hüssy, 2010; Hüssy et al., 2016). Due to the lack of practical alternatives and because they were not considered to substantially bias model results (Reeves, 2003; Hüssy et al., 2016; McQueen, 2019), age data were continuously used until 2014 when the age-based analytical stock assessment model did not convert anymore (Eero et al., 2015). Subsequently, a series of benchmarks was conducted and the influence of age data in the stock assessment reduced (ICES, 2015; ICES, 2019). Despite the uncertainties related to EBC ageing, age data are still being used as input data (in the form of age-length keys) without validation (ICES, 2020).

Validated, time-stamped otolith material never existed in suitable amounts for EBC (but see Hüssy et al., 2009) and thus Chapter 4 analyses a very valuable dataset of chemically marked otoliths for this stock. To leave a distinct fluorescent mark on the otolith of Baltic cod at the day of tagging, the intraperitoneal injection of tetracycline-hydrochloride was successfully tested (Stötera et al., 2018). After Krumme et al. (2020) successfully validated the zone formation of adult WBC in the Western Baltic Sea by a mark-recapture experiment with chemically marked individuals, the approved method and the gained knowledge was applied to cod in the southern Baltic Sea during the TABACOD project (Chapter 4). Although the return rate of 1.5% was relatively low compared to historic Baltic cod tagging projects in the 1960-1970s in which return rates ranged between 7 and 42% (e.g. Berner, 1962; Thurow, 1963; Berner, 1968; Berner, 1974; Berner, 1981), the otoliths of 258 recaptured cod provided a sufficient sample size to show that also EBC otoliths followed a seasonal pattern in zone formation. Chapter 4 revealed that the translucent zone in adult EBC otoliths is formed during summer and autumn. This, however, is not enough to return to a reliable age determination of Eastern Baltic cod because the age at first increment formation is still uncertain. Therefore, the next necessary step involves understanding the formation of the core area by analysing the zone formation of juvenile Eastern Baltic cod.

The slower growth rate of EBC compared to WBC (McQueen et al., 2020) was also visible in a reduced growth of the otolith (Chapter 4) and gives further evidence for the lower productivity of the EBC stock. Svedäng et al. (2020) observed an increased nitrogen content with increasing fish length deposited in the otolith of EBC in the period 2010-2015 compared to the period 1995-2005, which they interpreted as an indicator for an increased feeding rate. They concluded, against the common understanding that the reduced productivity of EBC is strongly interlinked with food limitation (Eero et al., 2012; Casini et al., 2016; Orio et al., 2019; Neuenfeldt et al., 2020), that the feeding rate in larger cod has increased (Svedäng et al., 2020). In contrast to this suggestion, the reduced daily otolith growth rate of adult EBC otoliths from Chapter 4 strongly suggests that daily increments are compressed, i.e. a given distance on a contemporary otolith of an EBC includes more daily increments than previously, so that the concentration of nitrogen content in a given spot size of a mass spectrometer would also increase. Hence, changes in feeding rate are not required to explain an increased nitrogen content in larger cod. Or in other words, increased nitrogen content from a given spot size of the mass spectrometer would indicate that cod grow slower because more days with nitrogen accretion are included in the measurement.

Linking environmental conditions to otolith formation

The zone formation and growth of otoliths combined with the seasonal movements of cod in the southern Baltic Sea provided new insights into the ecology of the two cod stocks in that area. Data from the TABACOD project allow linking data from the movement of individual, stock-assigned Baltic cod and the environmental conditions experienced during their time at liberty to the zone formation visible on the otolith between the tetracycline mark and otolith edge and to better understand the ecological context influencing patterns in ring formation in EBC. Previously, influencing factors such as temperature or food intake were mostly studied in controlled lab experiments (Høie et al., 2008; Neat et al., 2008) or modelled (Fablet et al., 2011) to understand the influence on otolith mineralization and opacity. A previous attempt in linking DST data which recorded temperature, depth and salinity to the opacity level of cod in the Bornholm Basin revealed a high variability and could not identify a clear pattern (Hüssy et al., 2009).

The bioenergetic modelling of cod otolith biomineralization presented by Fablet et al. (2011) predicts the formation of translucent otolith material during periods of warmer temperatures and under lower feeding conditions. Inversely, opaque material is deposited when temperatures are lower and feeding condition are better (Fablet et al., 2011). WBC in the western Baltic Sea avoid the warmed-up shallow and productive habitats during summer and descent into slightly colder waters (McQueen, 2019; McQueen et al., 2019; Funk et al., 2020b; Funk et al., 2020a; Funk, 2020; Krumme et al., 2020). However, given the shallowness of the Belt Sea and hypoxic conditions in deeper areas during summer (Snoeijs-Leijonmalm and Andrén, 2017; similar to patterns observed in a South-Norwegian fjord (Freitas et al., 2015; Freitas et al., 2016)), WBC in the Belt Sea cannot avoid the temperature stress. WBC otoliths form a translucent zone during this period. The summer period coincides with temporarily reduced feeding (Funk et al., 2020a) and, as a result, the somatic growth rate was slightly reduced (McQueen et al., 2018). Overall, the summer period is described as a stressful period for WBC in the western Baltic Sea (McQueen, 2019; Krumme et al., 2020). In late summer, water temperatures decrease, and WBC intensively use the shallower feeding habitats (Funk et al., 2020b; Funk, 2020). The somatic growth rate displays an annual peak (McQueen et al., 2018; Krumme et al., 2020). From late autumn to spring the opaque zone is formed. Recent results from an individual-based bio-energetic model of WBC suggest that the formation of the translucent zone during summer is primarily driven by water temperature and that reduced growth and reduced feeding are only associated phenoma (Funk, 2020). Overall, the pronounced seasonal fluctuation in water temperatures, exceeding optimum conditions during summer, seems to be the major driver of the distinct changes between opaque and translucent zones in WBC of the Belt Sea.

The WBC in the southern Baltic Sea, i.e. in the Arkona and Bornholm Basin, could potentially descent to deeper, colder waters during summer, but the DST profiles from Chapter 3 clearly showed that all individuals did not use this option but stayed in habitats shallower than 50 m and thus, during summer experienced both warming waters and a depletion of oxygen, however within normoxic conditions (Fig. 3, Chapter 3). Consequently, driven by the same conditions as in SD 22, also in SD 24 and 25 the translucent zone of WBC is formed during summer and zones are clearly distinct from each other (Fig. 3C, Chapter 4). Hence, the results of the validation of zone formation of WBC otoliths from SD 22 also apply to WBC otoliths from the SD 24 and 25 ranging from age 2 to age 4.

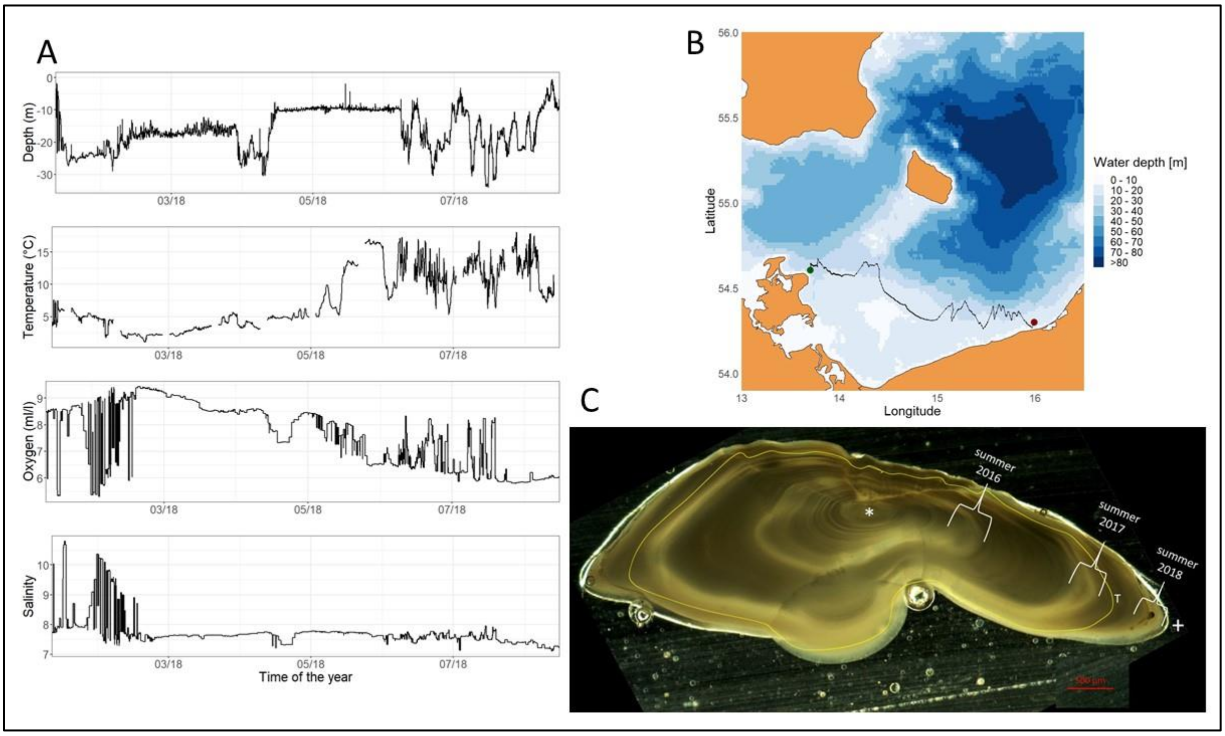

Figure 3: Information collected for the recaptured 2-year-old female Western Baltic cod ID U9187 released in February 2018 (release length: 316 mm) and recaptured in September 2018 (recapture length: 349 mm) with the maturity state "resting". (A) Depth and temperature profiles recorded by the DST and oxygen and salinity profiles matched based on estimated daily positions and water depth. (B) Reconstructed daily positions between release (green dot) and recapture (red dot). (C) Cross-section of the sagittal otolith of this individual taken under a transmitted light microscope. Translucent summer rings, marked with brackets, appear lighter than opaque zones. Hatching is marked with "*", the timing of tagging as revealed by the redrawn yellow band which can be seen under the fluorescent filter due to the tetracycline-hydrochloride tagging is marked with "T", and the timing of recapture is marked with "+".

For EBC, temperature and behaviour, i.e. whether or not an individual is participating in spawning, were assumed to be important drivers in otolith opacity (Hüssy et al., 2009). An interaction of several drivers such as reduced food availability during spawning time but also colder ambient water temperatures in the Bornholm Basin were assumed to result in less distinct changes between translucent and opaque zones in EBC (Hüssy et al., 2009) and were linked to a lack of seasonal variations in somatic growth rate (Mion et al., 2020a). Contrary to WBC, different seasons cannot be as clearly divided into periods of favourable and unfavourable conditions for EBC (McQueen, 2019, Chapter 3, 4, Fig. 4). But still, Chapter 4 demonstrated that the translucent zone in EBC with a fish length between 25 cm and 54 cm was predominantly formed during summer and autumn, when EBC accumulate on the spawning ground below the thermocline and experience higher salinity conditions and likely reduced feeding and hypoxic conditions (Chapter 3, Fig. 4, Hornetz (2020)). Spawning, food limitation and hypoxic conditions induce an increase in stress. Due to a strong stratification of the water column, a change in water temperature less likely plays an important role in the formation of the translucent zone in EBC (Chapter 3, 4). When EBC ascent into shallower waters in late autumn, feeding intensity increases again and peaks in late spring (Bagge, 1981; Baranova et al., 2011). During this period, the ambient water temperatures are at the minimum and might be too low for optimal food conversion and growth rate (Björnsson et al., 2001). Contrary to Hüssy et al. (2009), the DST profiles of the cod tagged within the TABACOD project between 2016 and 2019 did not indicate that almost half of the tagged individuals skipped spawning. Instead, only one of the 28 individuals might have skipped spawning (Chapter 3). This indicates an interannual variability in spawning participation and might be one explanation why seasonal patterns in opacity were more distinct in Chapter 4 than in Hüssy et al. (2009). Generally, changes between translucent and opaque zones might have become more distinct because the stock distribution of EBC contracted into the Arkona and Bornholm Basin in the southern Baltic Sea and thus experienced conditions have become more similar compared to former times when cod distribution included the central and northern Baltic Sea (Aro, 1989). Indeed, it has been reported that age readers from different regions of the Baltic used different interpretations of zone patterns (ICES, 1994; ICES, 2000; McQueen, 2019).

The same dataset of chemically marked otoliths tagged during the TABACOD project was also used to validate the periodicity of trace elements as revealed by microchemistry analysis (Hüssy et al., 2020b). Phosphorus concentration showed a high seasonal consistency over the years and was lowest in spring (Hüssy et al., 2020b). This indicates that the minima in phosphorus concentration might coincide with the formation of the opaque zone. Although microchemistry is rather costly and time-consuming and can thus not be a replacement of traditional age-reading at present, it might be a valuable supplement to aid visual age reading (Heimbrand et al., 2020).

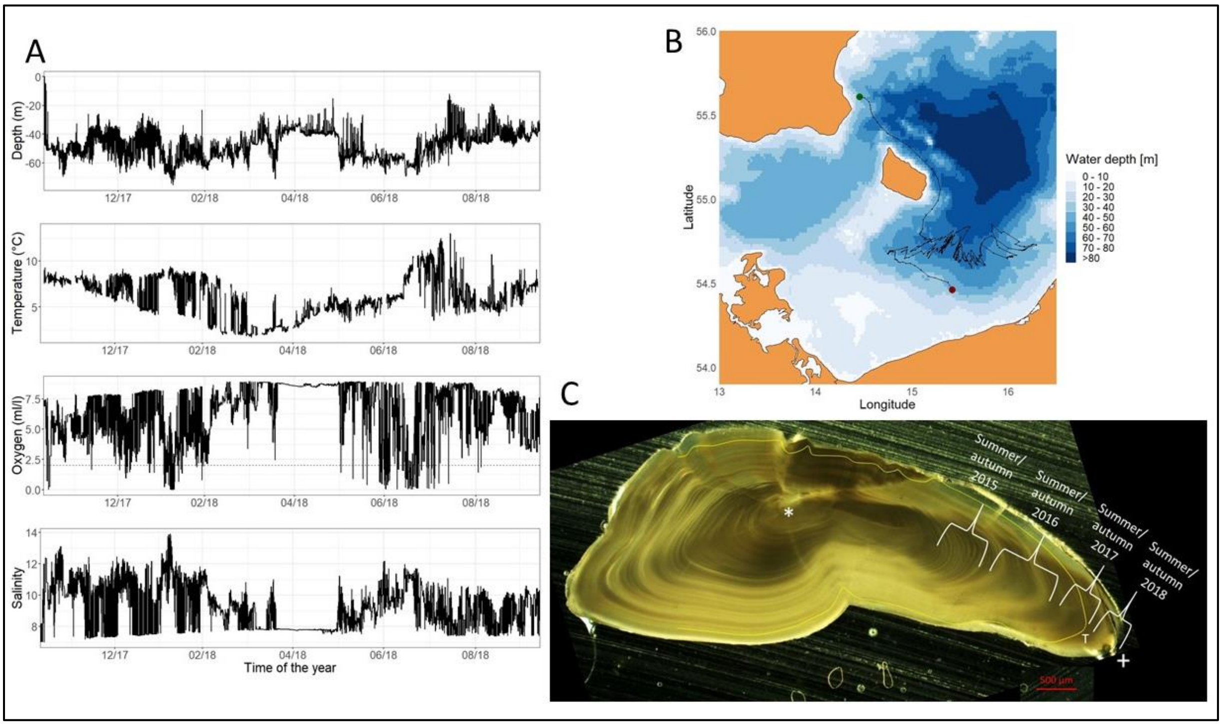

Figure 4: Information collected for the recaptured female Eastern Baltic cod ID U9232 released in November 2017 (release length: 400 mm) and recaptured in October 2018 (recapture length: 460 mm) with the maturity state "resting". (A) Depth and temperature profiles recorded by the DST and oxygen and salinity profiles matched based on estimated daily positions and water depth. (B) Reconstructed daily positions between release (green dot) and recapture (red dot). (C) Cross-section of the sagittal otolith of this individual taken under a transmitted light microscope. Translucent summer rings, marked with brackets, appear lighter than opaque zones. Hatching is marked with "*", the timing of tagging as revealed by the redrawn yellow band which can be seen under the fluorescent filter due to the tetracycline-hydrochloride tagging is marked with "T", and the timing of recapture is marked with "+".

Application of methods to other stocks or areas

Chapter 1 (post-release mortality) and chapter 2 (validation of the geolocation framework) are methodological chapters and form the basis for the result sections of this thesis (Chapter 3, DST profiles, and Chapter 4, otolith zone formation and otolith growth). While the results from these chapter are specific to a species (cod) and a region (southern Baltic Sea), the methods can be applied elsewhere if the species show similar behaviour as Baltic cod.

Baltic cod is decreasing in importance for the fisheries and the economic and ecological role of other species like flatfishes may come more and more into focus. Hence, a better understanding of the behaviour of these species may be required, given the current changes occurring in the Baltic Sea environment and fisheries. However, DSTs have not been used to study flatfish or other round fish species like whiting in the Baltic Sea.

The geolocation framework HMMoce was originally developed for large pelagics in the Atlantic Ocean (Braun et al., 2018). To apply it on Baltic cod tagged with temperature-depth DSTs, several adaptions were necessary. Cod are demersal species but in the southern Baltic they conduct daily vertical movements in the water column. The relatively heterogenous bathymetry and strong stratification of the Baltic Sea led to good performance of the two adapted likelihoods compared to other geolocation frameworks using DST profiles (Chapter 2). This geolocation framework could thus be directly applied to other demersal species regularly crossing stratified water masses which live in an environment with a heterogeneous bathymetry.

The adapted HMMoce could potentially be used for flatfish species inhabiting the Baltic Sea, or any other area with heterogeneous bathymetry, when more emphasis is put on the maximum depth likelihood. Flatfish are often closely related to the seafloor and therefore do not cross stratified water masses regularly, but for instance the flounder *Paralichthy dentatus* tagged with DSTs displayed vertical movements (Henderson et al., 2014) and regular vertical movements are also known from the European plaice (*Pleuronectes platessa*) and European flounder (*Platichthys flesus*) (Woodhead, 1960; Verheijen and De Groot, 1967; Jones et al., 1979). The temperature-depth likelihood therefore not necessarily leads to more constrains of positions. In the current maximum depth likelihood, fish are expected to stay within ± 5 m to the sea floor. For flatfish this could be even more limited, but the accuracy of the depth sensor of the tagged DST should be considered to not accidentally exclude possible positions. The uncertainty involved with geolocation flatfish will be likely larger than of cod (Chapter 2), since the bathymetry within the basins is rather similar and geolocation might be limited to the assignment to the separate basins. Still, already a general direction of movements and depth use patterns could bring new insights into flatfish behaviour. However, due to the relatively small body cavity of flatfish, external attachment of tags might be necessary. It is important to test how this external attachment, often associated with wound infection and altered swimming performance due to drag (Lewis and Muntz, 1984; Jadot, 2003; Cooke et al., 2012; Jepsen et al., 2015), can record representative behaviour of the species before experiments in the wild are started. Recently, tetracycline was confirmed to leave a fluorescent mark on the otoliths of the three flatfish species European plaice, European flounder and turbot (*Scophthalmus maximus*) (Meyer et al., under review) and thus DST tagging could be supplemented with time-stamped marks on the otoliths to link otolith patterns to environmental conditions in these species.

Contrary, for pelagic species like clupeids, under the assumption that there will be a suitably small tag which could be used, focus should rather be put on the temperature-depth likelihood since clupeids like herring and sprat stay off the ground and perform regular vertical movements across the thermocline (Nilsson et al., 2003). Again, the uncertainty of the geolocation model might be higher for clupeids because the maximum depth likelihood is probably not effective for pelagic clupeids. But since small pelagics like the Western Baltic spring spawning herring cross several, very different water bodies during the seasonal migration linking spawning grounds in brackish-water lagoons in the south and feeding grounds in the Kattegat-Skagerrak in the north of their distributional area, important findings e.g. about timing of movements and ambient water temperatures could be derived.

These theoretical considerations of necessary adaptions of the geolocation framework for fish groups of different behaviour should be validated, for example with the proposed validation methods in Chapter 2. The advantage for the validation experiments conducted in Chapter 2 was that previous DST studies on cod in the Baltic (Neuenfeldt et al., 2007; Hüssy et al., 2009; Righton et al., 2010; Nielsen et al., 2013) already gave a good indication on the behaviour, particularly how individuals make use of the water column within a day. If such information does not exist in the same environment, i.e. archival tagging has not been conducted on the other previously mentioned species in the Baltic yet, DST studies from comparable environments should be considered before applying validation studies to mimic the behaviour of the species of interest. Simulating an artificial track coupled with the environmental parameters which are going to be recorded by the DST and subsequently reconstructing daily positions can be conducted with the lowest effort, given that sufficient knowledge of the behaviour exists (Liu et al., 2017). But especially the application of both simulated and observed data is useful to adapt and subsequentely test the applicability of a geolocation framework. In summary, the adapted HMMoce geolocation framework can likely be also adapted to reconstruct the daily positions of other species and in other areas but further adaption of the likelihoods will be necessary.

In Chapter 1 the post-release short-term mortality associated with the process of catching fish for tagging and handling on board was evaluated. Chapter 1 revealed that tagging did not have a significant effect on the short-term mortality but the catch and handling itself caused some mortality. Contrary to Kock (1975) who observed higher mortality rates of Baltic cod during summer, capture month did not significantly influence the mortality rate in our experiment. Further, mortality increased with decreasing fish size indicating that smaller individuals were more vulnerable to the handling process than larger individuals. Specifically, for the TABACOD project, this led to relatively low return rate of individuals below 30 cm although tagging started at 15 cm (Fig. 5). Although it causes additional effort to conduct these short-term post-release mortality experiments in a tagging project, it is an important step to ensure the quality of the tagging technique, and if deemed necessary, adapted the tagging procedure. Every tagging project benefits from as many recaptures as possible - which ideally behaved naturally during their time at liberty (Smith et al., 2017).

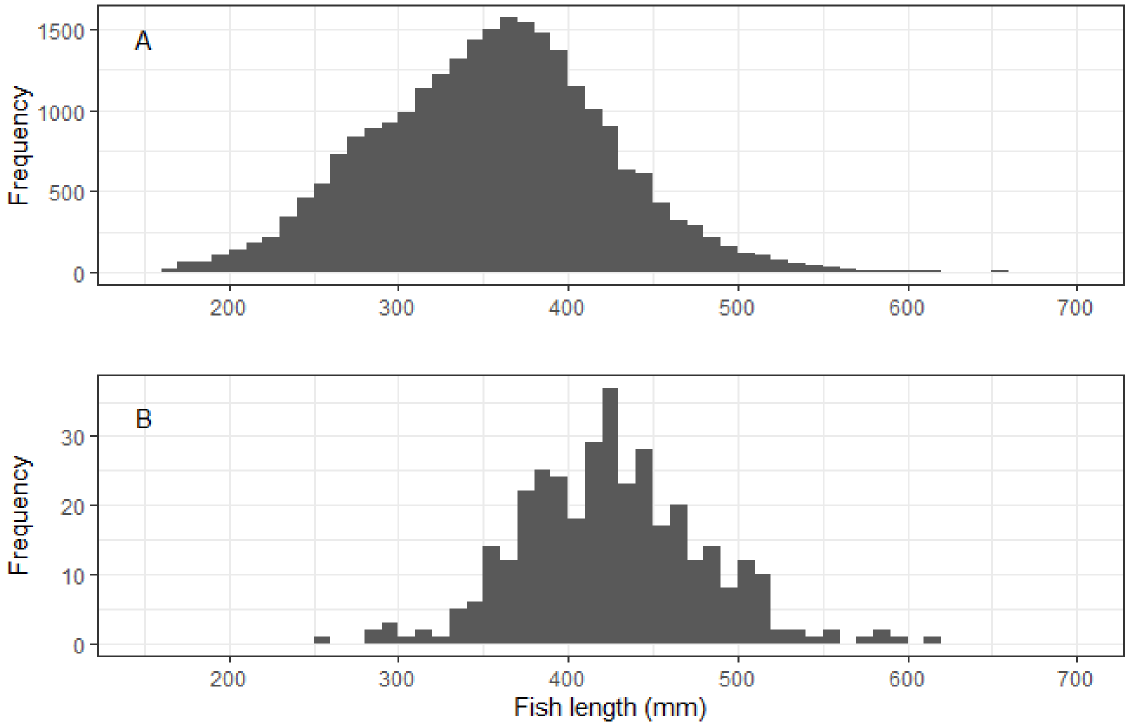

Figure 5: Length distribution of released (A) and recaptured (B) cod during the TABACOD project.

Conclusion and research perspective

In conclusion, this thesis highlights the distinct stock-specific movement patterns and behaviour of cod inhabiting the southern Baltic Sea. The movements of WBC and migratory EBC in the southern Baltic Sea demonstrated the importance of shallow-water habitats in this region, especially in in spring and autumn. The seasonal patterns in environmental conditions experienced throughout the year are laid down in very distinct zone patterns in WBC and also in distinguishable rings in EBC otoliths. Summer is a stressful period for both WBC and EBC and result in the formation of a translucent zone on the otoliths, but reasons for the formation of a translucent zone are obviously different between stocks: While WBC experience warm ambient water temperatures and likely limitations in food supply during the summer aestivation period, EBC suffer from periodic hypoxic conditions and food limitation during the spawning period. Temperature less likely plays a significant role in EBC because seasonal differences are rather low and the extent of regular short-term temperature changes as the result of daily vertical movements is much larger.

Tagging always only covers a subsample of the whole stock. Although it is commonly assumed that this subsample represents the stock as a whole this assumption might be biased by tagging and recapture positions. The conclusions drawn from both the T-bar and DST recaptures might only apply for cod in SD 24 and 25 for individuals above 25 cm and the transferability to other areas and length classes needs to be tested. The knowledge about the dynamics of juvenile cod is rather limited and spatial distribution including feeding grounds remain largely unknown. Size and robustness of the juvenile cod make them unsuitable for tagging. This lack of small individuals leaves the formation of the core area of EBC otoliths largely unclear. Age data should not be included in the current stock assessment before the formation of the first translucent zone has been validated.

The movement strategies of WBC and migratory EBC highlight the importance of shallow water habitats, especially during autumn and winter. This includes the shallow Rönnebank which was discovered to be an important habitat for WBC. These shallow habitats might be underrepresented in the demersal survey because water habitats < 20 m are not covered in the BITS and might lead to a bias in survey indices.

Further, this is the first time that DST data of WBC are presented. They were, however, released and recaptured outside the core area of distribution of WBC which is in SD 22 and 23. Based on the experiences from the TABACOD project, the Thünen Institute is now tagging WBC in the Belt Sea with DSTs recording temperature and depth. Due to the low return rate of tagged cod in recent tagging projects, DSTs coated with a positively buoyant floats are used. After the cod died due to natural causes or when the DST is overlooked during evisceration on-board commercial vessels and thrown overboard, the tag can float up to the water surface and ultimately to a shoreline where it can be found and reported.

References

Andersen N, Lundgren B, Neuenfeldt S, Beyer J (2017) Diel vertical interactions between Atlantic cod Gadus morhua and sprat Sprattus sprattus in a stratified water column. Mar Ecol Prog Ser 583: 195–209

Aro E (1989) Review of fish migration patterns in the Baltic Sea. Rapp P-V Reun Cons Int Explor Mer 190: 72–96

Bagge O (1981) The yearly consumption of cod in the Baltic and the Kattegat as estimated from stomach content. ICES CM 1981/J:27.

Baranova T, Müller-Karulis B, Šics I, Plikshs M (2011) Changes in the annual life cycle of eastern Baltic cod during 1950-2010. ICES CM 2011R10

Beamish RJ, McFarlane GA (1983) The Forgotten Requirement for Age Validation in Fisheries Biology. Trans Am Fish Soc 112: 735–743

Behrens JW, Seth H, Axelsson M, Buchmann K (2014) The parasitic copepod Lernaeocera branchialis negatively affects cardiorespiratory function in Gadus morhua: response to parasite infection by gadus morhua. J Fish Biol 84: 1599–1606

Berner M (1962) Die Ostseedorscherträge 1960/61 und die Fangaussichten 1962. Dtsch Fisch Zeitg 9: 126–131

Berner M (1968) Einige orientierende Untersuchungen an den Otolithen des Dorsches (Gadus morhua L.) aus verschiedenen Regionen der Ostsee. Fischerei Forschungs Wissenschaftliche Schriftenreihe: 119–129

Berner M (1974) Some results of cod tagging experiments of the GDR in the Baltic 1968-1971. 8

Berner M (1981) Dislocation parameters of tagging experiments on cod in the Baltic (subdivision 22-25) from 1959 to 1975. ICES CM 1981 J15 26 pp.

Björnsson B, Steinarsson A, Oddgeirsson M (2001) Optimal temperature for growth and feed conversion of immature cod (Gadus morhua L.). ICES J Mar Sci 58: 29–38

Bonsdorff E (2006) Zoobenthic diversity-gradients in the Baltic Sea: Continuous post-glacial succession in a stressed ecosystem. J Exp Mar Biol Ecol 330: 383–391

Brander K (2020) Reduced growth in Baltic Sea cod may be due to mild hypoxia. ICES J Mar Sci 77: 2003–2005

Brander KM (1995) The effect of temperature on growth of Atlantic cod (Gadus morhua L.). ICES J Mar Sci 52: 1–10

Braun CD, Galuardi B, Thorrold SR (2018) HMMoce: An R package for improved geolocation of archival-tagged fishes using a hidden Markov method. Methods Ecol Evol 9: 1212–1220

Campana SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J Fish Biol 59: 197–242

Carstensen J, Conley DJ, Bonsdorff E, Gustafsson BG, Hietanen S, Janas U, Jilbert T, Maximov A, Norkko A, Norkko J, et al (2014) Hypoxia in the Baltic Sea: Biogeochemical Cycles, Benthic Fauna, and Management. AMBIO 43: 26–36

Casini M, Käll F, Hansson MV, Plikshs M, Baranova T, Karlsson OM, Lundström K, Neuenfeldt S, ardmark AG, Hjelm J (2016) Hypoxic areas, density-dependence and food limitation drive the body condition of a heavily exploited marine fish predator. R Soc Open Sci. doi: 10.6084/m9.figshare.c.3518970.v1

Chabot D, Dutil J-D (1999) Reduced growth of Atlantic cod in non-lethal hypoxic conditions. J Fish Biol 55: 472–491

Cooke SJ, Hinch S, Lucas MC, Lutcavage M (2012) Biotelemetry and biologging. Fish. Tech. 3rd Ed Zale Parrish T Sutton Eds. American Fisheries Society, Bethesda, Maryland, pp 819–881

Davies JC (2021) 2020 Western Baltic Cod age reading exchange report (SmartDots ID 292 & 294).

Eero M, Hjelm J, Behrens J, Buchmann K, Cardinale M, Casini M, Gasyukov P, Holmgren N, Horbowy J, Hüssy K, et al (2015) Eastern Baltic cod in distress: biological changes and challenges for stock assessment. ICES J Mar Sci 72: 2180–2186

Eero M, Vinther M, Haslob H, Huwer B, Casini M, Storr-Paulsen M, Köster FW (2012) Spatial management of marine resources can enhance the recovery of predators and avoid local depletion of forage fish. Conserv Lett 5: 486–492

Engelhardt J, Frisell O, Gustavsson H, Hansson T, Sjöberg R, Collier TK, Balk L (2020) Severe thiamine deficiency in eastern Baltic cod (Gadus morhua). PLOS ONE 15: e0227201

Fablet R, Pecquerie L, de Pontual H, Høie H, Millner R, Mosegaard H, Kooijman SALM (2011) Shedding Light on Fish Otolith Biomineralization Using a Bioenergetic Approach. PLoS ONE 6: e27055

Funk S (2020) Spatio-temporal distribution, food intake and growth of cod (Gadus morhua L.) in the Western Baltic Sea. Räumlich-zeitliche Verteilung, Nahrungsaufnahme und Wachstum des Dorsches (Gadus morhua L.) in der Westlichen Ostsee

Funk S, Frelat R, Möllmann C, Temming A, Krumme U (2020a) The forgotten feeding ground: patterns in seasonal and depth-specific food intake of adult cod Gadus morhua in the western Baltic Sea. J Fish Biol. doi: https://doi.org/10.1111/jfb.14615

Funk S, Krumme U, Temming A, Möllmann C (2020b) Gillnet fishers' knowledge reveals seasonality in depth and habitat use of cod (Gadus morhua) in the Western Baltic Sea. ICES J Mar Sci fsaa071

Grabowski TB, McAdam BJ, Thorsteinsson V, Marteinsdóttir G (2015) Evidence from data storage tags for the presence of lunar and semi-lunar behavioral cycles in spawning Atlantic cod. Environ Biol Fishes 98: 1767–1776

Haase K, Orio A, Pawlak J, Pachur M, Casini M (2020) Diet of dominant demersal fish species in the Baltic Sea: Is flounder stealing benthic food from cod? Mar Ecol Prog Ser 645: 159–170

Haase S, Krumme U (2020a) Report of the autumn 2019 Western Baltic cod (Gadus morhua) age reading exchange – SD 22.

Haase S, Krumme U (2020b) Report of the spring 2019 Western Baltic cod (Gadus morhua) age reading exchange – SD 22.

Hansen AN, Visser AW (2016) Carbon export by vertically migrating zooplankton: an optimal behavior model. Limnol Oceanogr 61: 701–710

Heimbrand Y, Limburg KE, Hüssy K, Casini M, Sjöberg R, Bratt A-MP, Levinsky S-E, Karpushevskaia A, Radtke K, Öhlund J (2020) Seeking the true time: Exploring otolith chemistry as an age-determination tool. J Fish Biol 97: 552–565

Henderson MJ, Fabrizio MC, Lucy JA (2014) Movement patterns of summer flounder near an artificial reef: Effects of fish size and environmental cues. Fish Res 153: 1–8

Hernández-León S (2008) Natural variability of fisheries and lunar illumination: a hypothesis: Natural variability of fisheries and lunar illumination. Fish Fish 9: 138–154

Høie H, Folkvord A, Mosegaard H, Li L, Clausen LAW, Norberg B, Geffen AJ (2008) Restricted fish feeding reduces cod otolith opacity. J Appl Ichthyol 24: 138–143

Hornetz P (2020) Spatio-temporal distribution and food intake of cod (Gadus morhua) along a depth gradient in the western Bornholm Sea. Master Thesis. Hamburg

Hüssy K (2010) Why is age determination of Baltic cod (Gadus morhua) so difficult? ICES J Mar Sci 67: 1198–1205

Hüssy K, Eero M, Radtke K (2018) Faster or slower: has growth of eastern Baltic cod changed? Mar Biol Res 14: 598–609

Hüssy K, Krüger-Johnsen M, Thomsen TB, Heredia BD, Naeraa T, Limburg KE, Heimbrand Y, McQueen K, Haase S, Krumme U, et al (2020) It's elemental, my dear Watson: validating seasonal patterns in otolith chemical chronologies. Can J Fish Aquat Sci cjfas-2020-0388

Hüssy K, Nielsen B, Mosegaard H, Clausen L (2009) Using data storage tags to link otolith macro- structure in Baltic cod Gadus morhua with environmental conditions. Mar Ecol Prog Ser 378: 161–170

Hüssy K, Radtke K, Plikshs M, Oeberst R, Baranova T, Krumme U, Sjöberg R, Walther Y, Mosegaard H (2016) Challenging ICES age estimation protocols: lessons learned from the eastern Baltic cod stock. ICES J Mar Sci 73: 2138–2149

ICES (2015) Report of the Benchmark Workshop on Baltic Cod stocks (WKBALTCOD), 2-6 March 2015, Rostock, Germany. ICES CM 2015/ACOM:35. ICES Sci Rep 172 pp.

ICES (2019) Benchmark Workshop on Baltic Cod stocks (WKBALTCOD2). ICES Sci Rep 1: 310 pp.

ICES (2020) Baltic Fisheries Assessment Working Group (WGBFAS). ICES Scientific Reports. 2:45. 643 pp. http://doi.org/10.17895/ices.pub.6024.

ICES (1994) Report of the workshop on Baltic cod age reading, 6−10 June 1994, Gdynia, Poland. ICES CM 1994/J:5.

ICES (2000) Study group on Baltic cod age reading, 27−31 March 2000, Karlskrona, Sweden. ICES CM 2000/H: 01.

Jadot C (2003) Comparison of two tagging techniques for Sarpa salpa: external attachment and intraperitoneal implantation. Oceanol Acta 26: 497–501

Jepsen N, Thorstad EB, Havn T, Lucas MC (2015) The use of external electronic tags on fish: an evaluation of tag retention and tagging effects. Anim Biotelemetry 3: 49

Jones FRH, Arnold GP, Walker MG, Scholes P (1979) Selective tidal stream transport and the migration of plaice (Pleuronectes platessa L.) in the southern North Sea. ICES J Mar Sci 38: 331–337

Kock K (1975) Über die Haltung von Dorschen (Gadus morhua L.) in Netz-käfigen. Arch Fisch Wiss 26: 35–48

Köster FW, Huwer B, Hinrichsen H-H, Neumann V, Makarchouk A, Eero M, Dewitz BV, Hüssy K, Tomkiewicz J, Margonski P, et al (2017) Eastern Baltic cod recruitment revisited—dynamics and impacting factors. ICES J Mar Sci 74: 3–19

Krumme U, Stötera S, McQueen K, Pahlke E (2020) Age validation of age 0-3 wild cod Gadus morhua in the western Baltic Sea through mark-recapture and tetracycline marking of otoliths. Mar Ecol Prog Ser 645: 141–158

Kulke R, Bödewadt V, Hänselmann K, Herrmann JP, Temming A (2018) Ignoring the vertical dimension: biased view on feeding dynamics of vertically migrating sprat (Sprattus sprattus). ICES Journal of Marine Science, Volume 75, Issue 7, December 2018, Pages 2450–2462, https://doi.org/10.1093/icesjms/fsy136

Last KS, Hobbs L, Berge J, Brierley AS, Cottier F (2016) Moonlight Drives Ocean-Scale Mass Vertical Migration of Zooplankton during the Arctic Winter. Curr Biol 26: 244–251

Lewis AE, Muntz WRA (1984) The effects of external ultrasonic tagging on the swimming performance of rainbow trout, Salmo gairdneri Richardson. J Fish Biol 25: 577–585

Liu C, Cowles GW, Zemeckis DR, Cadrin SX, Dean MJ (2017) Validation of a hidden Markov model for the geolocation of Atlantic cod. Can J Fish Aquat Sci 74: 1862–1877

LLUR (2020) Sauerstoffmangel im bodennahen Wasser der westlichen Ostsee.

McQueen K (2019) Age Validation and Growth Estimation of Baltic Cod. Dissertation. Hamburg, Hamburg

McQueen K, Casini M, Dolk B, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Mion M, Mohr T, Radtke K, et al (2020) Regional and stock-specific differences in contemporary growth of Baltic cod revealed through tag-recapture data. ICES J Mar Sci fsaa104

McQueen K, Eveson JP, Dolk B, Lorenz T, Mohr T, Schade FM, Krumme U (2018) Growth of cod (Gadus morhua) in the western Baltic Sea: estimating improved growth parameters from tag-recapture data. Can J Fish Aquat Sci. doi: 10.1139/cjfas-2018-0081

McQueen K, Hrabowski J, Krumme U (2019) Age validation of juvenile cod in the Western Baltic Sea. ICES J Mar Sci 76: 430–441

Meyer S, McQueen K, Krumme U (under review) Suitability of calcein and tetracycline hydrochloride as otolith markers for European flounder (Platichthys flesus), European plaice (Pleuronectes platessa) and turbot (Scophthalmus maximus).

Mion M, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Krüger-Johnsen M, Krumme U, McQueen K, Plikshs M, Radtke K, et al (2021) Multidecadal changes in fish growth rates estimated from tagging data: A case study from the Eastern Baltic cod (Gadus morhua, Gadidae). Fish Fish. doi: https://doi.org/10.1111/faf.12527

Modig H, Ólafsson E (1998) Responses of Baltic benthic invertebrates to hypoxic events. J Exp Mar Biol Ecol 229: 133–148

Neat FC, Wright PJ, Fryer RJ (2008) Temperature effects on otolith pattern formation in Atlantic cod Gadus morhua. J Fish Biol 73: 2527–2541

Neuenfeldt S, Bartolino V, Orio A, Andersen KH, Andersen NG, Niiranen S, Bergström U, Ustups D, Kulatska N, Casini M (2020) Feeding and growth of Atlantic cod (Gadus morhua L.) in the eastern Baltic Sea under environmental change. ICES J Mar Sci 77: 624–632

Neuenfeldt S, Hinrichsen H-H, Nielsen A, Andersen KH (2007) Reconstructing migrations of individual cod (Gadus morhua L.) in the Baltic Sea by using electronic data storage tags. Fish Oceanogr 16: 526–535

Nielsen B, Hüssy K, Neuenfeldt S, Tomkiewicz J, Behrens J, Andersen K (2013) Individual behaviour of Baltic cod Gadus morhua in relation to sex and reproductive state. Aquat Biol 18: 197–207

Nilsson LAF, Thygesen UH, Lundgren B, Nielsen BF, Nielsen JR, Beyer JE (2003) Vertical migration and dispersion of sprat (Sprattus sprattus) and herring (Clupea harengus) schools at dusk in the Baltic Sea. Aquat Living Resour 16: 317–324

Orio A, Bergström U, Florin A, Lehmann A, Šics I, Casini M (2019) Spatial contraction of demersal fish populations in a large marine ecosystem. J Biogeogr 46: 633–645

Pecuchet L, Törnroos A, Lindegren M (2016) Patterns and drivers of fish community assembly in a large marine ecosystem. Mar Ecol Prog Ser 546: 239–248

Pinti J, Visser AW (2018) Predator-Prey Games in Multiple Habitats Reveal Mixed Strategies in Diel Vertical Migration. Am Nat 193: E65–E77

Plonus R, McQueen K, Günther C, Funk S, Temming A, Krumme U (2021) Is analysis of otolith microstructure a valid method for investigating early life history of Western Baltic cod? Mar Biol 168: 30

Reeves SA (2003) A simulation study of the implications of age-reading errors for stock assessment and management advice. ICES J Mar Sci 60: 314–328

Righton D, Quayle VA, Hetherington S, Burt G (2007) Movements and distribution of cod (Gadus morhua) in the southern North Sea and English Channel: results from conventional and electronic tagging experiments. J Mar Biol Assoc U K 87: 599–613

Righton DA, Andersen KH, Neat F, Thorsteinsson V, Steingrund P, Svedäng H, Michalsen K, Hinrichsen HH, Bendall V, Neuenfeldt S, et al (2010) Thermal niche of Atlantic cod Gadus morhua: limits, tolerance and optima. Mar Ecol Prog Ser 420: 1–13

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

Rojbek MC, Tomkiewicz J, Jacobsen C, Stottrup JG (2014) Forage fish quality: seasonal lipid dynamics of herring (Clupea harengus L.) and sprat (Sprattus sprattus L.) in the Baltic Sea. ICES J Mar Sci 71: 56–71

Schaber M, Hinrichsen H-H, Gröger J (2012) Seasonal changes in vertical distribution patterns of cod (Gadus morhua) in the Bornholm Basin, central Baltic Sea. Fish Oceanogr 21: 33–43

Schade FM, Weist P, Dierking J, Krumme U (under review) Living apart together: 43-year coexistence of shallow-water Western Baltic cod and deep-water Eastern Baltic cod in a mixing area.

Smith NJ, McCall PL, Sutton TM (2017) Effects of different tagging protocols on survival, growth, and tag retention in juvenile least cisco Coregonus sardinella. Fish Res C: 68–72

Snoeijs-Leijonmalm P, Andrén E (2017) Why is the Baltic Sea so special to live in? Springer, pp 23–84

Sokolova M, Buchmann K, Huwer B, Kania P, Krumme U, Galatius A, Hemmer-Hansen J, Behrens J (2018) Spatial patterns in infection of cod Gadus morhua with the seal-associated liver worm Contracaecum osculatum from the Skagerrak to the central Baltic Sea. Mar Ecol Prog Ser 606: 105–118

Stötera S, Degen-Smyrek AK, Krumme U, Stepputtis D, Bauer R, Limmer B, Hammer C (2018) Marking otoliths of Baltic cod (Gadus morhua Linnaeus, 1758) with tetracycline and strontium chloride. J Appl Ichthyol 35: 427–435

Sutherland WJ (1997) From Individual Behaviour to Population Ecology. Oxford University Press, Oxford; New York

Svedäng H, Thunell V, Pålsson A, Wikström SA, Whitehouse MJ (2020) Compensatory Feeding in Eastern Baltic Cod (Gadus morhua): Recent Shifts in Otolith Growth and Nitrogen Content Suggest Unprecedented Metabolic Changes. Front Mar Sci. doi: 10.3389/fmars.2020.00565

Takemura A, Rahman MS, Nakamura S, Park YJ, Takano K (2004) Lunar cycles and reproductive activity in reef fishes with particular attention to rabbitfishes. Fish Fish 5: 317–328

Thurow F (1963) Dorschmarkierungen in der Westlichen Ostsee. Informationen Für Fischwirtsch 10: 12–17

Verheijen FJ, De Groot SJ (1967) Diurnal activity pattern of plaice and flounder (pleuronectidae) in aquaria. Neth J Sea Res 3: 383–390

Woodhead PMJ (1960) Diurnal variations in trawl catches of plaice. ICES, C.M. 1960, Doc. No. 158.

Acknowledgements

Besonders möchte ich mich bei meinem Betreuer Dr. Uwe Krumme bedanken. Uwe, deine motivierende und enthusiastische Art haben mich durch die ganze Promotion unterstützend begleitet und die vielen Diskussionen haben die Arbeit weiter reifen lassen. Dein umfassendes Wissen und hilfreiches Feedback habe ich immer sehr dankend angenommen. Durch dich durfte ich in viele weitere Bereiche eintauchen und du hast mich so als Wissenschaftlerin wachsen lassen.

Mein weiterer Dank geht an meinen zweiten Betreuer Prof. Dr. Axel Temming von der Universität Hamburg. Ich schätze deine logische und kritische Denkweise seit der Betreuung meiner Masterarbeit sehr. Du hast Uwes Betreuung gut komplementiert.

Vielen Dank an Dr. Christopher Zimmermann für jegliche Unterstützung und alle Möglichkeiten und Herausforderungen, die du mir am Institut ermöglichst!

Mein besonderer Dank geht auch an Juan Santos und Dr. Daniel Stepputtis. Ihr begleitet mich von Anfang an auf meinem Weg in der Fischereiforschung und durch euch habe ich alle Grundlagen des wissenschaftlichen Arbeitens gelernt! Ich erinnere mich immer gerne an die Seereisen und alle Projekte mit euch zurück! Juan, vielen Dank für viele Kaffeepausen, Abendessen und wertvolle Gespräche!

Vielen Dank an viele weitere Kolleginnen und Kollegen, die mir die Arbeit und auch die Pausen sehr angenehm gemacht haben: Vor allem Dr. Kate McQueen für eine wunderbare und inspirierende Zusammenarbeit in vielen Projekten, Dr. Dorothee Moll, Annegret Finke und Steffi Meyer für viele tolle Gespräche in den Pausen, Dr. Sarah Kraak für die Einführung in die Populationsdynamiken und viele spannende Rosa-Lee Sessions und Fanny Barz für die gegenseitige Unterstützung, besonders in den letzten Monaten.

Danken möchte ich auch Ina Hennings, Titus Rohde, Britta Rotzoll und Gustav Basedow. Ohne eure Fischmarkierungen und Aufarbeitung wären die Ergebnisse dieser Arbeit gar nicht möglich gewesen. Außerdem ist es immer schön, euch auf den Fluren zu treffen!

Zusätzlich möchte ich Thomas Hogh, Aldo Raffaelli, Nakula Platena, Mario Koth, Annemarie Schütz, Sabine Kliem und Katharina Wolf herzlich danken. Ihr habt mich auf vielfältige Weisen während meiner Promotion und in anderen Projekten unterstützt.

Vielen Dank auch an alle Co-Autoren und Mitglieder des TABACOD Projektes. Ich habe viel von euch gelernt und es war mir eine große Freude mit euch zusammen zu arbeiten.

Ein ganz besonderer Dank geht an meine Freunde, die mich während der letzten drei Jahre begleitet haben und für die manchmal notwendige Ablenkung, viele schöne Abende und die vielen anderen Sichtweisen gesorgt haben. Besonders erwähnen möchte ich hier: Rike, mein Sonnenschein, danke, für die vielen wunderbaren Stunden der letzten Jahre. Lena, du bist das perfekte Beispiel, wie aus Kolleginnen sehr gute Freundinnen werden und ich bin dir sehr dankbar dafür. Johanna, du hast mich durch das gesamte Studium begleitet und besonders am Ende immer wieder für die nötige Motivation gesorgt.

Ein großer Dank geht an meine Familie. Mama, Papa, Sabrina, ihr habt mich immer unterstützt und mir alles ermöglicht! Danke, dass ihr mit „Wickie“ schon so früh die Liebe zum Meer in mir entfacht habt und mir immer einen Ort zum Entspannen gebt. Danke Tilia, du hast mir eine besondere Sichtweise des Lebens gezeigt.

Björn, du bist Familie für mich geworden und kennst jeden Trick, um mich zum Lachen zu bringen. Ich schätze es sehr, dass du mich besonders in den letzten Wochen der Promotion auf Händen getragen hast.

Funding

This research was partly funded by BalticSea2020 (http://balticsea2020.org) through the project “Tagging Baltic Cod” (TABACOD).

Declaration of author's contributions to manuscripts within this thesis

Chapter 1: Short-term tagging mortality of Baltic cod (*Gadus morhua*)

Stefanie Haase, Kate McQueen, Monica Mion, Magnus Andersson, Annelie Hilvarsson, Hans Jakob Olesen, Anders Svenson, Michele Casini, Karin Hüssy, Krzysztof Radtke, Uwe Krumme

Published in 2021 in Fisheries Research, DOI:10.1016/j.fishres.2020.105804

KMQ, MM, AH, HJO, MC, KH and UK were involved in the conception and design of the experiment. MA, AH, HJO and AS were involved in data collection. SH performed the statistical analysis and SH and KMQ wrote the original draft. All authors provided feedback on drafts of the manuscript.

Chapter 2: Validation approaches of a geolocation framework to reconstruct movements of demersal fish equipped with data storage tags in a stratified environment

Stefanie Haase, Uwe Krumme, Ulf Gräwe, Camrin Donald Braun, Axel Temming

Published in 2021 in Fisheries Research, DOI:10.1016/j.fishres.2021.105884

SH and UK developed the concept and experiments of the manuscript. UG provided the Baltic regional model. CDB guided SH in the functionalities of the geolocation framework. SH conducted all analyses and wrote the original draft of the manuscript under the guidance of UK. All authors provided feedback on drafts of the manuscript.

Chapter 3: Movement of cod (*Gadus morhua* L.) in the southern Baltic Sea: evidence from data storage tags

Stefanie Haase, Ulf Gräwe, Camrin Donald Braun, Axel Temming, Kate McQueen, Monica Mion, Franziska Maria Schade, Annelie Hilvarsson, Hans Jakob Olesen, Karin Hüssy, Michele Casini, Krzysztof Radtke, Uwe Krumme

Planned submission in June 2021 to Canadian Journal of Fisheries and Aquatic Sciences

SH developed the concept and objectives of the manuscript under the guidance of UK, AT and CDB. UG provided the Baltic regional model. KMQ, MM, AH, HJO, KH, MC and UK were involved in the tagging and collection of the data storage tag data. FMS conducted the otolith shape analysis. SH conducted the analysis and prepared the first draft of the manuscript. CDB, AT, KR and UW provided feedback on the current version of the manuscript.

SH presented the study in two posters at the 5th International conference on fish telemetry in Arendal in 2019. SH and UK wrote a public science article about this study in the magazine "Wissenschaft erleben" in 2019.

Chapter 4: Validation of otolith zone formation and otolith growth of adult wild cod (*Gadus morhua* L.) in the southern Baltic Sea through mark-recapture and tetracycline marking

Stefanie Haase, Kate McQueen, Axel Temming, Franziska Maria Schade, Karin Hüssy, Annelie Hilvarsson, Michele Casini, Monica Mion, Krzysztof Radtke, Uwe Krumme

Planned submission in June 2021 to Marine Ecology Progress Series

SH and UK developed the concept and objectives of the manuscript. SH, KMQ, KH, AH, MC, MM, KR and UK were involved in the collection and preparation of data collection. FMS conducted the otolith shape analysis. SH analysed the data and wrote the manuscript. UK, KMQ and AT gave feedback to the data analysis. All authors provided feedback on the current version of the manuscript.

Hereby, I confirm the accuracy of the statement above,

S. Haase

Uwe Kr.

Stefanie Haase
(Doctoral candidate)

Dr. Uwe Krumme
(on behalf of the supervisors)

The following manuscripts represent minor contributions to the thesis:

- Hüssy K, Krüger-Johnsen M, Thomsen TB, Heredia BD, Naeraa T, Limburg KE, Heimbrand Y, McQueen K, Haase S, Krumme U, Casini M, Mion M, Radtke K (2020) It‘s elemental, my dear Watson: validating seasonal patterns in otolith chemical chronologies. Can J Fish Aquat Sci: in press, DOI:10.1139/cjfas-2020-0388

- Mion M, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Krüger-Johnsen M, Krumme U, McQueen K, Plikshs M, Radtke K, Schade FM, Vitale F, Casini M (2020) Multidecadal changes in fish growth rates estimated from tagging data: A case study from the Eastern Baltic cod (*Gadus morhua,* Gadidae). Fish Fisheries 22:413-327, DOI:10.1111/faf.12527

- McQueen K, Casini M, Dolk B, Haase S, Hemmer-Hansen J, Hilvarsson A, Hüssy K, Mion M, Mohr T, Radtke K, Schade FM, Schulz N, Krumme U (2020) Regional and stock-specific differences in contemporary growth of Baltic cod revealed through tag-recapture data. ICES J Mar Sci 77(6):2078-2088, DOI:10.1093/icesjms/fsaa104

Additionally, the following peer-reviewed articles and working documents were published during the course of my PhD:

- Noack T, Stepputtis D, Madsen N, Wieland K, Haase S, Krag LA (2019) Gear performance and catch process of a commercial Danish anchor seine. Fish Res 211:204-211, DOI:10.1016/j.fishres.2018.11.012

- Kraak SBM, Haase S, Minto C, Santos J (2019) The Rosa Lee phenomenon and its consequences for fisheries advice on changes in fishing mortality or gear selectivity. ICES J Mar Sci 76(7):2179-2192, DOI:10.1093/icesjms/fsz107

- Santos J, Herrmann B, Mieske B, Krag LA, Haase S, Stepputtis D (2018) The efficiency of sieve-panels for bycatch separation in *Nephrops* trawls. Fish Manag Ecol 25(6):464-473, DOI:10.1111/fme.12323

- Kaljuste O, Amosova V, Degel H, Fedotova E, Fernandez R, Haase S, Karpushevskaia A, Larson N, Lilja J, Lövgren O, O'Malley M, Parner H, Plikshs M, Pönni J, Radtke K, Raid T, Rodriguez-Tress P, Schmidt B, Sepp E, Severin V, et al (2021) ICES Working Group on Baltic International Fish Survey (WGBIFS; outputs from 2020 meeting). Copenhagen: ICES, 539 p, ICES Sci Rep 3(2), DOI:10.17895/ices.pub.7679

- Haase S, Krumme U (2020) Report of the spring 2019 Western Baltic cod (Gadus morhua) age reading exchange – SD 22

- Haase S, Krumme U (2020) Report of the autumn 2019 Western Baltic cod (Gadus morhua) age reading exchange – SD 22

- Amosova V, Boje J, Brown E, Carlshamre S, Eero M, Gröhsler T, Gutkowska J, Haase S, Hommik K, Horbowy J, Jounela P, Kaljuste O, Karpushevskaia A, Krumme U, Lövgren J, Mirny Z, Neuenfeldt S, Schade FM, Stötera S, Strehlow HV, et al (2020) Baltic Fisheries Assessment Working Group (WGBFAS). Copenhagen: ICES, 643 p, ICES Sci Rep 2(45), DOI:10.17895/ices.pub.6024

- Hüssy K, Casini M, Haase S, Hilvarsson A, Horbowy J, Krüger-Johnsen M, Krumme U, Limburg K, McQueen K, Mion M, Olesen HJ, Radtke K (2020) Tagging Baltic Cod - TABACOD: Eastern Baltic cod: Solving the ageing and stock assessment problems with combined state-of-the-art tagging methods. National Institute of Aquatic Resources, Technical University of Denmark, 92 p, DTU Aqua Rep 368-2020

- De Oliveira JA, Bartolino V, Berges B, Björnsson H, Brooks ME, Butterworth D, Campbell A, Cardinale M, Carruthers T, Cerviño S, Chernega G, Cole H, Moor Cde, Deroba J, Die D, Duprey N, Fay G, Haase S, Kempf A, Rohlf N, et al (2020) The third Workshop on Guidelines for Management Strategy Evaluations (WKGMSE3). Copenhagen: ICES, 112 p, ICES Sci Rep 2(116), DOI:10.17895/ices.pub.7627

- Carbonara P, Coad Davies J, Damme CJG van, Aanestad Godiksen J, Allegaert W, Beier U, Bekaert K, Canha A, Farias I, Follesa MC, Gault M, Gillespie-Mules R, Haase S, Hilvarsson A, Hüssy K, Korta M, Krüger-Johnsen M, Krumme U, Stransky C, Ulleweit J, et al (2020) Working Group on Biological Parameters (WGBIOP). Copenhagen: ICES, 150 p, ICES Sci Rep 2(117), DOI:10.17895/ices.pub.7651

- Moesgaard Albertsen C, Amosova V, Andersen M, Behrens JW, Willestofte Berg C, Bergenius M, Brander K, Cardinale M, Carlshamre S, Casini M, Cormon X, Eero M, Gertseva V, Haase S, Krumme U, McQueen K, Pierce ME, Schade FM, Strehlow HV, Weltersbach MS, et al (2019) Benchmark Workshop on Baltic Cod Stocks (WKBALTCOD2). Copenhagen: ICES, 310 p, ICES Sci Rep 1(9), DOI:10.17895/ices.pub.4984

Declaration on oath

I hereby declare, on oath, that I have written the present dissertation “Interlinked patterns in movements and otolith formation of cod (*Gadus morhua*) in the southern Baltic Sea” by my own and have not used other than the acknowledged resources and aids.

Eidesstattliche Versicherung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift „Interlinked patterns in movements and otolith formation of cod (*Gadus morhua*) in the southern Baltic Sea“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

S. Haase

Rostock, 11.04.2021 Stefanie Haase

Appendix

This appendix includes the supplementary materials referred to within Chapter 2-4 of this dissertation.

Supplementary Material of Chapter 2

Validation approaches of a geolocation framework to reconstruct movements of demersal fish equipped with data storage tags in a stratified environment

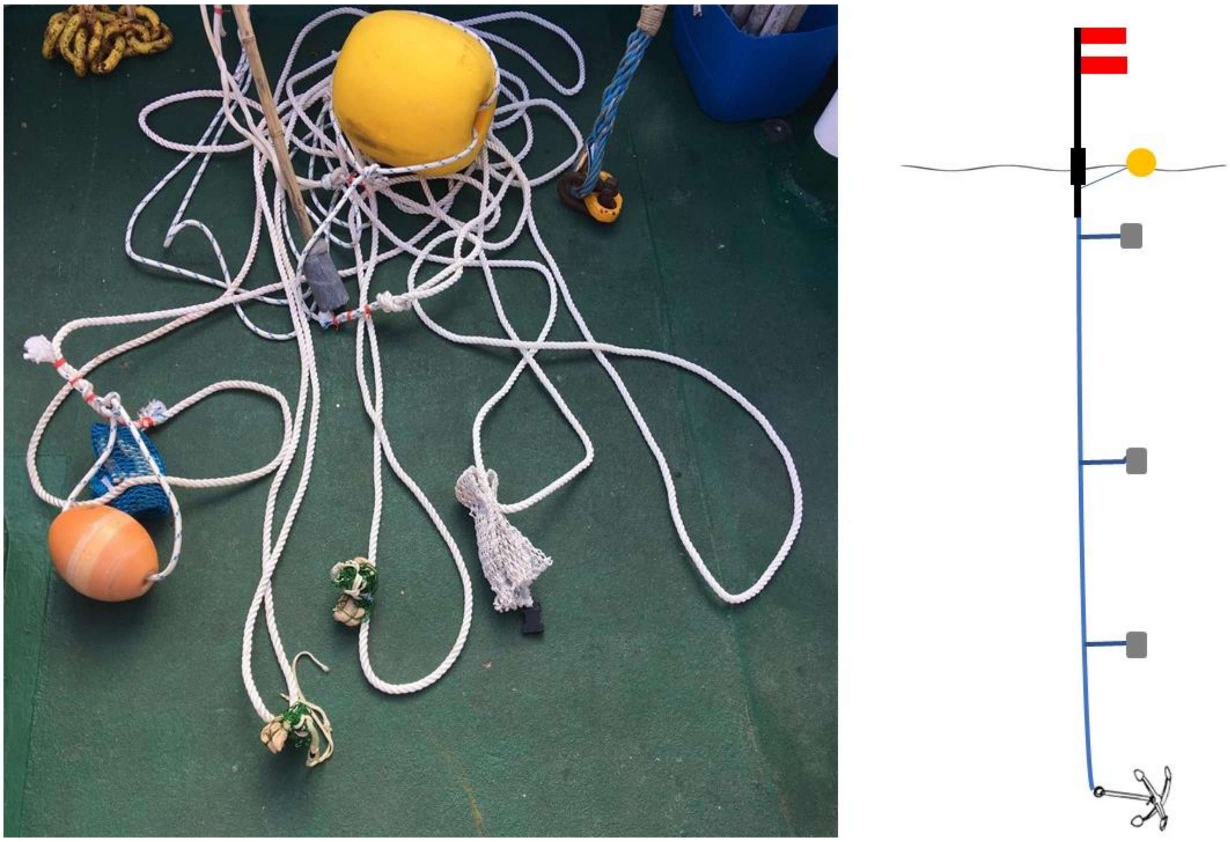

Figure S1: Photograph (left) and schematic drawing (right) of the setup of the stationary method. DSTs are mounted in small-meshed bags at around 2.5, 9 and 18 m along a rope which is marked at the surface and anchored to the bottom.

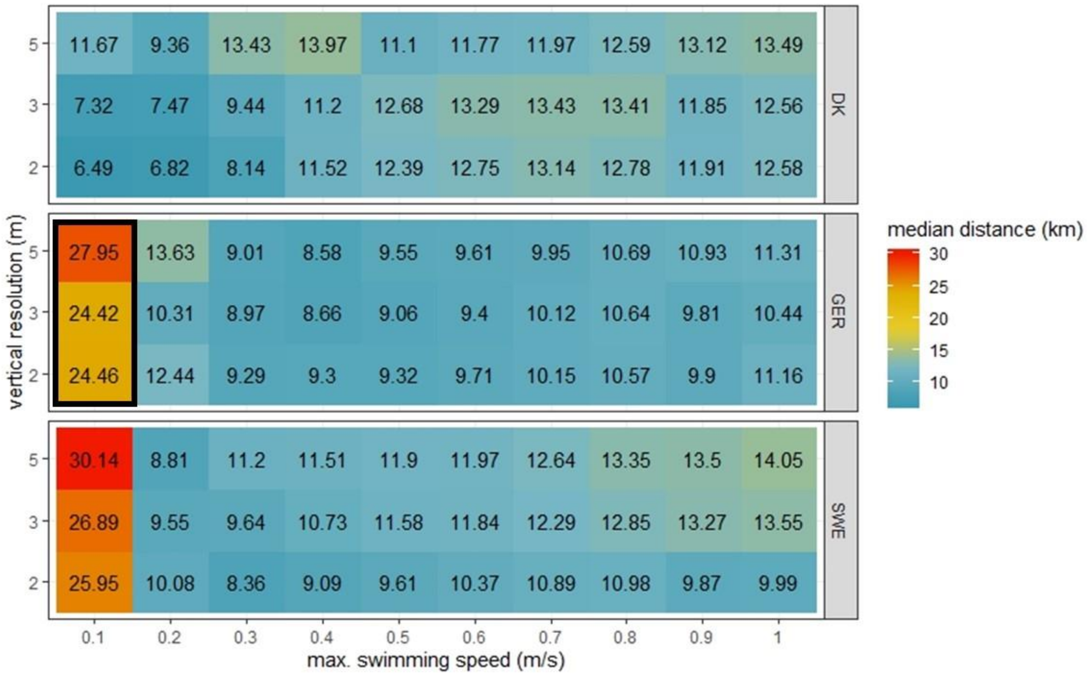

Figure S2: Median daily difference between known and reconstructed position for the Danish, German and Swedish release positions. The black square indicates that start and end-position were not modelled correctly.

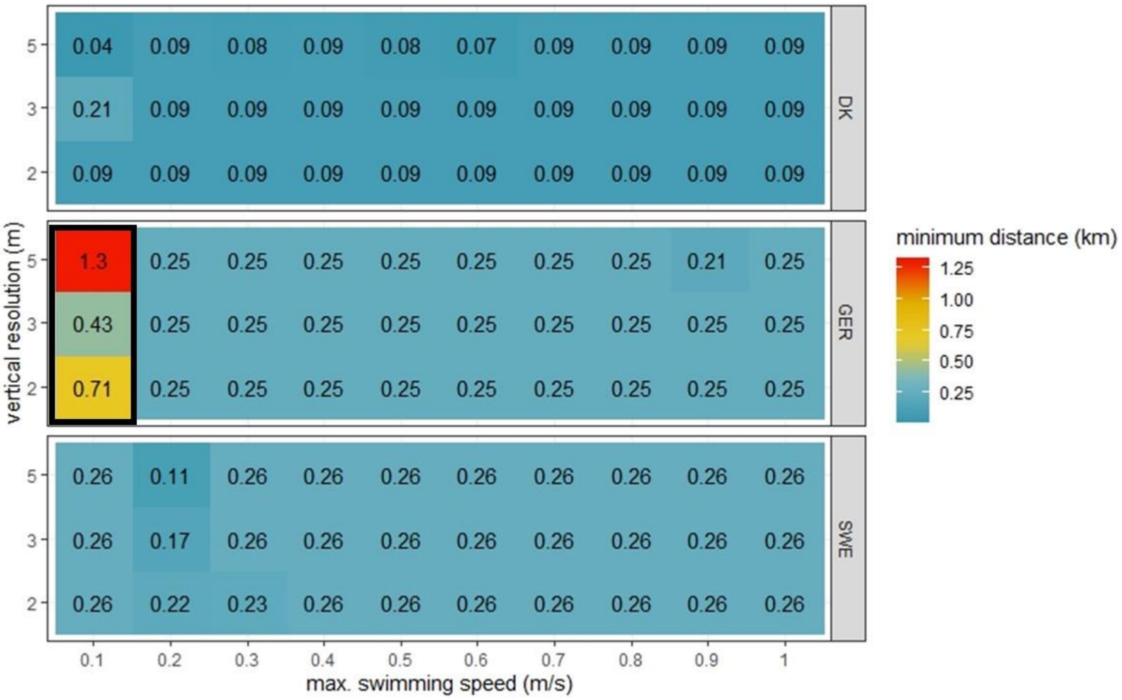

Figure S3: Minimum daily difference between known and reconstructed position for the Danish, German and Swedish release positions. The black square indicates that start and end-position were not modelled correctly.

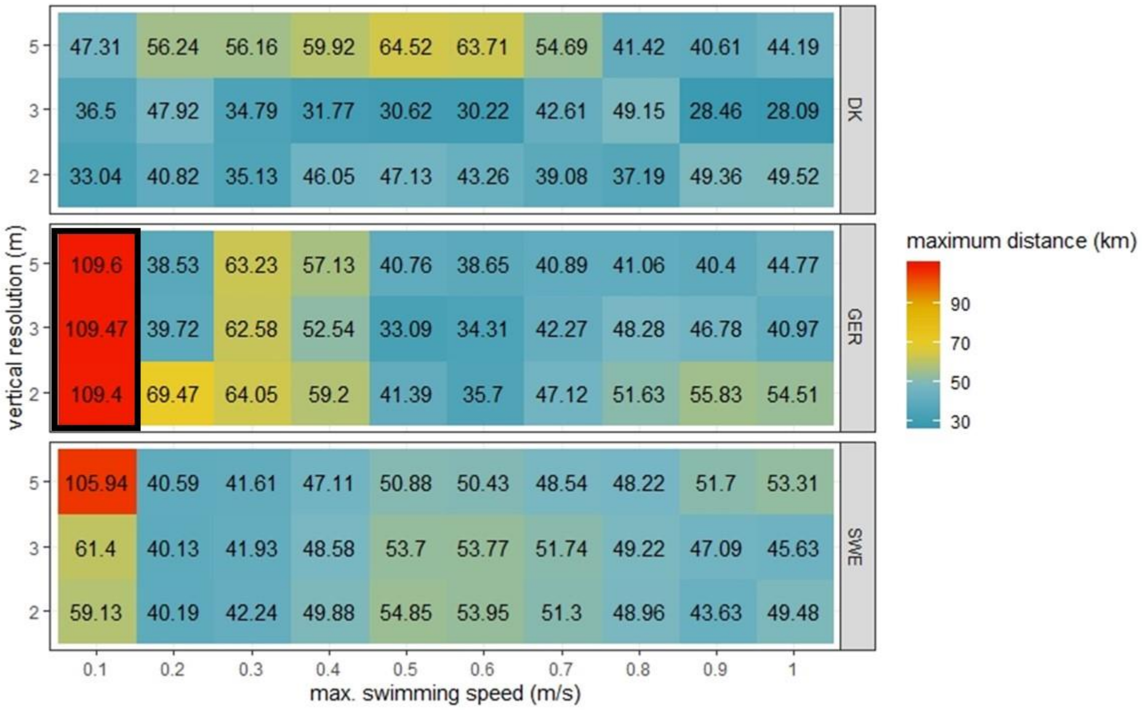

Figure S4: Maximum daily difference between known and reconstructed position for the Danish, German and Swedish release positions. The black square indicates that start and end-position were not modelled correctly.

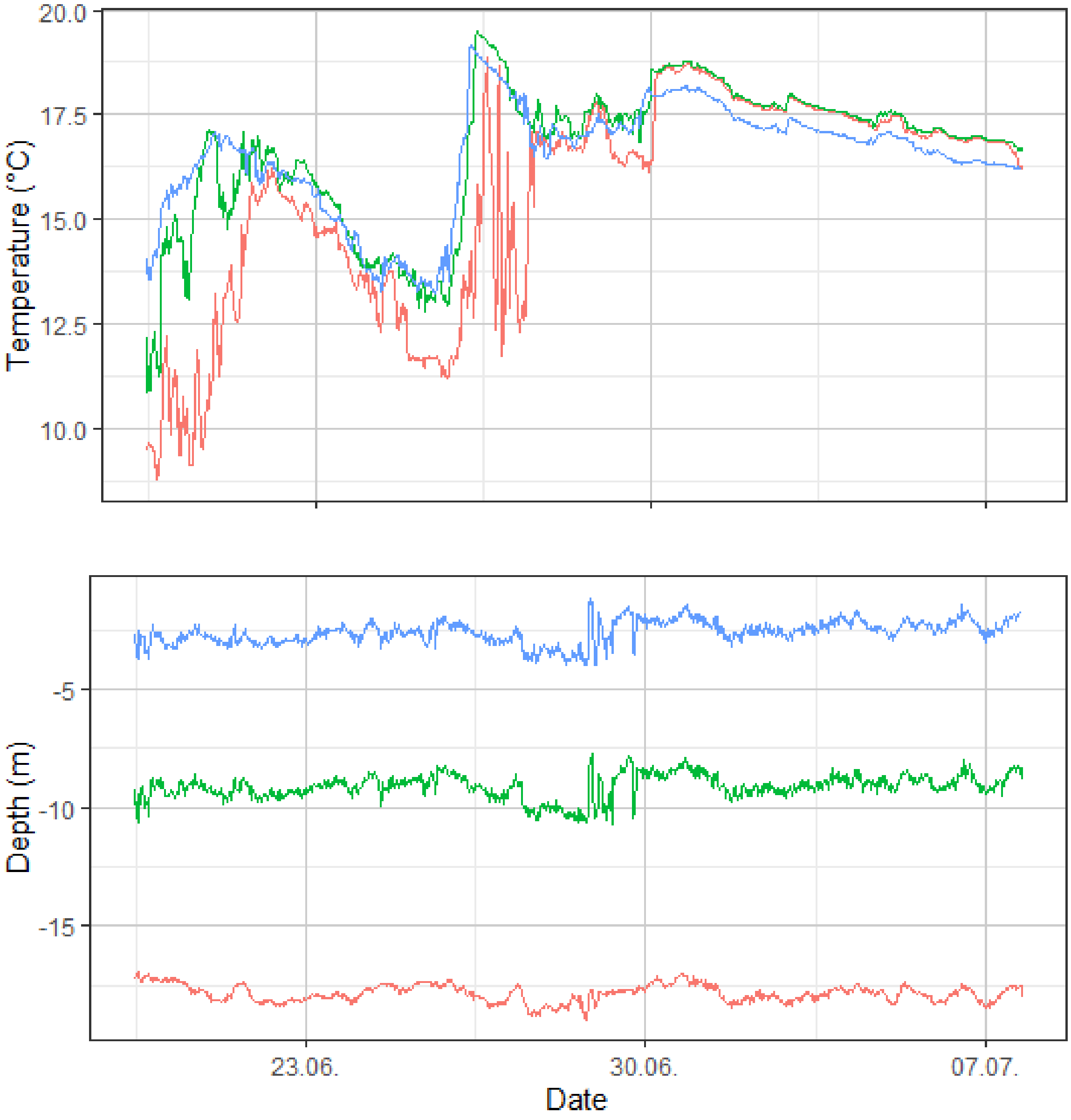

Figure S5: Temperature and depth profiles of the three stationary mounted DSTs (blue: close to the surface, green: medium and red: greatest depth). To reconstruct the location of the moored DSTs with geolocation model, two meters were substracted from the deepest DST since this one was deeper than the water depth at this position (original data presented here).

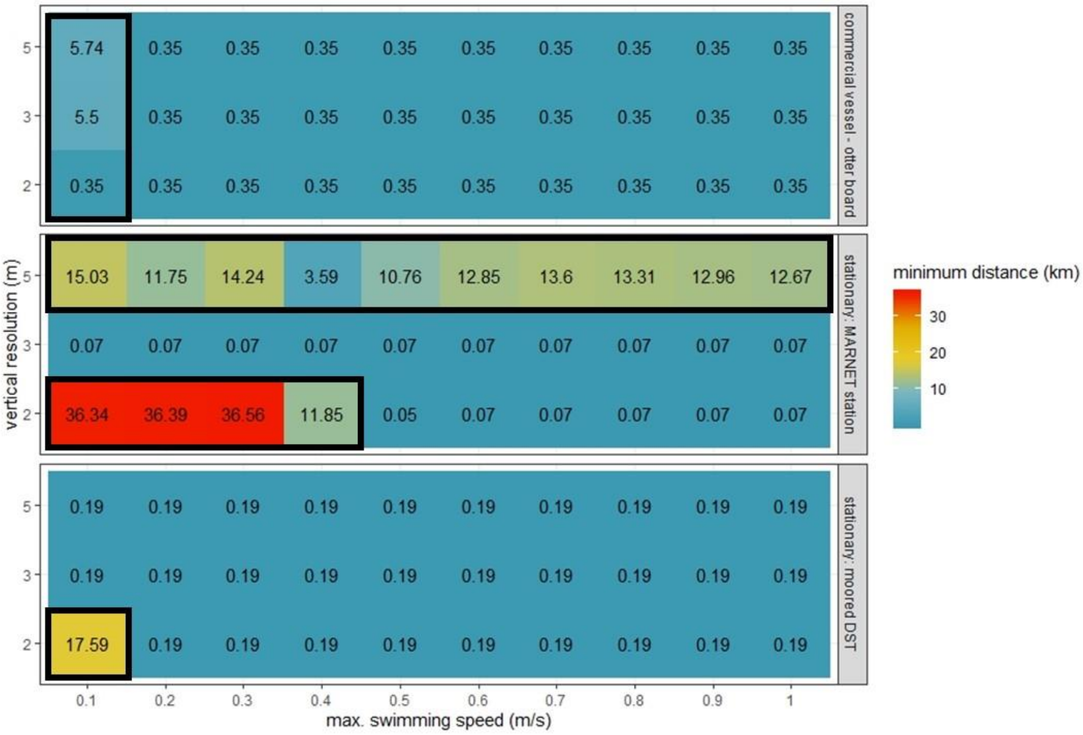

Figure S6: Minimum daily difference between known and reconstructed position from the temperature-depth data sampled with the probe attached to the otter board of the commercial vessel and from the stationary DSTs. The black square indicates that start and end-position were not modelled correctly.

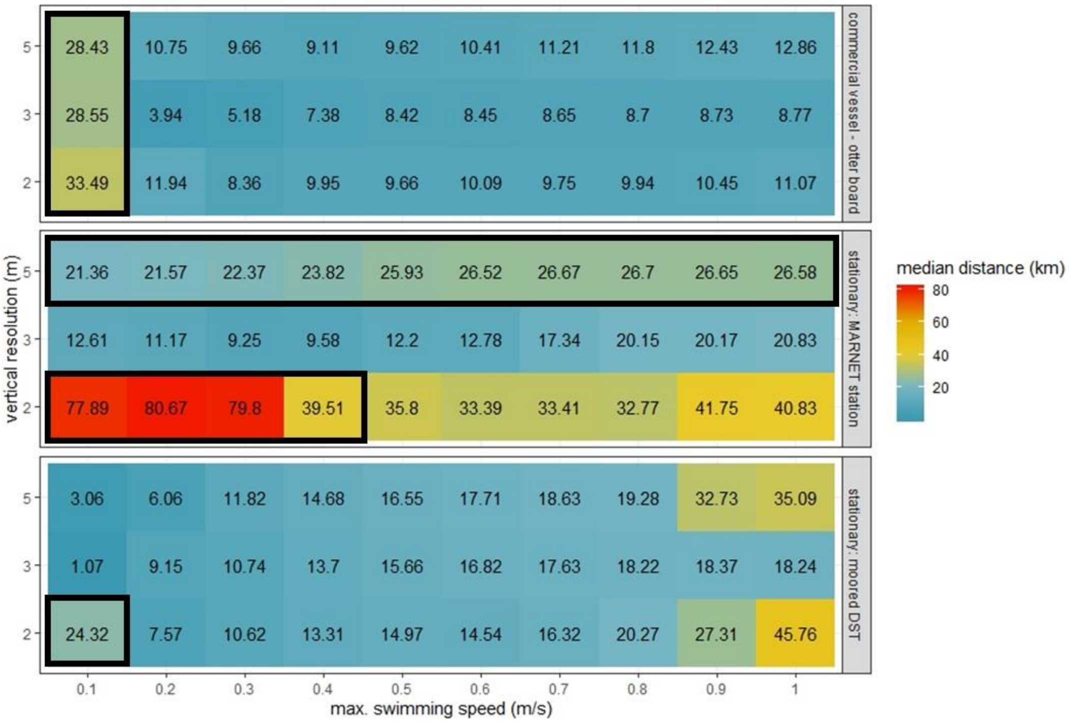

Figure S7: Median daily difference between known and reconstructed position from the temperature-depth data sampled with the probe attached to the otter board of the commercial vessel and from the stationary DSTs. The black square indicates that start and end-position were not modelled correctly.

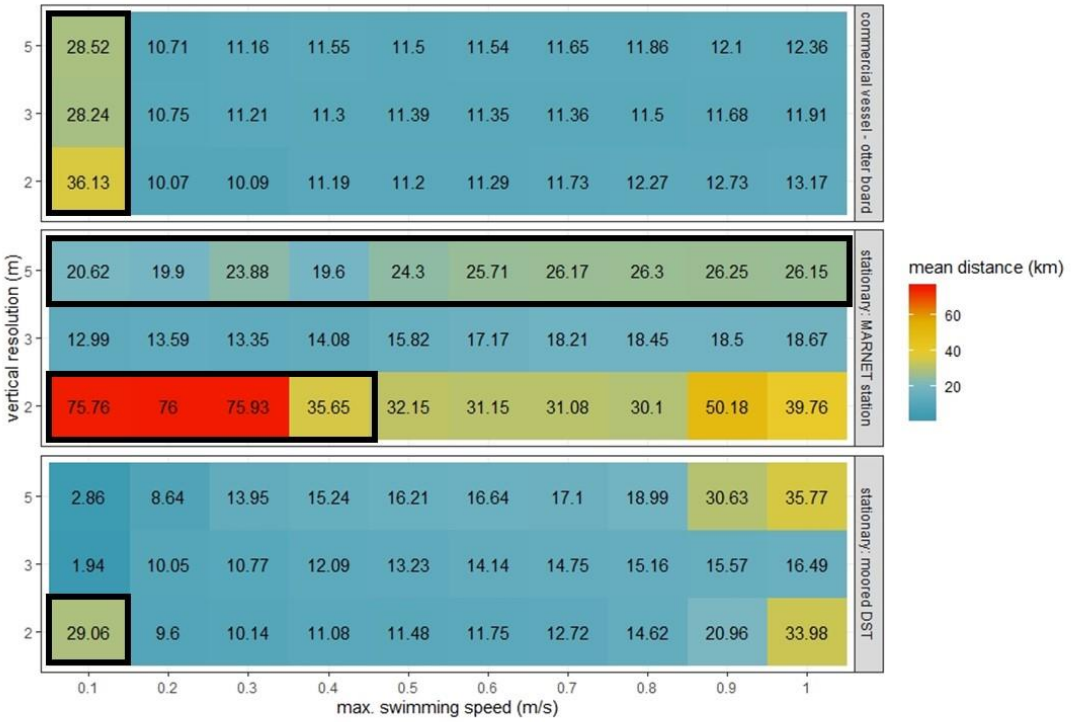

Figure S8: Mean daily difference between known and reconstructed position from the temperature-depth data sampled with the probe attached to the otter board of the commercial vessel and from the stationary DSTs. The black square indicates that start and end-position were not modelled correctly.

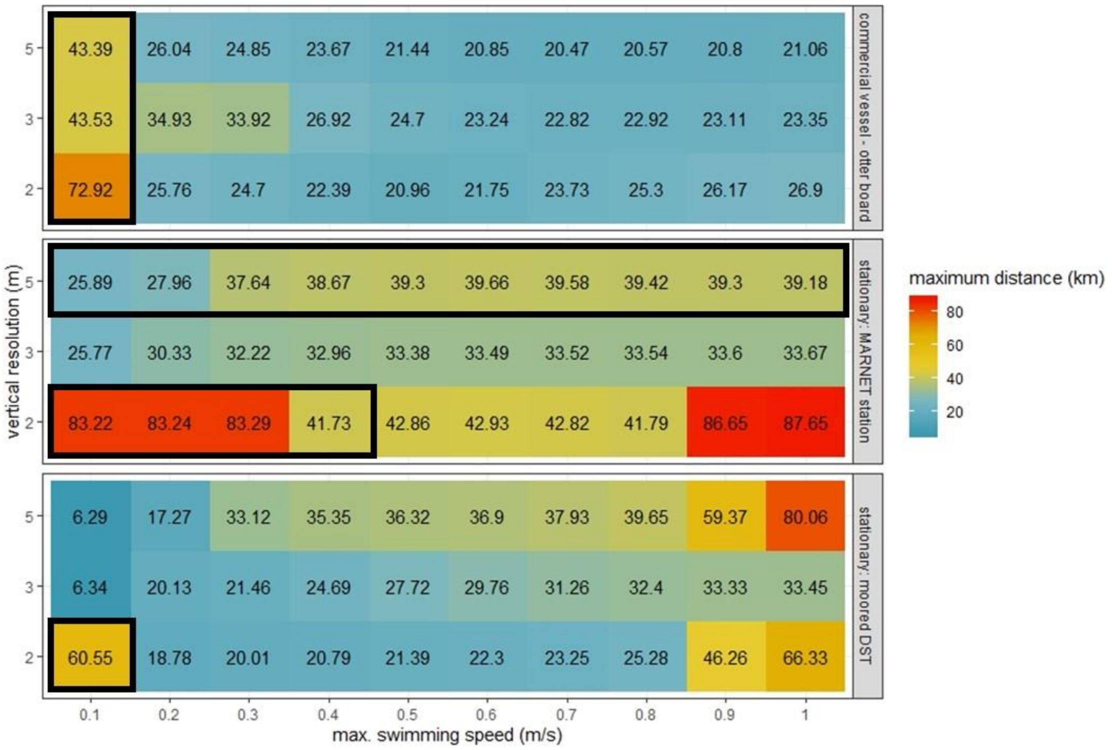

Figure S9: Maximum daily difference between known and reconstructed position from the temperature-depth data sampled with the probe attached to the otter board of the commercial vessel and from the stationary DSTs. The black square indicates that start and end-position were not modelled correctly.

Figure S10: Temperature profiles of the automated measuring station (MARNET) in the Arkona Basin. Temperature values below 7.5 °C were deleted from the dataset since they can be accounted as measurement errors.

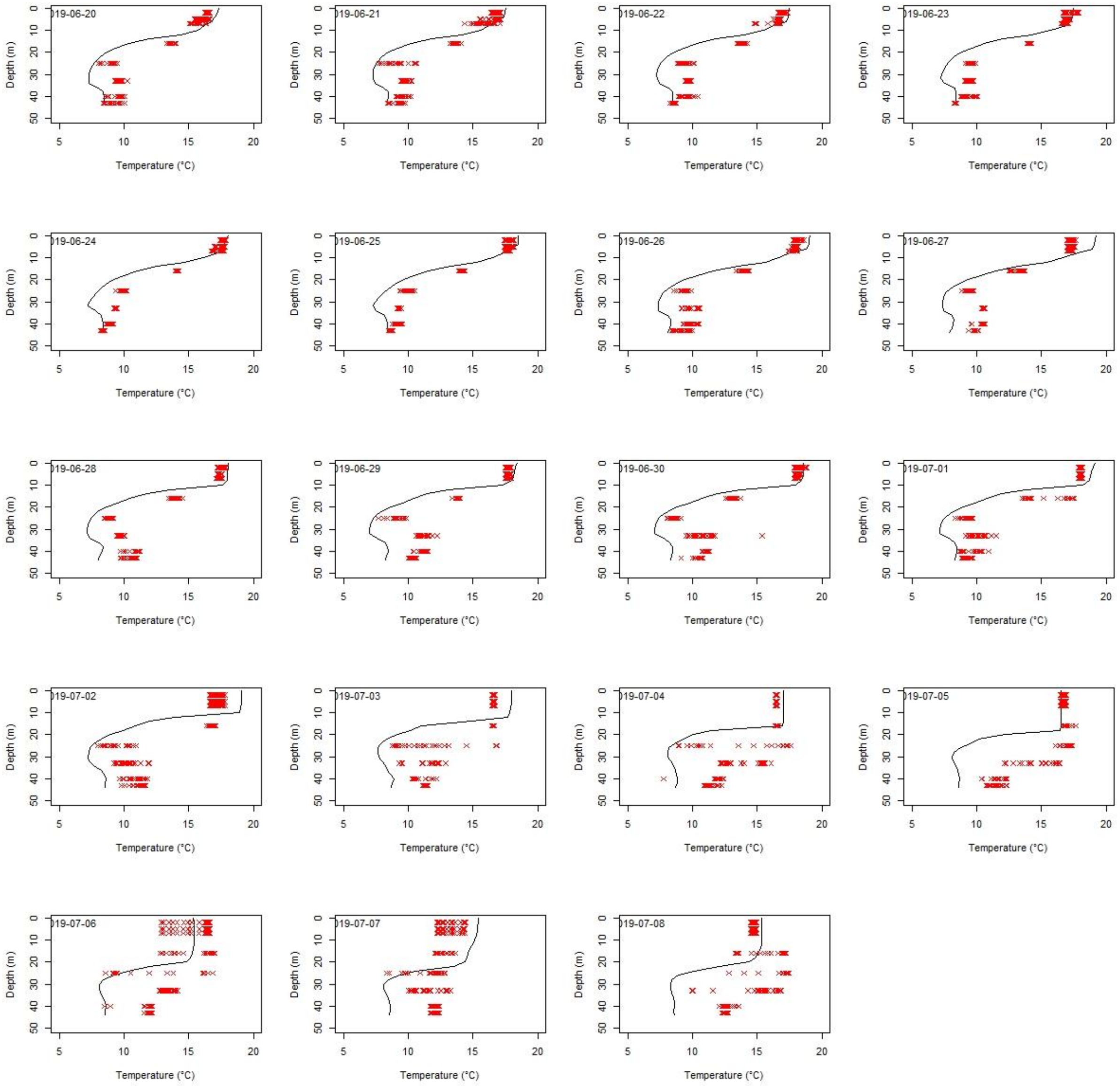

Figure S11: Vertical temperature profile extracted from the Baltic ROM at the true position of the MARNET-station (black) over the period of deployment. Red crosses indicate measurements from the MARNET station.

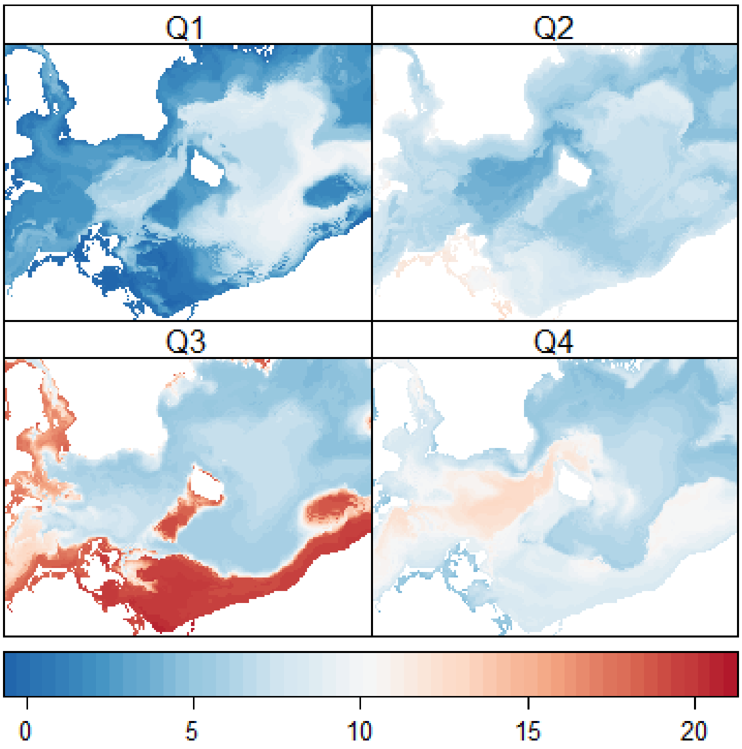

Figure S12: Quarterly bottom temperature (in °C) in the study area extracted from the regional ocean model (Gräwe, IOW) (Q1: 15.2.2019, Q2: 15.5.2019, Q3: 15.8.2019, Q4: 15.11.2019).

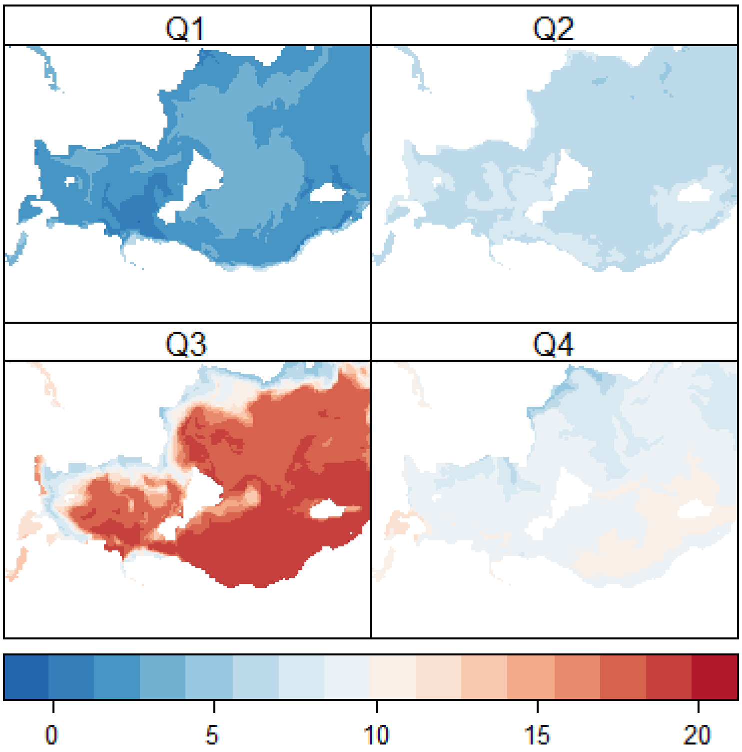

Figure S13: Quarterly temperature (in °C) at 20 m water depth in the study area extracted from the regional ocean model (Gräwe, IOW) (Q1: 15.2.2019, Q2: 15.5.2019, Q3: 15.8.2019, Q4: 15.11.2019).

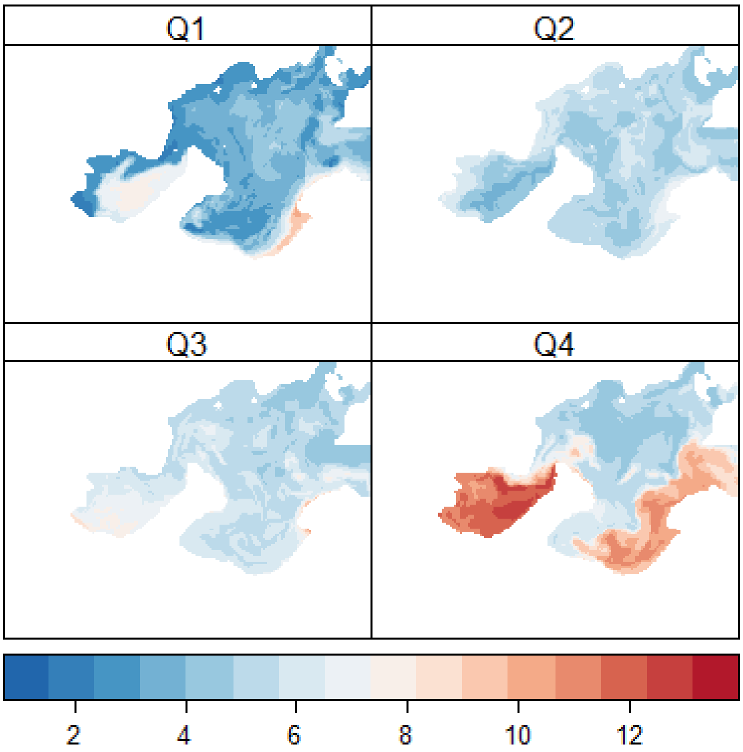

Figure S14: Quarterly temperature (in °C) at 40 m water depth in the study area extracted from the regional ocean model (Gräwe, IOW) (Q1: 15.2.2019, Q2: 15.5.2019, Q3: 15.8.2019, Q4: 15.11.2019).

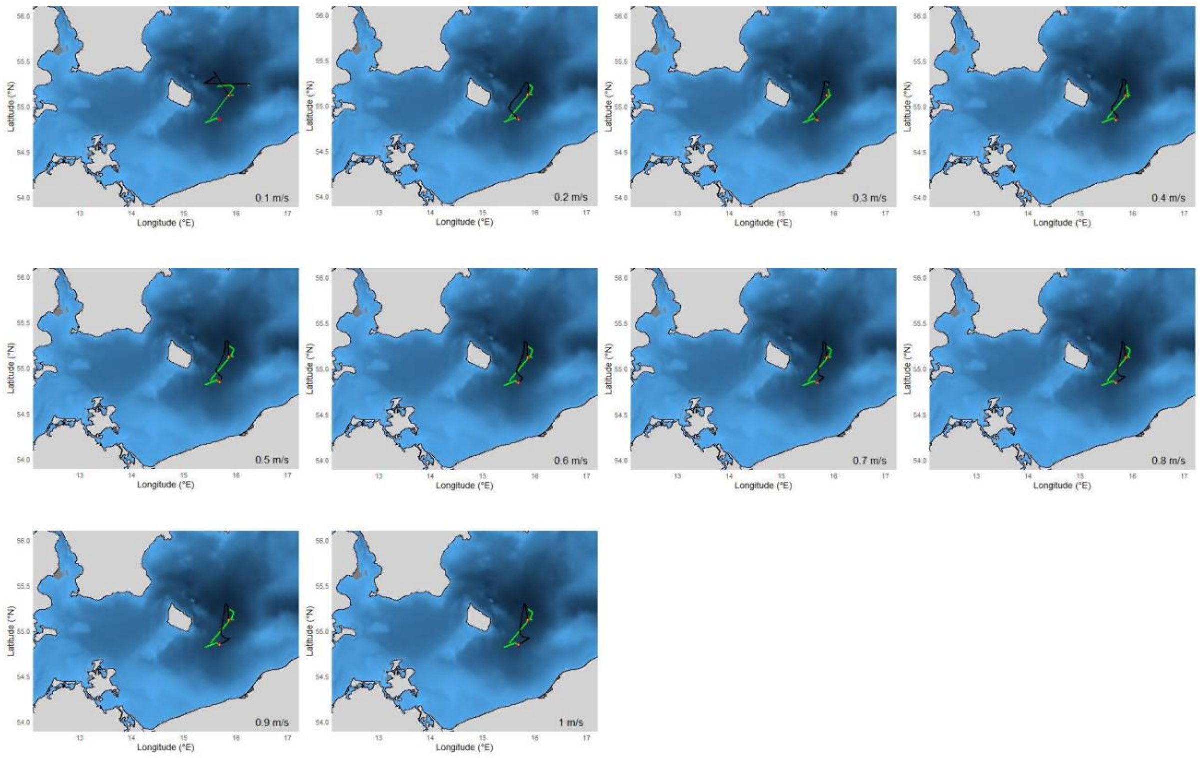

Figure S15: Known (green) and reconstructed (black) tracks with known start and end position (red dot) and reconstructed start and end position (yellow dot) for temperature-depth probe attached to the commercial vessel for a vertical resolution of 2 m and an increasing maximum swimming speed from 0.1 m/s until 1 m/s. While the track could not be reconstructed for a maximum swimming speed of 0.1 m/s, all other maximum swimming speeds could reconstruct the trend of the track correctly indicating a low sensitivity to the choice of input parameters.

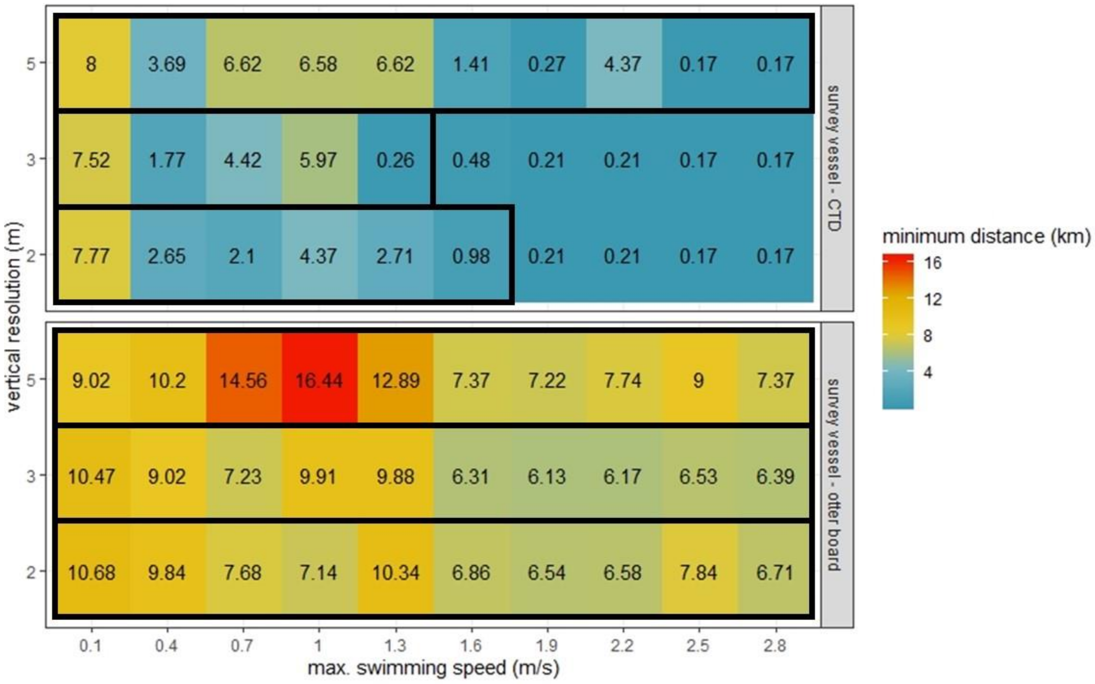

Figure S16: Minimum daily difference between known and reconstructed position from the temperature-depth data sampled with the DST attached to the CTD and otter board of the survey vessel. The black square indicates that start and end-position were not modelled correctly.

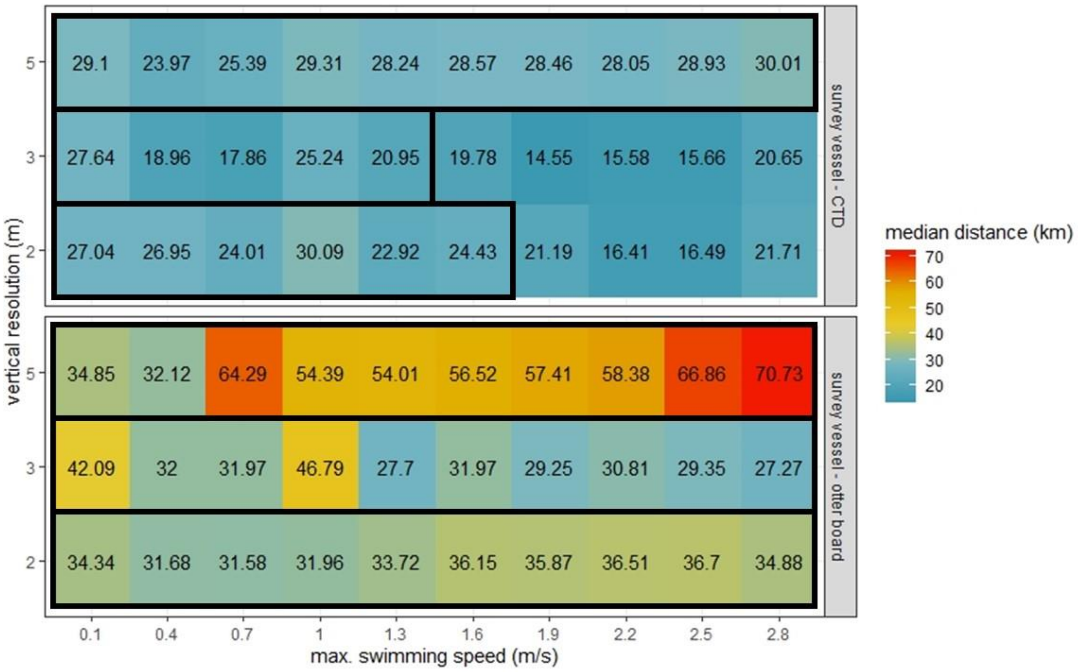

Figure S17: Median daily difference between known and reconstructed position from the temperature-depth data sampled with the DST attached to the CTD and otter board of the survey vessel. The black square indicates that start and end-position were not modelled correctly.

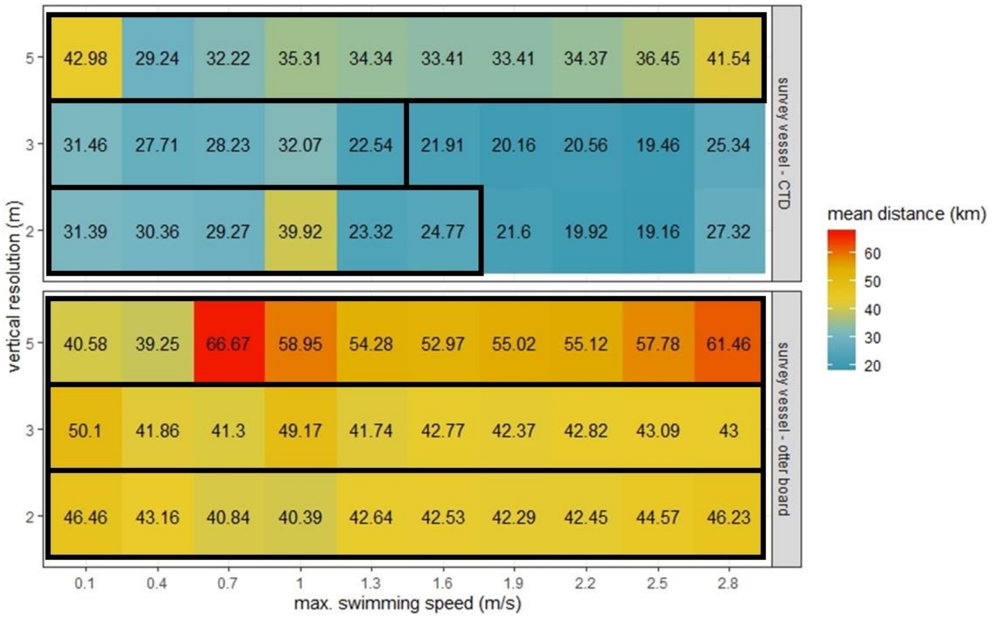

Figure S18: Mean daily difference between known and reconstructed position from the temperature-depth data sampled with the DST attached to the CTD and otter board of the survey vessel. The black square indicates that start and end-position were not modelled correctly.

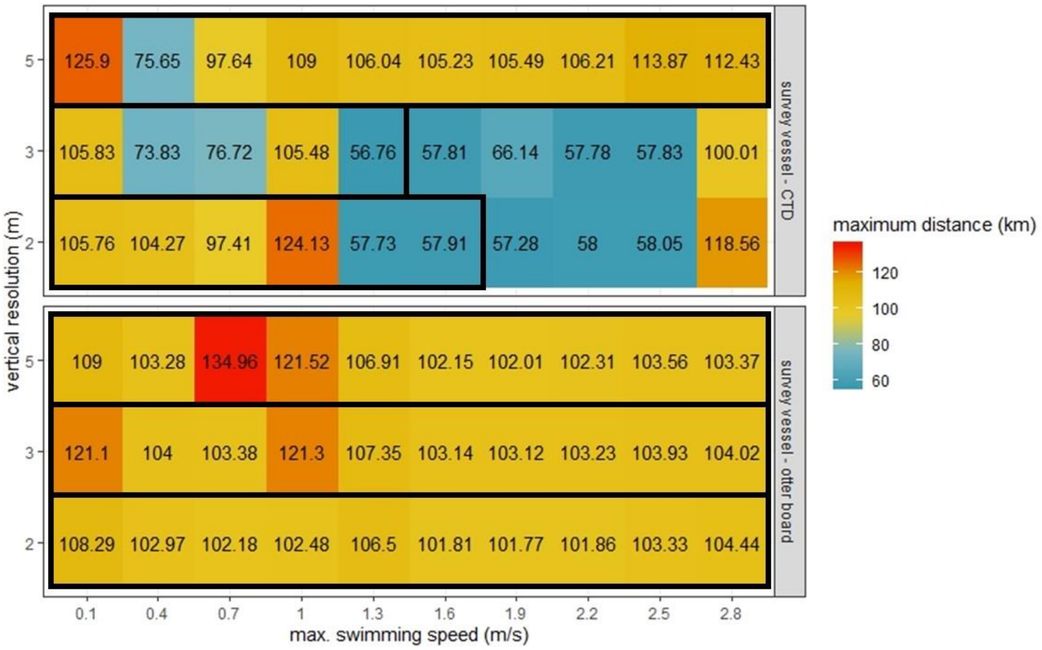

Figure S19: Maximum daily difference between known and reconstructed position from the temperature-depth data sampled with the DST attached to the CTD and otter board of the survey vessel. The black square indicates that start and end-position were not modelled correctly.

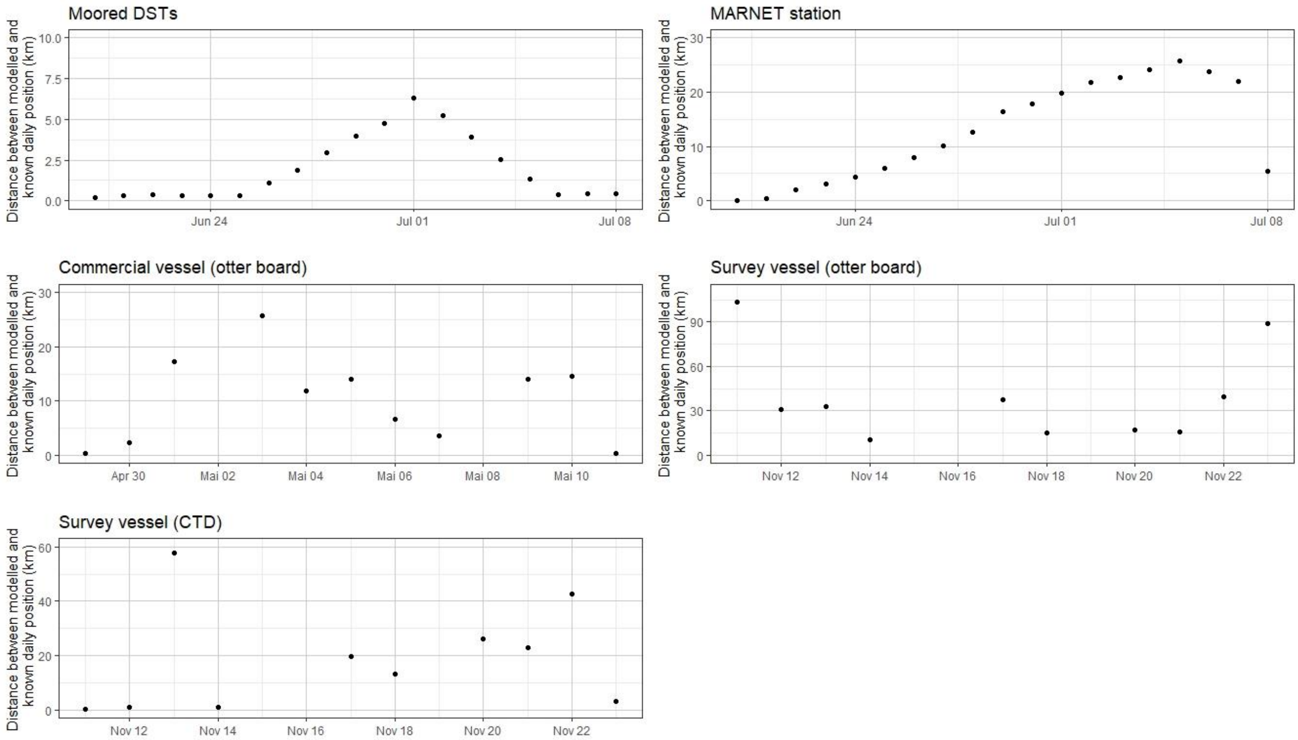

Figure S20: Distances between true and modelled daily positions. Mind the differences in the y-axis.

Table S1: Overview of the number of possible L_{TD} calculations for the different validation methods and scenarios.

Validation method	days of track	resolution	days with L_{TD}
artificial track: GER	367	2	227
		3	221
		5	199
artificial track: DK	367	2	291
		3	275
		5	252
artificial track: SWE	367	2	266
		3	256
		5	231
stationary track:	19	2	19
Moored DST		3	19
		5	19
stationary track:	19	2	19
MARNET station		3	19
		5	19
commercial track	13	2	7
		3	5
		5	5
survey track:	13	2	10
CTD		3	10
		5	10
survey track:	13	2	10
otter board		3	10
		5	10

Supplementary Material of Chapter 3

Movement of cod (*Gadus morhua* L.) in the Southern Baltic Sea: evidence from data storage tags

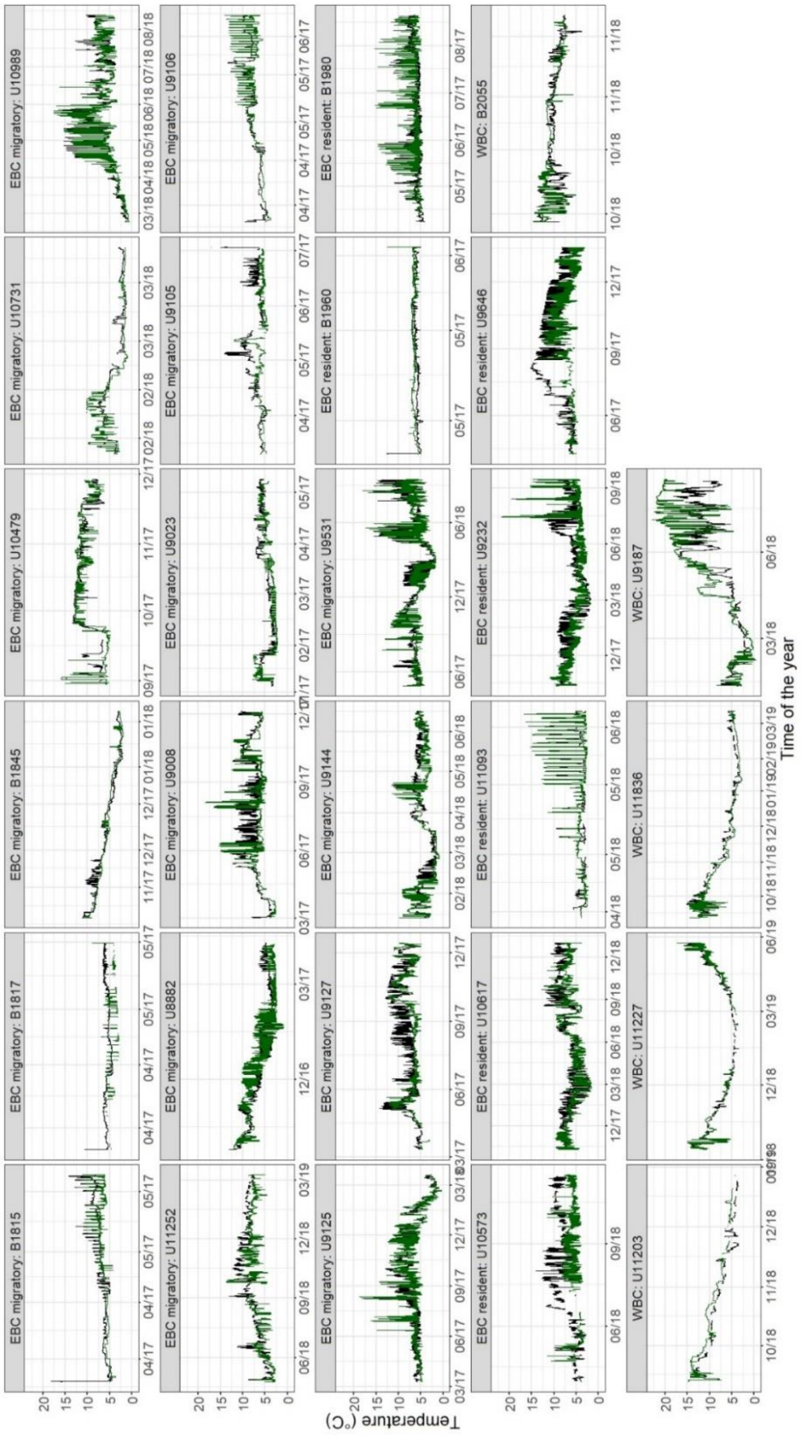

Figure S1: Temperature profile as recorded by the DSTs (black line) and estimated by the geolocation model (green line). ID U11227 was classified as Eastern Baltic cod by shape analysis but the temperature and depth profile suggested to reassign this individual to the Western Baltic cod stock.

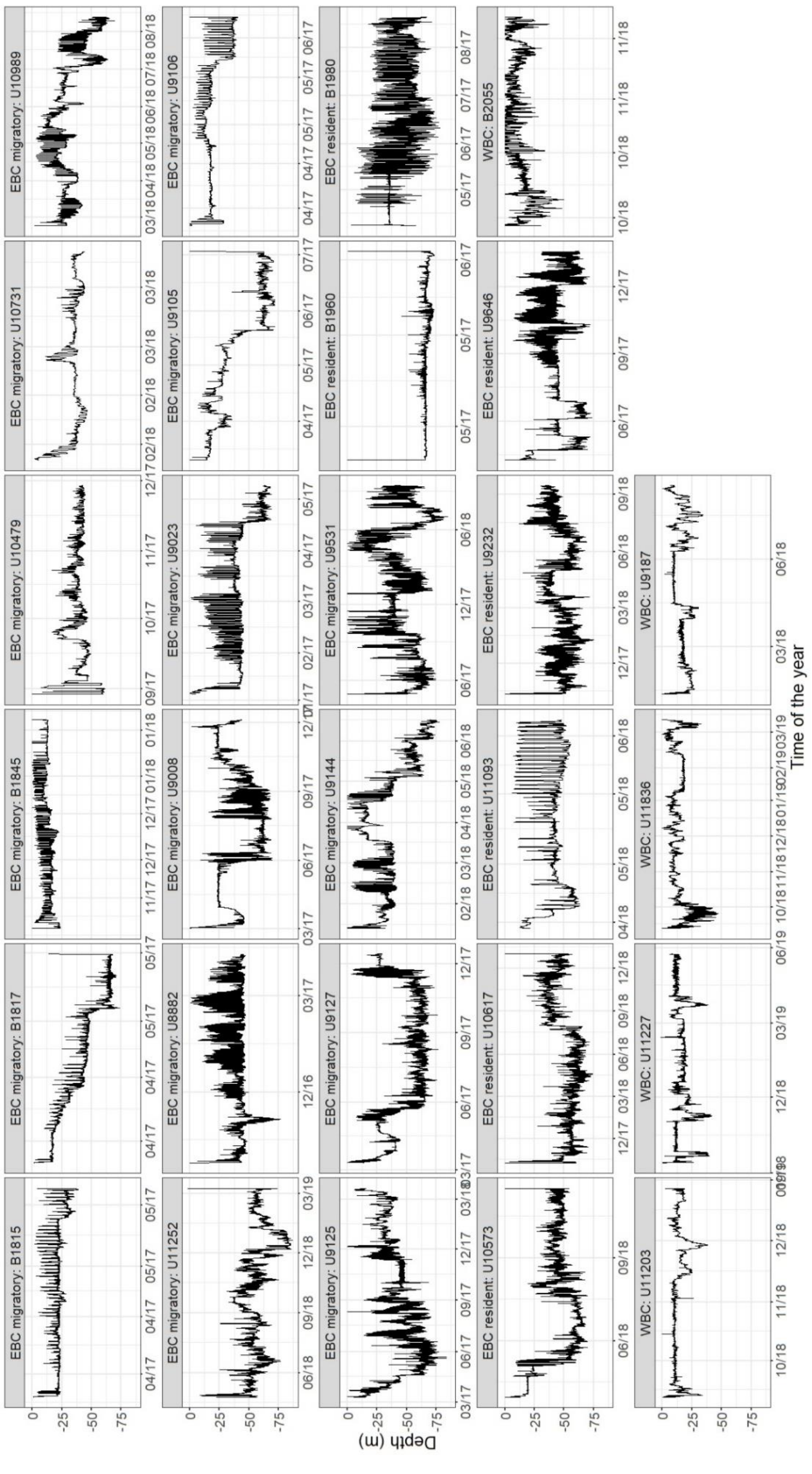

Figure S2: Depth profiles of cod tagged with DSTs in the southern Baltic Sea.

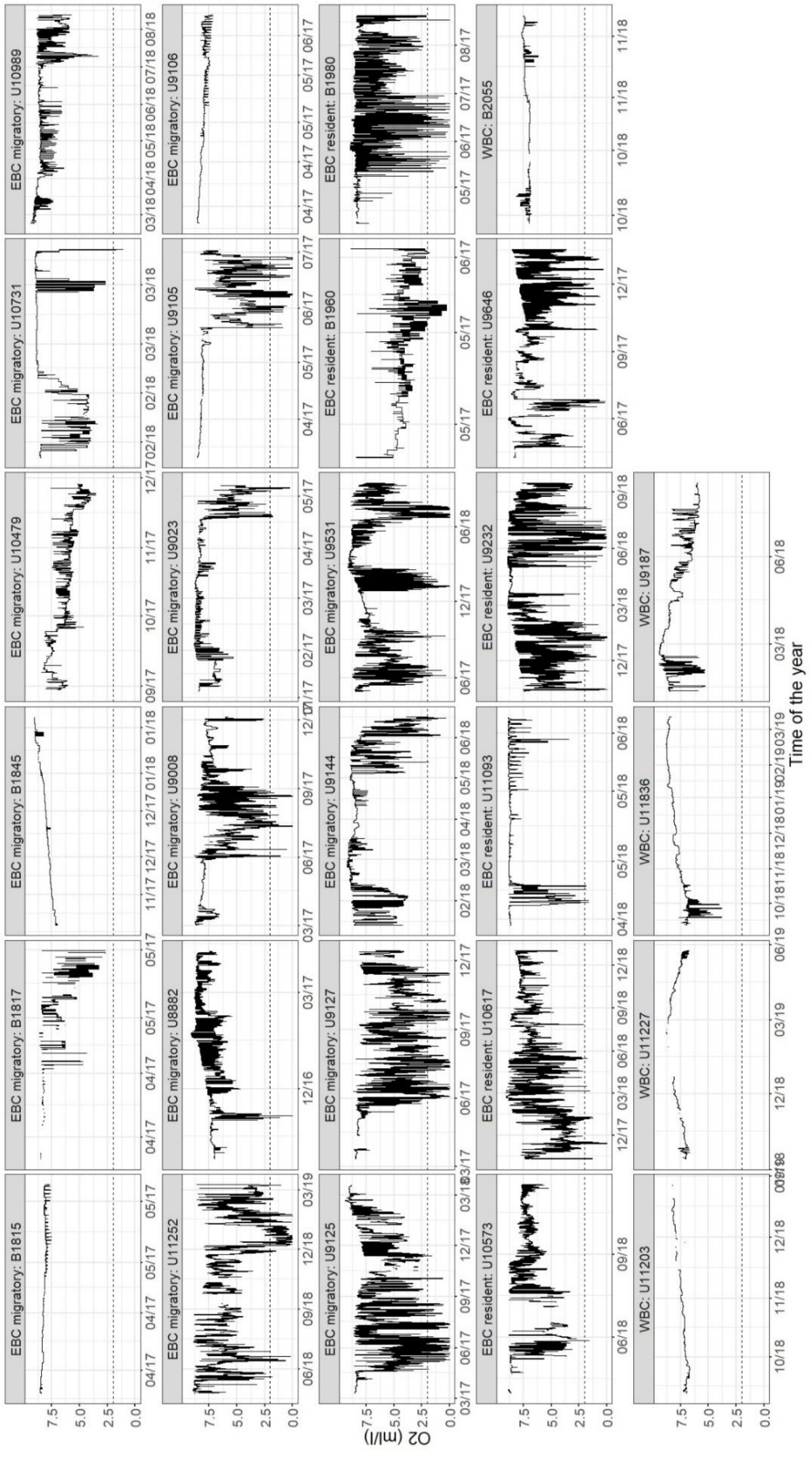

Figure S3: Oxygen content extracted from the Baltic regional ocean model based on the daily position and depth of cod tagged with DSTs (date format: mm/yy). The dashed line indicates the transition to hypoxic conditions (2ml/l).

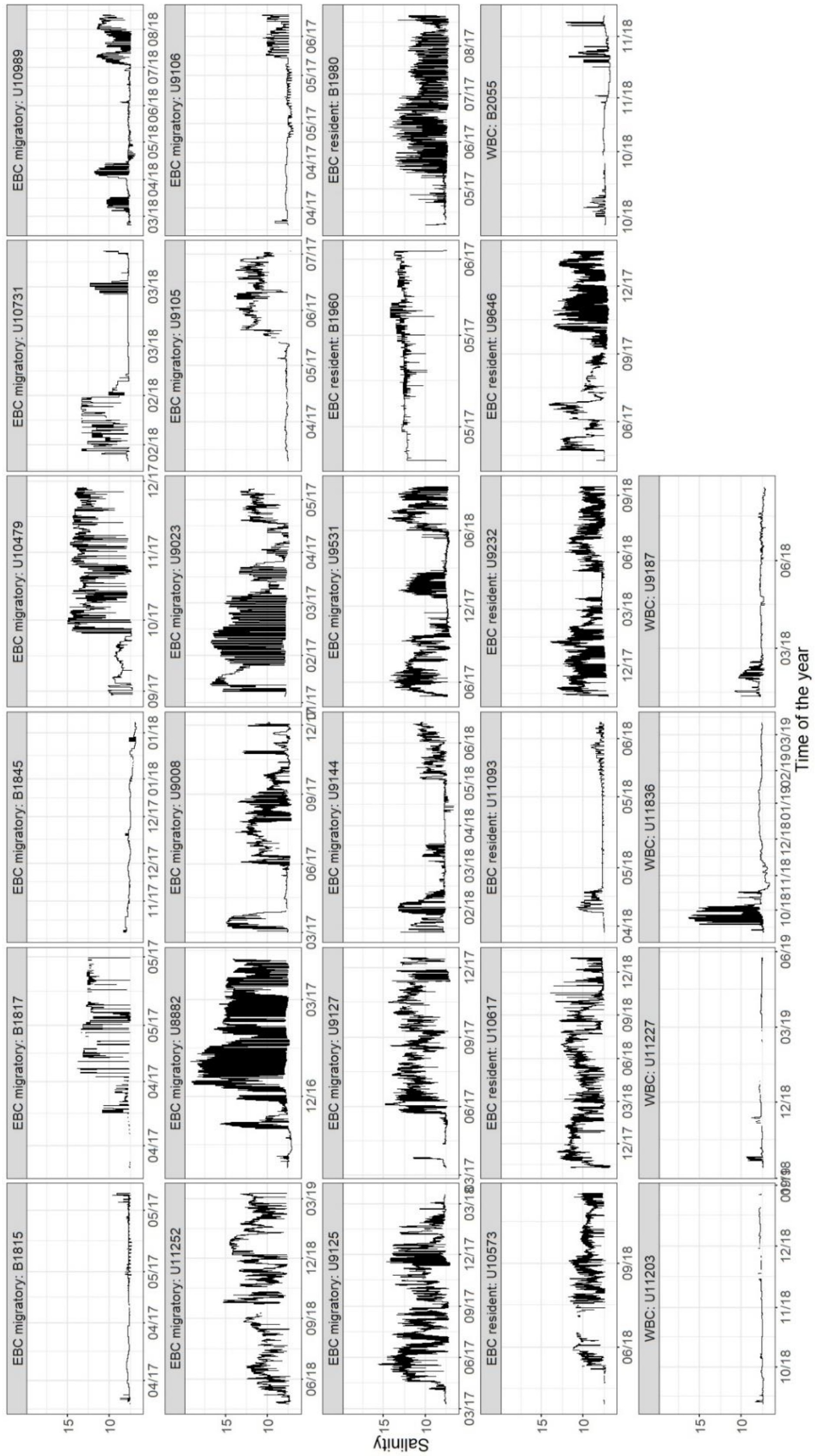

Figure S4: Salinity extracted from the Baltic regional ocean model based on the daily position and depth of cod tagged with DSTs in the southern Baltic Sea (date format: mm/yy).

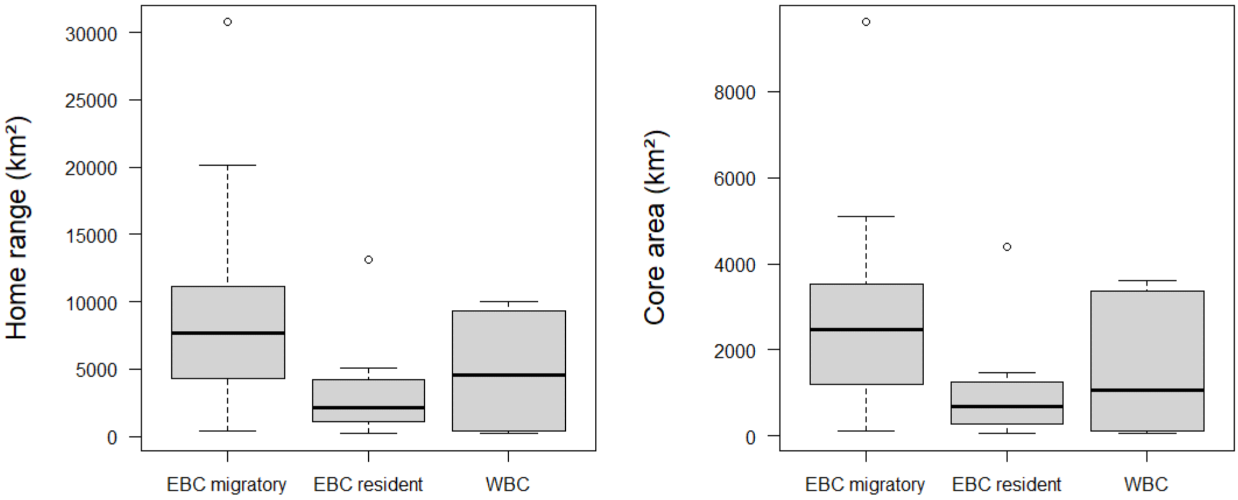

Figure S5: Home range (left) and core area (right) for the three behavioural modes EBC migratory, EBC resident and WBC.

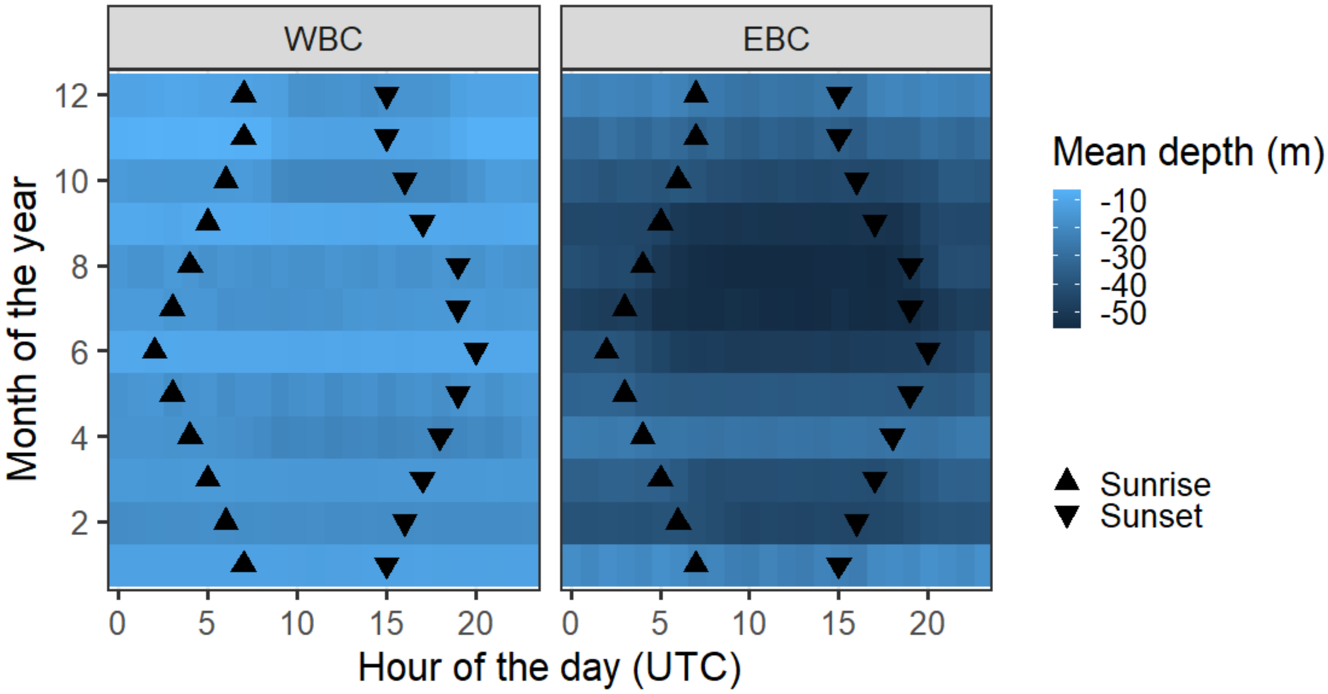

Figure S6: Mean depth per month and hour of the day separately for Western Baltic and Eastern Baltic cod. Since Bornholm was the area of gravity of the recaptures, the time of sunrise and sunset in Rønne (55.21 N, 14.97 E; www.timeanddate.com/sun/denmark/ronne-bornholm) was used and rounded to the nearest full hour for each month and indicated with arrows.

Supplementary Material of Chapter 4

Validation of otolith zone formation and otolith growth of adult wild cod (*Gadus morhua* L.) in the southern Baltic Sea through mark-recapture and tetracycline marking

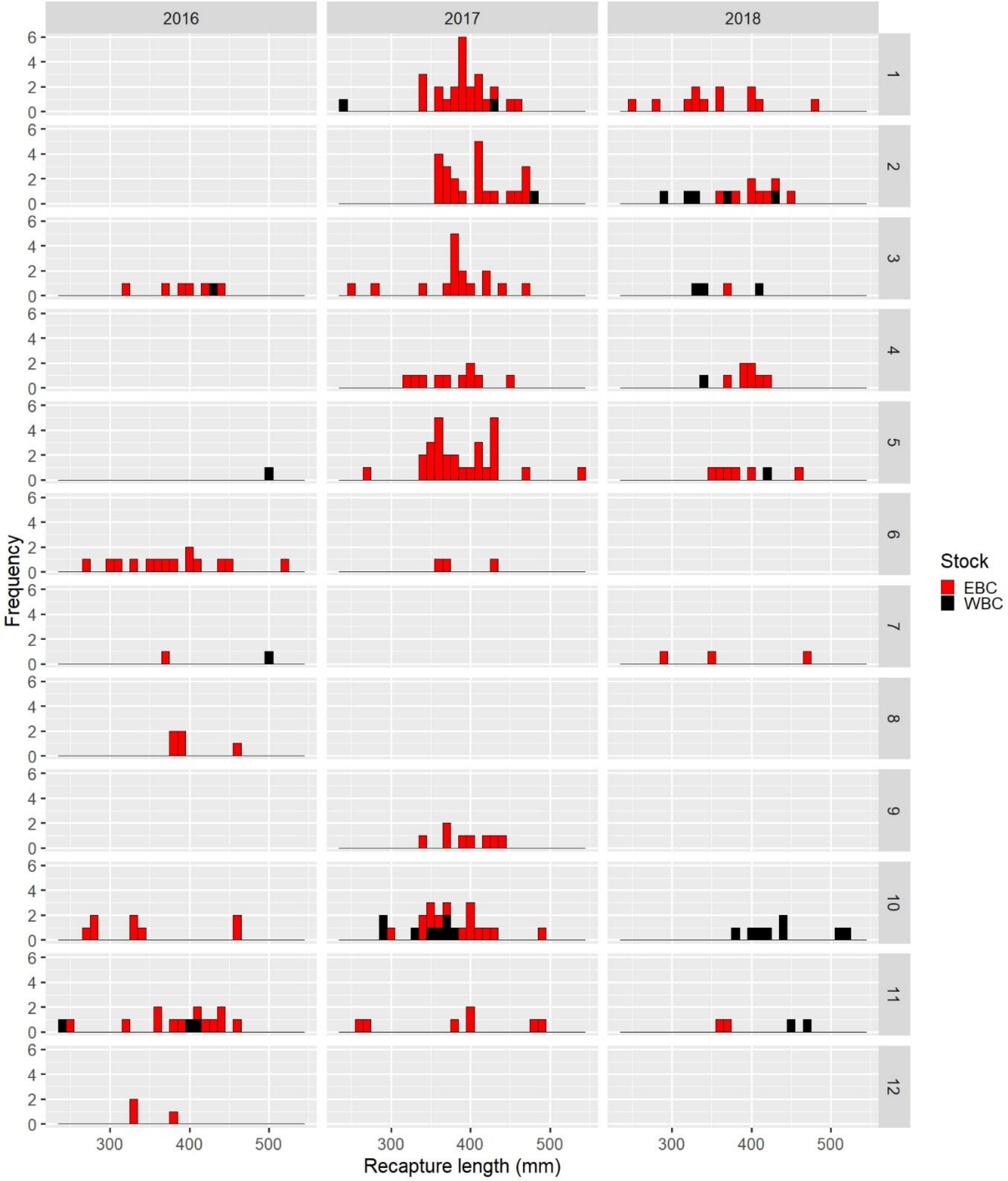

Figure S1: Length distribution at release of recaptured cod in the different months (1-12) and assignment to either the WBC or EBC stock.

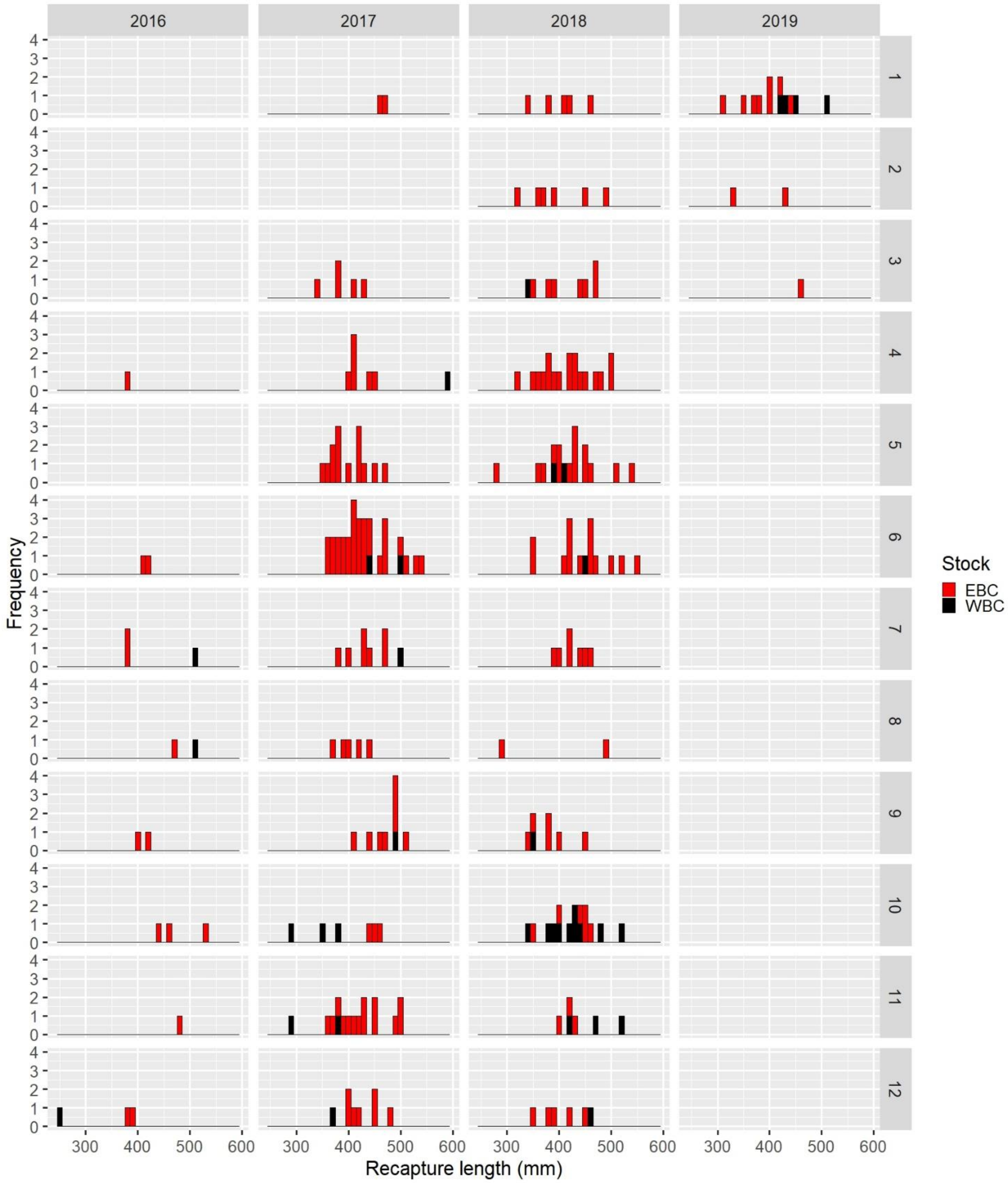

Figure S2: Length distribution at recapture of recaptured cod in the different month (1-12) and assignment to either WBC or EBC stock. The recapture length of individuals which were returned frozen were transformed by a shrinkage key (McQueen et al. 2019b).

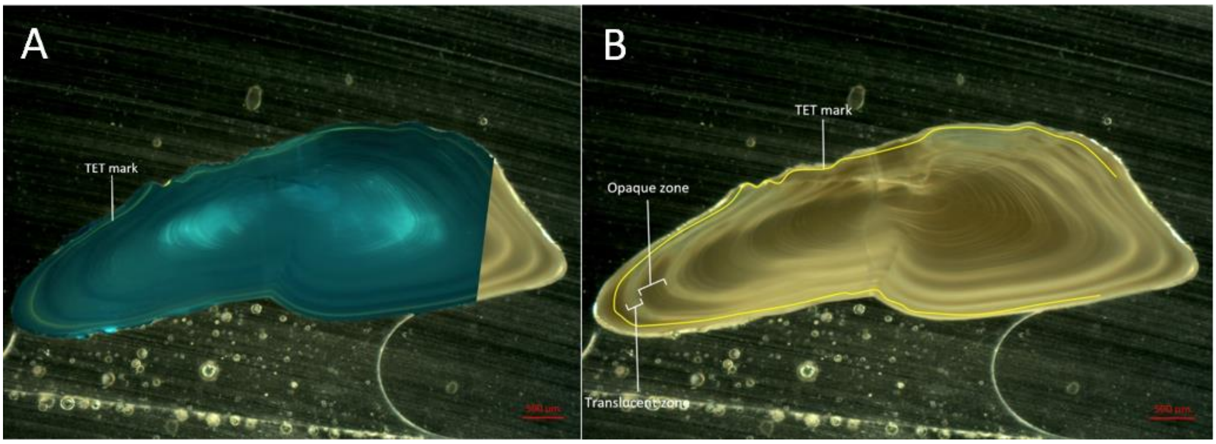

Figure S3: (A) Example of a cross section of an otolith taken under a fluorescent microscope overlapped to the same cross section taken under transmitted light to mark the TET mark. (B) Cross section of an otolith taken under transmitted light with one opaque and one translucent zone marked as an example, and with the TET mark. The release date was 17/03/2016 and the recapture date was 11/10/2016, the release length was 425 mm and the recapture length was 431mm, the DAL were 208 days.

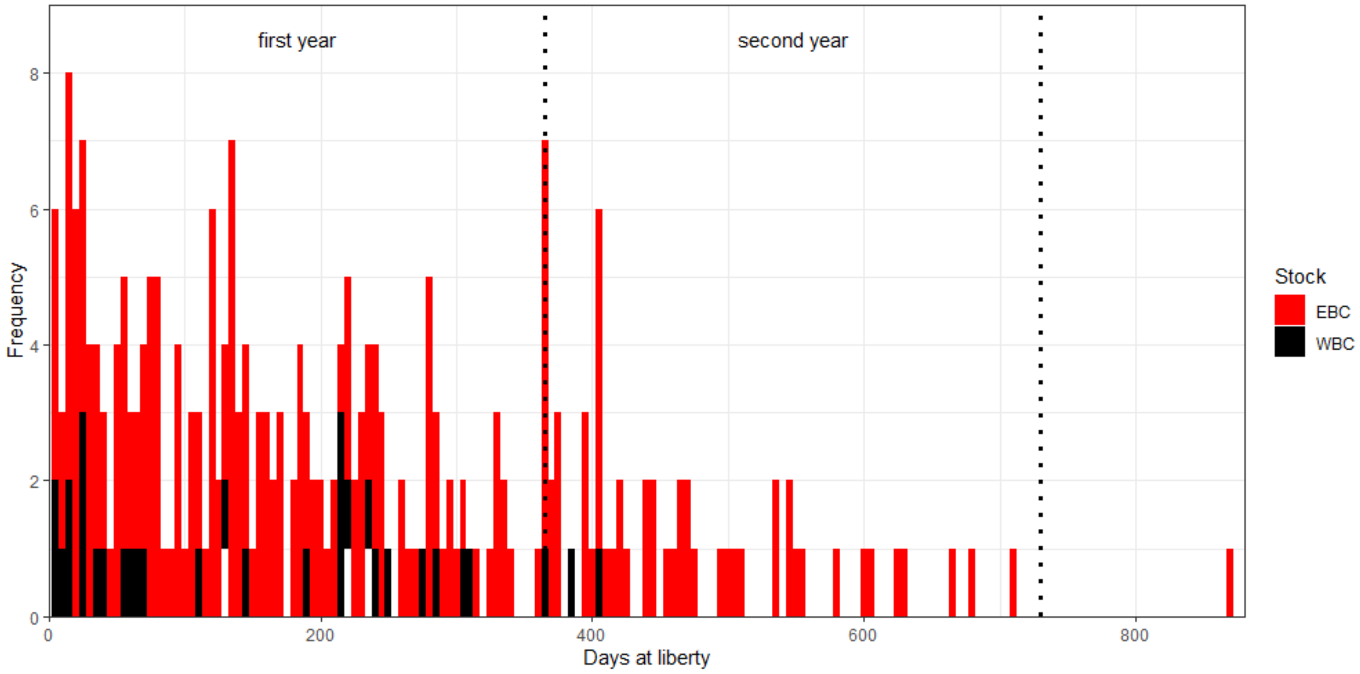

Figure S4: Distribution of the days at liberties of Eastern Baltic cod (EBC) and Western Baltic cod (WBC) stocks. Vertical dotted lines indicate the shift into a new year.

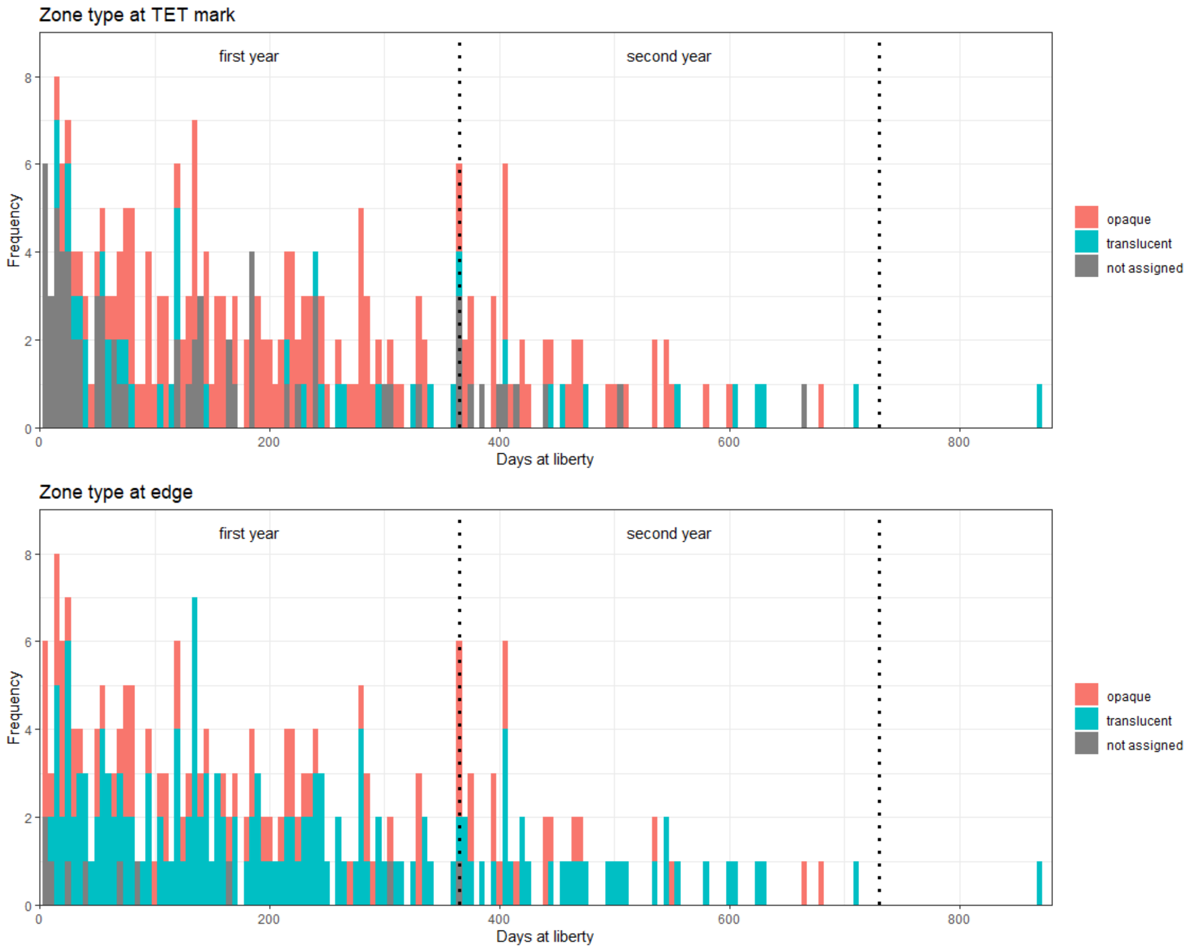

Figure S5: Number of otoliths were assigned to the zone types opaque and translucent or that could not be assigned at the time of release (=TET mark; n=179; above) and at the time of recapture (= at the otolith edge; n=247; below).

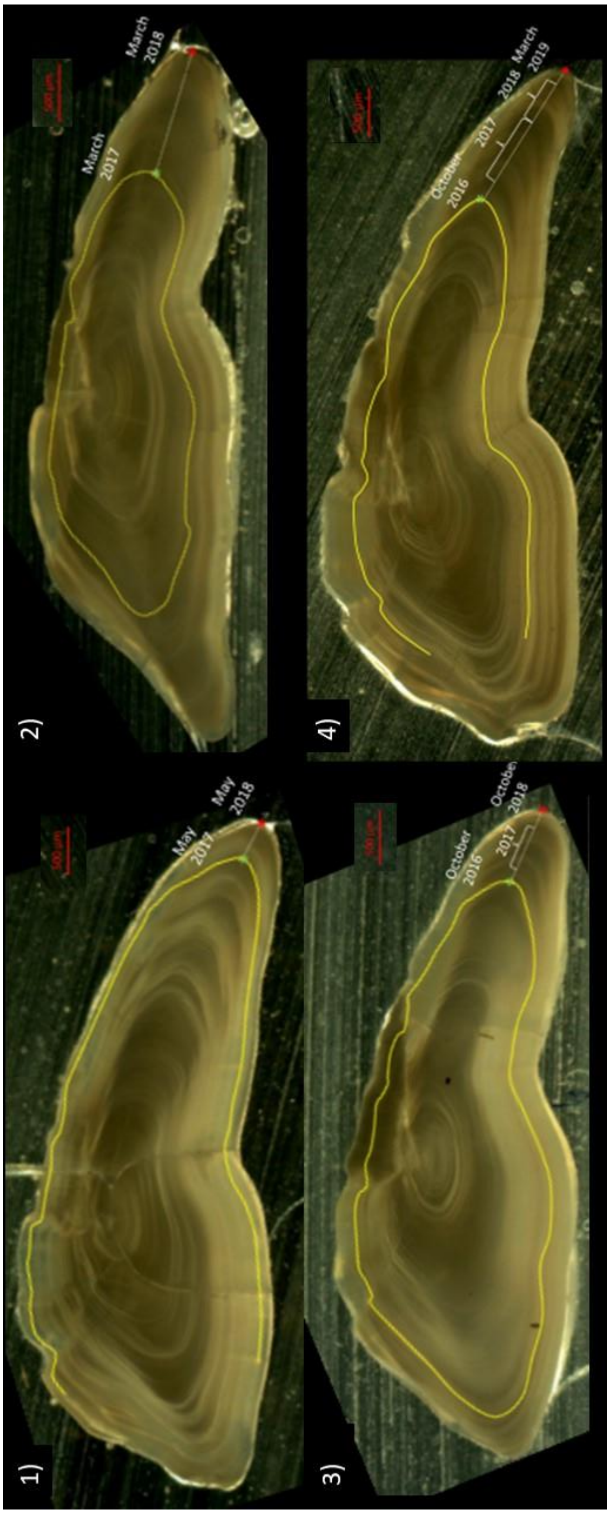

Figure S6: Cross section of otoliths from EBC at liberty for 364 (1) and 2)) and more than a year ((3) and 4)). The tetracycline-mark (yellow line) as marked on the image taken under the fluorescent filter is overlaid onto the image of the otolith viewed under transmitted light. Release (green star with month and year) and recapture (red star with month and year) are marked. The brackets in 3) and 4) indicate the approximation of one year of otolith growth with the corresponding year. Further information can be found in Table 2. Zoomed in otoliths can be found in Fig. 4.

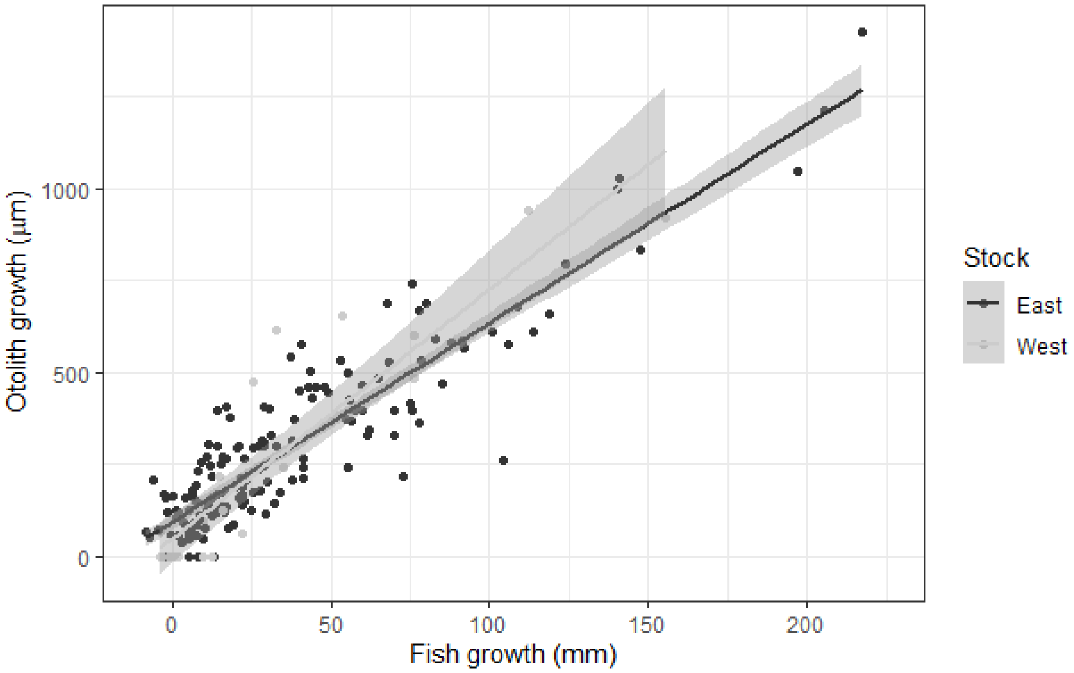

Figure S7: Otolith growth vs. fish growth after release with a linear model fitted with 95%-confidence intervals to the data (n=213) separately for Western Baltic cod (grey dots) and Eastern Baltic cod (black dots).

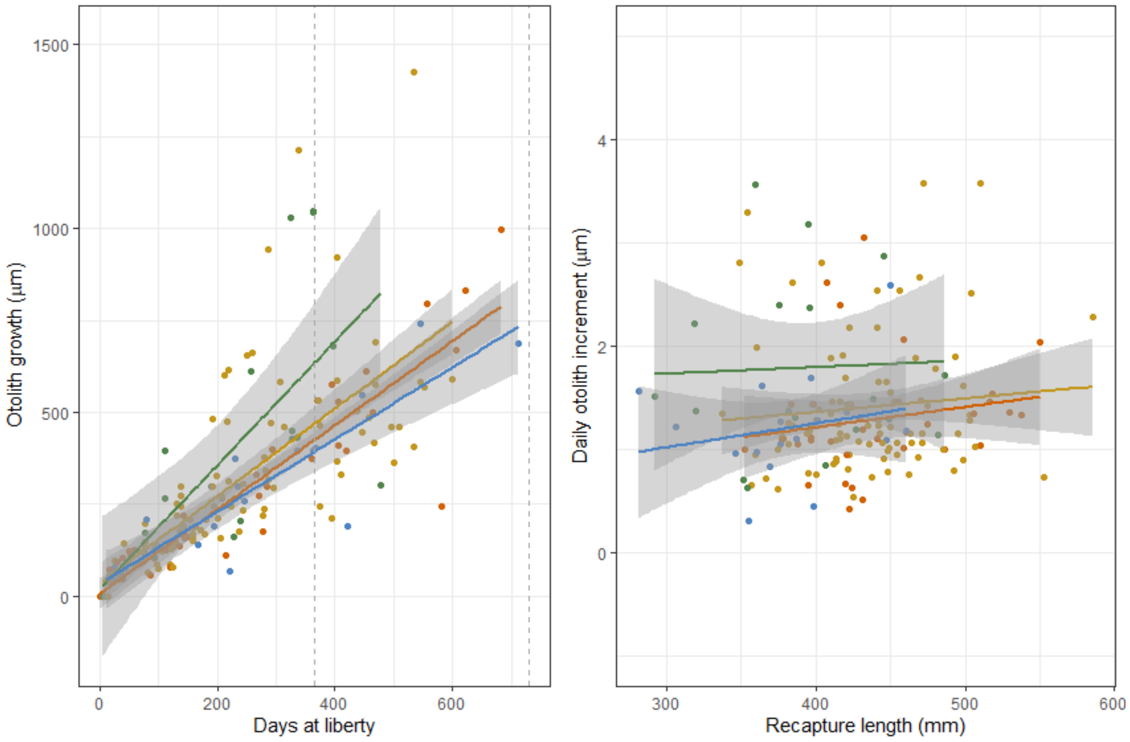

Figure S8: Otolith growth after release vs. days at liberty (left) and daily otolith increments vs. recapture length (right) separately for tagging countries (Germany: yellow, Denmark: orange, Poland: green, Sweden: blue). A linear model was fitted with 95%-confidence intervals (n=213).

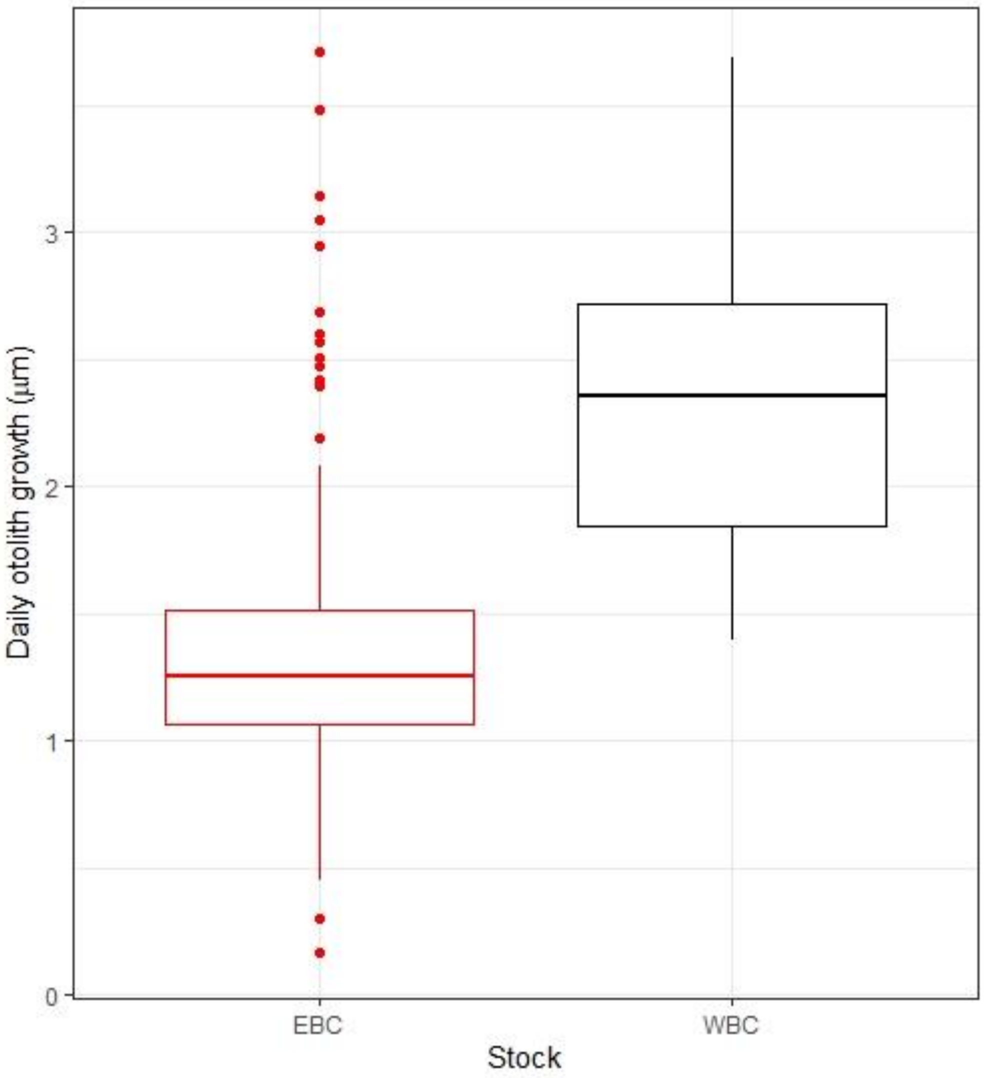

Figure S9: Comparison of daily otolith growth between Eastern Baltic cod (EBC) and Western Baltic cod (WBC) stocks for individuals with days at liberty >30 (n=175).

Table S1: Age distribution of Western Baltic cod at recapture. One of the otoliths which were not classified was broken, the other two had a low readability.

Age	Number
1	1
2	15
3	13
4	5
not classified	3