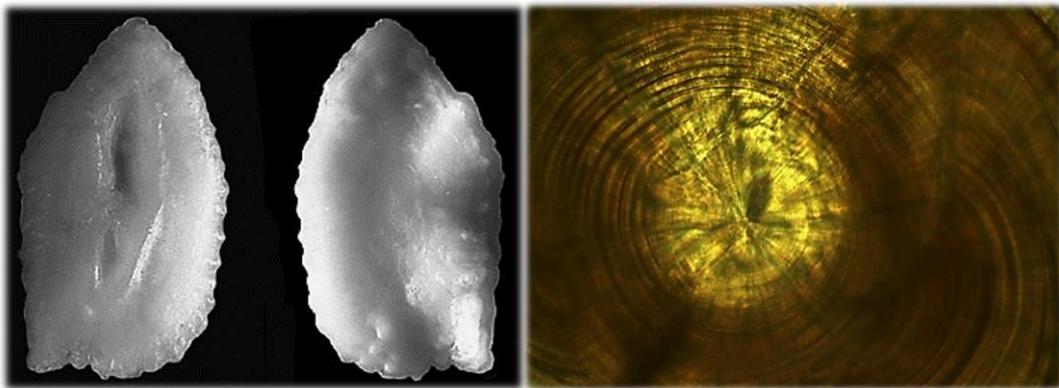


Seasonal variability in growth of 0-group European flounder at the Balgzand intertidal in the western Dutch Wadden Sea

GROWTH VARIABILITY IN 0-GROUP FLOUNDER *PLATICHTHYS FLESUS* AS REVEALED BY OTOLITH MICROSTRUCTURE ANALYSIS



Anne de Vries

HZ University of Applied Sciences
Delta Academy, Aquatic EcoTechnology
Bachelor Thesis Report
May 30, 2016

Netherlands Institute for Sea Research | Landsdiep 4, 1797 SZ Den Hoorn Texel

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A.N. de Vries (63183)

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Supervisor NIOZ: H.W. van der Veer

Supervisor HZ: A. van den Brink

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Mortelmans, n.d.; Gennadiy, 2015; Anne de Vries

Preface

This thesis is written as part of the completion of the bachelor Aquatic Ecotechnology, at the Hogeschool Zeeland University of Applied Sciences in Vlissingen, the Netherlands. The study program aims that water managers trained at the Hogeschool Zeeland have thorough knowledge and understanding of the supporting capacity of water and water systems, the ecological functions of water, the processes in water and their interrelationships. It focuses on water in broad terms and is unique in that students study marine systems, fresh water systems and the transitions between them. The programme is unique because it combines knowledge of water ecology with water technology.

Over the course of the study I came to know that my personal interest lies in the marine systems and how they function from an ecological perspective. When looking into Bachelor thesis topics in this area I found the research done at the Netherlands Institute for Sea Research (NIOZ) in the Wadden Sea on species interactivity and their interaction with the environment. Henk van der Veer has done a multitude of studies in the area and forwarded a subject on otolith microstructure analysis. With analysing the seasonal growth pattern of 0-group European flounder this research was built on previous work done here at the NIOZ on the functionality of an intertidal flat in the western Dutch Wadden Sea.

Before this thesis I had no knowledge about otoliths and little on individual growth in combination with Dynamic Energy Budget (DEB) models. At the completion of this report, on top of improving skills in setting up research, executing the plan and data analyses, I have now a thorough understanding about otolith analysis for aging fish, have gained knowledge in the workings of DEB simulations and how to manage an extensive dataset.

Now at the end of my stay here at Texel I can truly say that this has been an incredible personal and professional experience. Working with the many experts and student colleagues in a multidisciplinary setting was an eye opener on different interests, motivations, the work that is done currently and the extent of the many things still to be discovered in our world.

My sincere thanks goes out to my NIOZ supervisor Henk van der Veer whom has helped me throughout my stay at Texel with looking critical at my own work, and my HZ supervisor Anneke van den Brink for helping in all matters school related and guiding me through the bachelor thesis process. At the start of my thesis I was an unexperienced otolith microstructure analyst but with the help of Hans Witte I can now see myself as a starting expert in this field. Finally I want to thank Sarina Jung and Joana Cardoso for their help with the R scripts used in this research.

't Horntje Texel, the Netherlands, 19th of May, 2016

Anne de Vries

Summary

The coastal Wadden Sea system is an UNESCO World Heritage site with importance from both the human as well as wildlife perspective. The Wadden Sea is an ecotone forming a transition between open sea and the main land with a multitude of associated biotopes. The exceptional natural environment is the main driver for the tourism sector in the area. The Wadden Sea has a high productivity, which is a prerequisite for its function as turntable on the East Atlantic Flyway, providing a residence for maximum 6.1 million birds at the same time. The high productivity also forms the basis for recruitment of commercial and non-commercial North Sea fish species. European flounder and plaice are two of the fish species that uses the Wadden Sea as nursery area. The European flounder is a common fish species mainly of interest to fisheries in Baltic and Danish waters.

Population size increase from sampling campaigns at the Balgzand intertidal in the western Dutch Wadden Sea suggested a growth decrease in both plaice and flounder populations during summer months. Research performed at the Netherland Institute for Sea Research (NIOZ, Texel) into the seasonal growth of 0-group plaice, *Pleuronectes platessa*, confirmed this suspicion. The 0-group are those individuals that are in their first year of life. The years that were compared, 1995 and 1996, had contrasting winter temperature regimes. However, as both years showed summer growth reduction it is suggested that summer benthos activity might be the underlying mechanism. To further investigate the extent of this suspicion the seasonal growth patterns of 0-group flounder was researched.

Samples were taken on a grid of 36 stations using a 1.9m beam trawl (1 ticker chain, net mesh size of 5x5mm) at frequent intervals (2 to 4 weeks) from February to August in 1995 and from March to August in 1996. In the laboratory, samples were sorted by species within a few days, whereby all individuals were measured to the nearest mm total length and fixated in 96% ethanol afterwards. Per sampling campaign around 100 individuals of different size classes (mm) were selected in relation to their abundance. Otoliths were removed from the fish, fixed to microscope slides and daily growth increments were analysed using a light microscope. There are two zones of daily growth increments considered for this research. The inner zone are those increments that have formed since the hatching in the North Sea up till the metamorphoses and settlement of the juveniles in the Wadden Sea. The outer zone are those that have formed since settlement up till catch. The observed growth of the 0-group flounder population was compared to DEB simulations that show maximum potential growth based on prevailing environmental conditions.

Results show that water temperatures were much lower in February and March of 1996 compared to 1995 (ANCOVA, $p < 0.05$). Larvae settled about 4 weeks earlier in 1995, densities of the entire 0-group population was higher in 1995 compared to 1996, however, peak densities of newly settled flounder was about the same in both years ($\pm 200 \text{ ind./}10^3 \text{ m}^2$). The growth curves of 1995 and 1996 seem to be different from each other. Comparing observed growth to predicted growth from the DEB models it is seen that individuals in 1995 were able to reach maximum potential growth (realized growth ratio (RGR) over time ± 1), while in 1996 observed growth is even below the male DEB prediction line (RGR average max 0.7).

Data analysis shows that the 1995 DEB model is a good fit with the observed growth while in 1996 it is not. A possible explanation for the low observed growth compared to DEB average in 1996 is a skewed sex ratio in summer. This can, however, not be proven as sex was not determined in this research. A DEB sensitivity test validated that trends do not change independent of changing initial settlement length, which was thought to be an overestimation in the first DEB model. Therefore, it can be said that the upward trend in RGR of 1996 suggests that individuals were not able to reach maximum potential growth in the beginning but over time were moving more towards the predicted growth.

The differences seen between the plaice and flounder study might be explained by species specific factors such as settlement habitat selection of which it is known that juvenile flounder inhabit the more silty tidal flat systems. Movement variation between size classes in flounder along abiotic factor gradients such as salinity, temperature variations and depth, might also explain the differences. Lastly, it could be related to differences in prey preferences.

All in all, it can be said that for European flounder at the Balgzand summer growth reduction does not take place. Taking all findings of the plaice and flounder research into account the underlying mechanism remains elusive, but it is still suggested that the difference lies in the composition and activity of the benthos over time at the Balgzand intertidal flat. Further research into this topic should shed light on the underlying mechanism of population size decrease in the summer months.

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1. Introduction

Coastal and shelf ecosystems are among the most important marine ecosystems from the human perspective and are intensively used worldwide. The areas provide many opportunities for anthropogenic developments, consequently causing conflicting interests and changes at all trophic levels in coastal and shelf ecosystems all over the world during the last centuries and especially decades. Currently the Wadden Sea food web lacks species and complexity compared to the desired rich environment as defined based on the past (Lotze, 2005). Therefore, the Dutch government initiated the ambitious goal and program “Towards a rich Wadden Sea” (Programma Naar Een Rijke Waddenzee, 2010) to improve the present status of the Wadden Sea and to re-establish a healthy Wadden Sea, rich of species, with lots of mussel beds and sea grass areas by 2026 (Waddensleutels magazine, 2015).

The Wadden Sea is an important nursery area for both commercial and non-commercial fish species (Zijlstra, 1972) and the expectation has always been that growth conditions would be optimal for young fish (Zijlstra, Dapper, & Witte, 1982; Van der Veer & Witte, 1993; Van der Veer, 1986). However, growth analysis of 0-group plaice *Pleuronectes platessa* in the Wadden Sea, using population size increase validated by otolith microstructure analysis, indicated indeed optimal and maximum growth in the beginning of the season, but a slowing down during the summer months (Van der Veer, Freitas, Koot, Zuur, & Witte, 2010; Freitas, Van der Veer, & Kooijman, 2012; Cardoso, Freitas, de Paoli, Witte, & van der Veer, 2016). The 0-group fish are individuals that have not yet completed their first year of life.

Besides juvenile plaice, juvenile flounder also occur in the Wadden Sea, however, they have a more restricted distribution compared to juvenile plaice and inhabit the more silty tidal flat systems (De Vlas, 1979). In juvenile flounder, population size increase suggests a similar phenomenon with a growth decrease during the summer months (Freitas, Van der Veer, & Kooijman, 2012). However, it cannot be excluded that this trend resulted from the migration of the larger flounders out of the shallow and silty parts of the Balgzand Wadden Sea area. Growth analyses based on length-age relationships established by otolith microstructure analysis of individual fish do not suffer from such methodological problems and offers an independent unbiased check.

This project ties into the research about the functioning of the marine populations and communities by focussing on a more specific part of the ecosystem: the growth of juvenile flounder. The aim is to find out whether summer growth reduction is a general phenomenon occurring not only in juvenile plaice but also among various species (gobies, shrimps, shore crabs) in the western Dutch Wadden Sea, starting with the flounder research presented in this report.

The main question in this research is “Does summer growth reduction occur annually in young 0-group flounder in the western Dutch Wadden Sea?”, whereby the following aspects are addressed:

1. What are the length-age relationships over time established by otolith microstructure analysis of individual fish for 0-group flounder?
2. Are length-age relationships different among years?
3. What are the growth patterns based on the length-age relationships and do they reflect maximum possible growth as predicted by a dynamic growth model (DEB)?

For this research 0-group flounder was collected from the Balgzand during two years, 1995 and 1996 with a contrasting temperature regime. Water temperatures in spring in February and March were much lower in 1996 than in 1995. By using these contrasting years growth pattern of flounder under different temperature regimes could be analysed.

The purpose of this thesis report is to provide evidence for acquiring the study competences for the study Aquatic Ecotechnology at the HZ University of Applied Sciences. Therefore, this scientific report is more than the general scientific report layout. The report starts with background information on the internship provider NIOZ, a description of the Wadden Sea to give an idea about the importance of this area and is followed by information about the European flounder. Before continuing with the general scientific report layout the uses of otoliths and how the otoliths are analysed for research purposes is discussed.

2. Thesis Internship Provider NIOZ

2.1 History

With the founding of the Dutch Zoological Society in 1872 coordinated research on animal life at sea began. The society owned a dismantlable building which would be used as field station until 1890. Generally no more than five people were at work here, including the first directors, who would carry out their research unsalaried. In 1877 the building was stationed in Vlissingen from where a schooner departed for the first scientific cruise to the English coast and Helgoland. During this cruise the five crew members collected marine animals with a 'schrobnet'. In 1890 a permanent building was opened for the Zoological Station in Den Helder.

From 1931 onwards the Dutch government financially supported the Zoological Station. This significantly strengthened its ties with the biological faculties of universities by organizing student courses. Unfortunately the economic crisis set a limit to the expansion. After the Second World War the workforce expanded with researchers on temporary positions. The scope of research was widened after director Verweij submitted a proposal in 1957 to include chemistry, physics and geology. In 1960 the Zoological Station was renamed the 'Netherlands Institute for Sea Research'. A year later the first issue of Netherlands Journal for Sea Research was released. As the institute expanded the accommodation became too small for the 150 employees. Besides, Den Helder had a limitation of sea water intake. Therefore, in 1969, the NIOZ moved to the 'provisorium' a temporary housing in polder 't Horntje at the island of Texel opposite Den Helder and only in 1977 the new building was opened. Since 1990 NIOZ is part of NWO (Dutch Organisation for Scientific Research) and has been disincorporated from the Dutch Zoological Society. On the 1st of January 2012 NIOZ merged with the former NIOO-CEME (Centre for Estuarine Marine Ecology) in Yerseke. As a consequence, NIOZ has now two different locations from which they operate, Texel and Yerseke. Each location has their own research group(s) and focus areas.

2.2 Mission and financial support

The mission of NIOZ Royal Netherlands Institute for Sea Research is to gain and communicate scientific knowledge on seas and oceans for the understanding and sustainability of our planet (NIOZ, 2015). Its task is also to facilitate and support marine research and education in the Netherlands and Europe.

NIOZ is owned by the NWO and since the reorganisation in January 2016 Utrecht University is co-shareholder (Figure 1). After the reorganisation especially the salary costs have been reduced. There are several sources of annual income for the NIOZ. The main three are approximately 14 million euros basic funding by the NWO, more or less 16 million from the free market by open competition for projects and now also 2.4 million from the Utrecht University. The basis funding of NWO is fixed whereas project money can fluctuate depending on the projects being brought in. The funding by Utrecht University is solely for research.

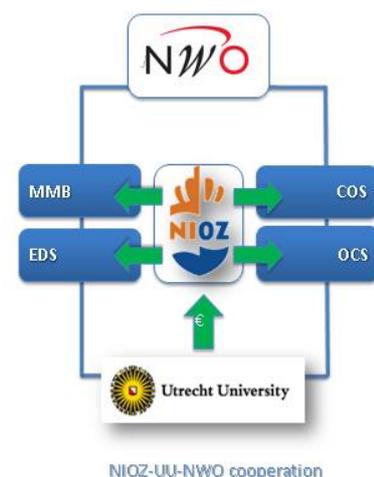


Figure 1: The structure of the different shareholders, NIOZ and main stream of subsidies.

2.3 NIOZ departments

From January 1st 2016 a reorganization was set in motion as a result of the financial situation at NIOZ. Costs increased 2% per year in the last decade, without an inflation correction from the basis subsidy by NWO. The North Sea ship Pelagia became more expensive to maintain and influx of external project with additional funding declined. With the reorganisation a change in the department structure was realized in which different departments merged together. For example, the old Department of Marine Ecology, under which this flounder project originally grouped, merged with part of the Department of Physical Oceanography into the newly established multi-disciplinary department Coastal systems.

Coastal Systems (COS) mainly focuses on key physical, chemical and biological processes that determine the productivity and ecological functioning of coastal, open marine areas, especially in terms of population dynamics of higher trophic levels. For example, the role of coastal systems as fuelling stations for migrating birds and nursery and feeding areas for fishes, marine mammals and other species. The principal goal of COS is to understand how these generally highly productive systems function, and how changes in trophic interactions, in response to natural and anthropogenic environmental changes in hydrological, morphological and environmental regimes, influence the richness and productivity of coastal marine ecosystems and the ecological functions they provide. To realize this goal a combination of field observations, experiments and modelling activities are used.

Besides COS there are three other departments. Ocean Systems (OCS) aims to investigate open ocean processes ranging from physical oceanography at multiple scales to chemical oceanography, and deep-sea ecology to palaeoceanography and paleoclimatology. The oceans are studied through a variety of scientific disciplines of experiment and data collection during sea-going oceanographic research as well as through home-based laboratory experiments and analysis. The involved scientists rely on modern observational techniques including moorings, landers, thermistor strings, trace-metal clean samples, and novel underwater autonomous observations.

Estuarine and Delta Systems (EDS), currently the only department housed in Yerseke, researches the understanding of the complex interactions of organisms and dynamic estuarine and deltaic environments in the context of natural and anthropogenic induced environmental changes. This entails among others the analysis of how the environment is shaped by 'ecoengineering' organisms (including the 'sea' floor), how biological, physical, chemical and morphological processes and fluxes affect general ecosystem functioning, and how these interactions shape community structure and biodiversity in estuarine and deltaic systems. As in the other departments the approach is multidisciplinary, the departments key-strength is the integration of observational, experimental and modelling approaches, covering the disciplines ecology, (deltaic and coastal) hydrodynamics, biogeomorphology, physics and chemistry.

The dark ocean, a remaining terra incognita in terms of microbial diversity, is one of the focal points of the Marine Microbiology and Biogeochemistry department (MMB). The department performs detailed studies on microbial communities in the marine environment by field and experimental approaches. Using a variety of techniques including modern genomic techniques MMB studies the diversity, physiology and activity of prokaryotes (archaea and bacteria), viruses and phytoplankton in diverse marine environmental settings. Lipids produced by eukaryotic phytoplankton and prokaryotes are used in reconstruction of environmental and climate parameters such as marine productivity and sea surface temperature in the geological past.

2.4 Organization structure

NIOZ employs about 200 people permanently. Temporary employees included make this 300, at Texel 225 and Yerseke 75. The scheme below (Figure 2) shows how NIOZ is organized. The management

team supervises the different activities of the institute. The team consists of the NIOZ managing director, who also acts as the department head of the GES (General Support), the department heads of the research groups, department head of NMF (National Marine Facilities) and department head of FFI (Finance, Facility and Information). Generally the department head of the research groups further communicate to the employees in their department, the scientists and assistants. The intern students, PhD students and Postdocs are supervised by a scientist. Supporting departments are ICT, Data Management, HRM (Human Resource Management) and Facility Management.

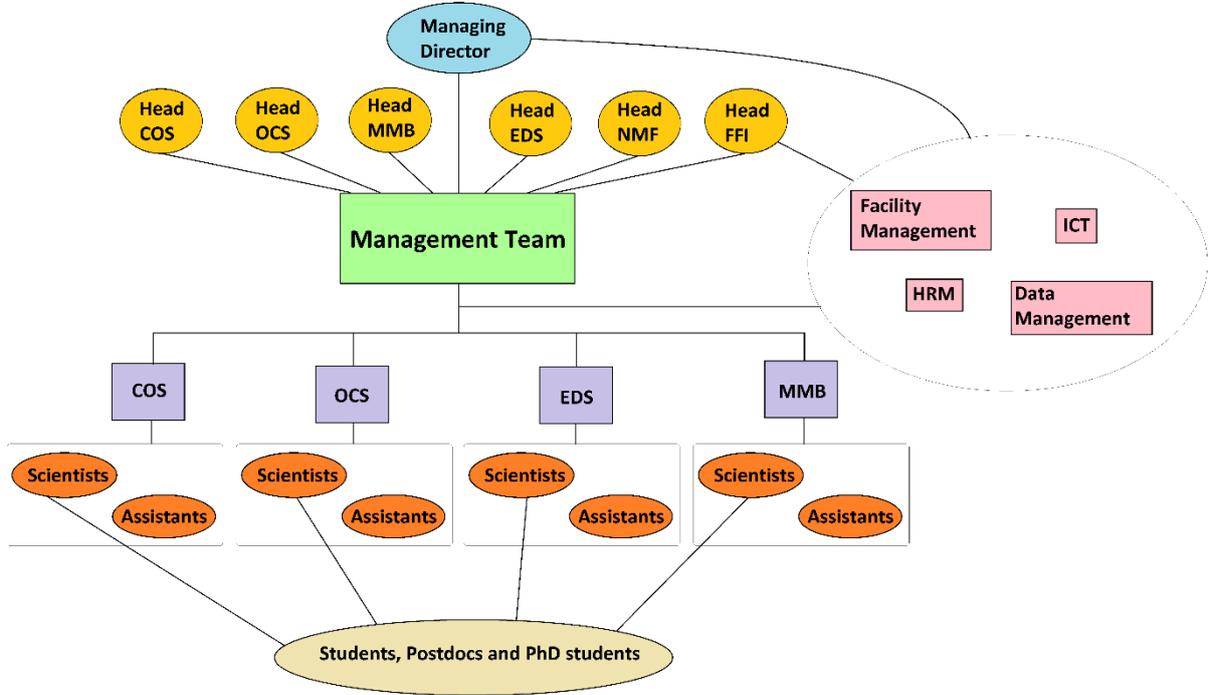


Figure 2: Main organisation structure of NIOZ.

3. The Wadden Sea

Covering 1.143.403 ha of World Heritage property along the coasts of three countries in the south-eastern part of the North Sea makes the Wadden Sea the largest unbroken system of intertidal sand and mud flats in the world (UNESCO, n.d.). It stretches over a distance of about 500km from Den Helder in the Netherlands to Esbjerg in Denmark (Figure 3). The Wadden Sea is shaped by the intricate interactions between physical and biological factors. These form the large, temperate, relatively flat coastal wetland environment with its multitude of transitional habitats such as tidal channels, sandy shoals, mussel beds, mud flats, sea-grass meadows, sandbars, salt marshes, estuaries, dunes and beaches.



Figure 3: The Wadden Sea with indication of Den Helder and Esbjerg by red dots. (Wadden Sea, n.d.)

3.1 History

The Wadden Sea as we know it was formed during the last glacial period, also known as the last Ice Age. The Ice Age lasted from approximately 110.000 to 12.000 years ago. During this period Canada was nearly completely covered by ice as well as the northern part of the United States and Eurasia. Some areas such as Alaska remained ice free due to arid conditions. The Arctic Ocean between the ice sheets of America and Eurasia was not frozen throughout (Spielhagen, et al., 2004). More likely is that it was only covered by relative shallow ice, subject to seasonal changes.



Figure 4: Europe during its last glaciation, about 20,000 to 70,000 years before present, in northern Europe. The North Sea is almost completely dry. (Jose, 2006)

As a result of the massive ice sheets coverage in the northern hemisphere during the coldest phase of the last Ice Age, 20.000 to 18.000 years ago, the North Sea was almost completely run dry (Figure 4) (Reise, 2013). The global sea level was some 120m lower than it is today. After the cold peak sea level rising was picking up quickly with a rate of about 100 meters in 10.000 years. This flooded the flat plain in the southern North Sea and a channel opened between England and the continent. The human population in the area retreated further inland, following the sea level rise. When the ice sheets of Canada and Scandinavia had largely melted and entered the oceans about 8.000 years ago the sea level rise began to slow down to 15cm per century. Only during this last period did the Wadden Sea start to emerge as the slow sea level rise allowed sedimentary deposits to accumulate in coastal plains. Figure 5 shows a schematic diagram of the sea level rise and its consequences in the North Sea area over the past 20.000 years.

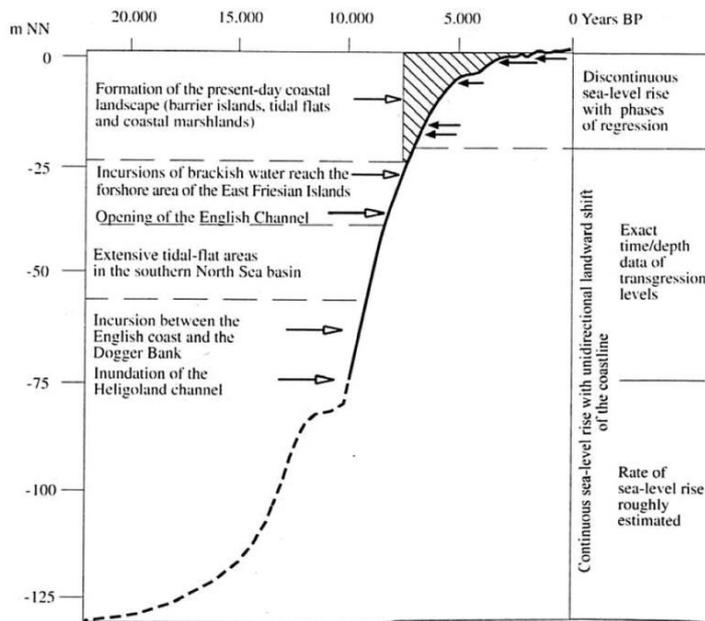


Figure 5: The sea level rise in a time/depth diagram from 20.000 years to recent times with changes in the North Sea estuaries. As displayed in: (Hamer, et al., 2013)

The sea level rise is not the only process that contributed to the creation of the Wadden Sea. The coincidence of four other conditions and processes had a major play in the Wadden Sea's formation (Reise, 2013).

Firstly, the shallow North Sea is a very rough sea with low air pressure systems approaching from the North Atlantic, generating heavy surf, particularly in winter. When the waves touch the ground, sand and other debris is transported towards the shore. In the past during storm surges water levels could rise 3m or more above normal, flooding far inland across the flat terrain. This in combination with the slowly rising sea the land could grow.

Secondly, the tidal range increased with the sea level rise. At places where tidal range is less than 1m currents and wind generate long, sandy spits. Where tidal range is higher and the currents are stronger the long sandy spits break into chains of islands or sand bars (southern and northern Wadden Sea). When tidal range exceeds 3m the currents become too strong for permanent islands to form (as in the central part of the Wadden Sea).

Thirdly, the North Sea has filled with huge amounts of sand in preceding millions of year causing an ample sediment supply to the coastal zone. The North Sea is most likely a more reliable source of sediment than rivers, especially when river basins become modified by human activities.

Finally, besides the deposition of sediment the tidal flats and salt marshes grew through the deposition of suspended matter generated by North Sea plankton, and the deposition of suspended composite particles which formed out of dead organic material, bacteria and minute mineral pieces in the brackish river mouths.

In addition to these main conditions there were some minor involved processes that took part in the Wadden Sea's formation. The region continued to develop with much temporal and sub-regional variation. The rather stable climate over the last 11.000 years, exceptional storm events and rainfall, slow tectonic subsidence (mainly in the central part of the Wadden Sea), shifts in vegetation and fauna and the lifestyle of the coastal human population all had a significant effect on the Wadden Sea.

3.2 Natural environment

The Wadden Sea is an ecotone forming a transition from the open sea to the mainland (Oeschger, 2000). It consists of a multitude of associated biotopes such as supralittoral or spray zone (the dunes and salt meadows that only flood at high tidal levels), the intertidal (tidal flats covered by water twice daily) and the subtidal (the tidal creeks and channel that are permanently covered by water). The species and communities of the Wadden Sea are adapted to its highly dynamic processes such as storm surges and ice winters, and are interlinked with the neighbouring terrestrial and marine ecosystems. The high productivity of the area forms a basis for recruitment of North Sea fish stocks. This is also a prerequisite for its function as turntable on the East Atlantic Flyway. This makes the Wadden Sea a component of ecological structures from the Arctic to South Africa. Annually on average about 10-12 million birds pass through the Wadden Sea with a maximum of 6.1 million birds residing at the same time (UNESCO, 2016).

The Wadden Sea has a high resilience to change, it has a great ability for self-organization (as cited in Oeschger (2000)). Due to the 'natural disasters' causing enormous fluctuations in flora and fauna populations are a typical feature of the Wadden Sea ecosystem. No other ecosystem has a species inventory as that of the Wadden Sea. With about 30 different biotopes the biological biodiversity is very high. There are about 4800 marine and semi-terrestrial species. Although only encompassing 3% of the surface area the salt meadows have the highest species diversity, containing nearly half of the plant and animal species. Many of these species are endemic. In the Wadden Sea the ecosystem is at risk by overexploitation (fisheries and tourism), but also by changes in the species spectrum from introduction of alien species.

3.3 UNESCO

In June 2009, the Dutch and German parts of the Wadden Sea were inscribed on UNESCO's World Heritage List. Five years later in June 2014 the Danish part was added. The Wadden Sea is seen as of universal value, it represents a coastal wetland ecosystem of great international significance. The area fulfils at least three of the four criteria of the operational guidelines for the inclusion in the World Heritage List (World Heritage Centre, 2015). The area that falls under the World Heritage property is shown in Figure 6.

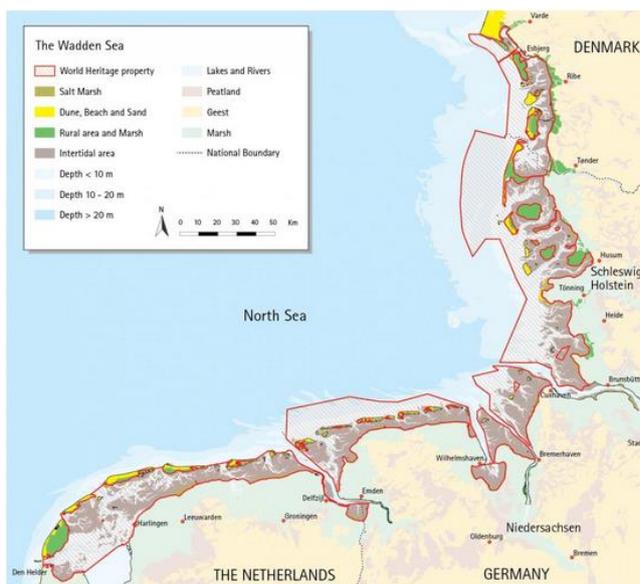


Figure 6: The Wadden Sea World Heritage property is shown in the areas outlined with red. (Common Wadden Sea Secretariat, n.d.)

Firstly, the Wadden Sea is a depositional coastline of unparalleled scale and diversity (criterion viii) (UNESCO, 2016), consisting of almost entirely of a tidal flat and having a barrier system with minor river influences. The highly dynamic natural processes are uninterrupted across the majority of the World Heritage property, creating the variety of different barrier islands, flats, gullies, channels, saltmarshes and other coastal and sedimentary features. The Wadden Sea is an excellent example of large-scale development of an intricate and complex temperate-climate sandy barrier coast under sea level rise conditions.

Additionally, the Wadden Sea includes some of the last remaining natural large-scale intertidal ecosystems where natural processes continue to function largely undisturbed (criterion ix) (UNESCO, 2016). The geological and geomorphologic features intertwined with biophysical processes provide an invaluable record of the ongoing dynamic adaptations of coastal environments to global change. The basis of the species richness of the area is formed by the multitude of transitional zones between land, sea and fresh water. The productivity of biomass in the Wadden Sea is one of the highest in the world. It supports a numerous amount of fish, shellfish and is a key site for migratory birds.

Lastly, the coastal wetlands are not always the richest sites in relation to faunal diversity. The Wadden Sea is an exception with around 2300 species of flora and fauna on the salt marshes, and hosts a further 2700 species in the marine and brackish areas, with an addition of 30 breeding bird species (criterion x) (UNESCO, 2016). Its importance to migratory birds as a staging, moulting and wintering area is demonstrated by the presence of 6.1 million birds at the same time, and an average of 10-12 million annually pass through the property. The Wadden Sea is the essential stopover that enables the functioning of African-Eurasian and East Atlantic migratory flyways which makes the biodiversity on a worldwide scale reliant on the Wadden Sea.

3.4 Socio-economics

Coastal areas are one of the most densely populated areas around the world. Half of the world’s population lives within 60km of the coast, and 75% of all large cities are located on the coast (UNEP, 2016). The Wadden Sea is no exception with its many activities on- and offshore. As far as the Dutch Wadden Sea is concerned there are 13 coast municipalities and 5 island municipalities. The population of the Wadden area as of 2013 was about 270.000 inhabitants and shows a declining trend (Sijtsma, et al., 2014). There is an increase of elderly people, and a decrease in all other demographic groups, especially on the islands. In the Dutch region there is an employment growth, about 1.2% of all jobs in the Netherlands are located in the Wadden area. Figure 7 shows the distribution of jobs over the three countries bordering the Wadden Sea. What stands out is that tourism is the sector with the highest job percentage on the islands compared to the other sectors.

	Jobs in 2011 x 1000	Job growth 2002-2011 annual %	Percentage of jobs in 2011 by sector				
			agric.	manuf.	tourism	edu.	health
NL Wadden area	103.1	0.8	6,8	10,4	6,2	5,5	13,9
Wadden islands	12.9	0.8	7,7	4,1	25,7	4,8	7,3
Netherlands	8483.5	1.2	2,7	9,7	4,2	10,5	15,3
DE Wadden area	341.9	0.7	5,8	18,2	7,8	3,0	14,1
Wadden islands	30.7	0.0	7,0	2,5	36,5	1,5	11,7
Germany	41098.0	0.5	4,2	22,6	3,1	3,8	12,8
DK Wadden area	98.9	-0.4	5,6	14,7	3,6	7,3	18,9
Wadden islands	1.0	-0.7	1,3	2,1	11,0	8,8	17,1
Denmark	2600.0	-1.0	2,7	8,7	3,2	8,4	19,6

Figure 7: Employment figures of the Netherlands, Germany and Denmark with comparison between Wadden area in total and Wadden islands per country. (Sijtsma, et al., 2014)

3.5 Balgzand

The description of the area is according to a paper by Van der Veer et al. (1991). As the specimens are from 1995 and 1996 this reference is accurate in describing the situation at the Balgzand around that time.

The small nursery area Balgzand is an intertidal of about 52 km² in the most southwestern part of the Wadden Sea. On the south and west the Balgzand is surrounded by dikes and to the north and east by 5 to 20m deep tidal channels. The area is mostly barren tidal flats with a few mussel beds in the north-western part. Sediment composition varies over the flats. In the northern more exposed part the sediment is coarse sand and in the southern sheltered areas it is more mud. Median grain size is about 150µm and mean silt content is about 5%. The flats are submerged for 8 to 10 hours of the tidal cycle and for 2 to 4 hours they run dry, exposing the flats to the air. At high water the water depth varies between 0.8 and 1.5m. The actual depth depends on prevailing weather conditions. The flats are intersected by numerous small gullies through which water recedes to deeper tidal channels during the later phase of the ebb tide.

4. The European flounder, *Platichthys flesus*

4.1 Description

European flounder (Figure 8) is a flatfish species that is specially designed, as all flatfish species, for benthic habitats. The flounder are deep-bodied fish with both eyes on one side of the head and a twisted mouth for feeding on the bottom. The European flounder is closely related to plaice (*Pleuronectes platessa*) (Luna, n.d.). *Platichthys flesus* does not have distinct spots such as plaice but rather large patches of darker brown and some irregular reddish spots. Another difference in external features is that flounder has small tubercles along the lateral line (Figure 9). The body of flounder is also thicker than plaice and the meat is finer in structure.

The body of *P. flesus* is oval-shaped with a width about half its length (Luna, n.d.). Length at maturity is about 25-30cm. The maximum recorded age is 15 years, maximum recorded weight 14kg and maximum recorded length is 60cm. Although, they seldom grow larger than 30cm (FAO, 2016).



Figure 8: *Platichthys flesus* (Freshwater Diversity Identification for Europe, n.d.)

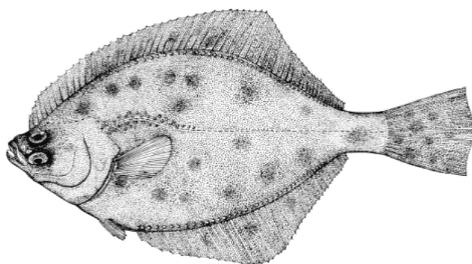


Figure 9: Right eyed flounder illustration with indication of rough skin along the lateral line and base of the dorsal and anal fins. (FAO, 2016)

The upper surface is fawn, olive green or pale brown with patches of darker brown (Luna, n.d.). The under surface is opaque pearly-white. The lateral line is nearly straight running along the middle of the upper surface where it curves round the short pectoral fins. The dorsal and anal fins run along the length of the body to the caudal peduncle. The skin is rough along the basis of the dorsal and anal fins (Figure 9). The caudal peduncle width is about half the length of the tail with at the end the caudal fin that has a squared-off end.

During the metamorphoses flatfish can either become left or right eyed. However, there is no universal agreement on how to perceive whether the fish is right or left eyed. An observer can choose to either turn the head or tail towards oneself. The orientation of the eyes are then determined if they are located on the right or left side from the lateral line (Figure 10). When using the tail as starting point most of the flounder are left eyed, which is also seen in this study. In some areas one-third of the population is reversed (FAO, 2016).



Figure 10: European Flounder left eyed (left) and right eyed (right). (Van Emmerik, 2009)

4.2 Habitat and biology

Platichthys flesus is a migratory catadromous fish (Riede, 2004) that spawns in marine environments, but which can live in marine, freshwater or brackish waters. They are found at shallow depths between 1-100m (Muus & Nielsen, 1999). The pH tolerance range lies between 7.5 and 8.2 and temperatures

between 5°C and 25°C (Baensch & Riehl, 1997). *Platichthys flesus* is a euryhaline species that tolerates salinities from 0-35 ‰ (Hemmer-Hanson, Nielsen, Grønkjær, & Loeschcke, 2007). The natural range of *P. flesus* extends from the White Sea to the Mediterranean and the Black Sea (Figure 11). Due to accidental introductions in ballast water, it may also be found around North America (Munroe, 2010).

The adults occur on mud and sandy substrate in shallow water where it burrows itself in the substrate. During the winter months the mature adults enter deeper warmer waters, where they spawn in spring between February-June (Cooper & Chapleau, 1998) being later in more northerly latitudes. However, a recent study showed that flounder also appears to spawn in estuarine areas and not just in coastal areas as was previously widely accepted (Morais, Dias, Babaluk, & Antunes, 2010). Growing planktonic larvae from coastal spawning grounds drift towards coastal areas where they migrate upstream rivers using selective tidal transport (Bos, 1999) triggered by for example salinity, prey density and water temperature. The juveniles live in shallow coastal waters and estuaries (Bos, 2000), which are also the summer feeding grounds of adult fish (Cooper & Chapleau, 1998). In some areas, e.g. western Baltic, flounder hybridizes with plaice (*Pleuronectes platessa*) (FAO, 2016).



Figure 11: Distribution of *P. flesus* along the coasts of Western Europe. (Munroe, 2010)

The juveniles of the 0-group feed on plankton, larvae of insects and juvenile crustaceans, juveniles older than one year and adults feed on benthic fauna such as bivalve molluscs and other invertebrates such as shrimps, polychaete worms, gastropod molluscs and small fish (Rochard & Elie, 1994). Juveniles are susceptible to predation by small crustaceans such as the brown shrimp *Crangon crangon* (Van der Veer, Bergman, Dapper, & Witte, 1991).

As mentioned before the adults migrate offshore during the winter and spawn there between February and June. Spawning occurs in the water column (Balon, 1990), fertilization is external and the parents are nonguarders. The females produce 400.000 to 2.000.000 buoyant eggs (Rochard & Elie, 1994) of about 0.8-1.4mm in diameter (Muus, 1966). The eggs are spherical, transparent, smooth and not sticky (Russell, 1976). The egg hatch after 5-7 days at water temperatures of 10°C (Muus, 1966).

4.3 Human interactions

Platichthys flesus has been assessed as Least Concern on the red list (Munroe, 2010). As this species has a broad geographic range it is reported as common. Despite some significant threats such as harvesting and chemical pollution, these are not thought to pose a significant threat to the global population at this time. It is recommended by the IUCN to undertake systematic monitoring to determine stock status across the entire range.



Figure 12: Global capture of *P. flesus* from 1950 to 2013. On the y-axis are the tonnes (*1000). (FAO, 2016)

The European flounder are mainly of interest to fisheries in Baltic and Danish waters (FAO, 2016). The total catch in recent years was about 20000 tons. The countries with the largest catch were Denmark, with 3528 tons, and the Netherlands, with 3159 tons. The most common fishing techniques used for European flounder are "demersal bottom trawling" and "small flatfish (flounders, soles) bottom trawling". Figure 12 shows the global capture of *P. flesus* from 1950-2013.

5. Otolith

5.1 Description

Otoliths are bony granules of calcium carbonate in the inner ear of vertebrates. They have been identified in both extinct and extant vertebrates (Sahney & Wilson, 2001). Otoliths are used as gravity, balance, movement, and directional indicators in all vertebrates. In higher aquatic and terrestrial vertebrates the inner ear and otoliths have a second function in sound detection (Sheykholeslami & Kaga, 2002; Jones, Lukashkina, Russell, & Lukashkin, 2010).

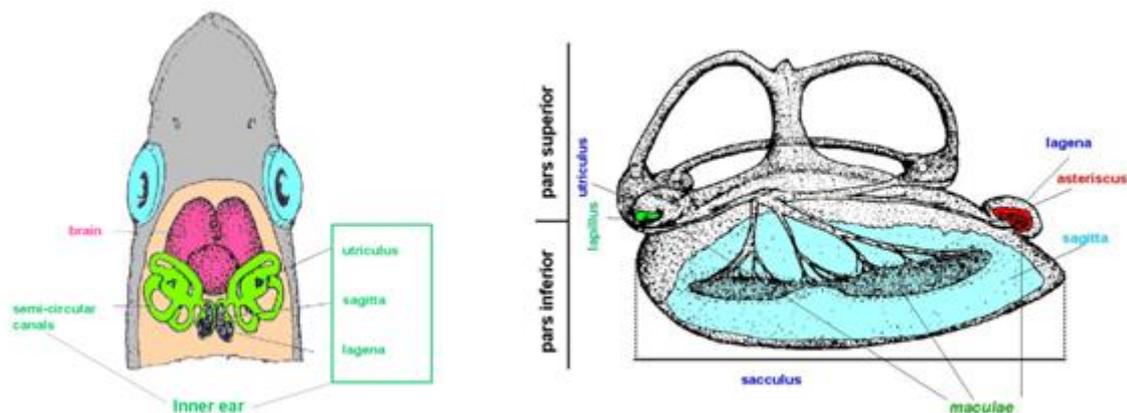


Figure 13: Location of the inner ear behind the brain in a round fish (left) and a close up with colour indication of the otoliths in the inner ear (right). (Lombarte, et al., 2006)

In teleost, bony fish, the inner ear, generally located behind the brain, is composed of three pairs of otolithic organs which include otoliths and sensory epithelia as well as three semi-circular canals (Figure 13). Each of the otolithic organs is associated with a certain type of otolith. The lapillus is located in the utricle, the asteriscus in the lagena and the sagitta in the sacculus (Lombarte, et al., 2006). The inner ear has two functions: to perceive sounds and to perceive angular acceleration and gravity. These functions correspond to two different parts of the inner ear. The upper part (utricle and semi-circular channels) mainly controls the equilibrium functions. The lower part (sacculus and lagena) is specialised in receiving sound. Each of the otoliths in the inner ear has besides a different function also a different size, shape and microstructure, interspecific (Figure 14) and intraspecific (Figure 15).

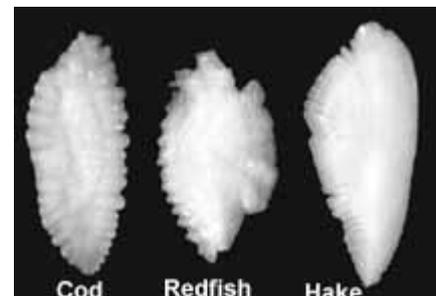


Figure 14: Differences in otolith shape of sagitta otolith between three species. (Online Otolith Lab, 2006)

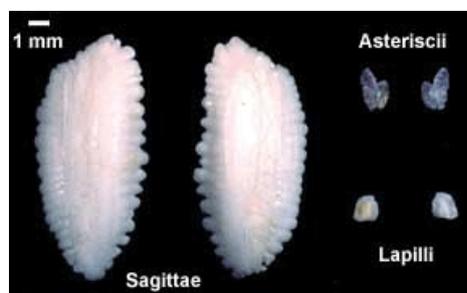


Figure 15: Differences in otolith size within one species. (Online Otolith Lab, 2006)

5.2 Otolith study applications

One of the main applications of otoliths in fish studies is aging fish. Knowing the age of fishes allows fishery managers an understanding of dynamics of fish stocks and how fish population react to environmental stresses. Aging on the basis of annual increments (the rings) is similar to counting tree rings as otoliths also form annual marks (Alaska Fisheries Science Center, n.d.). With otoliths it is also possible to analysis daily increments with young individuals. The largest otolith of the three, the sagittae, is often used in the analysis (Stevenson & Campana, 1992). Resolution and differentiation of daily increments in fast growing otoliths can be difficult, and also shifts in growth axes commonly occur causing accurate reading problems. Same as with daily increments annual marks are not always clearly visible. Environmental factors causing stress such as food supply, water temperature, water chemistry, salinity, and population density mark differently on the otolith from those of annual rings. This can cause a complicated pattern that the researching biologist must interpret. Therefore, depending on species and the type of otolith analysis, sectioning/polishing plane (sagittal, frontal, transverse, or oblique) should be selected to increase visibility in the analysis (Figure 16).

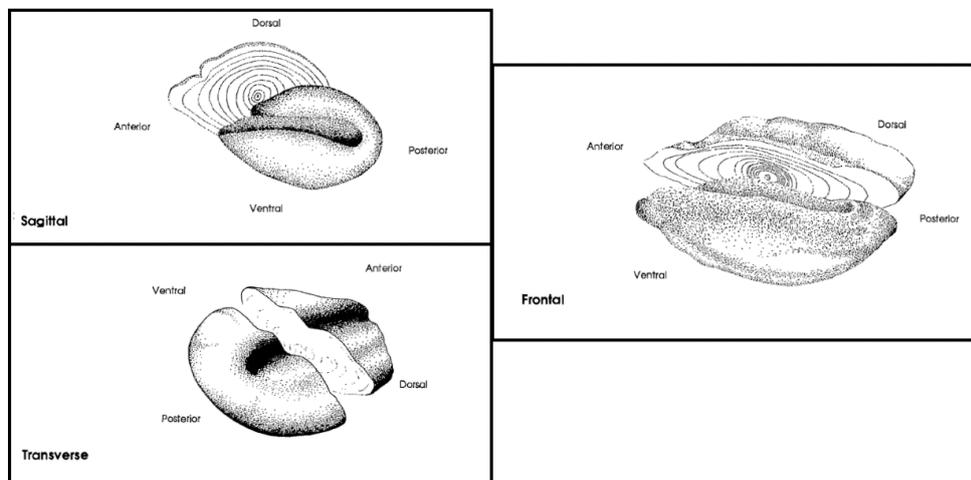


Figure 16: Different section/polishing planes (Stevenson & Campana, 1992)

The chemical composition of fish otoliths can give an indication of the water bodies the fish has previously occupied (Patterson, Smith, & Lohmann, 1993). As the otolith grows new calcium carbonate crystals form. In crystal structures lattice vacancies exist during the formation allowing trace elements from the water to bind in the otolith structure. Studying these trace elemental composition or isotopic structures of trace elements within the fish otolith in combination with aging gives insight in the environment the fish occupied over time and potential migration route information.

Otoliths can also be used in marine mammal diets. As the compounds in otoliths are reasonably resistant to digestion they are found in the digestive tracks of piscivorous marine mammals. As the sagittae otolith shape is species specific many fish can be identified to genus and species level. This can, to some extent, be used to reconstruct prey composition of marine mammals. Besides the prey composition also the size and biomass of the prey can be reconstructed as otolith size is proportional to both length and weight of a fish (Arim & Naya, 2003). However, otoliths are subject to degradation and damage by acidic solutions which can give skewed prey number and biomass measurements (Bowen, 2000).

In palaeontology dispersed otoliths are one of the many microfossils which can be found through micropaleontological analysis of fine sediment (Sahney & Wilson, 2001). However, their stratigraphic significance is minimal (rock layers and stratification studies). The rare cases in which fossil otoliths are found *in situ* are of special significance. The presence, composition, and morphology of the material

can clarify the relationship of species and groups. Various fossil material in primitive fish show that endolymphatic infillings (fluid contained in the membranous labyrinth of the inner ear) were similar in elemental composition to the rock matrix. However, they were restricted in coarse grained material which presumably is better for the detection of gravity, displacement and sound.

5.3 Daily growth increment analysis

There are two zones of daily growth increments considered for this research. The inner zone are those increments that have formed since the hatching of the flounder in the North Sea up till the metamorphoses and settlement of the juveniles in the Wadden Sea. The outer zone are those that have formed since settlement up till the catch. The increments are recorded separately, added together they form the age of the fish after hatching in days.

When the fish are young it is generally quite simple to distinguish the line between the inner and outer zone and between increments. Most of the time the increment indicating settlement is different from the others in means of homogeneity. It can be darker than the other rings immediately before and after the settlement ring. This is probably due to the fact that during the metamorphoses the fish does not grow much and the rings that are formed during this period are packed closer together. Another feature that easily indicates the settlement ring is the onset of bulges. At the centre of the bulges there is a small notch. At the centre of this notch the last increment that goes around completely without interruptions is the settlement increment. All these indicators combined give a profound estimation of the location of the settlement ring. Examples of these indicators are shown in Figure 17.

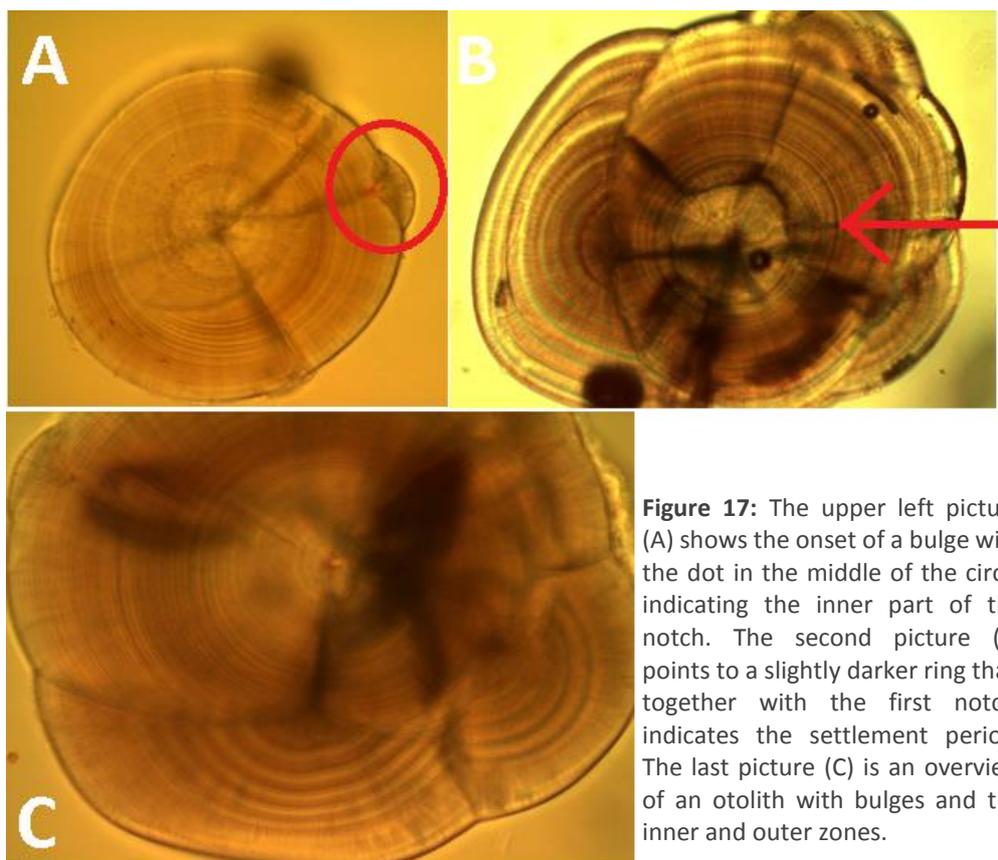


Figure 17: The upper left picture (A) shows the onset of a bulge with the dot in the middle of the circle indicating the inner part of the notch. The second picture (B) points to a slightly darker ring that, together with the first notch, indicates the settlement period. The last picture (C) is an overview of an otolith with bulges and the inner and outer zones.

6. Materials and methods

6.1 Sampling

0-group *Platichthys flesus* was collected from the Balgzand, a large intertidal area in the western Dutch Wadden Sea (Figure 18), at frequent intervals (2 to 4 weeks) from February to August in 1995 and from March to August in 1996. Fishing was done during a period of 3 hours around high tide since in this time frame the population is distributed randomly over the area (Kuipers, 1977). Fishing was carried out on a grid of 36 stations distributed over the study area. Using a 1.9m beam trawl (1 ticker chain, net mesh size of 5x5mm) towed by a rubber dinghy with a 25HP outboard motor at a speed of approximately 35m/min nine transacts, each consisting of 4 stations of about 100m in length, were sampled during daytime. The location of the hauls was established by GPS. The water temperature was measured at each sampling date and compared to the daily temperature series of the Marsdiep (Van Aken, 2008). Catches were transported to the laboratory and stored at 20°C awaiting further processing.

In the laboratory, samples were sorted by species within a few days, whereby all individuals were measured to the nearest mm total length. Thereafter they were fixated with 96% ethanol. Some otoliths were lost over time due to deterioration of the sample before or during storage.

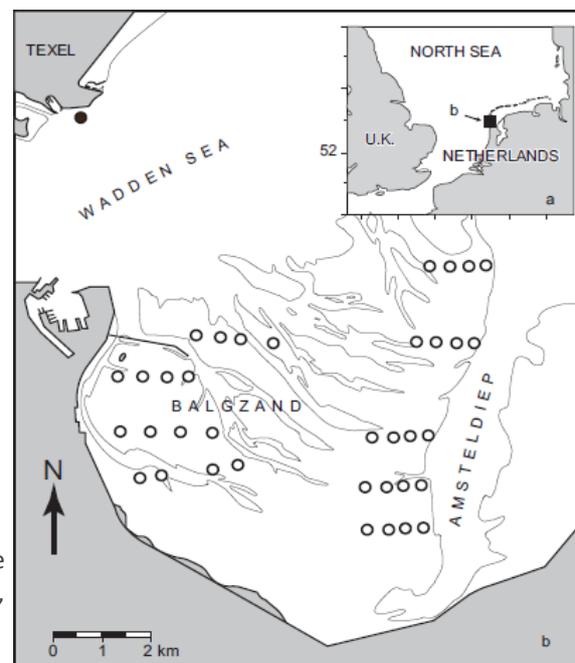


Figure 18: Sampling locations (o) on the Balgzand in the western Dutch Wadden Sea. (Cardoso, Freitas, de Paoli, Witte, & Van der Veer, 2016)

6.2 Otolith microstructure analysis

Per sampling campaign around 100 individuals of different size classes (mm) were selected in relation to their abundance. These 100 individuals therefore are a representation of the different fish sizes caught from the sampling campaign.

The materials used in the otolith microstructure analysis are listed in Appendix 1. Appendix 2 and 3 describes the otolith removal and otolith daily growth increment analysis method, respectively, applied in this research.

Table 1 indicates which recordings were made during the research and which parameter they are registered in. These were used in the analysis of the growth of the 0-group flounders.

Table 1: Recordings for otolith analysis research in 0-group Flounder.

Recordings	Parameters
Total length	Millimetres from head to end of the caudal fin
Standard length	Millimetres from head to end caudal peduncle
Eye orientation fish	Location of the eyes from the middle of the body when the fish is positioned with the tail towards one self
Inner rings	Amount of rings from settlement ring inwards
Outer rings	Amount of rings from settlement ring outwards

As the student was inexperienced with the otolith microstructure analysis there was first a period of training before the values were acceptable compared to the analysis of the otoliths by an expert. How this was achieved is described in Appendix 4.

6.3 DEB model

DEB stands for Dynamic Energy Budget and is based on the specification of the uptake of energy from the environment and the mass flows of an organism, via feeding and digestion, and the usage of this energy for various purposes (Kooijman, 2010). The mechanic basis of the different processes that together constitute the DEB model are many interrelationships that make the model more than just a collection of independent submodels (Kooijman, 2000). The processes the energy is regulated to are divided into maintenance, development, somatic growth and reproduction. In a static energy budget these fluxes represent a snapshot of an individual in a given state, while a dynamic energy budget follows the changes of these fluxes during its life cycle.

It all starts with the ingestion of food by an animal which is transformed into faeces and egested. Food uptake is proportional to organism surface area and converted into reserves with a constant efficiency (Van der Veer, Freitas, Koot, Witte, & Zuur, 2010). Energy derived from food is taken up via the blood which exchanges energy with the storage. Blood has a low capacity for energy but high transportation rate. The principle is that energy is first stored in the reserves after which it is again transported via the bloodstream to somatic and reproductive tissues (Figure 19).

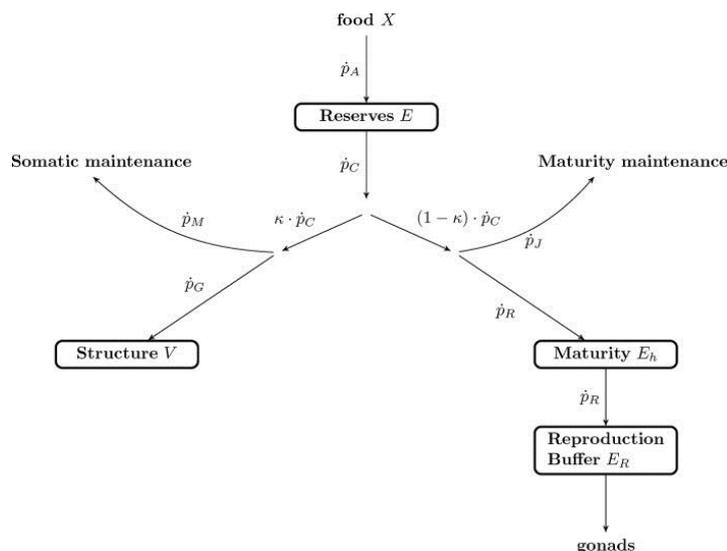


Figure 19: Schematic representation of the standard DEB model. Energy enters the organism as food (X), is assimilated at a rate of P_A into reserves (E). The mobilization rate, P_C , regulates the energy leaving the reserves to cover structural growth (P_G), somatic maintenance (P_M), maturity maintenance (P_J), maturation (P_R) in immature individuals and reproduction (P_R) in mature individuals. (Smale & Barnes, 2008)

A fixed part of the energy, κ , from the reserves is used for (somatic) maintenance and growth, the remaining part, $1 - \kappa$, can be used for development in juveniles and reproduction in adults (Kooijman, 2000). Maintenance has priority over growth so that growth ceases if all energy available for κ is solely used for maintenance. Embryos and juveniles put a significant amount of energy in development. This

energy is similarly partitioned into maintenance of a certain degree of maturation and an increase in the degree of maturity. Energy spend on increasing the degree of maturity in juveniles is allocated to reproduction in adults.

With a set of species-specific parameters the DEB model can be applied for all combinations of fish size, food conditions and temperature. The values for the different parameters for flounder in this research were taken from a publication by Freitas *et al.* (2012) (Table 2). At constant food densities, the reserves are in equilibrium with the environment. Consequently, the growth of structural biovolume represents a weighted difference between surface area specific intake rate and volume growth, which corresponds with the von Bertalanffy growth curve:

$$(1) \quad d/dt L = r_b * (L_{inf} - L)$$

in which the DEB parameters can be combined to give:

$$(2) \quad L_{inf} = f * L_m / dM = f * v / (g * k_M * dM)$$

$$(3) \quad r_b = 1 / \left(\left(\frac{3}{k_M} \right) + 3 * f * \frac{L_m}{v} \right) = \left(\frac{k_M}{3} \right) / (1 + f/g)$$

where L_{inf} is the ultimate size (cm) and r_b the von Bertalanffy growth rate constant (d^{-1}), f is the scaled functional response (dimensionless), δ_M is the shape coefficient (-), L_m the maximum volumetric length an individual can reach (cm), v is the energy conductance ($cm d^{-1}$), a parameter related to reserve dynamics, g is the investment ratio (-) that stands for the costs of new biovolume relative to the maximum potentially available energy for growth and maintenance, and k_M is the maintenance rate coefficient (d^{-1}), which stands for the ratio of somatic maintenance costs to structure (Van der Veer, Freitas, Koot, Zuur, & Witte, 2010).

Table 2: Dynamic Energy Budget model parameters for *Platichthys flesus* at a species-specific reference temperature, and temperature dependence parameters. For parameters that differ between sexes, values for males are shown in (). (Freitas, Van der Veer, & Kooijman, 2012)

Parameter	Units	Interpretation	Flounder
T_1	K	Reference temperature	283
V	$cm d^{-1}$	Energy conductance	0/156
G	-	Energy investment ration	3.590(4.211)
k_M	d^{-1}	Maintenance rate coefficient	0.0035
L_m	cm	Maximum physical length	56 (47)
δ_M	-	Shape coefficient	0.224
T_A	K	Arrhenius temperature	7500
T_L	K	Lower boundary of tolerance range	277
T_H	K	Upper boundary of tolerance range	296
T_{AL}	K	Rate of decrease at lower boundary	35000
T_{AH}	K	Rate of decrease at upper boundary	75000

When food conditions change (f) it is expected to affect both L_{inf} and r_b , while temperature conditions affect r_b only, due to the temperature dependence of physiological rates. The temperature effect in the DEB model is based on an Arrhenius type relation (a formula for the temperature dependence of reaction rates) that describes the rates at ambient temperature, $k(T)$, as follows:

$$(4) \quad k(T) = k_1 e^{\left(\frac{T_A}{T_1} - \frac{T_A}{T} \right)} * \frac{1 + e^{\left(\frac{T_{AL}}{T_1} - \frac{T_{AL}}{T_L} \right)} + e^{\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T_1} \right)}}{1 + e^{\left(\frac{T_{AL}}{T} - \frac{T_{AL}}{T_L} \right)} + e^{\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T} \right)}}$$

where T is absolute temperature (K), T_{AL} and T_{AH} are Arrhenius temperatures (K) for the rate of decrease at the lower (T_L) and upper (T_H) boundaries, respectively. T_i is the reference temperature (K), T_A the Arrhenius temperature (K), and k_i is the rate at the reference temperature.

6.4 Data analysis

In this research a combination of previous and current data was used. For the data analysis the same method is used as executed in the previous research by NIOZ on plaice environment (Cardoso, Freitas, de Paoli, Witte, & Van der Veer, 2016). The statistical analysis of the plaice research was carried out in the R package version 3.1.0 programming, whereas for this research a more recent version was used (version 3.2.3) (R Core Team, 2015). The script written for the plaice research was reused for the flounder research by replacing input data of plaice with those of the flounder and fitting specifications in the script to flounder.

An additional R script was created to obtain data from the dataset about the sampling campaigns such as mean length and mean biomass over time, mean density, and length-frequency per sampling campaign.

Water temperatures have been compared to long-term temperature series of a nearby site, Marsdiep, with analysis of covariance (ANCOVA), using the `lm` function in R. Water temperatures at the start of the settlement period were compared between both years in the same way.

The settlement date per individual fish was estimated by subtracting the number of increments after settlement from the sampling date. Otolith daily increment counts were compared between observers during the entire analysis to validate the data (max. difference of ± 5 increment counts was accepted). To identify the number of settling cohorts, individuals were grouped into two-week age classes. Model validation was done following Zuur *et al.* (2009). For all tests, model assumptions regarding homogeneity, normality, independence, and absence of influential observations were met.

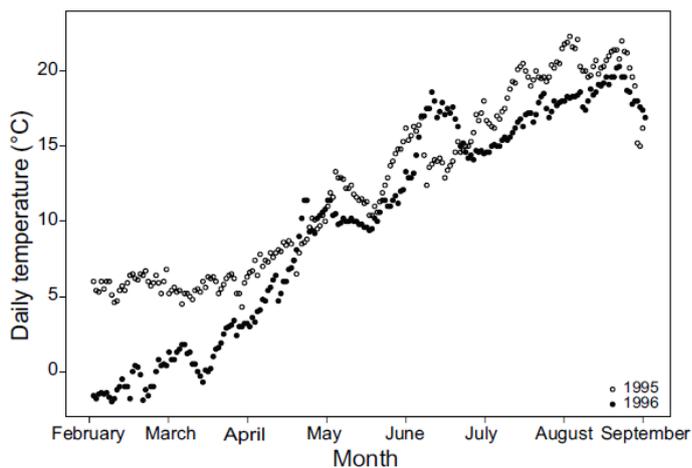
With the equations described in Chapter 6.3 a DEB simulation excel file was set up. The growth rates predicted by the DEB model with reference temperature of 10°C and maximum food conditions ($f=1$) at various temperatures were estimated. Parameters between males and females vary due to difference in food intake and energy participation, and hence, growth characteristics vary as well. In this study no difference was made between sexes for the observed growth. However to get a complete overview of the DEB simulation the male, female but also average predicted growth was modelled.

7. Results

7.1 Field data

7.1.1 Water temperatures

No significant differences in temperatures between Balgzand and Marsdiep were found in 1995 and 1996 (ANCOVA $p > 0.05$). Therefore, the daily water temperature series of Marsdiep was used in further analysis. The samples were collected from February until August in 1995 and March until August in 1996. During the sampling period the temperatures increased as is shown in Figure 20. Daily temperatures in February, March and April were significantly different between years. Average monthly temperatures were much lower during the months February and March between years, it was

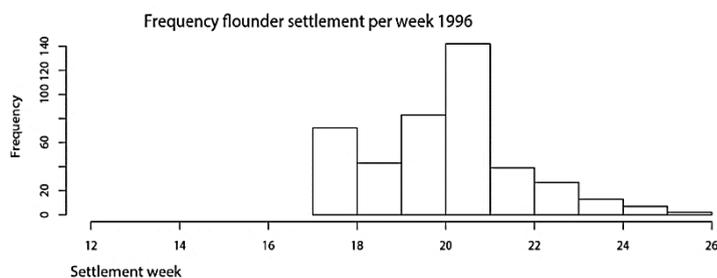
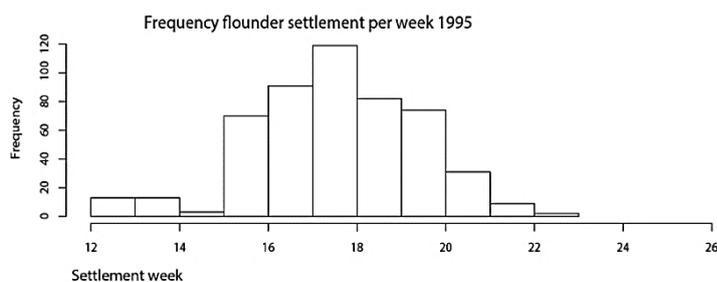


5.8 and 5.6°C in 1995 respectively, and -0.9 and 1.5°C in 1996. As the year continued the temperatures in 1996 increased to similar heights as in 1995 (ANCOVA, for all months $p < 0.05$).

Figure 20: Mean daily temperatures in Marsdiep jetty from February to September in 1995 and 1996.

7.1.2 Settlement at Balgzand

In 1995 the first 0-group flounder sampled in 1995 was on the 3rd of April. Compared to this in 1996 the first occurrence of 0-group flounder was on the 6th of May. Using back calculation of settlement date by otolith daily increment analysis showed that in 1996 settlement occurred four weeks later than



in 1995. Figure 21 shows an occurrence frequency histogram of 0-group flounder over time for both years. Here the difference in settlement week is clearly seen.

Figure 21: Settlement frequency of flounder per week for 1995 (top) and 1996 (bottom). There is a time difference of 4 weeks of settlement between the years.

Besides arriving later in the season, Figure 22 shows that newly settled flounder, which are between 7 and 15mm long, settle at the Balgzand up to about a month later in 1996 compared to 1995. Additionally, the figure shows the settlement peaks for both newly settled individuals and for the entire 0-group population combined. In both cases the peaks for 1995 falls halfway May and at the

beginning of June in 1996. The densities of newly settled individuals <15mm is about the same in both years (± 200 ind./ 10^3m^2). On the other hand, flounder densities of the entire 0-group show a higher density in 1995 (± 400 ind./ 10^3m^2) compared to 1996 (± 250 ind./ 10^3m^2).

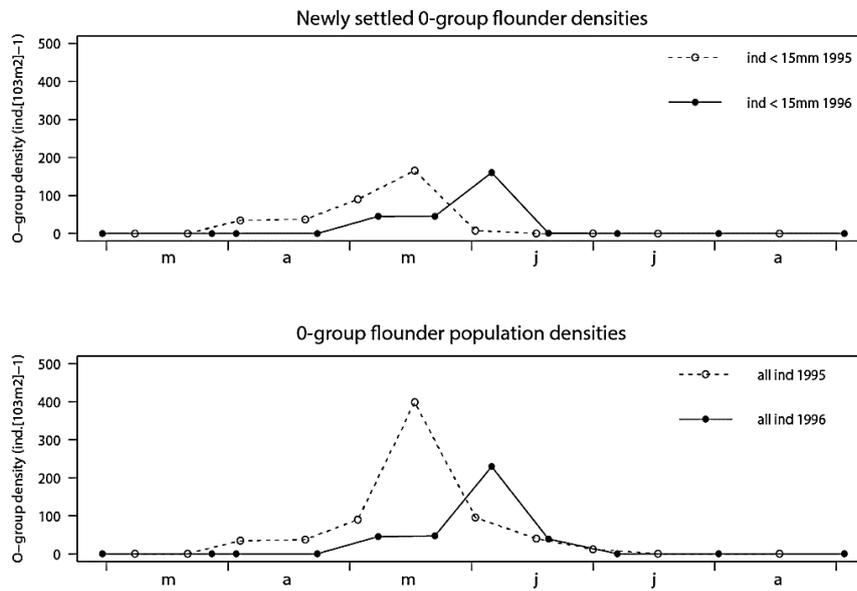


Figure 22: 0-group flounder density (ind./ 10^3m^2) for 1995 and 1996 over time (months). In the upper graph the newly settled flounders per year are displayed. The graph below shows all individuals of both years.

The size-frequency graphs in Figure 23 give an idea of how the length of the sampled population was divided up over the months. It also shows that although in 1995 the flounder arrived earlier the size frequency of the first three sampling campaigns occupies only a few small size classes. From the fourth campaign onwards, 17th of May, population starts to distribute more out over different sizes and grows in length accordingly. Comparing 1995 and 1996 in length classes it is also seen that in 1995 there were larger individuals than in 1996.

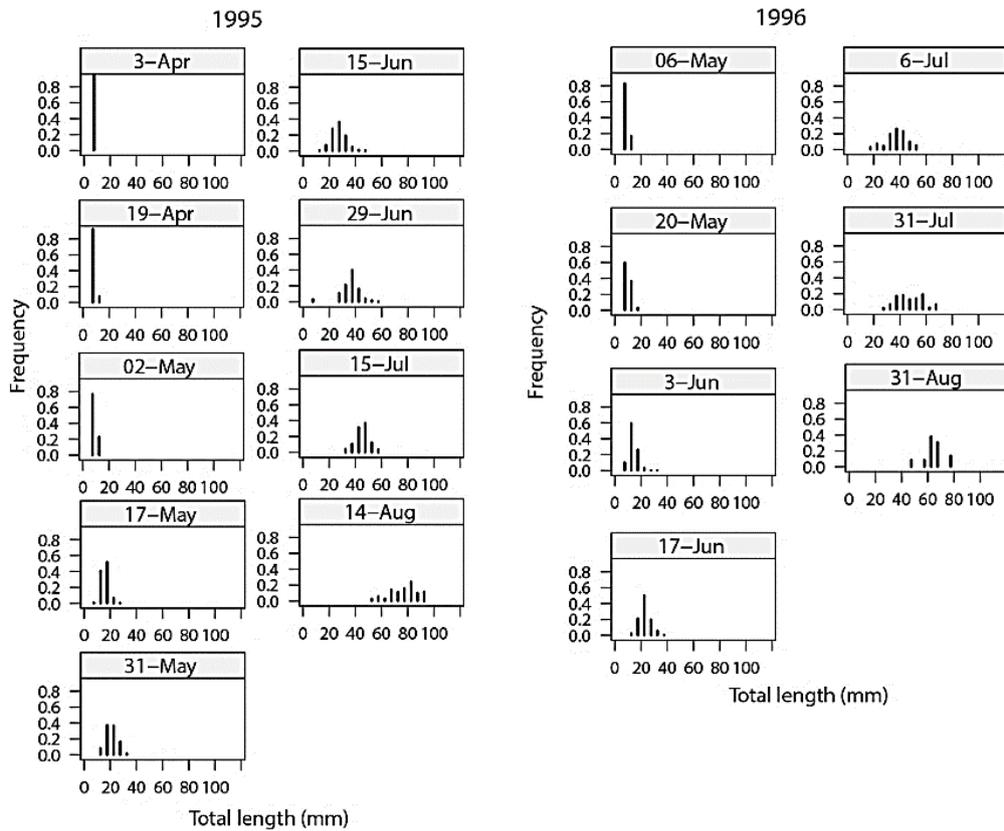


Figure 23: Size-frequency distributions of 1995 (left) and 1996 (right) per sampling campaign. On the x-axis the size classes are given in total length (mm) with on the y-axis the frequency of the population that reached that size.

7.1.3 Average length

As already suggested by Figure 23 during the sampling period larger individuals were found in 1995, which becomes even more evident from the graph in Figure 24. Over the entire sampling period the mean population length in 1996 is below that of 1995. The smallest individual in 1995 was 6mm and in 1996 7mm. At the end of the sampling period in August the largest individual in 1995 was 98mm while in 1996 it was 64mm.

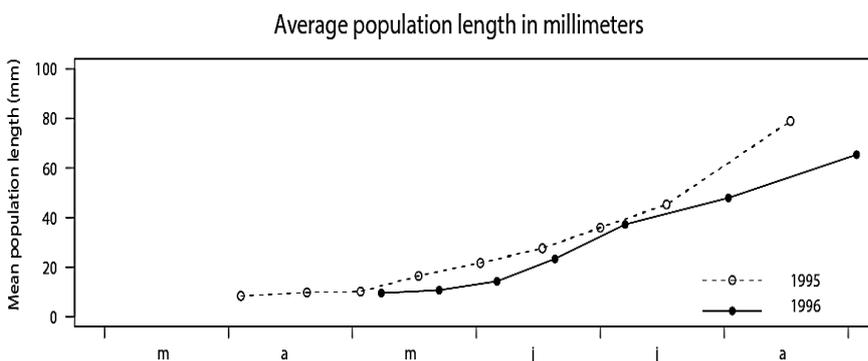


Figure 24: Mean population length of 1995 and 1996 in mm set out against the sampled months.

7.2 Length-age relationships

Using results of individual otolith microstructure analysis plotted against the individual length the growth curves of the population for both years were constructed. The graph in Figure 25 suggest a linear growth curve for both years. At the beginning there is a lag phase in growth, this is due to inhibition in growth for a few days after metamorphosis.

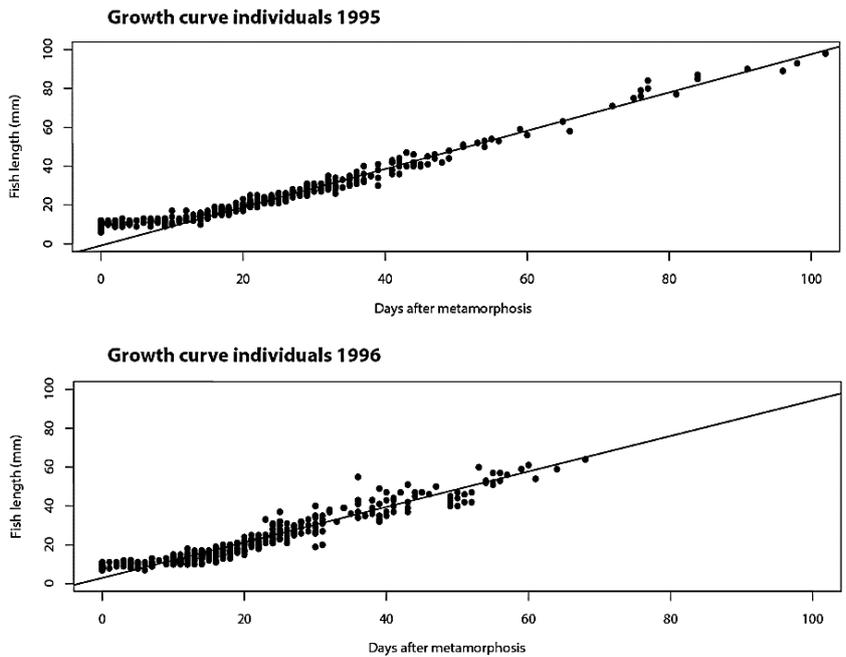


Figure 25: Length age relationship for 1995 (top) and 1996 (bottom), both showing a linear regression with a lag phase up to approximately 15mm.

In the graph of Figure 26 the growth curves for both years are plotted so that the overlap can be seen. It seems there might be growth difference between the years.

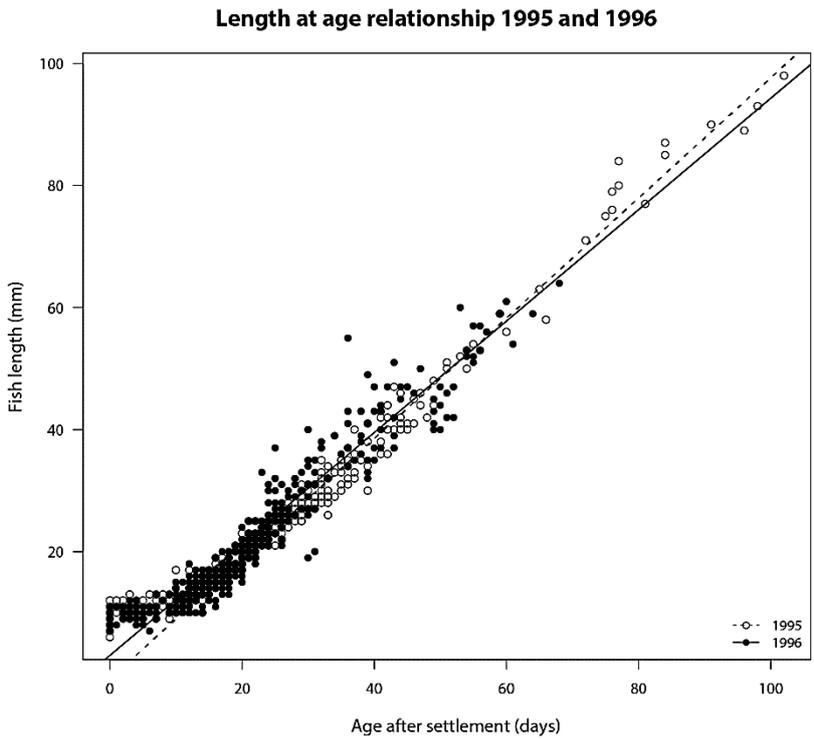


Figure 26: The graphs of Figure 25 combined to see the overlap in length age relationships of both sample years.

7.3 Observed growth as compared to DEB growth

The individuals are divided into settlement batches of 2 week periods. After setting up a DEB simulation model in Excel the maximum potential growth per batch was plotted against observed growth. DEB predictions were made assuming ad libitum food conditions and using prevailing water temperatures in 1995 and 1996. The individuals were not tested for sex so besides the female and male predicted growth curves an average is taken as well.

7.3.1 Field observations versus DEB 1995

In the graphs of Figure 27 the average DEB model predictions are shown as a red line, females with a green line (upper line) and males with a purple line (lowest line). The individuals of the batch are shown as blue bullets. These graphs shows similarities with previous graphs in that over the months (increasing batch number) more individuals are found at the Balgzand with a slight decrease in the last batches. Additionally, individuals in later batches are able to increase in size over the entire sampling period since settlement. When looking at the graphs the DEB model shows a good fit with the observed growth curves for 1995. The observed growth is at the start of the growth curves per batch are close to the average DEB line, as the individuals become larger the bullets start to overlap more with the female DEB line.

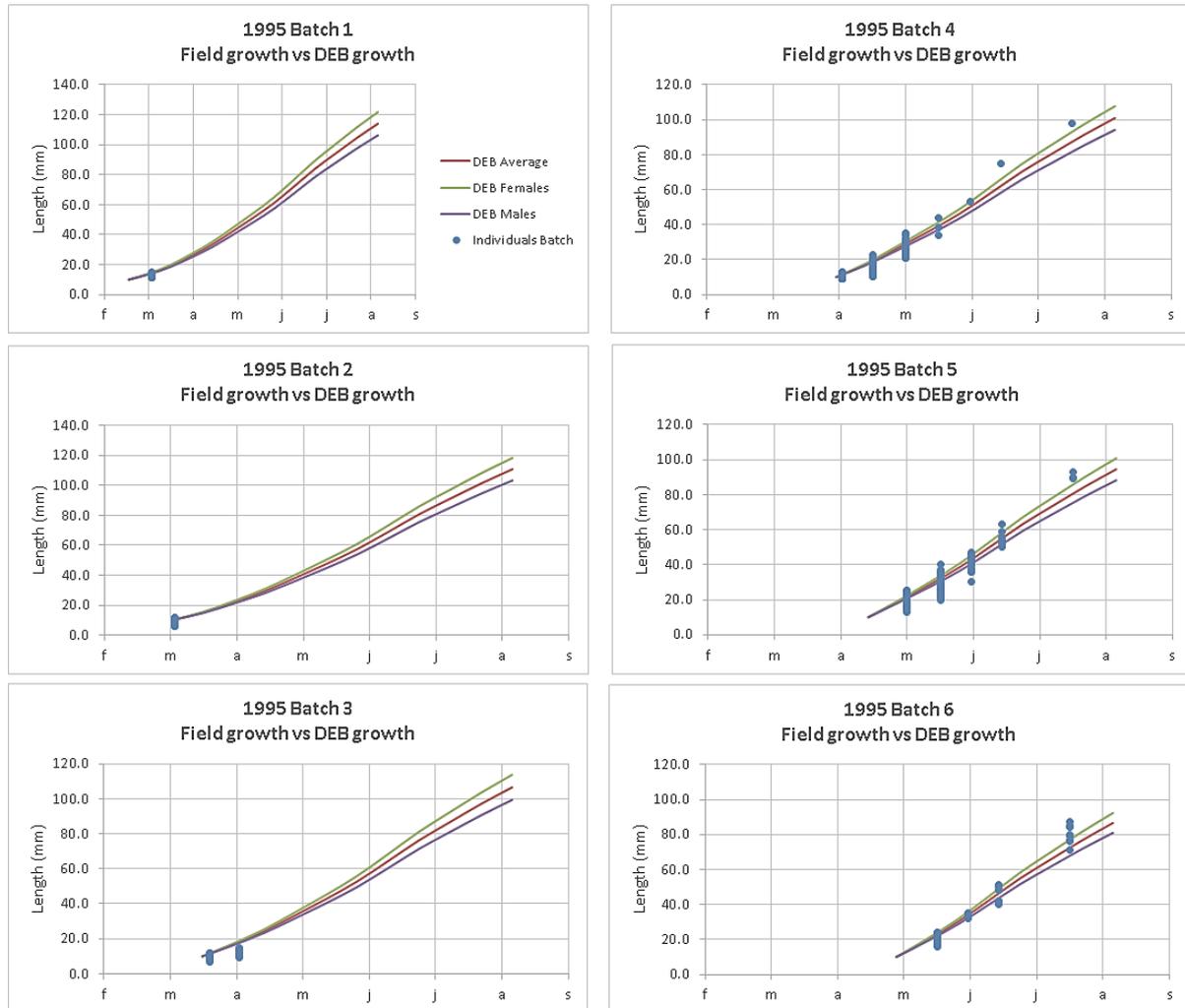


Figure 27: Theoretical growth trajectories predicted by a DEB model (green is females, red is average, purple is males) and field observations (blue bullets) for 1995.

With the realized growth ratio (RGR) (Figure 28) it is seen that over the months the differences between predicted growth and realized growth is more or less stable (values approximately 1). When RGR is plotted against length of individuals per batch (Figure 29) the graphs show that over the entire length range the ratio stays around 1 as well for each batch.

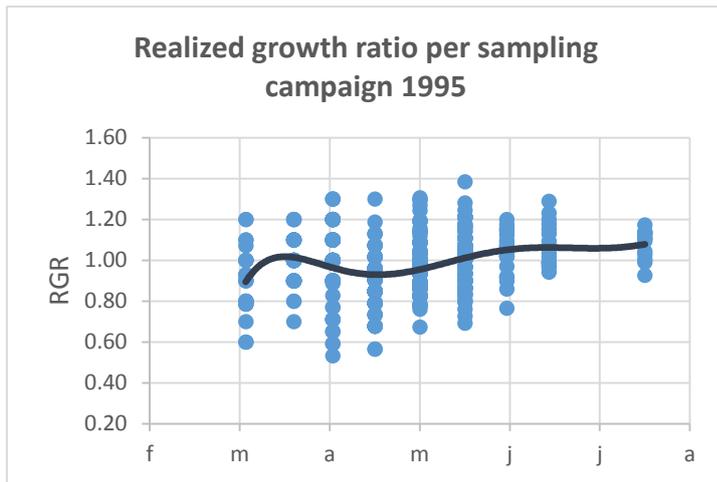


Figure 28: Realized growth ratio per sampling campaign over the different months for 1995.

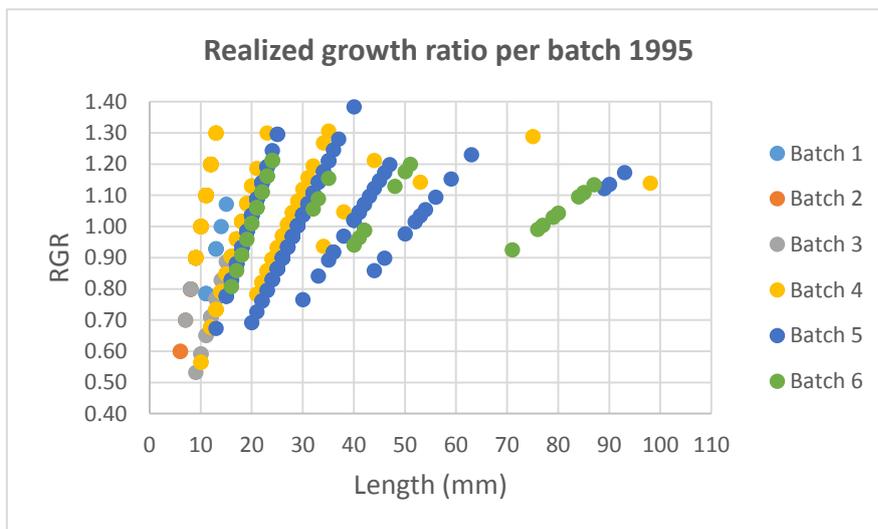


Figure 29: Realized growth rate against length per settlement batch in 1995.

7.3.2 Field observations versus DEB 1996

In Figure 30 the predicted growth compared to observed growth are shown in graphs per batch for 1996. The observed growth does not meet the female, average nor the male DEB growth curves. However, over the course of time observed growth and DEB growth follows the same trend. When looking at the graphs of 1996 the DEB model shows no good fit with the observed growth curves.

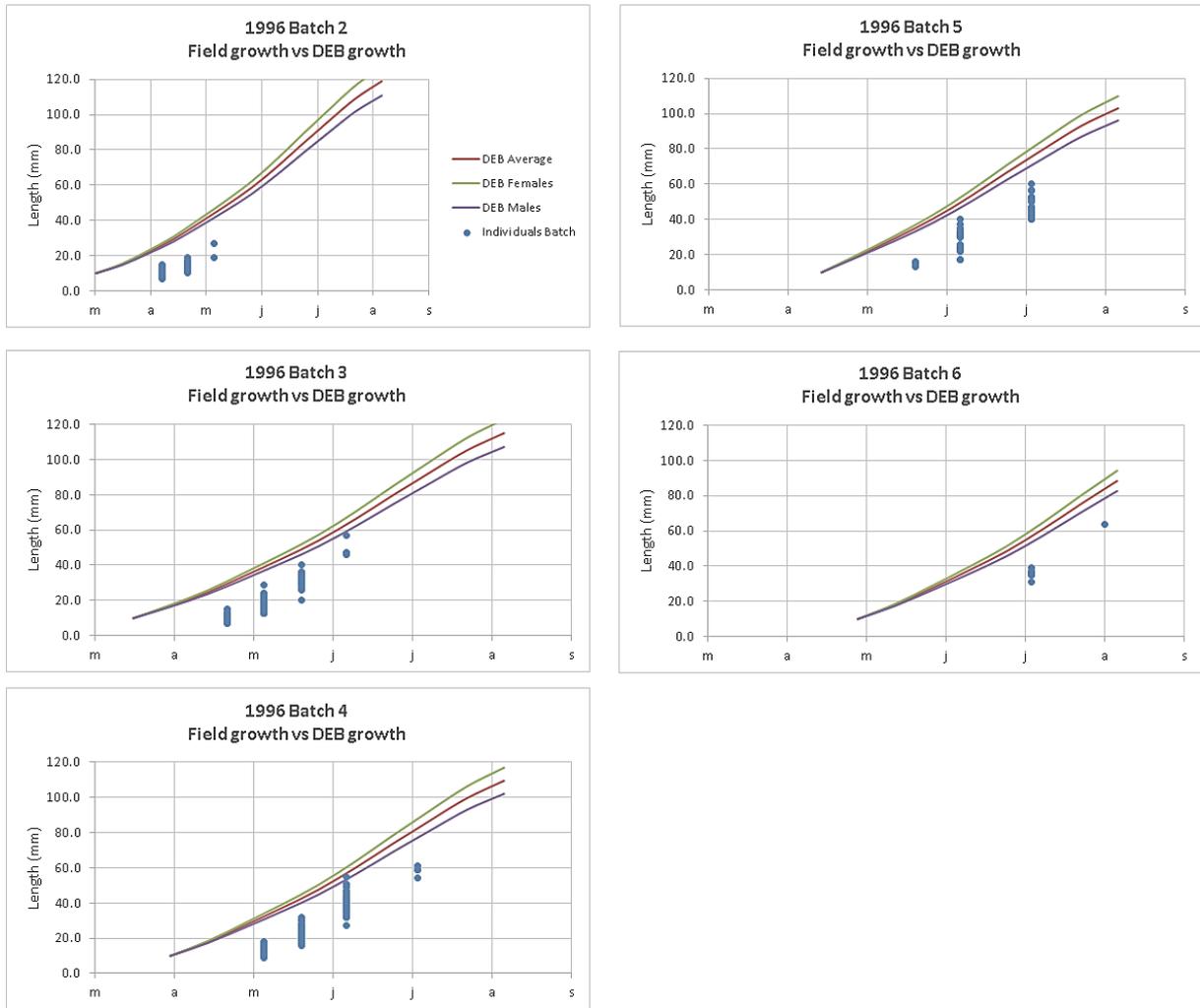


Figure 30: Theoretical growth trajectories predicted by a DEB model (green is females, red is average, purple is males) and field observations (blue bullets) for 1996.

Values for the RGR are always below 1 (Figure 31 and 32). The RGR graphs of 1996 show an upward trend towards the 1 line for both the ratio over time and ratio plotted against fish length.

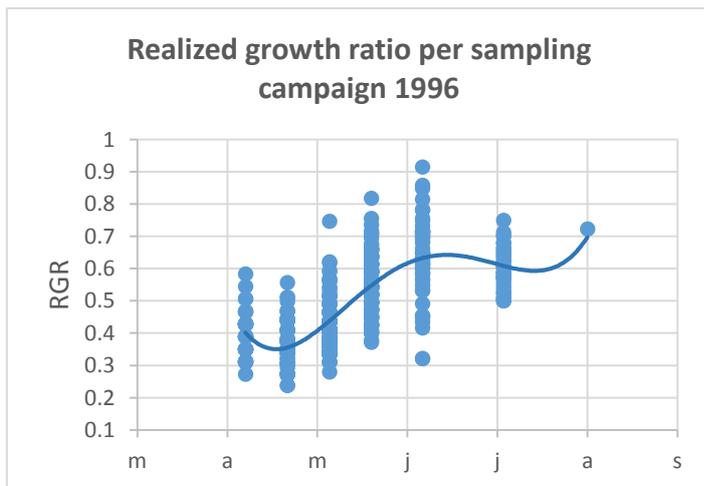


Figure 31: Realized growth ratio per sampling campaign over the different months for 1995.

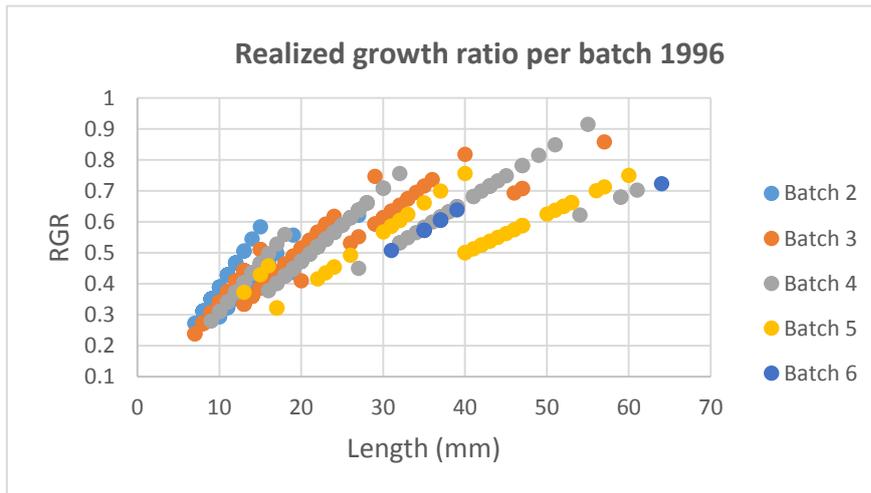


Figure 32: Realized growth rate against length per settlement batch in 1995.

7.4 DEB sensitivity control

In the original DEB model the initial starting length at settlement was taken to be 10mm. However, as is evident from Figures 30, 31 and 32 this might not be a correct assumption. The differences in realized growth pattern between years left aside it would have been expected that at least the values should be closer to 1, as was seen in 1995. With the conclusion and discussion in perspective the next test will be to verify if the DEB model is sensitive to the change of the initial settlement length for the growth patterns of the 1996 population. The parameters will be changed to 7, 8 and 9mm respectively (indicated between brackets in the graph titles).

The graphs of predicted growth compared to observed growth gathered after the execution of the sensitivity analysis are attached in Appendix 5 (Figures 38 and 39). The realized growth ratio analysis is shown in Figure 33 below. What might not be apparent in the graphs of Figures 38 and 39 but can be seen by looking at the ratio patterns of the different sensitivity control graphs is that the ratio changes. With a lower initial settlement length (e.g. 7mm) the ratio values come closer to 1. What does not change is the predicted growth pattern of the DEB models, whichever initial settlement length is used.

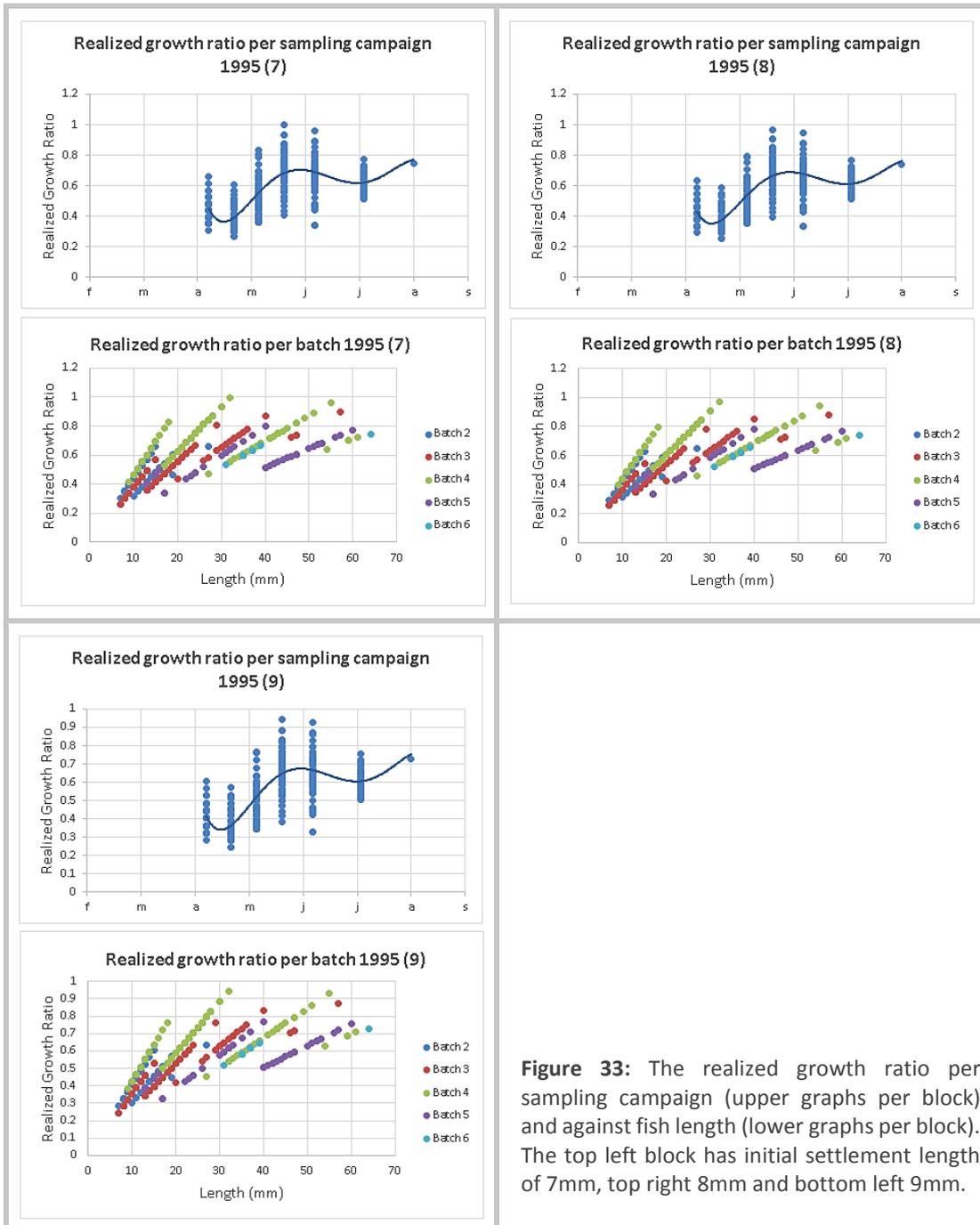


Figure 33: The realized growth ratio per sampling campaign (upper graphs per block) and against fish length (lower graphs per block). The top left block has initial settlement length of 7mm, top right 8mm and bottom left 9mm.

8. Discussion

Previous research on seasonal growth of European plaice, *Pleuronectes platessa*, indicated an optimal and maximum growth in the beginning of the season, but a slowing down during the summer months (Cardoso, Freitas, de Paoli, Witte, & Van der Veer, 2016). This research was dedicated to investigate if similar patterns were seen for *Platichthys flesus*. Although plaice and flounder are closely related they differ in some perspectives such as that juvenile flounder is more restricted in distribution compared to juvenile plaice and inhabit the more salty tidal flat systems at the studied area Balgzand (De Vlas, 1979). Additionally, while adult flounder migrate offshore to spawn the postlarvae are generally found nearer to shore than those of other pleuronectids (Russell, 1976). Also, juvenile plaice and flounder differ in their prey preferences (De Vlas, 1979). More on the differences between the species later on.

The 0-group flounder collected for this research at the Balgzand during two years were taken due to the contrasting temperature regime between the years. Using these contrasting years growth pattern of flounder under different temperature regimes could be analysed. It was expected that 0-group flounder would reach optimal growth at the beginning of the season but a decrease in growth compared to maximum potential growth over the summer.

This study has its strength in that population size increase was validated by using otolith microstructure analysis. In combination with DEB simulations the field growth was analysed standardized to a certain temperature which made comparison between years possible.

8.1 Results summarized

When comparing both years all results of 1996 were lower or later in time than in 1995; temperatures in spring, settlement frequency over time, population densities, biomass and mean length. Water temperatures were much lower in February and March of 1996 compared to 1995 (ANCOVA, $p < 0.05$), afterwards it increased to about similar temperatures as in 1995 for the rest of the season. Larvae settled about 4 weeks earlier in 1995, which is mostly caused by a longer larval development period due to exposure to low water temperatures during drift (Van der Veer & Witte, 1999). Densities of the entire 0-group population was higher in 1995 compared to 1996. However, peak densities of newly settled flounder was about the same in both years ($\pm 200 \text{ ind./}10^3 \text{ m}^2$).

The growth curves of 1995 and 1996 seem to be different when looking solely at the observed growth. This however does not mean that the growth in 1996 is less than in 1995 as the growth curves are not comparable due to the difference in water temperature regimes. To solve this the DEB model was standardized to 10°C and using the prevailing environmental conditions a specified DEB simulation was set up. This way observed growth rate could be compared to the specific maximum potential growth. When plotting 1995 the individuals seem to be able to reach maximum potential growth (realized growth ratio (RGR) over time ± 1), while in 1996 observed growth lies well below the DEB average and even below the male DEB prediction line (RGR average max 0.7).

8.2 The DEB model

Although the observed growth and DEB simulation do not show a good fit for 1996 a DEB sensitivity test validated that trends do not change even though initial settlement length changes. The RGR of 1996 shows an upward trend which suggest that at the beginning individuals were not able to reach maximum potential growth but over time were moving more towards the predicted growth.

This does not explain why values of observed growth in 1996 are systematically below the DEB average line. It might potentially be due to a skewed sex ratio. In the Minho estuary in Portugal it was found that over a sample period of 12 months in summer there was a higher sex ratio (1.30) male/female

compared to other seasons (Souza, et al., 2013). In 1995 the average DEB model and field growth show a good fit which then would mean that the population consisted of about 50-50 males and females. Unfortunately, no information was available on the sex of individuals in this study to prove this.

Another explanation for the differences in the DEB models compared to observed growth in 1996 and between 1995 and 1996 might be due to the inexperience of the otolith microstructure analyst. This is, however, unlikely as the analyses was checked by an expert along the entire execution process to ensure reliability of the data (maximum allowed difference in daily increment analysis was 5 increments).

8.3 Flounder versus plaice

The population size increase of juvenile flounder suggested a similar phenomenon of growth decrease during summer months as is seen in plaice (Freitas, Van der Veer, & Kooijman, 2012; Cardoso, Freitas, de Paoli, Witte, & Van der Veer, 2016). This study, however, shows that this is not the case for flounder as results from 1995 show that individuals can reach maximum potential growth and RGR in 1996 indicates an upward growth trend towards maximum predicted growth over time.

A possible explanation can be that larger flounder migrated out of the sampled area. It is known for flounder to move around estuaries along abiotic factor gradients such as salinity, temperature variations and depth (Souza, et al., 2013; Mendes, Ramos, & Bordalo, 2014). Overall, size classes associated differentially with environmental variables in the Minho study. At the Balgzand this will not be much different as it has open access to the rest of the Wadden Sea with its wide variety in abiotic factors. Flounder are thus free to move around to different areas over time. This could have caused the illusion of a decrease in summer growth based on the population size measurements while actually the remaining flounder in the study area were able to reach maximum potential growth.

The difference seen between the results of the plaice research by Cardoso et al. (2016) and this research could also be due to the difference in prey composition between juveniles of plaice and flounder. Juvenile flounder eat a wide variety of Polychaeta, Oligochaeta, Crustaceans and Gastropoda (as cited in Rasmussen (2005)) while juvenile plaice's food organism include small benthic invertebrates such as harpacticoid copepods and the appendages of larger organism including bivalve siphons and polychaeta tentacles (as cited in Burrows (1994)). At the Balgzand especially juvenile crustaceans are very abundant.

9. Conclusion and Recommendation

At the start of this study three aspects were set out to be addressed in the form of questions. Firstly the length-age relationships over time for both 1995 and 1996 had to be established by otolith microstructure analysis. Next could be tested if length-age relationships differed among years (which seemed it did) and finally whether the growth patterns based on length-age relationships reflected maximum potential growth as predicted by a dynamic growth model. For 1995 this is true as the observed growth and DEB model showed a good fit. As for 1996 it was less of a good fit although patterns of increased observed growth compared to maximum potential growth over time remained the same, independent of the initial settlement length parameter in the DEB simulation.

To come back to the main question “Does summer growth reduction occur annually in young 0-group flounder in the western Dutch Wadden Sea?”, after answering the subsidiary questions and interpreting the results it can be said that for flounder summer growth reduction does not take place.

Taking all findings of the plaice and flounder research into account the underlying mechanism remains elusive, but it is suggested that the difference might still lie in the composition and activity of the benthos over time at the Balgzand intertidal flat. The first step to be undertaken is to add a third year to the flounder sequence to validate the conclusion of no summer growth reduction occurrence. If this is confirmed it can be hypothesised that species dependent on infauna prey have summer growth reduction while species dependent on epifauna do not. This because infauna of the tidal flat can be very active during spring blooms but in summer less so that predators of infauna will have less food available. Species that depend on epifauna that is highly abundant at the Balgzand will have less influence on growth over the summer months.

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Appendices

Appendix 1: Materials Otolith Analysis

Apparatus

- Hot plate
- Binoculars with light source
- Light microscope with mounted camera (magnification objective lens 2.5, 5, 10, 32 and 40x)

Liquids

- 96% ethanol
- Mounting Wax, crystalbond 509
- Nail polish
- Metadi Supreme Diamantsuspension 3 μ m polykristallin 40-6631F
- Metaprep AIO5

Laboratory equipment

- Storage jars
- Erlenmeyer flask
- Glass petri dish
- Millimeter measuring paper
- Scalpel
- Forceps
- Dissection needles (one with bend tip, other straight tip)
- Dropper pipet
- Microscope slides
- Waterproof marker
- Microscope slide storage boxes
- Carbon silicium sanding discs (P1200, P2500 and P4000)
- Two polish discs from Keat 3 Autimatsche Le –8 and Polijstmachine

Appendix 2: Method Otolith Removal

Preparation

The labeling of the samples was according to the code on the jar with each individual fish assigned its own number (label: FishingLocation-SamplingYear-SamplingCampaign-FishNumber). The hot plate was set on 150°C so the mounting wax could melt in the Erlenmeyer flask.

Otolith removal

The removal of the otoliths was done using an adapted version of the *Up through the gills method* (Stevenson & Campana, 1992). This method is suitable for the removal of sagittae (largest of the three otoliths) in juvenile and young adult flatfishes. The picture in Figure 34 gives an overview of the first part of the procedure. Figure 35 shows the microscope slides and hot plate settings.

1. Using a petri dish with mm measuring paper in it, the total length and the standard length were measured. At the same time the orientation of the eyes, either left or right from the middle of the body when the tail is towards oneself, were noted down.
2. Next the fish was put in a glass petri dish and the head cut halfway through at the second gill cover with a scalpel. The head was bend back away from the gills and the rest of the body to expose the otoliths. With fish <15mm the gill cover was flipped back to work through the tissue from the top of the fish as it was too small to hold the fish and remove the otoliths otherwise.
3. The otoliths were located in the bulla (gas filled sphere located ventral and anterior to the inner ear, part of the auditory capsule). The bulla and surrounding bone tissue were cracked or chipped when the otoliths were not exposed yet.
4. The largest pair of the otoliths, the sagittae, were taken out and transferred to the two microscope slides (one for the original, generally the cleanest of the two, and one for 6-8 spare otoliths together) using dissection needles. The spare sagitta was fixed with nail polish. The other microscope slide was transferred to the hot plate and the sagitta fixed with mounting wax.
5. Before storing the otoliths the sagitta with mounting wax was checked for bubbles and to see if it was positioned correctly. If this was not the case the microscope slide was reheated on the hot plate and using the dissection needles the problem was solved.
6. The microscope slides were stored in microscope slide storage boxes.

All fish that were cut open were stored in a new jar with a label similar to the old one with additional information that the otoliths were removed from the fish. These fish might be used for other purposes in the future.

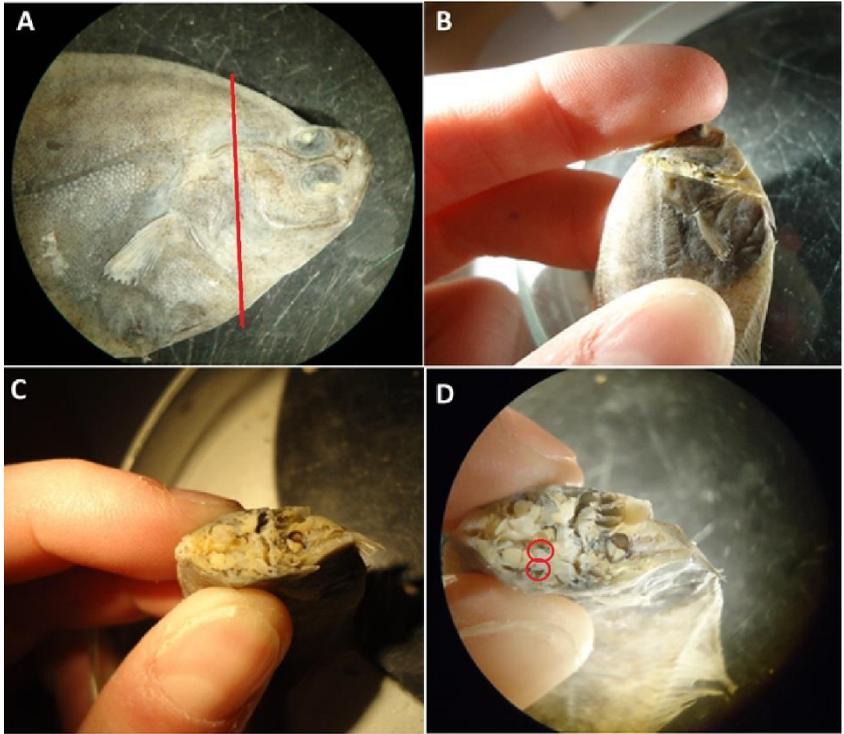


Figure 34: The first three pictures illustrate where the incision is made (red line in A), and how the head is bend backwards (B and C). The last picture (D) shows the inside of the flounder head with the small red circles the location of the otoliths.

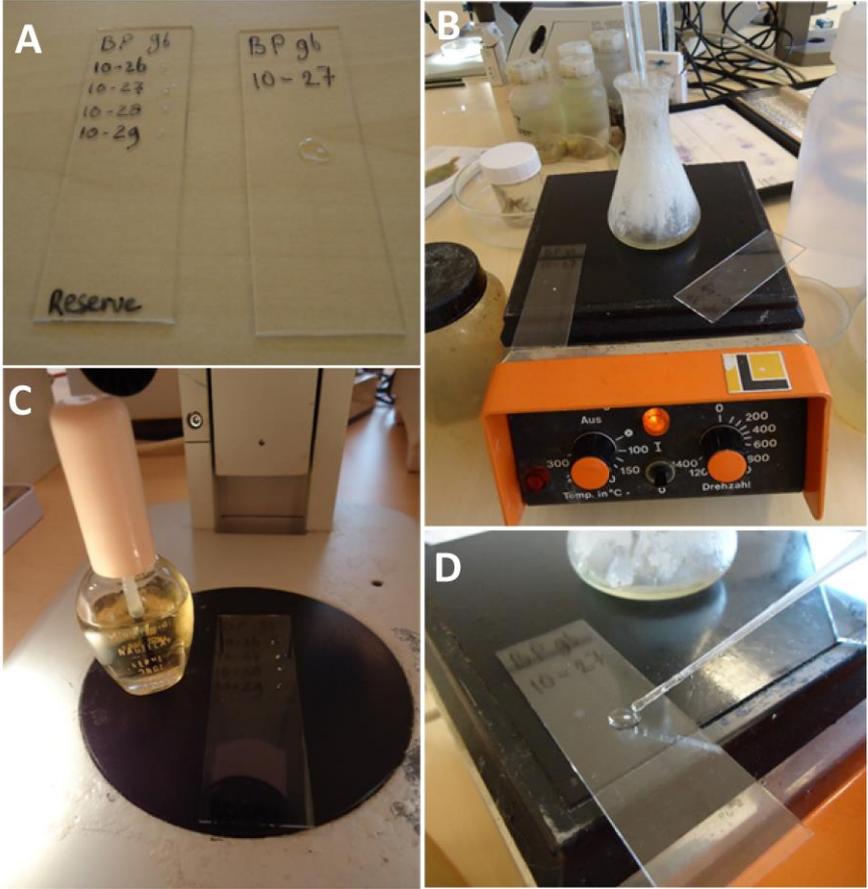


Figure 35: In the first picture (A) the two kind of microscope slides are shown. The second and fourth picture (B and D) display the hot plate with microscope slides and the Erlenmeyer flask filled with mounting wax. The third picture (C) is the spare microscope slide with nail polish as fixating liquid.

Appendix 3: Otolith Daily Growth Increment Analysis

The program AxioVision by Zeiss was used so that the otolith could be seen on the computer screen. The mounted camera made it possible, if needed, to take pictures. The steps for the daily growth increment analysis are as follows:

1. A microscope slide was slid underneath the microscope. Generally a magnification of 320x or 400x was used to assess the increments, dependent on the size of the otolith.
2. The transition between the inner and outer zone was determined using the indicators as mentioned in Chapter 5.3.
3. The outer rings were counted first as these are generally easier to see. The inner rings were counted when possible.
4. The otolith analysis was repeated by an expert, generally on a 1 in 10 basis. Whenever the analysis was unclear, large differences between analyses, the method was discussed to get to a logical and theoretical appropriate outcome.
5. Whenever the rings were not easily distinguishable the otoliths were polished before reanalysis. This was done as follows:
 - Three sanding discs and polishing discs were placed down in sequence; P1200, P2500, P4000, and two polishing discs from the Keat 3 Autimatsche Le –8. Water was set ready for on the sanding discs, and metadi supreme diamantsuspension and metaprep AIO5 for the polishing discs.
 - The otolith was first grinded on P1200 carbon silicium paper and checked if the sagitta midplane was attained (just slightly). Grinding was done by circling the microscope slide with the otolith face down on the grinding disc manually while applying mild pressure. Next the otolith was grinded on the P2500 and P4000 papers. In between any residue resulting from the grinding was wiped off.
 - The otolith were polished in the same way as with the sanding discs but instead of water using a few drops of the metadi supreme diamantsuspension and metaprep AIO5 respectively.

Appendix 4: Student-Expert Margin of Error Calibration

Over a 5 week period the student was trained in the otolith microstructure analysis discipline so that the margin of error became acceptable for the research (maximum accepted difference between student and expert ± 5 increments). In the beginning no record was taken on which day which otoliths were analysed. From the 3rd of March (otolith analysis day 15 (working days since the start of otolith analysis)) record was taken so that it could be judged when to accept the otolith analysis data gathered by the student, and which data had to be re-examined.

During the first two weeks the student and expert sat together for several days (about 1 hour a day) to discuss the features of the otolith. As an unexperienced otolith microstructure analyst the student had to be introduced into which features of the otolith are important to establish the settlement increment and whether all increments seen should be taken into account during the analysis. Generally, when otoliths grow an illusion of more increments appears due to double vision. This is because the otoliths besides growing in length also grow in height. On top of that in the inner zone the microstructures becomes more difficult to see with larger otoliths.

During this first period whenever the expert was not available the student would continue with flounder otoliths that had been counted previous year from the same sampling campaign years. The otolith analysis records of this sampling campaign served as an 'expert counting' to which the student could measure the margin of error.

As time continued the student went on with the analysis more independently from the expert up to the point where both worked blindly from each other. The student would first analyse a series of otoliths and the expert would afterwards analyse the same otoliths. Now that the otolith analysis was more independent the records of the student were set out against those of the expert (difference between observers against length).

Figure 36 shows the difference in analyses between the student (Anne) and the expert (Hans). The graph shows that in general the student counts more increments in an otolith than the expert. In the inner zone the maximum difference is ten, and for the outer rings this is only five. This is not unexpected as the inner part of the otolith becomes more difficult to see when the otoliths become larger.

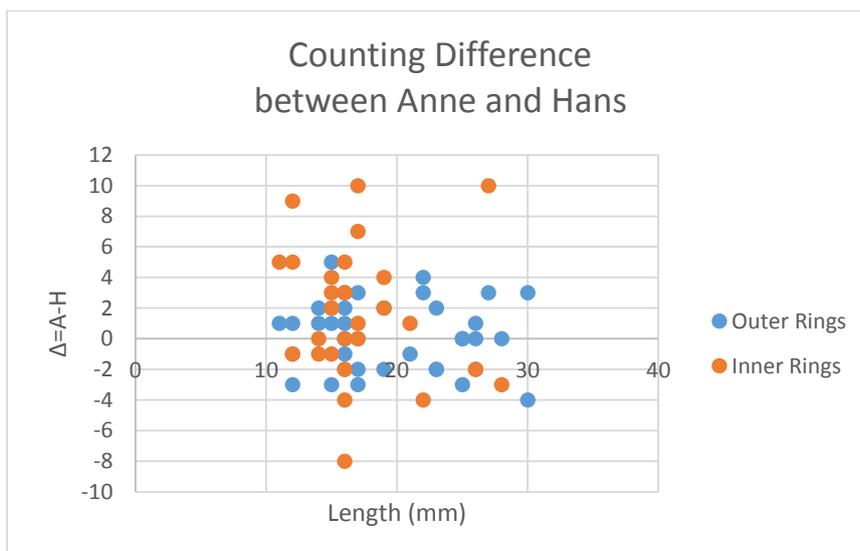


Figure 36: The results of the counting difference between student and expert are plotted against the length of the fish.

Figure 37 shows the difference between the student and expert in yet another graph. Here the $x=y$ line indicates the line on which the dots should be located (no difference between expert and student). Again it is clearly seen that there is a larger difference between the analysis of the inner and outer microstructure. For this study the outer zone is most important as they indicate when the flounder have settled in the Wadden Sea. Using the two graphs it was decided that after 5 weeks of otolith microstructure analysis (26 analysis days) the margin of error between the student and expert was small enough to use the data for the research purposes.

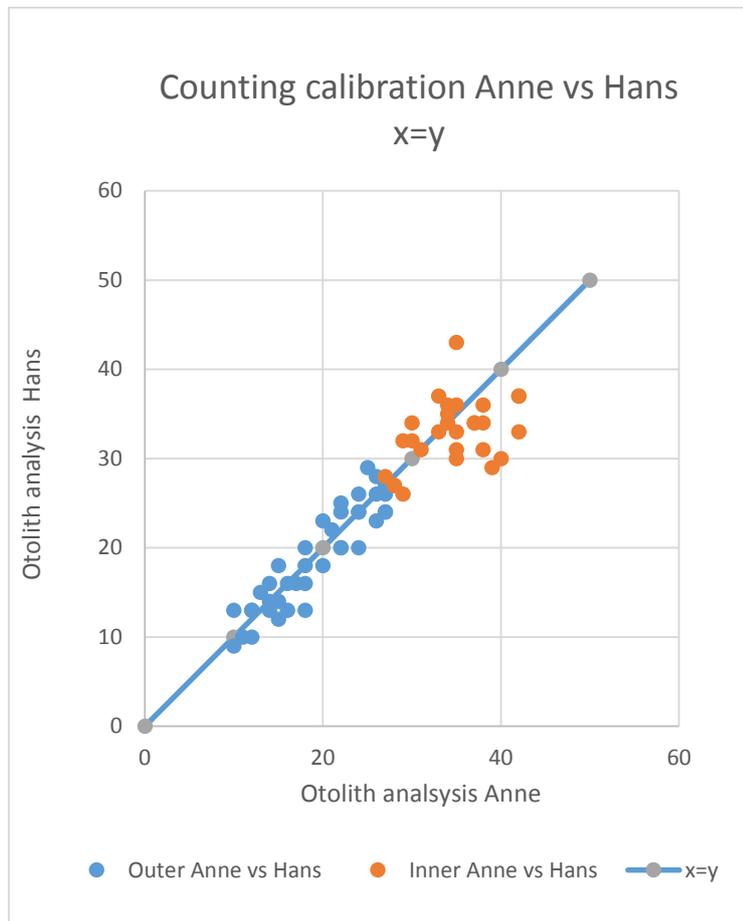


Figure 37: Graph about the difference in counting between the student and expert. Ideal scenario is when all points are on the $x=y$ line.

Otoliths analysed independent of the expert from the first five weeks were recounted. Otoliths analysed during the first 5 weeks together with the expert were re-examined on a 1/10 basis, if the difference was too large all those in that sampling campaign were reanalysed. The otoliths counted last year (used in another research and in this research) were reanalysed on a 1/10 basis as well. Again if the difference was too large all those in that sampling campaign were reanalysed. Additionally to these measures those that showed inconsistency with the rest of the growth curve were reanalysed to reduce the margin of error. The data was checked by the expert on a 1/10 basis to ensure reliable data during the entire execution phase of the research.

Appendix 5: DEB Model Sensitivity Control

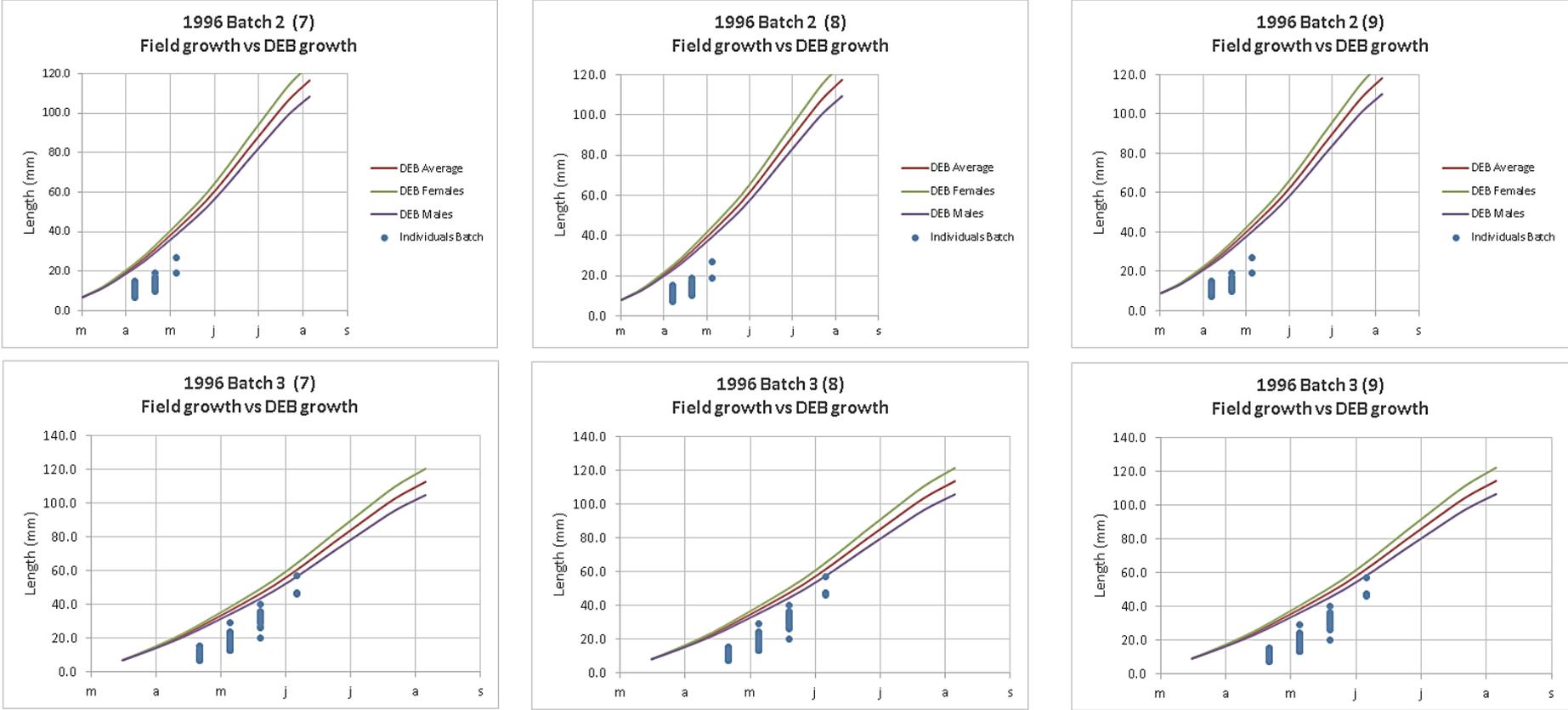


Figure 38: The graphs shows the predicted growth curve against observed growth for 1996. The DEB model was tested for sensitivity by using initial settlement size of 7, 8 and 9mm (indicated in the graph by the number between () in the title). From top row is batch 2, the bottom row is batch.

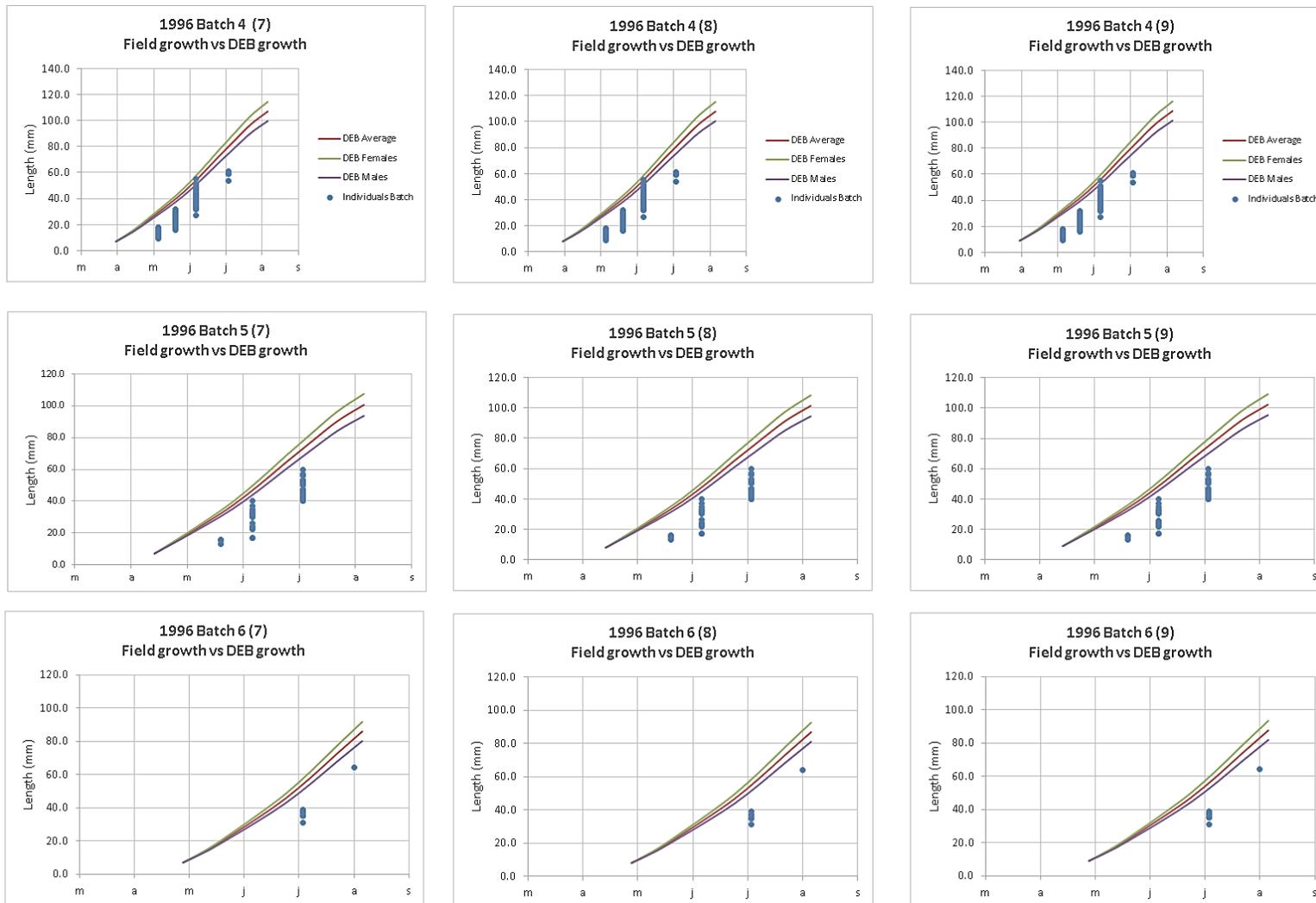


Figure 39: The graphs shows the predicted growth curve against observed growth for batches 4, 5 and 6 of 1996 (in ascending order from top to bottom). The DEB model was tested for sensitivity by using initial settlement size of 7, 8 and 9mm (indicated in the graph by the number between () in the title).