The Voordelta as a transitional habitat for sandeel populations (Ammodytes sp. & H. lanceolatus)

DA Final Thesis Report

Author: Brecht Vanoverbeke
Course: DA Final Thesis
Institution: HZ University of Applied Sciences
Supervisor: Tim van Oijen
Date: 3rd June 2016
Publication: IJmuiden
Version: 2.0

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Final Report

CU 11020 DA Final Thesis

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IJmuiden, 3 June 2016
Version: 2.0

Author:

B. Vanoverbeke	(student nr: 00065090)
Institution:	HZ University of Applied Sciences
Address:	Edisonweg 4, 4382 NW Vlissingen
E-mail:	vano0001@hz.nl

Client: IMARES Wageningen UR

In company supervisor:	dr. Ingrid Tulp
Institution:	IMARES Wageningen UR
Address:	Haringkade 1, 1976 CP IJmuiden
E-mail:	ingrid.tulp@wur.nl

HZ Supervisor:	dr. Tim van Oijen
Institution:	HZ University of Applied Sciences
Address:	Edisonweg 4, 4382 NW Vlissingen
E-mail:	tim.van.oijen@hz.nl

Cover images: Sandeel buried in the sand (topleft, from www.wnf.nl), sandeel foraging in the water column (bottomleft, from www.aqua.dtu.dk), and Sandwich terns with sandeel (right, from natuurpareltjes.skynetblogs.be and www.waddenacademie.nl).

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PREFACE

Along the course of my study, it became clear that marine ecology is one of my main interests. To fulfil my graduation, I also knew I wanted to become acquainted with marine resources and fisheries research since this topic has always been of great interest to me. Therefore, I was very pleased to be able to conduct my final thesis project at IMARES. I want to thank Adriaan Rijnsdorp for providing me the opportunity, and for getting me in contact with the right person.

I had a great experience working at IMARES and living in the charming city of Haarlem for the past 5 months. For the first time during my study, I was able to experience multiple aspects of the research cycle from data gathering in the lab through the data analysis with specialized software and finally the reporting and presenting of my findings.

I particularly enjoyed the practical work, where I got hands on experience with identifying, processing and measuring sandeel species. It was a nice experience to collect my own data, and to feel the curiousness about the potential following discoveries. Also, I can now proudly say I am one of the few sandeel experts at IMARES! The following period of R programming to analyse and process my data was challenging, but I gained a lot of valuable experience and it felt even better when I finally cracked the code after a long time of puzzling.

First of all, I want to thank Ingrid Tulp, my in company supervisor. I am very thankful for her professional and motivational supervision that contributed substantially to the quality of my thesis, and for incorporating me in the group as a colleague. You are definitely a role model to me! She also gave me the opportunity to get out of the office to join research vessels for fish monitoring fieldwork on the Western Scheldt and shellfish monitoring along the Dutch coast, and to share my work with researchers from the Flemish Research Institute for Nature and Forest (INBO), which were very exciting experiences. Thereby, I also want to thank Jack Perdon and Ingeborg de Boois for taking me with them in the field.

Furthermore, I want to thank Bram Couperus, Loes Bolle and Nicola Tien for their active commitment and for sharing their expertise regarding fisheries ecology, otolith analysis and statistical analysis respectively. I also want to thank Peter Groot and Kees Groeneveld for their time and effort they put into otolith reading, and Ineke Penock for her help with otolith digitization. Finally, I want to thank Tim van Oijen my HZ supervisor and examiner, for his trust and careful supervision from a distance.

To conclude, I was surprised so little is known about sandeel in Dutch coastal waters, yet it is an abundant and important fish in the marine ecosystem, as I experienced with my own eyes during the surveys I joined. Therefore, I hope I've made a valuable contribution to understand this keystone species better.

Brecht Vanoverbeke,

29th of May 2016, Oudenburg, Belgium

SUMMARY

This study aimed to get more insight into the habitat use and distribution of three sandeel species (*Ammodytes sp.* & *H. lanceolatus*) in the Voordelta and describes the findings regarding the Voordelta as a transitional habitat for sandeel populations.

As compensation for loss of habitat and foraging area of a number of species due to the expansion of the Rotterdam harbour (PMR), a seabed protection area was established in the Voordelta. In the area beam trawl fisheries was excluded in order to improve the quality of the benthic community. During a monitoring program by IMARES sandeel was found to be one of the few species experiencing a negative effect of bottom disturbing fisheries, which alternates between living buried in the sand and swimming in the water column. Furthermore, sandeel was found to be an important food source for Sandwich tern, an important species for the compensation measures. This way, the mode of life of sandeel provided a positive link between the exclusion of bottom disturbing fisheries and food availability for Sandwich terns. This link was not expected beforehand as the importance of sandeel for terns was underestimated. However, the general ecology of sandeel (*Ammodytes tobianus, A. marinus and H. lanceolatus*) in Dutch waters is still poorly understood since it is of no commercial importance.

In the Voordelta, sandeel was caught in a benthos survey (buried fish) and in a fish survey (swimming fish) allowing to investigate several aspects of sandeel ecology including species composition, length composition, age – length ratio, the spatial distribution of different species and sizes and the proportion of sandeel buried in the sand versus swimming in the water column. In addition, data collected further offshore was analysed allowing to identify possible differences in population structure between shallow coastal and deeper offshore regions. The combination of these data sources provided valuable information regarding the use of the Voordelta by sandeel, the prey availability for seabirds and to estimate the vulnerability of sandeel to bottom disturbing fisheries better.

The main findings of this study suggest the Voordelta as a transitional habitat for the *Ammodytes sp.* and *Hyperoplus lanceolatus.* Although both *Ammodytes* species have a similar mode of life a preference of *A. marinus* for the deepest areas and of *A. tobianus* (in particular small <10cm) for the shallow parts was observed without signs of competition. *A. tobianus* appears to fulfil its lifecycle in the Voordelta, while the *A. marinus* population might be dependent on larval drift from offshore breeding grounds beside the possibility of local reproduction. Sandeel in the Voordelta were younger (0-2 years old) than offshore (1-7 years old) suggesting migration of older fish or a shorter lifespan. However, there was high uncertainty in the age determinations because the otolith structures were often vague. Therefore the PMR fish could contain older individuals as well. Furthermore, sandeel were found both buried in the sand and in the water column during the day in autumn (Sept. – Oct.) suggesting a transitional period between activity and hibernation. This also suggests high vulnerability to bottom disturbing fisheries during this time of the year. Finally, the data is considered not optimal for the purpose of this study. A large amount of sandeel caught in the bottom dredge was damaged and small fish are likely to escape the fishing net used for the fish survey. Therefore, some recommendations are given to produce more representative and complete data.

INTRODUCTION

Background

My project is part of an extensive monitoring program of a new seabed protection area in the Voordelta serving as a nature compensation initiative for the large scale expansion of the Rotterdam harbour. The Project Mainport Rotterdam (PMR) involved a number of activities, including the construction of the “Tweede Maasvlakte” (MV2). For the latter project 2.000 hectares of land was reclaimed from the sea in order to facilitate the expanding industry.

MV2 is situated in the Voordelta, a protected nature reserve part of the Natura 2000 network. Areas within the Natura 2000 network are protected by the European Bird and Habitat Directive. The land reclamation activities of PMR are associated with irreversible alteration of the seabed and loss of habitat and foraging area for a number of plants and animals. To compensate for this, PMR initiated the institution of a 25.000 hectare seabed protection area southwest of MV2, and a new dune area near Delfland providing protected habitat and resting area for the affected species including benthic fauna, seabirds and seals.

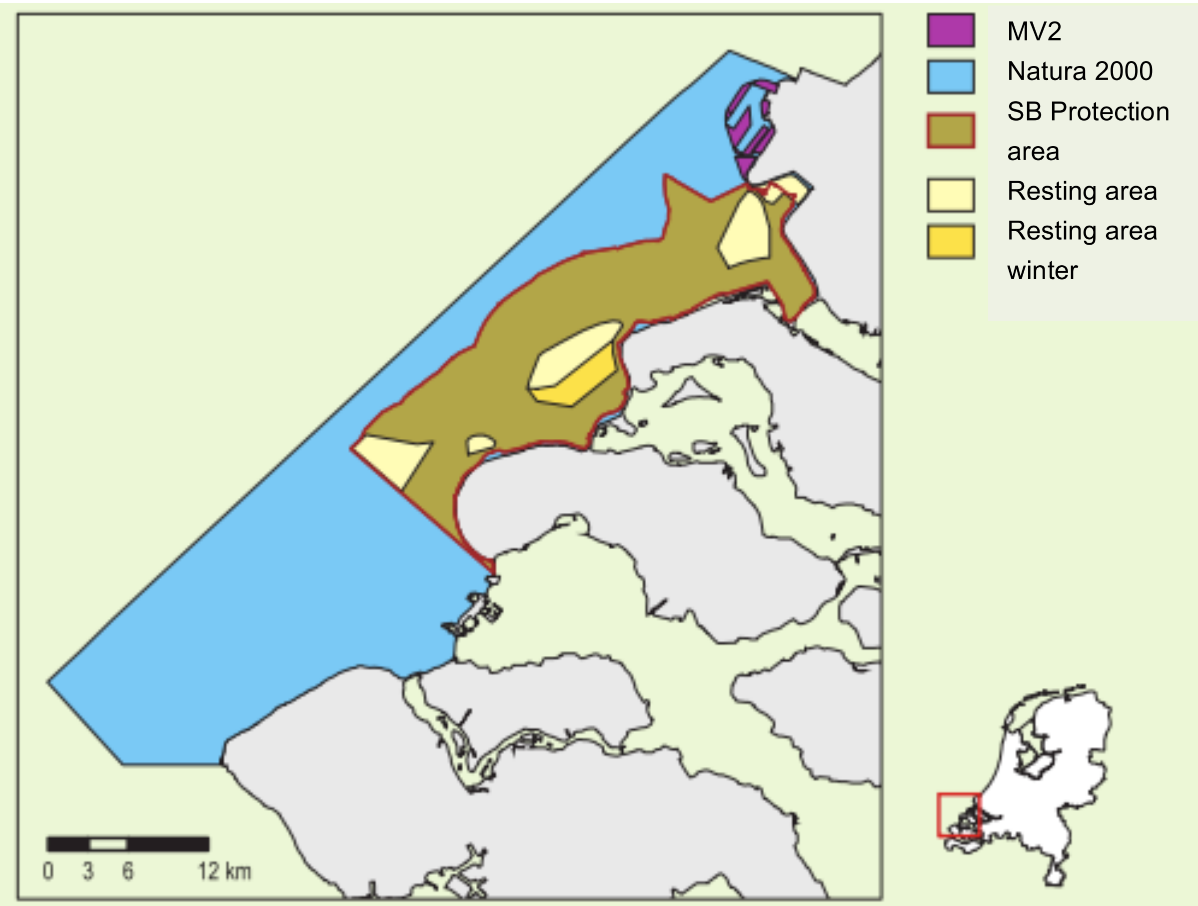

Figure 1: Map of the Voordelta (blue) situated in the southwest of the Netherlands. The new seabed protection area southwest of MV2 (purple) is indicated in brown, along with additional resting areas for birds and seals (yellow).

Figure 1 depicts the location of MV2 in the Voordelta marine reserve along with the compensatory seabed protection area (van Leeuwen, 2008). The PMR nature protection measures are targeting the bird species Common scoter, Sandwich tern and Common tern in particular since these species were expected to experience significant negative effects (Heinis, Vertegaal, Goderie, & van Veen, 2007).

Beam trawl fisheries targeting flatfish are prohibited in the newly protected area in order to improve living conditions for benthic fauna. The heavy beam trawls and tickler chains used for this fishery scrape off several centimetres of the upper seabed significantly damaging the benthic environment. However, beam trawl fisheries targeting shrimp are still allowed. Due to the use of lighter gear these fisheries are assumed to have less impact on the environment. Nevertheless, little is known about the effects of shrimp fisheries on the bottom fauna.

In 2005, a consortium of several environmental research agencies including IMARES was assigned to monitor the development of the seabed protection area coordinated by Rijkswaterstaat. IMARES was responsible for monitoring of the fish and benthic community in order to assess the effectiveness of the implemented measures.

Problem statement and goal

During the first years of the monitoring program sandeel was found to be one of the few species experiencing an adverse effect of flatfish and shrimp fisheries (Tien et al., in press). This species is affected by bottom disturbing fisheries because it exhibits the remarkable behaviour of burying in the seabed to rest and to escape from predators. In addition, these oil rich nutritious fish were found to be an important constituent in the diet of breeding Sandwich terns in the Voordelta, an important target species for the nature compensation measures (figure 2) (Fijn et al., 2015).

Figure 2: A pair of Sandwich terns feeding sandeel to their young. Sandeel is found to be an important food source in the Voordelta during certain times of the year.

This way, the mode of life of sandeel provided a positive link between the exclusion of bottom disturbing fisheries and food availability for Sandwich terns. This link was not expected beforehand as the importance of sandeel for terns was underestimated and provided an opportunity to learn more about sandeel ecology in the Voordelta.

Furthermore, recent research shows that sandeel contributed substantially to pelagic fish biomass in the Marsdiep suggesting its presence in other areas along the Dutch coast as well (Couperus et al., 2016). There are three species of sandeel occuring in the North Sea, namely *Ammodytes tobianus*, *A. marinus* and *H. lanceolatus*. *A. marinus* is the best studied species since it is the subject of a substantial fishmeal industry in Scandinavia and the British Isles. However, little is known about the distribution of sandeel in Dutch coastal waters because no important sandeel market exists in the Netherlands and fisheries research is mostly market driven. Yet it is apparent that this species plays an important role in the ecosystem and this study aims to provide a better insight into the use of the Voordelta by sandeel.

For this study I used sandeel data caught in a benthos survey (buried fish) and in a fish survey (swimming fish) in the Voordelta. In addition, data from a survey further offshore is analysed allowing to identify possible differences in population structure between regions. Finally, I did some research only sideways related to my thesis work including otolith reading for age determination and the collection of snout – eye and snout – gill measurements of sandeel (box 1).

The main question in this study is stated as the following:

What is the habitat use and distribution of three species of sandeel (*Ammodytes sp. & H. lanceolatus*) in the Voordelta?

The sandeel catch from the different surveys allowed to examine the following aspects of sandeel ecology:

- What is the species composition in the different surveys?
- What is the length composition & age – length ratio in the different surveys?
- What is the spatial distribution of the different species and sizes in the different surveys?
- What is the difference between male and female regarding the above parameters?
- What is the proportion of sandeel burrowed in the substrate versus sandeel in the water column?

This way, this study aims to provide a better insight in into the ecology of sandeel in the Voordelta which can provide valuable information to better estimate the sandeel availability for seabirds and the vulnerability of sandeel to bottom disturbing fisheries.

Reading guide

The first chapter provides an overview of the current knowledge about sandeel biology and ecology in literature. Next, the collection of the data and the method for sample processing are described followed by the results of the data analysis. The discussion puts the results into perspective and tries to explain the observed trends. Also shortcomings of the research method are put forward. Finally, the main findings of this study are outlined in the conclusion. In the attachments, the results of additional work regarding the snout – eye/gill measurements and otolith reading is presented.

Box 1: Snout – eye and snout – gill measurements of sandeel

More than half of the sandeel caught in the benthos survey with the bottom dredge was damaged (cut in half by the knife), which makes length estimation and species identification impossible resulting in a significant loss of important data. A linear relationship between the size of the head of the sandeel and its total body length is expected since the head of an organism is expected to grow proportionally to its body size. This correlation can be used in the future to estimate the length of damaged individuals (Attachment 1).

This chapter provides some theoretical background about the general biology and ecology of the three species of sandeel investigated. First, the role of sandeel in the marine ecosystem is sketched. Then, some taxonomy and morphology is described and furthermore topics regarding distribution and habitat, behaviour, growth, reproduction and population ecology are covered.

The role of sandeel in the marine ecosystem

Sandeel is a keystone species in the North Sea marine foodweb, forming a link between marine zooplankton and top predators (Frederiksen, Edwards, Richardson, Halliday, & Wanless, 2006). They are an important prey species with a high energetic value for a wide variety of seabirds, fish and marine mammals. Some examples are gadoids (cod related species), mackerel, sharks, rays, seals, porpoises, dolphins, gannets, terns and auk species like puffins. During the early 1900s, sandeel became abundant in Scottish waters and a highly lucrative industrial sandeel fishery for fish oil and fishmeal developed into the largest single species fishery in the North Sea between 1960 and 2000 (Gaaf, 2015). This large scale exploitation was subsequently linked with a collapse of several seabird breeding colonies on the Scottish coast (Frederiksen et al., 2005; Furness & Tasker, 2000; Wanless, Harris, Redman, & Speakman, 2005). After implementation of management measures, sandeel populations recovered.

In Dutch coastal waters, little is known about sandeel, but recent research showed that sandeel contributed substantially to pelagic fish biomass during spring in the Marsdiep, the gateway to the Wadden Sea, suggesting its presence in other areas along the Dutch coast as well (Couperus et al., 2016). Furthermore, sandeel is also found to be an important prey species for sandwich tern and common tern breeding colonies in the Voordelta during spring (Fijn et al., 2015) (figure 3). PMR nature compensation monitoring for birds in the Voordelta revealed that breeding success is influenced by the availability of sandeel and herring. When available, sandeel is a particularly important energy source for adult sandwich tern and sometimes also for juveniles.

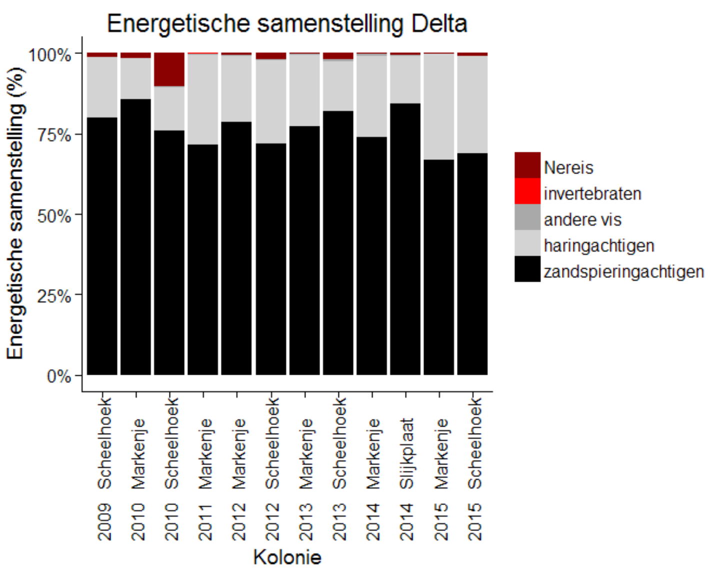

Figure 3: Species composition (%) of the diet of adult Sandwich tern colonies in the Voordelta expressed in energetic value (2009 – 2015), based on faeces analysis. Sandeel is indicated in black and herring in grey. Adopted from Fijn et al. (2015).

Taxonomy and morphology

In this study three species of sandeel of the *Ammodytidae* family were investigated. Two belong to the genus of *Ammodytes,* namely *Ammodytes tobianus* (Lesser sandeel, Linnaeus, 1758) and *Ammodytes marinus* (Raitt's sandeel, Raitt, 1934). The third species, *Hyperoplus lanceolatus* (Greater sandeel, Le sauvage, 1824) is part of the genus *Hyperoplus*.

Figure 4: The three species of sandeel found in the Voordelta. *A. tobianus* (top), *A. marinus* (middle) and *H. Lanceolatus* (bottom). The images are made of frozen and defrosted fish.

Sandeel are relatively small, elongated silver coloured fish (figure 4). *A. tobianus* reaches lengths up to 20 cm. Its mouth is characterised by a protrusible upper jaw and a lack of teeth. Its belly scales are symmetrically arranged in tight chevrons, and the tail is characterised by the presence of scales at the base of the caudal fin. It has a long dorsal fin with 61 – 66 fin rays (Raey, 1970). In general its colour appears slightly greenish, in particular when fresh.

A. marinus is slightly larger, reaching a length up to 25 cm. its mouth is also characterised by a protrusible upper jaw and a lack of teeth. *A. marinus* can be distinguished form *A. tobianus* by loosely, asymmetrically arranged belly scales, and the absence of scales at the base of the caudal fin. The dorsal fin ray number ranges between 56 and 75. In addition, its colour has a somewhat more dark blue appearance when fresh.

H. lanceolatus is considerably larger, reaching a length up to 40 cm. The upper jaw is not protrusible and it has a few teeth. The snout is characterised by a black spot on either side.

Distribution and habitat

All three species are inhabitants of the north-east Atlantic Ocean, mainly found in relatively shallow water over sandy areas of the continental shelf. Atlantic sandeel are roughly distributed between 36°N and 73°N, including the North Sea and Dutch coastal waters. In general, *A. marinus* is distributed more northernly than *A. tobianus* (Reay, 1970).

Sandeel larvae are planktonic and can be distributed widely by ocean currents. Larvae are generally found further offshore than were adults normally live (Reay, 1970). At a length of 30-40mm, juvenile sandeel become demersal, where they settle on shallow sandbanks. Different species of sandeel seem to occur in different, distinct areas. In general, *A. tobianus* is found relatively close inshore on sandy areas in bays and estuaries, even on intertidal beaches, at depths ranging between approximately 0 – 30m. Autumn and spring spawning populations are found to occupy the same habitat (Reay, 1970).

A. marinus is considered the most abundant sandeel species in the North Sea and even the most abundant fish species in general, accounting for 10-15% of the total fish biomass of the North Sea (Sparholt, 1990). *A. marinus* occurs mainly on offshore banks at depths between 30 and 150m, with a preference of depths between 20 and 45m (Wright, Jensen, & Tuck, 2000). Three main stocks are distinguished by Macer (1966) in the eastern North Sea (at 15-37m depth), in the western North Sea (south west Dogger Bank (27-37m) and Norfolk Banks (12-22m)) and in the southern Bight.

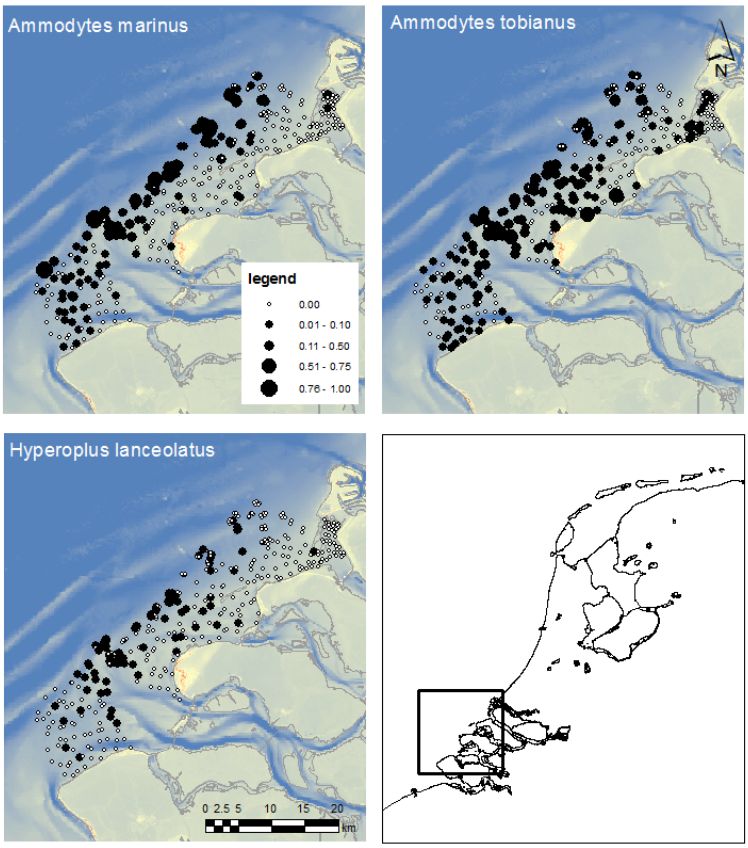

Figure 5: Distribution of *A. tobianus*, *A. marinus* and *H. lanceolatus* in the Voordelta expressed as average number/m2, based on the PMR monitoring data of 2009 - 2012. *A. marinus* is generally found further offshore than *A. tobianus*. *H. lanceolatus* is found much less frequent. Retrieved from (Tien, Craeymeersch, van Damme, Adema, & Tulp, in press).

H. lanceolatus is considered to have a wider distribution, occurring inshore, in estuaries and the intertidal zone as well as offshore between 0 - 150m depth However, mostly juveniles are found inshore (van Deurs et al., 2012).

Recent research shows that sandeel contribute substantially to the fish biomass in Dutch coastal waters in spring (Couperus et al., 2016).

Furthermore, results from the first years of the PMR monitoring also report significant densities of both *Ammodytes* species in the

Voordelta. *H. lanceolatus* was found much less frequent. *A. marinus* is mainly found in the deepest part of the Voordelta, while *A. tobianus* and *H. lanceolatus* also occurs closer inshore (Tien et al., in press) (Figure 5).

Since sandeel lack a swim bladder and large fins to swim long distances pelagically, they developed a remarkable characteristic. To save energy when not feeding, and to escape from predators, they bury in the sand (Raey, 1970) (figure 6).

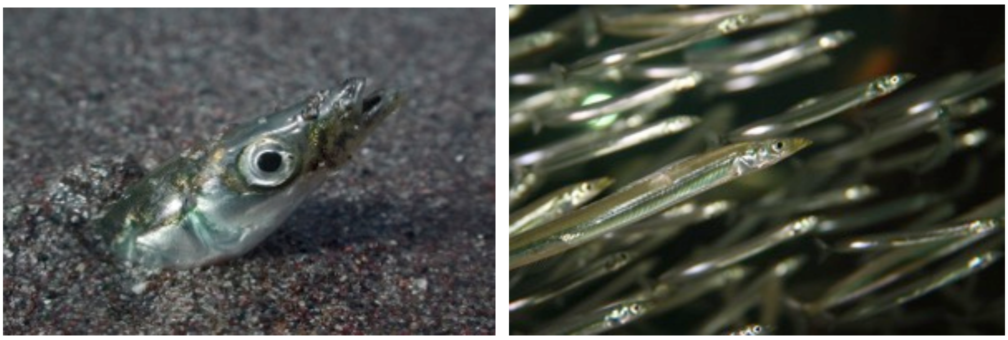

Figure 6: Sandeel partly buried in sandy substrate (left) and sandeel shoaling and foraging in the water column (right).

Due to this behaviour, sandeel require specific habitat features, that allows them to penetrate into the substrate. Macer (1966) describes the preferred substrates as clean (little organic matter), coarse sand or fine gravel; theyavoid of muddy areas. Buried sandeel have no opening in the sand to breath, requiring a well oxygenated substrate, with large interstitial spaces. Small particle size may constrain gill ventilation and clog the gills. The preferred substrate is associated with high current (tide) and wave energy. Therefore, sandeel are thought to prefer turbulent areas like the edges of sandbanks (Macer, 1966).

Wright et al. (2000) related decreasing *A. marinus* abundance with increasingly fine sediments (high silt/clay content). Sandeel was completely absent in areas with a silt/clay content >10%. Sediment choice experiments confirmed the results showing sandeel preference for medium to very coarse sand with median particle size of 0,25 – 2mm. Also Holland, Greenstreet, Gibb, Fraser, and Robertson (2005) revealed a clear selection of *A. marinus* for habitat with increased levels of coarse and medium size particles. Variation in sandeel abundance was mainly influenced by the silt content. In addition, the coarseness of the sediment also influenced the size of the sandeels living in the area.

A study of van der Kooij, Scott, and Mackinson (2008) linked the effect of a number of environmental factors on the daytime distribution and abundance of *A. marinus* on the Dogger Bank using a generalised additive model (GAM). This study showed that suitable burying habitat and seabed temperature where of highest influence on local sandeel distribution.

In the Voordelta, Tien et al. (in press) generally found all three species of sandeel on locations with medium to coarse sand particle size and low silt content. Moreover, these sites had high average flow velocity and relatively high salinity.

Behaviour

Diurnal activity

As already mentioned, sandeel spend their life partly burrowed in the seabed, and partly swimming in the water column. In general sandeel are known to burrow during the night and during a prolonged period of hibernation in winter. They leave the sandbank to feed and during the spawning period to spawn. Reay (1970) found *A. tobianus* to be feeding from late march to early November, suggesting a burrowing period during winter with no feeding activity. Cameron (1958) added proof to this behaviour by catching *A. marinus* on an offshore bank in winter with a scallop dredge, when they could not be caught with a trawl net.

Kühlmann and Karst (1967) provided underwater observations of *A. tobianus* diurnal behaviour in inshore waters of the western Baltic Sea. When foraging, the sandeel formed schools, where *A. tobianus* and *H. lanceolatus* regularly occurred in the same school, often together with young herring. Schools generally consisted of similarly sized fish.

The sandeel emerged from sandbank at sunrise in small groups, and formed large schools (>1000) to move towards the feeding grounds in the vicinity of the inshore sandbank, about 1 km away from their burrowing sites. Feeding behaviour was characterised by vertical and horizontal dispersal of the school in the water column. The school stayed relatively stationary while each individual searched for food suspended in the water column. The sandeel returned to the shallows in the afternoon, followed by a resting period and e second feeding period in the evening. With decreasing light, the fish returned again to the sandbank and separated into small groups to burrow in the sand during the night.

Popp Madsen (1963) suggested a relation between feeding activity of *A. marinus* and tidal strength, bringing increased food concentrations over the sandbank. However, this finding is not clearly confirmed by other studies. A more recent study by Freeman, Mackinson, and Flatt (2004) investigated diel patterns of habitat use of *A. marinus* based on integrated acoustic surveys on the Dogger Bank in June. A diurnal pattern of activity associated with day length as described above is confirmed, however, no correlation between sandeel distribution and the tidal cycle is found.

Furthermore, tank experiments conducted on the effect of several environmental factors on the behaviour of *A. marinus* showed a diurnal activity cycle dependant on food availability, light intensity and temperature respectively, suggesting sandeel remain buried during winter with low temperature, light intensity and food availability, after the spawning period (December – January) (Winslade, 1974a, 1974b, 1974c). Furthermore, swimming activity in the summer half year during the day also depended on food concentrations. In absence of prey, most sandeel remained partially burrowed in the sand, waiting for prey. Raey (1970) suggests that sandeel may respond to periods of food scarcity by remaining in the sand for longer periods, which is more energy efficient and safer.

Research by (van Deurs, Behrens, Warnar, & Steffensen, 2011) revealed similar drivers of foraging activity of *A. tobianus* from laboratory tank experiments with wild caught fish schools. However, the amount of food ingested is indicated as a primary driver of foraging activity. Temperature, light intensity and prey concentration are only considered secondary drivers, which trigger feeding activity in spring, when temperature rises and plankton concentrations increase rapidly.

Migration

Seasonal migration of sandeel to coastal water in summer and offshore in winter was reported, but without clear evidence. There is suggested some sort of migration between North Sea stocks by juveniles at recruitment (Raey, 1970). On the basis of tagging 900 fish in the southern North Sea, Popp Madsen (1963) found *A. marinus* to be very stationary without undertaking large distance feeding migration. However, another tagging study by Gauld (1990) found that *A. marinus* is capable of travelling distances of at least 64 km.

By linking suitable burying habitat (considering depth, high bottom current speed and the occurrence of coarse sand) of the Pacific sandeel (*A. hexapterus*) to catch data of pelagic schools, Robinson, Hrynyk, Barrie, and Schweigert (2013) identified key foraging areas in close proximity (majority 4.9 km) to adjacent burying habitats. Results from van der Kooij et al. (2008) report maximum daily movement of *A. marinus* away from their night time burrowing sites of about 5 km in the North Sea.

Laugier, Feunteun, Pecheyran, and Carpentier (2015) provide a more in depth study about population mixing and migration patterns of *A. tobianus* in the south – western English Channel (France) inferred from otolith microchemistry. Much variation in otolith chemistry was found between sampling sites, while less variation existed within sampling sites, suggesting high sand bank fidelity, after switching to a bentho – pelagic mode of life. This sedentary mode of life on sandbanks makes sandeel particularly vulnerable to bottom disturbing fisheries.

Feeding

Both *Ammodytes* species are visual feeders, consuming mainly marine zooplankton including copepods, crustacean larvae and annalids. Some difference exists between *A. marinus* and *A. tobianus* as a result of their different habitat. Juvenile *H. lanceolatus* (>15cm) feed on fish eggs and larvae, copepods and crustacean larvae. The larger individuals however, feed on fish, mainly the other sandeel species (Macer, 1966).

Growth and reproduction

All three species generally reach sexual maturity when they are two years old (Macer, 1966; Raey, 1970). The larger individuals may mature after one year.

Spawning occurs once every season, for *A. marinus* in the winter months (January – December). *A. tobianus* has two distinct sympatric spawning populations, spawning in spring (February – April) and autumn (September – November). Spawning generally takes place within the inhabited area of the population. There is no evidence of migration to specific spawning grounds (Raey, 1970). For *A. marinus*, three different spawning grounds are distinguished in the southern North Sea, namely south west of the Doggersbank, the eastern part of the North Sea and in the southern Bight (Macer, 1966). Also Coull, Johnstone, and Rogers (1998) and the Scottish Fisheries Research Service produced maps of widest known spawning areas of *A. marinus* in

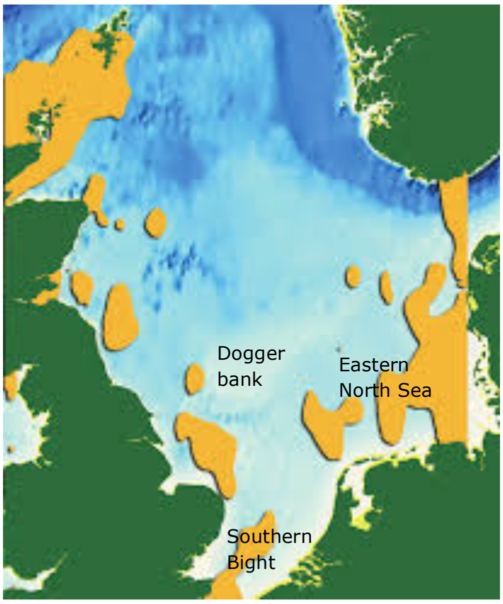

Figure 7: *A. marinus* spawning spwaning grounds in the North Sea. Retrieved from Scottish Fisheries Research Services.

the North Sea based on the distribution of eggs and larvae (figure 10). *H. lanceolatus* spawns during a more prolonged period in summer (April – August) (Macer, 1966).

Sandeel eggs are demersal and are deposited on the substrate where they attach to sand particles (Williams, Richards, & Farnsworth, 1964). After hatching the larvae become planktonic. At about 40mm length, the pelagic larvae undergo a metamorphosis and become demersal again, usually at an age of about 1,5 years old (Raey, 1970).
Adult sandeel have a short lifespan and experience a high mortality rate. The average life span of an adult fish is estimated on 0,9 years. The oldest year class found by Macer (1966) was 7 years for *A. tobianus* and 9 years for *A. marinus*. *H. lanceolatus* attains greater length than the other two species, but reaches about the same age (oldest age group of 8 years). Intraspecific variation in growth rate between populations of the same year – class on different sandbanks was found, most likely through different current regimes supplying variable densities of food (Macer, 1966). Given their relatively sedentary behaviour, sandeel depend on sufficient quality and supply of copepods in spring.
van Deurs, Christensen, and Rindorf (2013) suggests that smaller habitats can sustain higher growth rates and densities of sandeel per unit area due to less feeding competition.

Population ecology

In general the sex – ratio in a population of sandeel is about 1:1. There can be up to 7 – 9 year classes, but class 1 – 3 is usually the most abundant. Due to influx of juvenile fish the age composition can vary through the season. For *A. marinus* and spring spawning *A. tobianus*, recruitment of pelagic juveniles to the demersal population takes place between May and August, four to six months after the spawning period. For autumn spawning *A. tobianus* some recruitment takes place already in November and some larvae appear to overwinter until April of the next year (Macer, 1966; Raey, 1970).

A sandeel population usually has 1 or 2 length classes occurring most frequently, indicating the dominant year classes. There is also a seasonal variation in length composition due to rapid growth of the recruit fish (Raey, 1970). Macer (1966) indicated geographical variation in length of the same year class.

The literature study described the different aspects of sandeel biology and ecology including the specific habitat preferences of the different species and their remarkable burying behaviour. In this study all available sandeel data at IMARES from the Voordelta and offshore is analysed to be able to assess to what extent these findings are true for Voordelta.

METHOD AND MATERIALS

This chapter describes the available data for this study and the sandeel processing of the 2015 September – October PMR benthos survey and the IBTS survey executed in February 2016. In order to get more insight into the habitat use and distribution of sandeel in the Voordelta a number of parameters were determined. Every animal was identified to species level, sexed, measured (to the nearest mm), weighted (to the nearest g) and the otoliths were extracted for age determination. Furthermore, the additional activities are described including snout – eye/gill regression analysis with total length and digitisation of otolith images. Finally, the content and procedure for data analysis is described and some information is given regarding fieldwork activities.

Available data

Sandeel data from the Voordelta is available from 2009 – 2015. Since 2015 additional otolith data is available for age determination. Because sandeel burry in the seabed they are collected both in a benthos survey using a bottom dredge and a fish survey using a shrimp trawl (figure 8). Sandeel from the October 2015 benthos survey was processed during this project. The benthos surveys has approximately 400 sample locations in the Voordelta while the fish survey only covers about 20 locations. From the benthos data also sediment particle size data is available collected with a boxcorer at approximately the midpoint of each survey transect.

Figure 8: Illustration of the gear used for sandeel sampling in the Voordelta. Sandeel buried in the sand is caught in a benthos survey using a bottom dredge (left) and sandeel swimming in the water column is caught in a fish survey using a shrimp trawl (right).

In addition, sandeel from the IBTS (International Bottom Trawl Survey) executed in February 2016 was processed to provide a contrast with deeper areas further offshore. The IBTS is an annually executed survey for demersal and pelagic fish stock assessment in the ICES (International Council for Exploration of the Sea) area covering the North sea and the North Atlantic. Sandeel caught during this survey originate from further offshore including areas along the Dutch coast, the Danish coast, the Dogger Bank and the Scottish coast (figure 9). The contrast with the PMR data of the Voordelta is interesting because it allows to identify possible differences between regions in population structure.

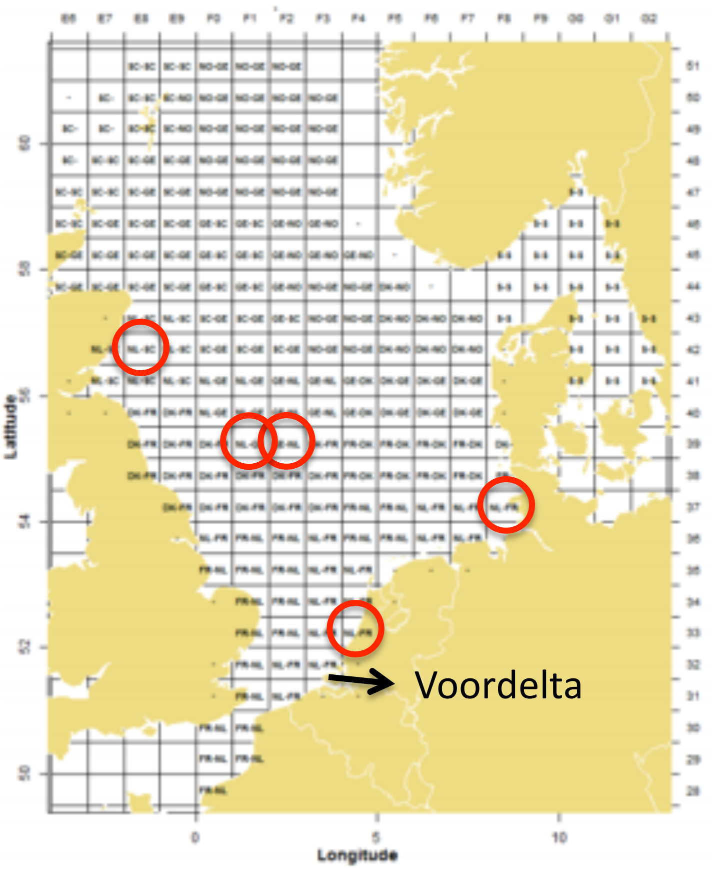

Figure 9: A map of ICES statistical quadrants showing locations were sandeel was caught during the 2016 IBTS survey, indicated by the red circles.

Species determination

PMR benthos sandeel were delivered deep frozen at – 20°C and individually sealed in order to facilitate species identification. IBTS sandeel were delivered in bags with all specimens packed together per sampling location. However, the bulk storage (IBTS) didn't pose any significant species identification problems. After defrosting, the sandeel are determined to species level on the basis of several characteristics described in the theoretical framework and listed in table 1.

At first sight, the pigmentation on the base of the caudal fin best explained the difference between *A. tobianus* and *A. marinus*. In *A. tobianus* a dark M-shaped band follows the caudal end of the scales and remains visible after removal of scales (e.g. due to physical impact during the catch process). In *A. marinus* a slightly darker (compared to *A. tobianus*) band indicates the edge of the base and the actual fin (figure 10, left). This characteristic was identified using a magnifying glass or a stereo microscope. *A. marinus* is found to have 1 sharply shaped scale in the middle of the base of the caudal fin and two, elongated spots without pigmentation (figure 10, right).

The arrangement of the belly scales could also be used to distinguish between the species examined with the aid of a stereo microscope. However, due to the small size and transparency of the scales distinguishing between different patterns was difficult. Finally, when in doubt, the number of dorsal fin rays was counted of a few individuals.

Tabel 1: Meristic characteristics used to distinguish between *A. marinus*, *A. tobianus* and *H. Lanceolatus*, described by van Deurs et al. (2012), based on Reay (1970) and Macer (1966) and H. Jensen from the Danish Institute for Fisheries Research (DIFRES) which discovered a new characteristic *.

	A. marinus	*A. tobianus*	*H. lanceolatus*
Spawning time	Dec – Jan	Feb – Apr & Sep - Nov	Summer
Premaxillae protrusible	Yes	Yes	No
Dark spot on both sides of the snout	Absent	Absent	Present
Vomerine teeth	Absent	Absent	Present
Belly scales organization	Loosely arranged	In tight chevrons	-
Scales at the base of the caudal fin	Max. 2 to 3 (very rare)	Min. 6	-
M – band at the base of the caudal fin *	Absent	Present	Absent
Dorsal fin ray number	56 – 75	61 – 66	65 – 69

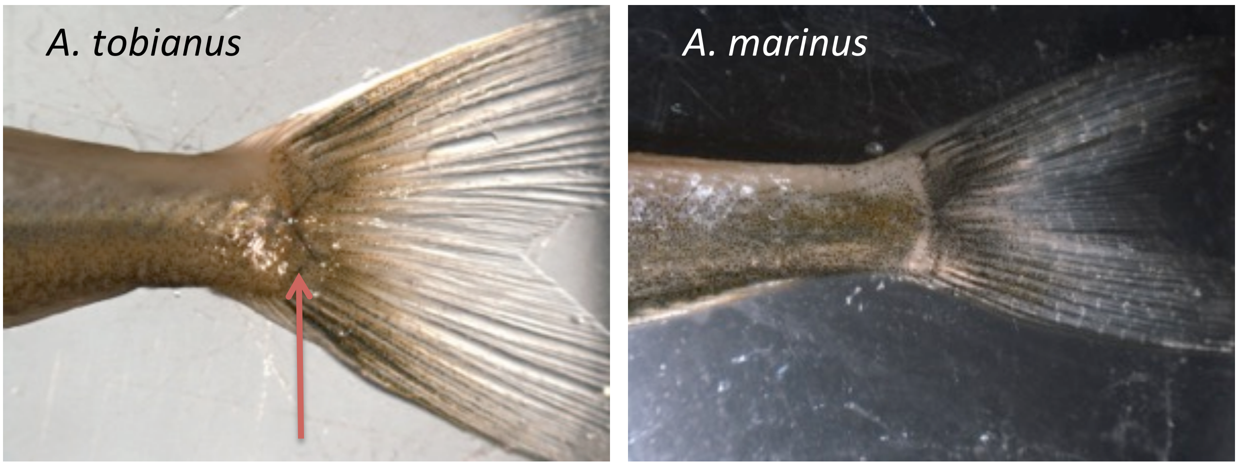

Figure 10: The caudal fin of *A. tobianus* (left) characterised by the M - shaped pigmentation band indicated by the red arrow, in comparison with the caudal fin of *A. marinus*, lacking the pigmentation band but having two, more pronounced white spots (absence of pigmentation) on the base of the caudal fin (right).

Length & weight measurement

Subsequently, the fish were measured using a measuring board (figure 11, bottom left). In addition to the total length of the fish, the snout – eye and snout – gill distance is measured using a digital calliper (figure 11, top). The size of the head is measured as the distance between the tip of the lower jaw and the eye (snout – eye, SE) and/or the gill (snout – gill, SG). Subsequently, total body length is modelled as a function of SE/SG distance using a linear regression model in R. Hereby, an equation is derived which can be used to estimate the total length of damaged sandeel. All data from different surveys are combined since no structural differences between surveys were expected.

The two different measures were taken to provide the option to select the parameters with the strongest correlation. Sandeel can only be identified to species level if the fish is undamaged. Because damaged sandeel are hard to determine to the species level, also a regression equation for both *Ammodytes* species combined is derived apart from the species-specific regressions. This equation can be used for total length estimation on board of the research vessel, without the need for species identification.

Furthermore, the sandeel were weighed using a digital balance for eventual condition estimation (figure 11, bottom right).

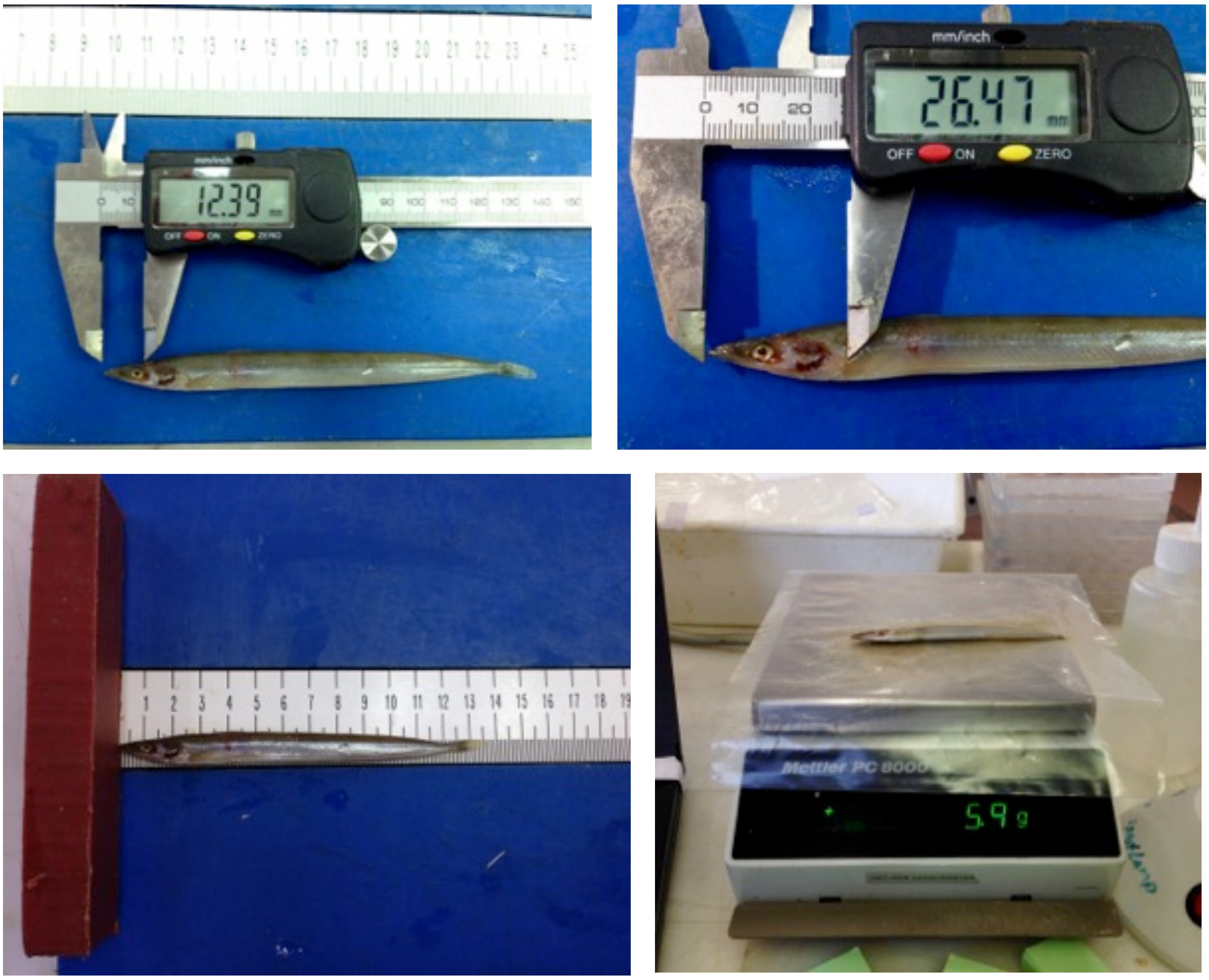

Figure 11: Total length measurement (bottom left), snout – eye (top left) and snout – gill (top right) measurements and weight determination (bottom right).

Determination of sexe

Determination of sex is done to identify possible differences between male and female regarding the examined parameters and is carried out by gonad examination. Small gonads are examined with a stereo microscope. The male and female gonads are distinguished on the basis of a number of characteristics. Male gonads are generally milk coloured and have a homogenous structure where no separate oocytes (eggs) can be observed. The shape is generally quite flat with sharp edges (figure 12, right). Female gonads are generally more stuffed, and have a more pronounced orange colour with a granular structure and distinguishable oocytes (figure 12, left). However, these characteristics are not straightforward and clear identification depends on the size of the fish and stage of maturity.

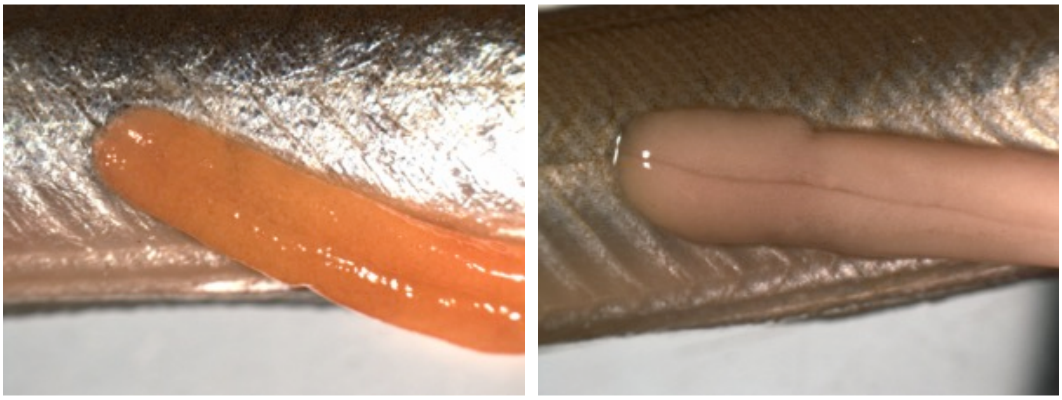

Figure 12: A picture of a female gonad, characterised by its dark orange colour and somewhat bulky, granular structure (left), and a male gonad, characterised by a more milky colour and homogenous structure, and a more flat and sharp shape (right).

Otolith extraction for age determination

For the purpose of age determination, the otoliths were extracted. Otoliths are small bony structures centrally located in the head of the fish fulfilling several functions including hearing and orientation. During growth of the fish, the otoliths grow by the deposition of aragonite (a form of calcium carbonate). Hereby, annual growth rings are formed which can be used for age determination.

The otoliths are extracted by making an incision right behind the cheek with a scalpel knife. Care must be taken not to break off the tips of the otoliths, since this is an important part for age determination. It is also important to preserve both otoliths since inspection of the second otolith may help identifying false growth rings when reading them. If done properly, the otoliths can now easily be extracted using forceps, with the aid of an illuminated magnifier (figure 13).

Otolith reading

Otolith reading requires a lot of experience. Therefore I assisted the Imares technicians in the otolith analysis instead of doing this completely myself. Age determination is done by counting the growth rings (annuli) using a stereo microscope (Bolle et al., 2013). Each annulus is characterised by an opaque band (white when using reflected light) and a translucent band (dark when using reflected light) (figure 14). The opaque core and the inner translucent band correspond to the first year of life. Furthermore, a birthday of January 1st is assumed, and the fish are classified in age groups. 0 group includes fish from hatching till December 31st. 1 group fish are thus in their second year of life.

Figure 13: Otolith extraction by making a small incision right behind the cheek of the fish using a scalpel knife (left). The otoliths are extracted with forceps under an illuminated magnifier (right).

Figure 14: microscopic photograph of sandeel otoliths (*A. marinus*) using reflected light. This individual is estimated to be two years old.

Otolith image digitisation for growth rate estimation

After removal, the otoliths are preserved in a labelled rack with small containers in a little bit of water for further otolith reading and image digitisation (figure 15). For future otolith measurements regarding growth rate estimation, a length and age stratified selection of the extracted otoliths was digitised using a microscope with a camera attached and the program ImageJ.

Figure 15: The otoliths are preserved in a labelled a rack with small containers (left). The otoliths are digitalised for growth rate estimation using a microscope with a camera attached (right).

Data analysis

The data analysis of this study includes all available PMR sandeel data from 2005 onwards, in addition to the PMR 2015 and IBTS 2016 data. Age data is only available for 2015. The gathered data is processed and visualised in figures, using the open software statistical computing program, Rstudio. Computed figures consist of species composition, length composition, age composition and age – length ratio. The latter is used to identify differences in growth rate. Furthermore, the data is checked for a correlation between densities of both Ammodytes species which could indicate competition for space, and for a correlation between the amount of *A. marinus* in the Voordelta and the stock size estimate in offshore areas.
Subsequently, the data were processed into maps, giving insight in the spatial distribution of the different species, age and sizes of sandeel in the Voordelta, thereby possibly revealing important habitat areas.

Sandeel sampling

During the 3rd week of May 2016 I joined the WOT coastal survey in the Voordelta. This survey is a benthos survey and up until now sandeel has not been collected. Because of the growing interest in the species, IMARES wants to start to collect sandeel from this survey as well. The resulting data will not be included in this study due to the limited timeframe, but some recommendations are given for efficient sandeel data collection (see recommendations).

RESULTS

This section provides the results from the data analysis of all available sandeel data from the PMR benthos and fish survey (September – October) including the comparison with offshore IBTS data (February 2016). Analyses include species composition, length composition, age – length ratio and the spatial distribution of different species and sizes of sandeel in the Voordelta. Also the sexe was determined to identify possible differences between male in the female in the relevant parameters, however, the poor quality did not allow to use the results in further analysis. Furthermore, the results of the SE/SG relationship with total body length are presented in attachment 1, based on the 2015 PMR and 2016 IBTS data. Finally, some results regarding otolith analysis or "otolith reading" for age estimation are described and presented in Attachment 2.

Species composition

The species composition of the PMR fish survey consisted mainly of *A. tobianus* and smelt, while the PMR benthos survey contained a larger proportion of *A. marinus*. Also the IBTS catch consisted mainly of *A. marinus*. *H. lanceolatus* (smelt) was found mainly in the fish survey catch (figure 16). Moreover, species composition varied between years.
Due to the small proportion of *H. lanceolatus* caught, this species is neglected in further analysis.

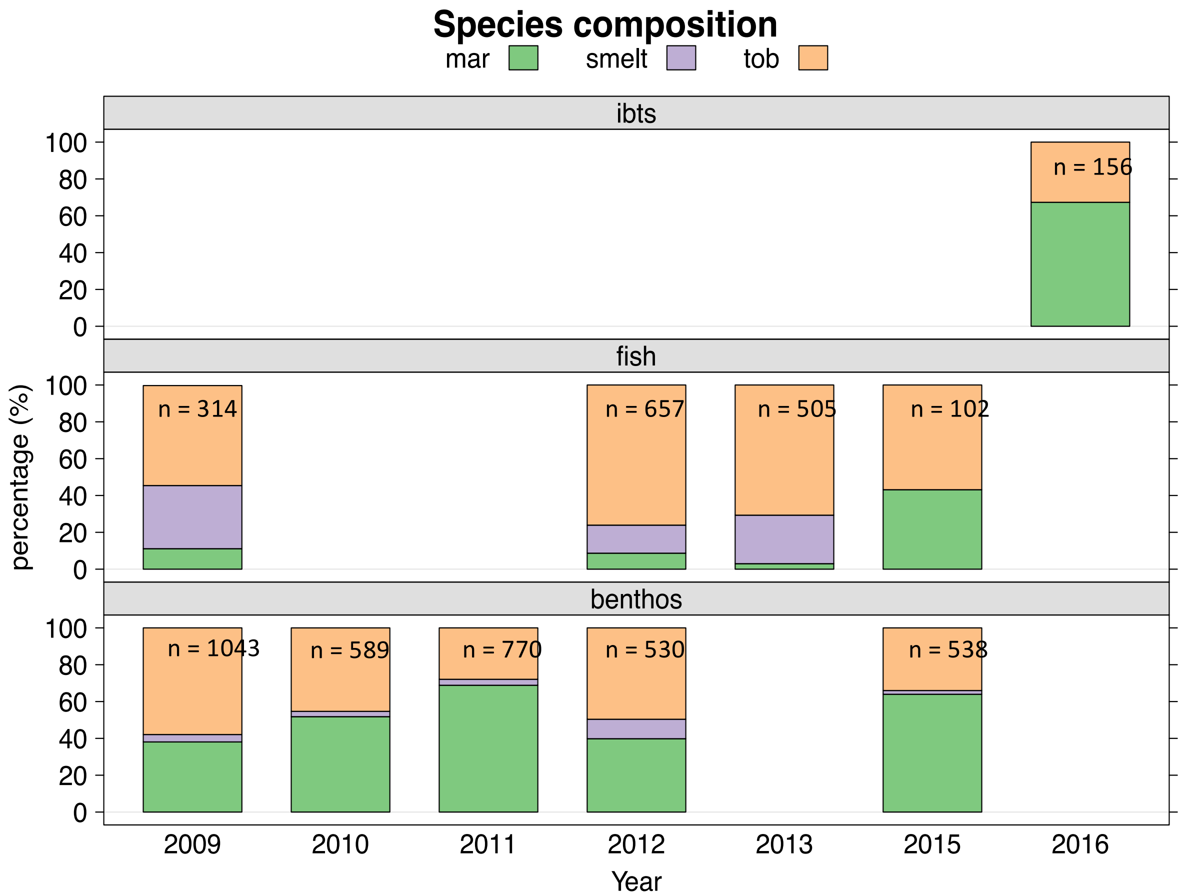

Figure 16: Sandeel species composition of all available PMR benthos and fish survey data and 2016 IBTS data, expressed in percentage (%). Colours represent different species and n – values indicate the sample sizes.

Length composition

Figure 17 and 18 show the length composition of both *Ammodytes* species compared between the three different surveys for 2015 and 2016.

A. marinus and *A. tobianus* from both PMR surveys had similar lengths between 7 and 18 cm, but the benthos catch contained considerably more small sandeel in the range of 7 – 11 cm, mainly *A. tobianus*. The IBTS catch contained considerably larger *A. marinus* (figure 17 & 18).

When comparing length class frequency distribution of the benthos catch between years, two main cohorts of sandeel were distinguished. There is a cohort generally between 8 and 13 cm and between 13 and 18 cm (figure 19). However, this pattern is more consistent for *A. tobianus* since the cohort of small *A. marinus* is absent in 2012 and only hardly present in 2015.
The fish survey only contains the cohort of large fish (mainly *A. tobianus*), and the absence of small fish is recognised every year (figure 20).

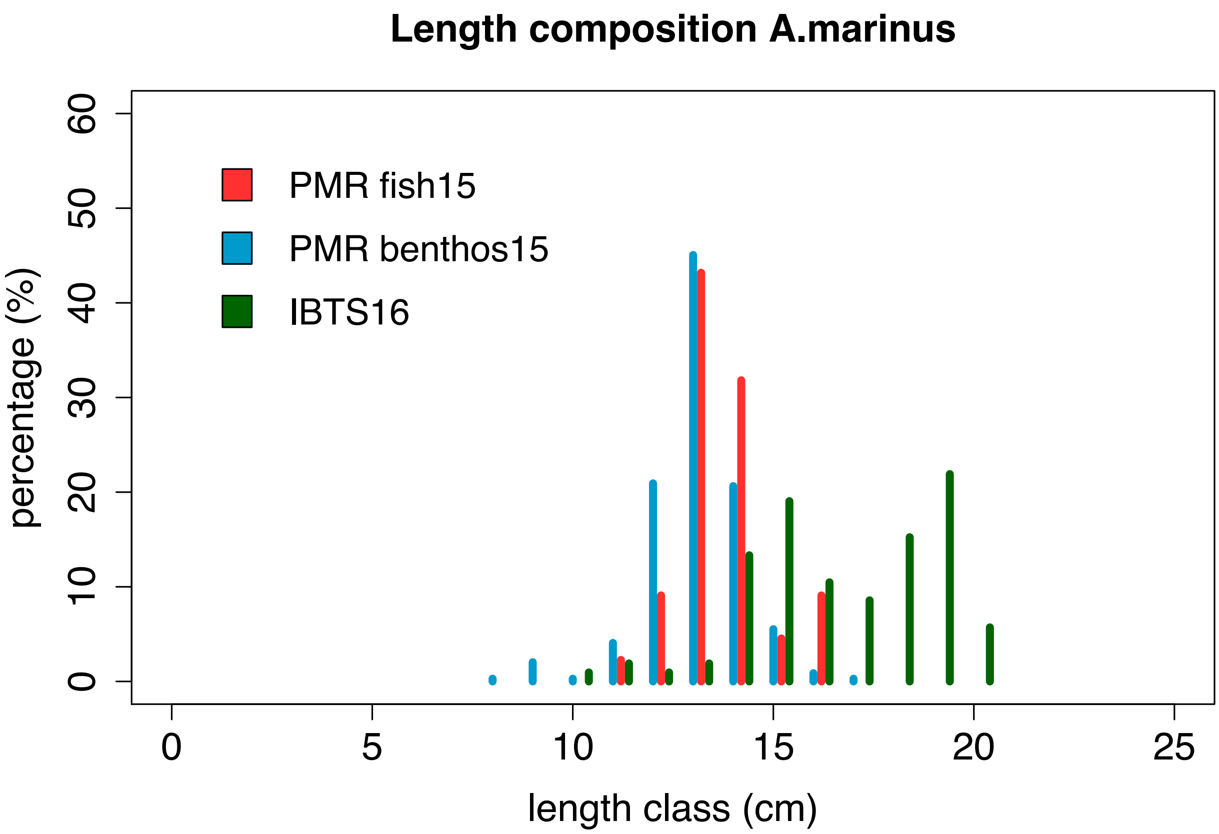

Figure 17: Length class frequency distribution of *A. marinus* caught in the 2015 PMR benthos and fish survey, and the 2016 IBTS survey, expressed in percentages (%).

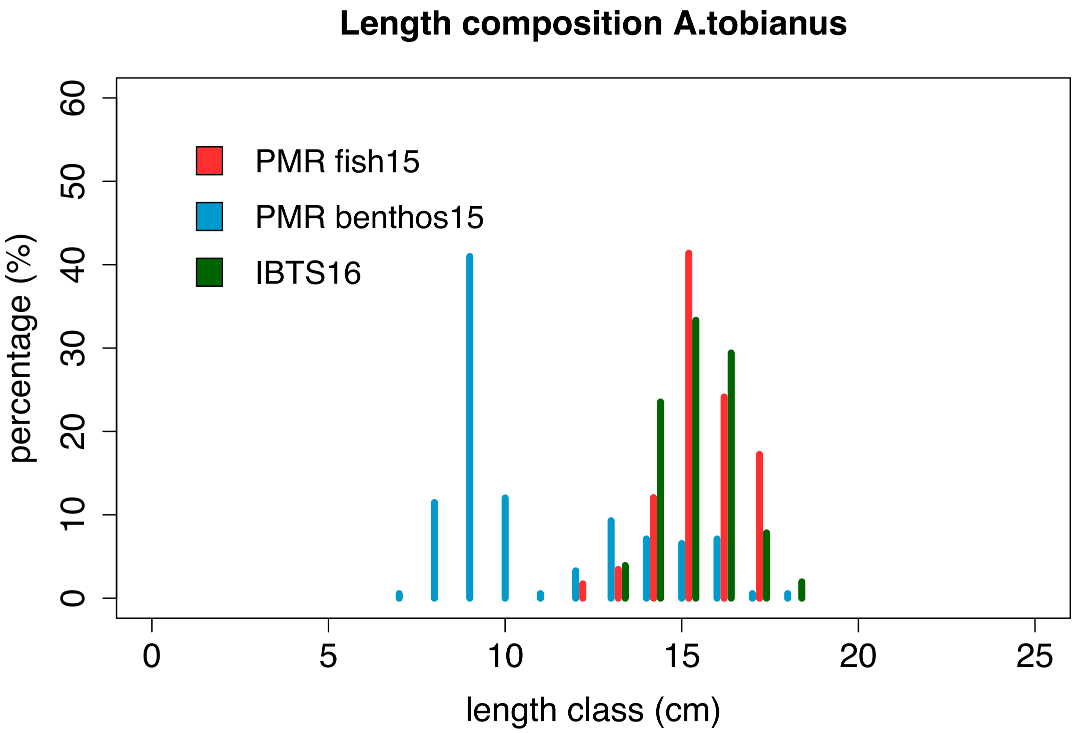

Figure 18: Length class frequency distribution of *A. tobianus* caught in the 2015 PMR benthos and fish survey, and the 2016 IBTS survey, expressed in percentages (%).

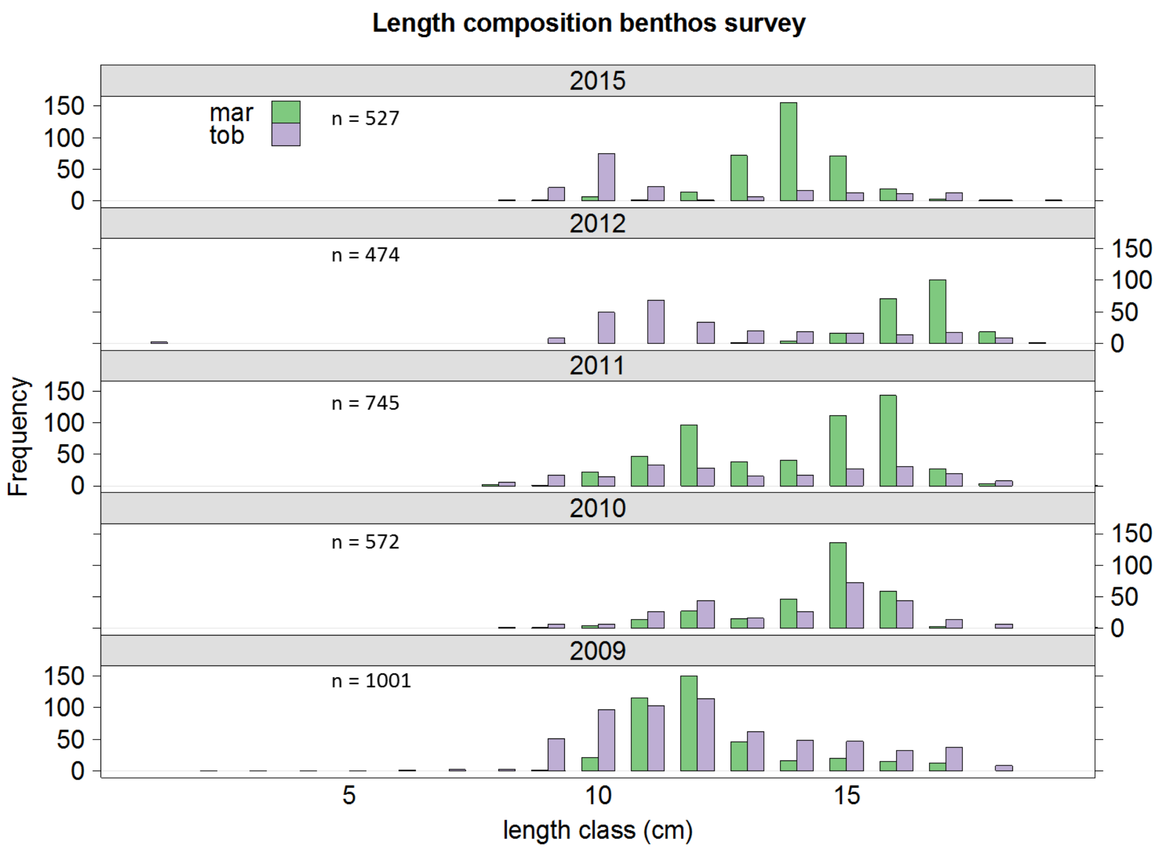

Figure 19: Length class frequency distribution of both *Ammodytes* species caught in the PMR benthos surveys. N – values indicate sample sizes.

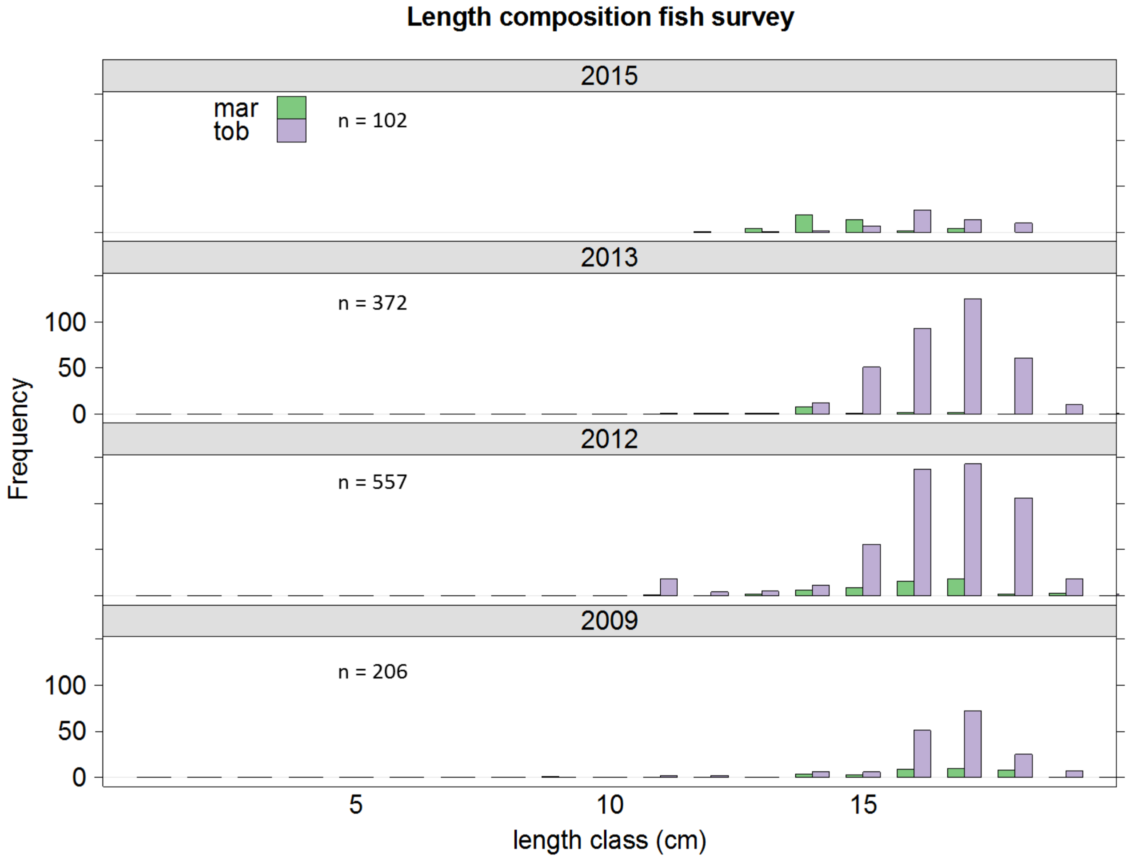

Figure 20: Length class frequency distribution of both *Ammodytes* species caught in the PMR fish surveys. N – values indicate sample sizes.

Age – length ratio

The cohort of small sandeel (7 – 11cm) caught in the benthos survey consisted of 0 group fish (figure 21). The larger fish were mainly 1 year old with some A. marinus of two years old (12 – 14 cm).

A. tobianus caught in the fish survey consisted exclusively of 1 year old fish (12 – 17 cm) (figure 22). *A. marinus* mainly consisted of 1 group fish (11 – 16 cm), but with some 2 group fish (13 – 16 cm). Age – length ratio shows minor difference between both species and between surveys.

A. marinus caught in the 2016 IBTS survey consisted mainly of 1 and 2 group fish between 10 and 16 cm. Sandeel between 17 and 20 cm were mostly between 3 and 5 years old with some fish reaching an age up to 6 and 7 years old (figure 23). Also *A. tobianus* comprised mostly of fish between 2 – 3 years old, with a length between 13 and 18 cm. Some *A. tobianus* also reached ages up to 7 years old.
In general, the IBTS survey caught remarkably older sandeel than the PMR surveys. No clear pattern was observed regarding length at age. Remarkably, a fish of the same length could be of highly variable age, for example *A. marinus* of 18 cm could have six different ages. However, there was high uncertainty regarding age determination and the PMR fish may also be older (see Attachment 2).

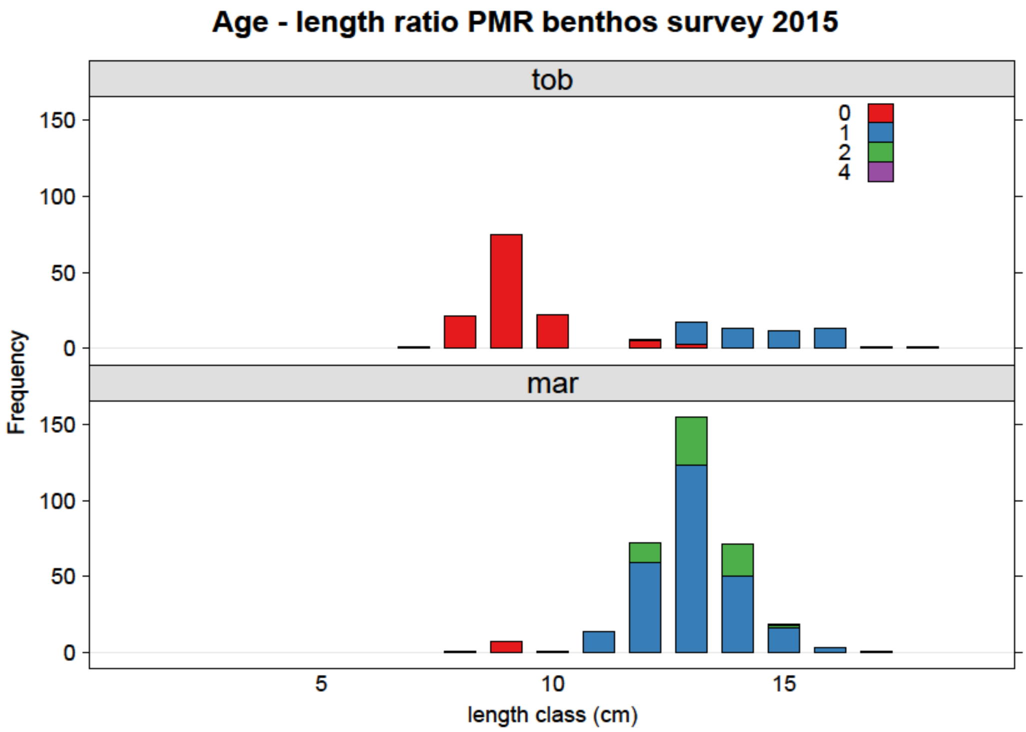

Figure 21: Length class frequency distribution of *A. marinus* and *A. tobianus* caught in the 2015 PMR benthos survey, including age composition of each length class. Different colours represent year classes.

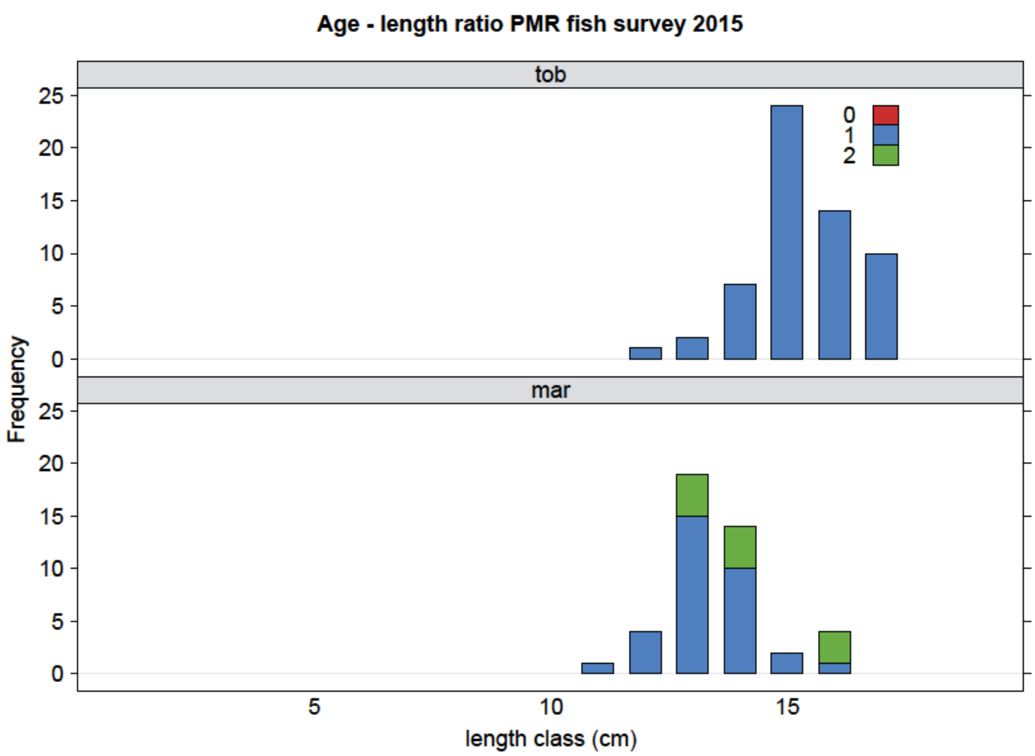

Figure 22: Length class frequency distribution of *A. marinus* and *A. tobianus* caught in the 2015 PMR fish survey, including age composition of each length class. Different colours represent year classes.

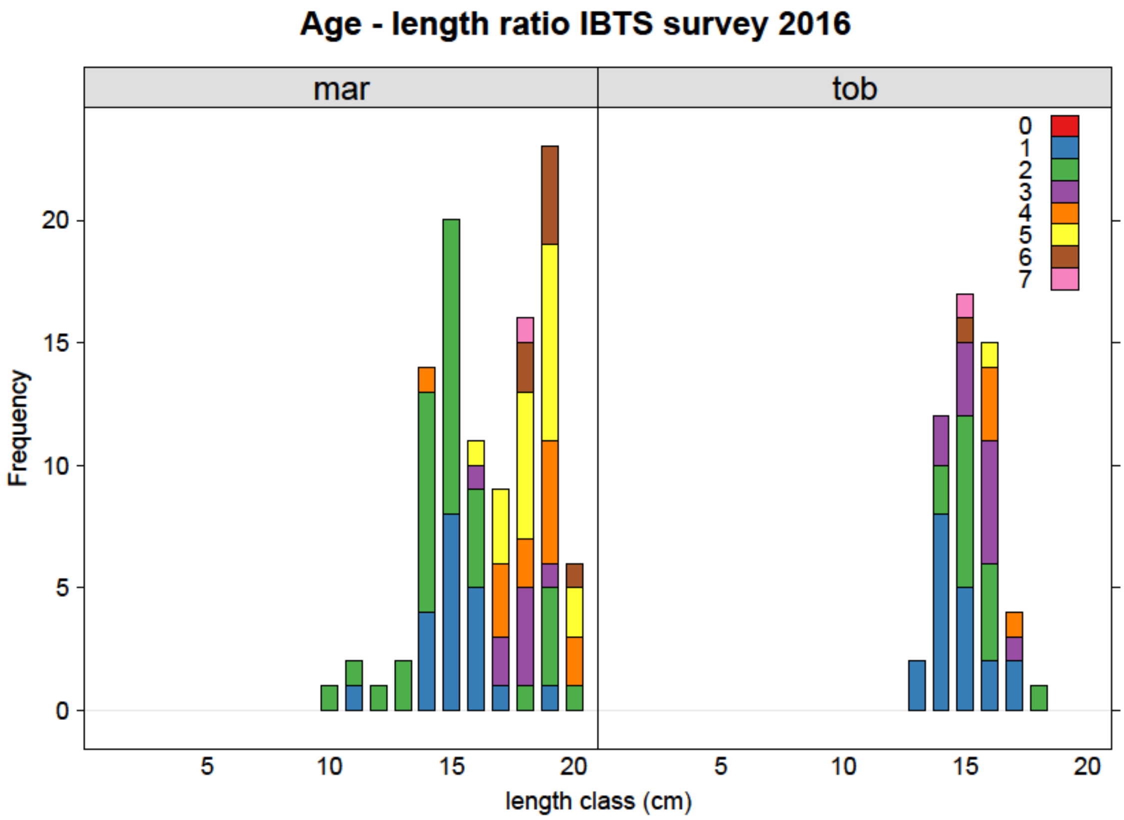

Figure 23: Length class frequency distribution of *A. marinus* and *A. tobianus* caught in the 2016 IBTS survey, including age composition of each length class. Different colours represent year classes.

Spatial distribution

The following results present the spatial distribution of sandeel caught in the Voordelta, allowing to examine the specific use of the area by different species and sizes.

In the fish surveys both species were caught mixed in the same areas scattered across the entire Voordelta (figure 24).

In the benthos survey *A. marinus* occurred mainly in the deepest part of the Voordelta, on the transition zone with deeper water of about 20m depth (figure 25). This pattern was fairly consistent through time, with abundance fluctuating between years. *A. tobianus* appeared to occur mostly on shallow sandy areas of less than 10m deep, in the vicinity of sandbanks. In addition, *A. tobianus* was mainly concentrated in the area in front of Schouwen – Duiveland, near gullies formed by inflowing water from the Eastern Scheldt in the south, and from Lake Grevelingen in the north.

A length structured analysis showed the smallest fish of <10cm were generally found in shallow water close inshore, which were mainly of *A. tobianus* (figure 26 and 27). The average density of *A. tobianus* is notably higher in 2009 than in the following years (figure 28). *A. marinus* average density is fluctuating more evenly with the lowest density in 2011.

Furthermore, the data was checked for a correlation between densities of both *Ammodytes* species in the Voordelta, which could indicate competition for space in years of higher *A. marinus* abundance, but no clear relationship was found (figure 29). Finally, the data was also checked for a correlation between the amount of *A. marinus* found in the Voordelta and the stock size in the North

Sea, which may a shift in habitat selection for shallower water dependant on the North Sea stock size (figure 30). However, the result showed no clear correlation. The North Sea stock was characterised by a large drop in 2012, while the proportion of *A. marinus* in the Voordelta was still high (40%). Although the largest ICES catch corresponded with the highest percentage *A. marinus* in the Voordelta, similar ICES catches in previous year also result in much lower percentages (2010 and 2009).

Since sandeel shows a specific preference for coarse sand, also the spatial distribution of sediment particle size composition on the benthos sampling points was plotted (figure 31). The range of silt and medium – coarse sand particle size used best explained the distribution of sandeel (Tien et al., in press). In general, the sediment is characterised by a small silt fraction. Furthermore, the southwestern part of the Voordelta, and the transition zone with deeper water, contained a higher fraction of medium – coarse sand, while the northeastern part of the Voordelta contained a larger fraction of fine sand.

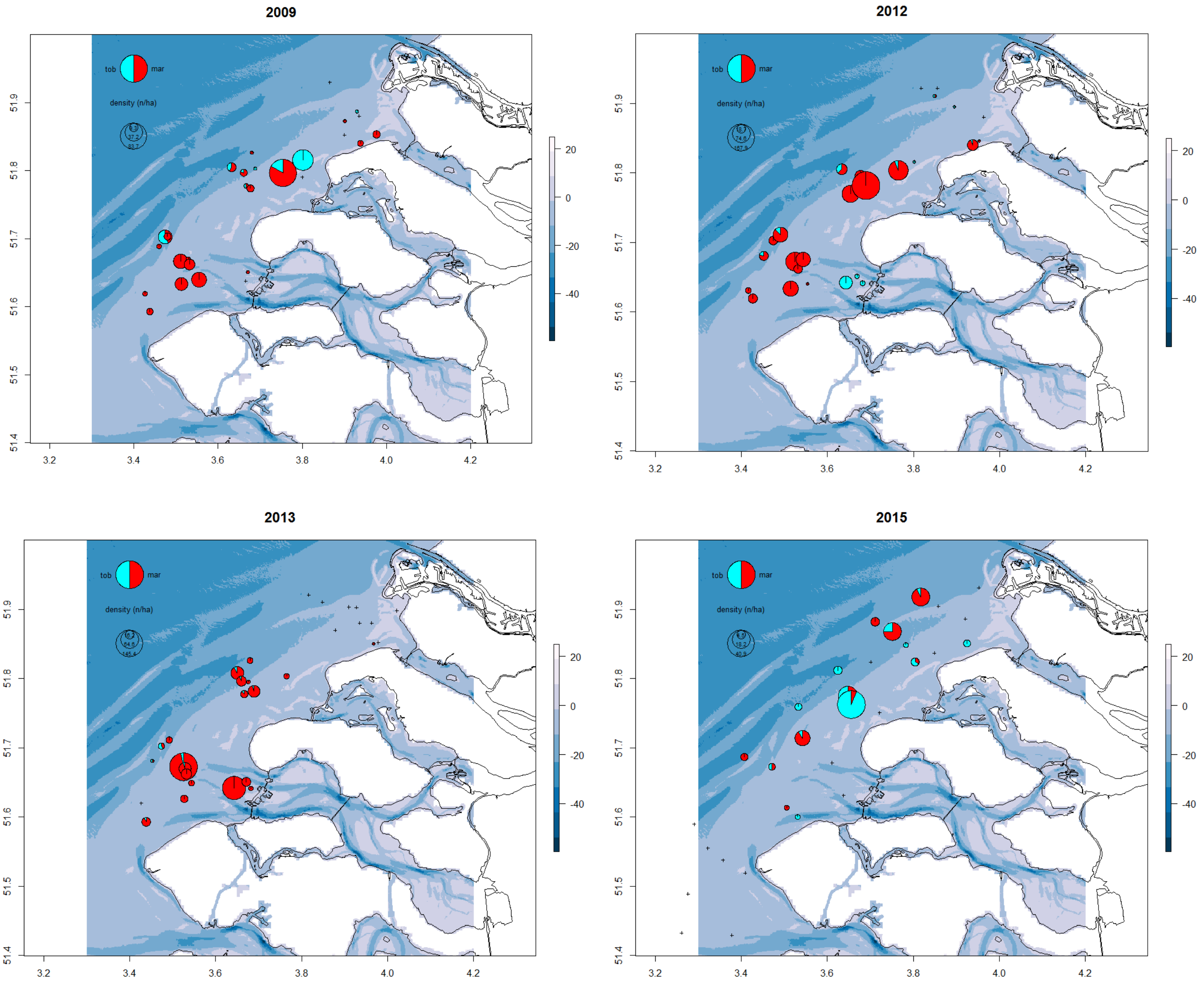

Figure 24: The spatial distribution of both *Ammodytes* species caught in the PMR fish survey in four years. Pie slices display the species composition, bubble size indicate densities (n/ha). Sampling locations where no sandeel is caught are indicated by a + symbol. Note that that bubble sizes are not proportional compared between years in these figures, while the maximal density or amount varies considerably, which may distort the image to some extent.

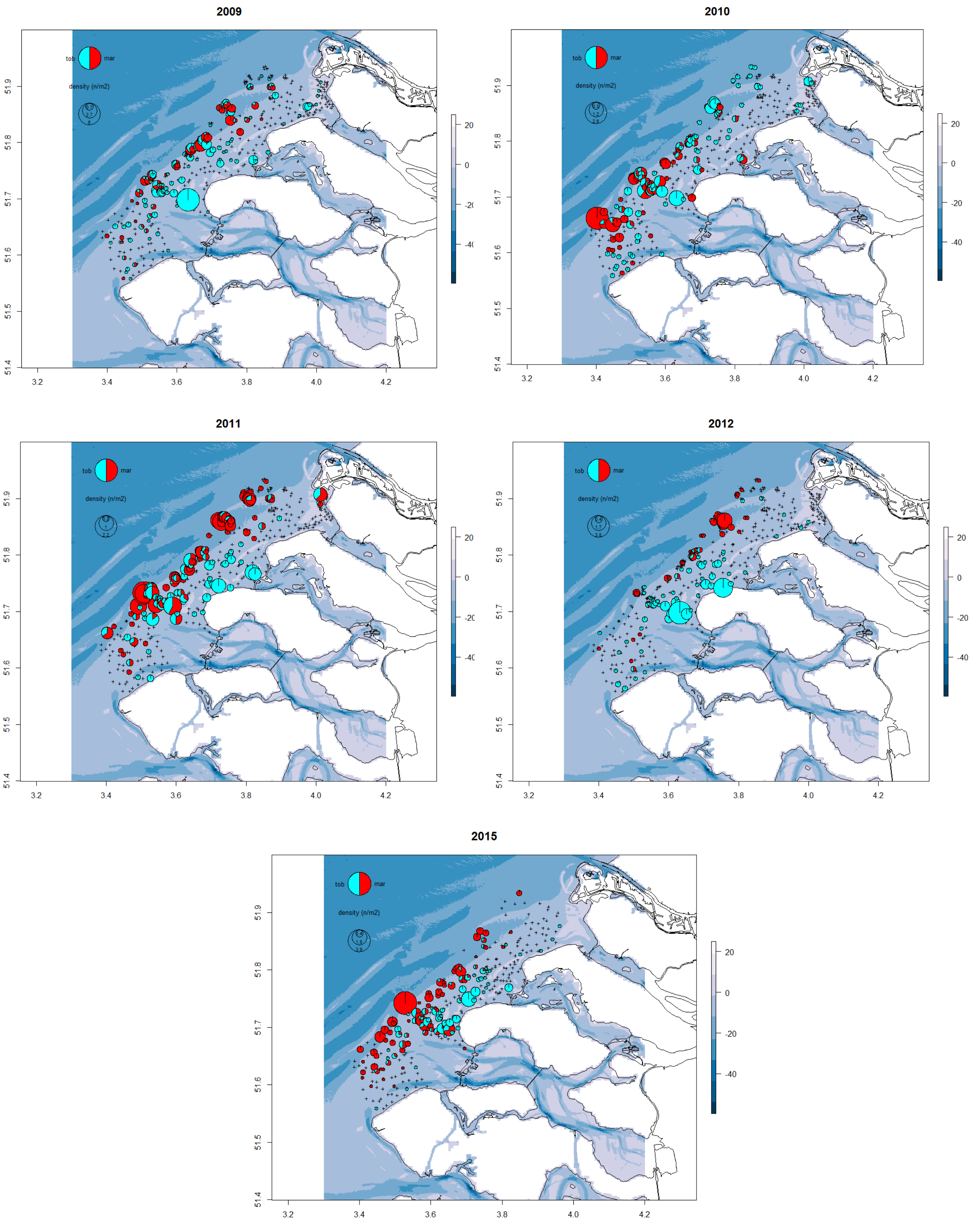

Figure 25: The spatial distribution of both *Ammodytes* species caught in the PMR benthos survey in 5 years. Pie slices display the species composition, bubble size indicate densities (n/m^2). Note that bubble sizes are not proportional compared between years. Sampling locations where no sandeel is caught are indicated by a + symbol.

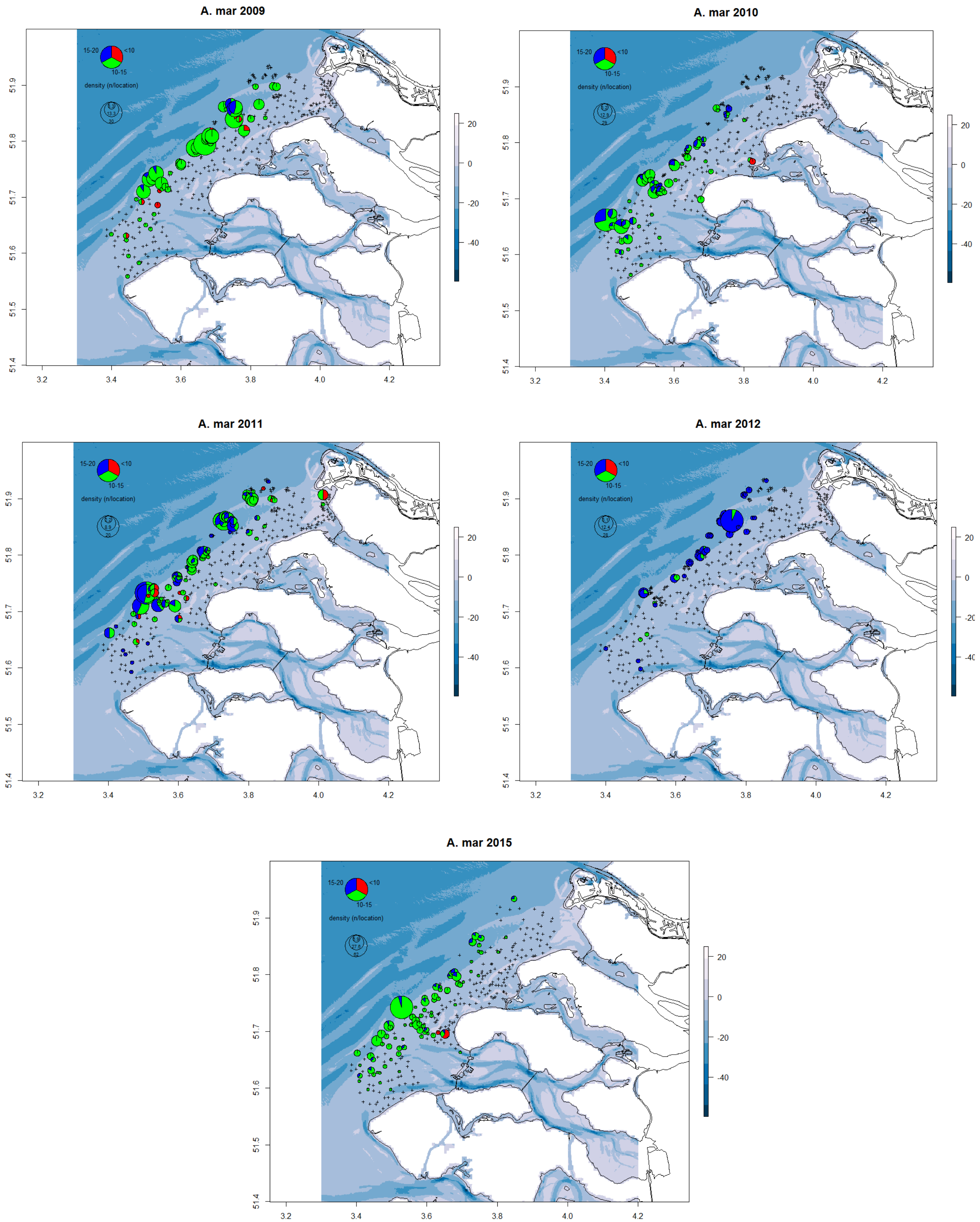

Figure 26: The spatial distribution of different length classes of *A. marinus* caught in the PMR benthos survey in 5 years. Pie slices represent length classes in cm (<10, 10-15 and 15-20). Bubble size indicates density in number of fish per location. Note that bubble sizes are not proportional compared between years. Sampling locations where no sandeel is caught are indicated by a + symbol.

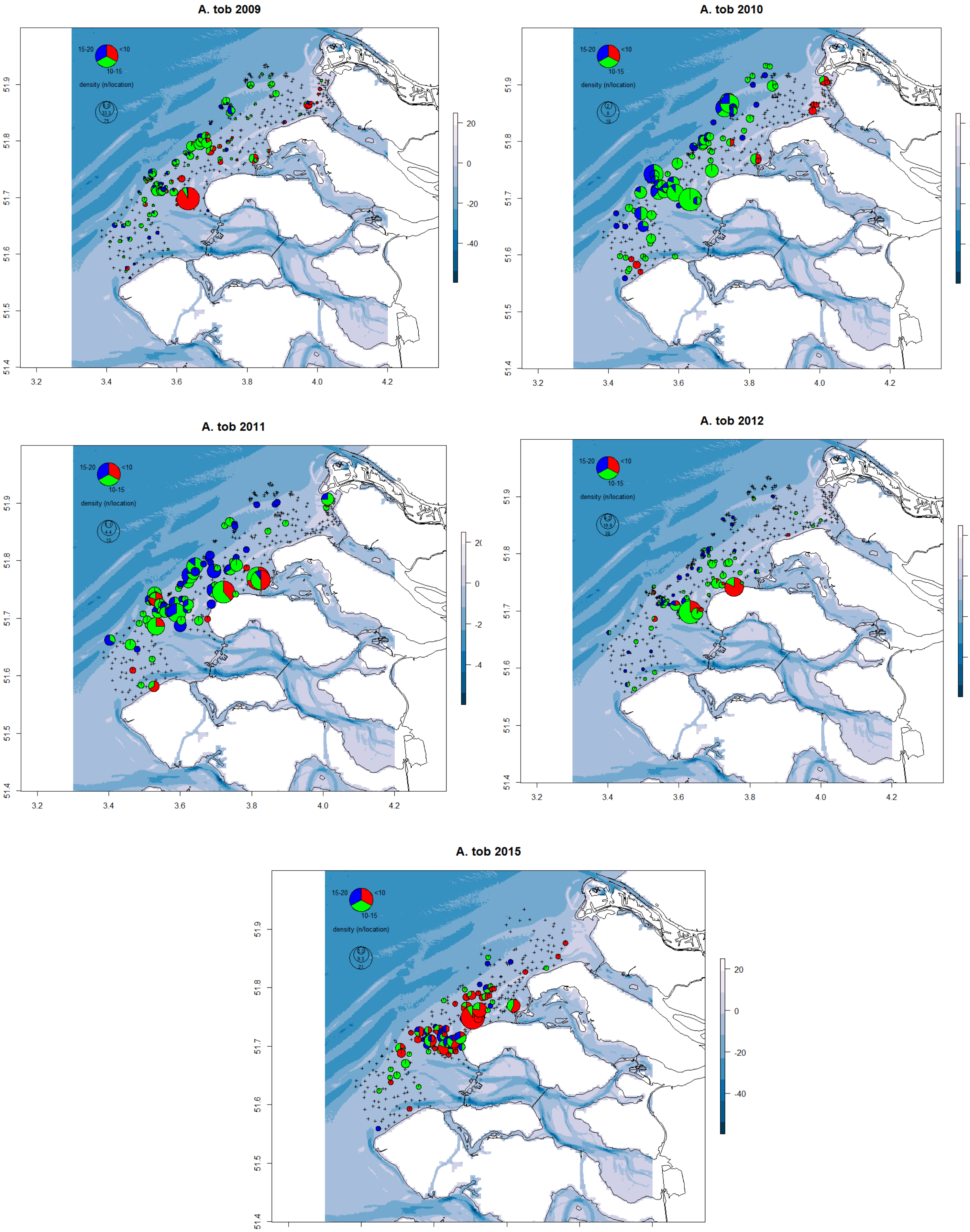

Figure 27: The spatial distribution of different length classes of *A. tobianus* caught in the PMR benthos survey in 5 years. Pie slices represent length classes in cm (<10, 10-15 and 15-20). Bubble size indicates density in number of fish per location. Note that bubble sizes are not proportional compared between years. Sampling locations where no sandeel is caught are indicated by a + symbol.

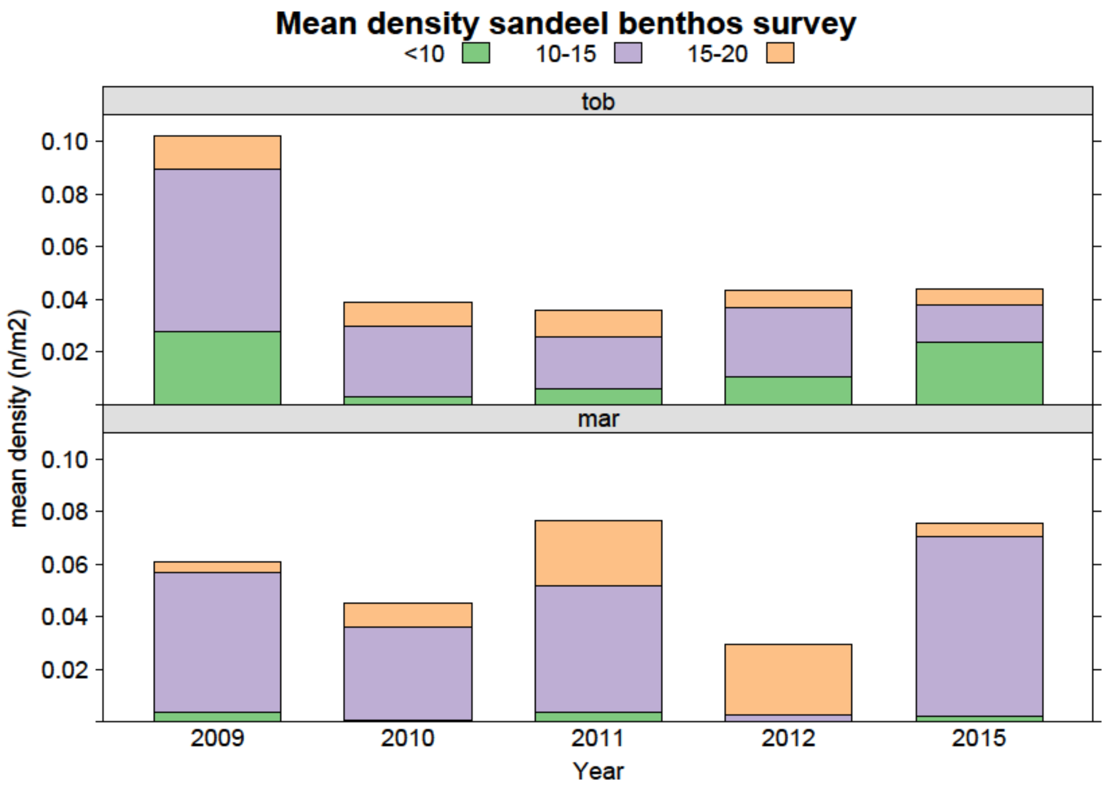

Figure 28: Mean sandeel density (n/m^2) per year caught in the PMR benthos surveys. Bar sections represent length classes in cm.

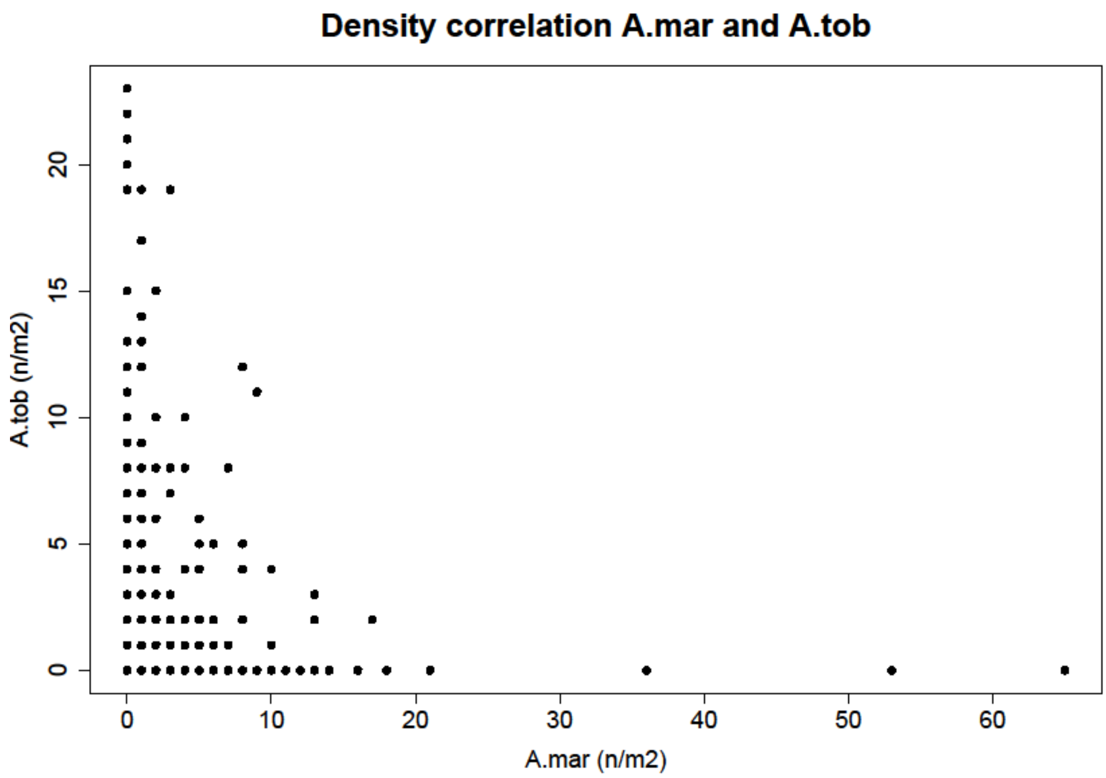

Figure 29: Density of *A. marinus* against density of *A.tobianus*. The data consist of all available PMR benthos data.

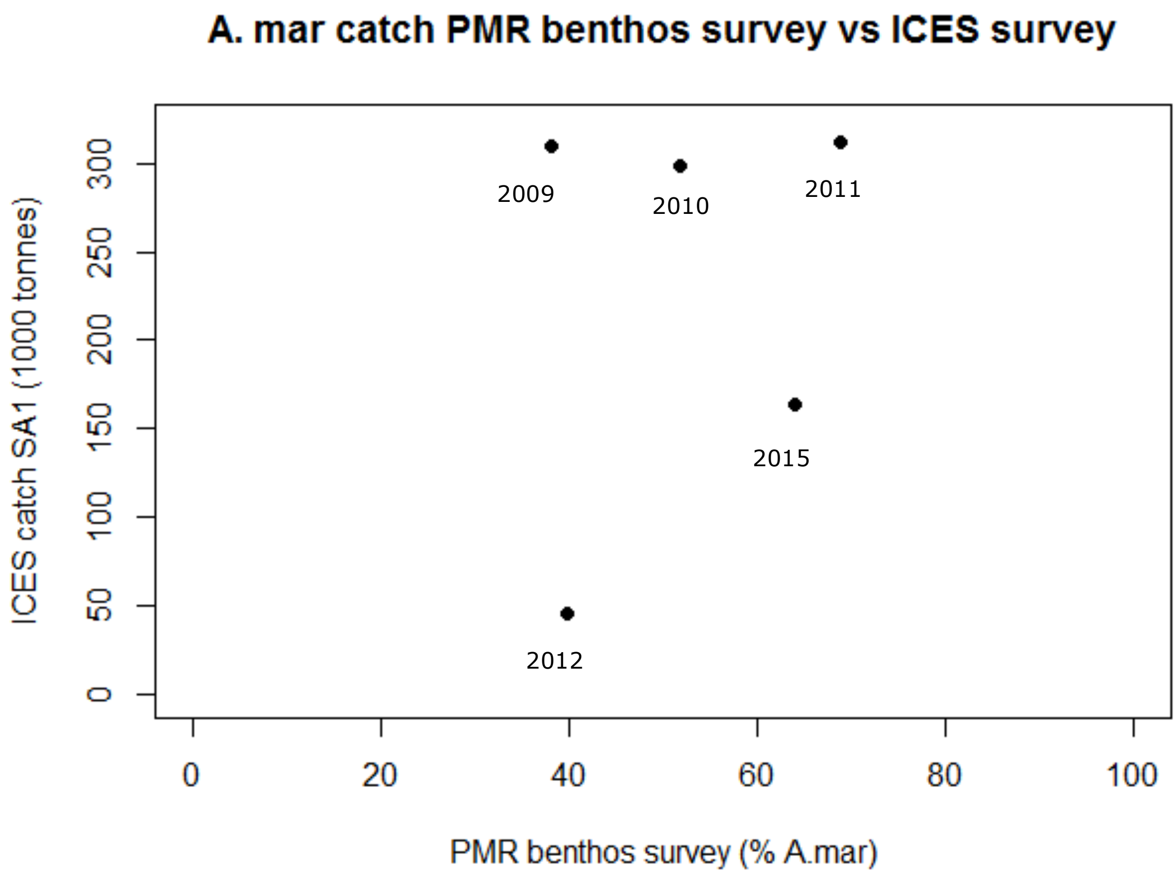

Figure 30: The amount of *A. marinus* caught in the Voordelta vs the ICES catch in the North Sea.

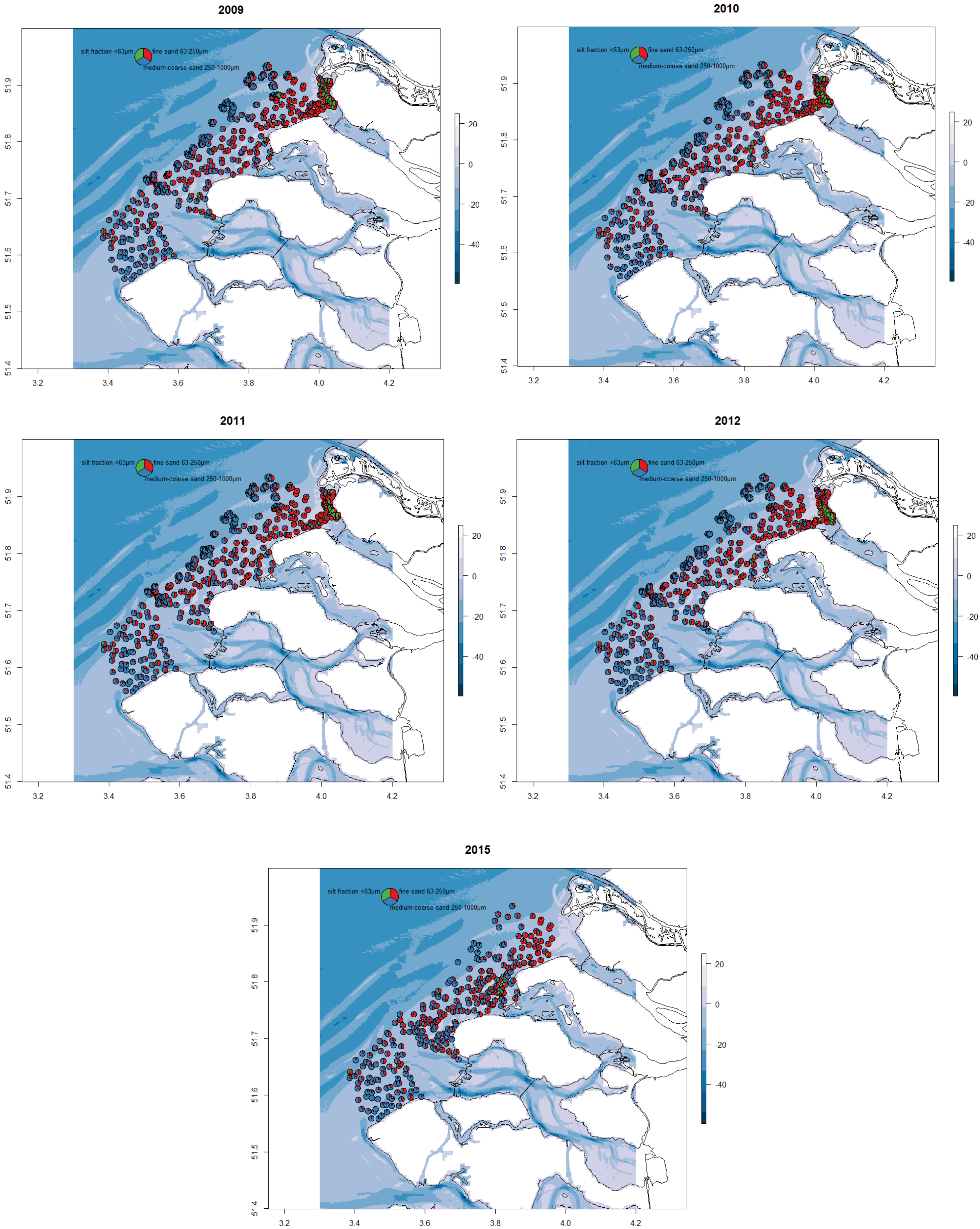

Figure 31: The spatial distribution of sediment particle size composition in the Voordelta in 5 years. Pie slices represent the percentage of silt (0-63 µm), fine sand (63-250 µm) and medium-coarse sand (250-1000 µm).

DISCUSSION

The following discussion puts the results into perspective. Thereby, possible explanations for observed trends are put forward along with a description of uncertainties and shortcomings regarding the collected data.

The role of the Voordelta for sandeel

As compensation for loss of habitat and foraging area of a number of species due to the expansion of the Rotterdam harbour (PMR), an number of measures were implemented including the established of a seabed protection area (where beam trawl fisheries are excluded) and resting areas for seabirds. During a monitoring program sandeel was found to be one of the few species experiencing a negative effect of bottom disturbing fisheries, providing a link between bottom disturbing fisheries and prey availability for seabirds. This way sandeel was found to be more important than previously thought. This study aimed to get more insight into the habitat use and distribution of this unknown species in Dutch waters.

The PMR surveys contained a mix of *A. marinus, A. tobianus and H. lanceolatus*, indicating the Voordelta as transitional habitat, suitable for different sandeel species. The IBTS catch also contained a considerable portion of *A. tobianus*, while one would expect mainly *A. marinus* offshore since this is its preferred habitat. However, all *A. tobianus* were caught in coastal quadrants and not further offshore.

Length composition results show that *A. marinus* in the Voordelta is relatively smaller (and younger) than *A. marinus* found further offshore, while *A. tobianus* reaches similar lengths in both areas . This suggests the Voordelta as suboptimal habitat for *A. marinus*, but optimal habitat for *A. tobianus*. The variation of species composition per year may indicate a shift in habitat selection of *A. marinus*, depending on the population size in the North Sea. A larger sandeel population in the North Sea, resulting in more competition for space, may lead to more *A. marinus* moving towards suboptimal coastal areas. However, this explanation is not supported by the ICES sandeel stock estimates for the North Sea (figure 30).

The PMR surveys only contained fish between 0 – 2 years old while the IBTS contained considerably older fish. Despite the uncertainty regarding age determination, this may suggest that the Voordelta is mainly used by young sandeel, and migration to other areas in the North Sea as they grow older. On the other hand the lifespan of sandeel in the Voordelta may be simply shorter than offshore. Moreover, length composition doesn't show recruitment of small *A. marinus* every year, but it does for *A. tobianus*. This can be the result of an unfavourable hydrodynamic regime by which *A. marinus* larvae could not reach the Voordelta. This may suggest that *A. tobianus* reproduces in the Voordelta (in its optimal habitat) while *A. marinus* doesn't, and whereby recruitment is dependent on influx of fish originating from breeding grounds on offshore banks (Southern bight and Doggerbank off south eastern English coast, see theoretical framework and figure 7). These offshore populations in the Southern Bight also seem to be located more or less within the migration range of *A. marinus* (at least 64 km) as found by Gauld (1990), although sandeel are believed to be very stationary.

Spatial distribution

The preference of *A. marinus* for the deepest part of the Voordelta while *A. tobianus* prefers the shallow sandbanks in the vicinity of the coast suggests a niche separation between both *Ammodytes* species, as suggested by the research of Tien et al. (in press). In addition, this study has shown a consistent pattern of habitat use between species and between years, and the presence of particularly small *A. tobianus* close inshore. Although both *Ammodytes* species have a similar mode of life, a different habitat seems to be preferred. There were also no signs of competition (figure 29). The observed habitat preference is less clear from the fish survey catch indicating a wider and more mixed foraging area.

Furthermore, the sandeel distribution appears to be related to areas with a high fraction of medium – coarse sand and a low silt fraction, which is also in accordance with the findings of Tien et al. (in press). In addition, the overall concentration of *A. tobianus* in an area off Schouwen – Duiveland may indicate optimal conditions through nutrient rich water inflow from inlets of the Eastern Scheldt and Lake Grevelingen.

Finally, the spatial distribution is also negatively linked to areas with high shrimp fishing by Tien et al. (in press), taking the influence of environmental factors like sediment particle size into account.

Age and size structure

All years surveyed, the PMR benthos survey contained a distinct group of small sandeel (mainly 0 group *A. tobianus*), which is hardly present in the fish survey. This may suggest small fish stay buried in the seabed. Another explanation may be a difference in catch efficiency between the surveys, where small fish systematically escape the net used for the fish survey. However, results for spatial distribution provide another clue. The fish survey only has a few sampling locations in the specific area of high density of small *A. tobianus* close inshore, which may explain the absence of small fish.

Apart from the 0 group cohort caught in the benthos survey, length – age ratios vary strongly. This may indicate different growth rates, however, there is considerable uncertainty regarding age determination. From our age determination we concluded that an *A. marinus* of approximately 19 cm can be 1, as well as 7 years old, thereby covering the complete age range of the IBTS data set. Although this might be correct, it needs additional investigations.

In the Recommendations (see below) some suggestions are given for age reading validation. However, such validation studies require a lot of effort and are not easily financed.

Sandeel in the water column vs buried in the sand

The presence of sandeel in both surveys shows that sandeel may be found actively swimming in the water column during the day (in autumn), but also buried in the sand, suggesting this time of the year as a transitional period between activity and hibernation. The density of buried sandeel is much higher (a few sandeel per square metre) compared to the density of sandeel in the water column (approximately a hundred per hectare on average). However, the comparison is biased due to the considerably higher number of sampling locations of the benthos survey.

Buried sandeel during the day may also suggests that burying behaviour is not only restricted to the day – night cycle, but also influenced by other factors. As already mentioned in the theoretical framework, results from controlled lab experiments with *A. tobianus* by van Deurs et al. (2011) suggest that sandeel foraging activity is primarily determined by the amount of food in their stomach. This means that sandeel may remain buried during the day with an optimal temperature, because of low food availability and empty stomachs. Or food availability may be high, but low visibility may restrict foraging activity. Likewise, large densities of buried sandeel in the Voordelta during the day may be as a result of suboptimal feeding conditions.

The larger proportion of *A. tobianus* in the fish survey, and of *A. marinus* in the benthos survey suggests a behavioural difference. However, the benthos survey covers a lot more sampling points which may obscure the comparison. *H. lanceolatus* seems to occur mainly in the water column, but they can get damaged easier in the benthos survey because of their larger size.

Assuming that buried sandeel are more vulnerable to bottom disturbing fisheries than sandeel swimming in the open water, a high number of buried sandeel also during the day can be associated with a high vulnerability to bottom disturbing fisheries during autumn.

The role of sandeel as food for other species

As already mentioned, to compensate for loss of habitat and foraging area due to the expansion of the Rotterdam harbour (PMR) a number of management measures were implemented. These included resting areas for Common Scoter, resting areas for Common tern and Sandwich tern and a bottom protection area where beam trawl fisheries are excluded to compensate for loss of Natura 2000 habitattype 1110. Sandeel was originally not included in the management plan. It was not taken into account that the exclusion of bottom disturbing fisheries could indirectly benefit terns because the importance of sandeel for terns was underestimated. It is important to consider these findings in further management plans.

The results of this study can be linked to studies of Sandwich tern breeding success. Breeding success of Sandwich tern colonies is highly dependent on the distance between the foraging area and the breeding colony, since Sandwich terns can only bring one fish at a time to their chicks. The results of the 2015 annual report on birds in the Voordelta shows several years of a higher breeding success of the colony on Markenje, compared to other colonies (Fijn et al., 2015). This result can be linked to sandeel distribution in the Voordelta since this colony is closest to the area of highest sandeel density. However, this pattern is not recognisable every year.

In addition, some other factors need to be taking into account. Breeding success also depends on the time when sandeel start to be active in spring and become available for seabirds. Furthermore, sandeel data from this study is obtained in autumn while tern research is conducted in spring. It is assumed that these results are more or less representative for the situation in spring. Finally, sandeel is only caught near the bottom in the PMR surveys, which is outside the foraging area of the Sandwich tern. A survey covering the water column closer to the surface is needed to be able provide more information about sandeel availability for seabirds.

Data collection

There are several factors that need to be taken into account when interpreting the results because of differences in sampling protocols between surveys. The benthos survey covers a considerably larger number of sampling locations compared to the fish survey, which may bias the comparison. Thereby, the benthos data is considered more reliable due to the higher number of sampling locations, although the data may be biased due to the absence of damaged sandeel. More than half of the sandeel catch is damaged resulting in a considerable loss of information. Thereby, it is likely that the larger fish are cut in half more easily than small fish. Furthermore, there is no data available from the 2013 benthos survey and the 2010 and 2011 fish survey, due to loss of data or a lack of species determination, which may hide important information. In addition, it is important to note that the fish survey employs a shrimp trawl, which is towed over the seabed surface. Consequently, this method is not a complete representation of pelagic fish biomass, and a pelagic net should be used to produce a better estimate of pelagic sandeel biomass. Regarding length composition, some additional factors need to be taken into account, on top of the differences between both PMR surveys. The IBTS survey, which catches generally larger fish than the PMR surveys, also uses a larger net (GOV trawl). However, a same mesh size of 20 mm is used, which makes the comparison more reliable.

There are several reasons that resulted in suboptimal data. At first, sandeel was not included in the PMR nature compensation monitoring program, and the collection of sandeel data takes place during surveys with other purposes with different protocols. In the final chapter some recommendations are given to produce better data for sandeel (see recommendations). However, the establishment of a specific monitoring program for sandeel in the Voordelta is considered unlikely since it is not funded by the Government. Sandeel appears to be considered unimportant because there is no commercial value and no direct threat to the stock.

To conclude, knowledge about sandeel ecology is very limited because sandeel is of no commercial importance in Holland, although this is fish plays a major role in the ecosystem as one of the most important forage fish. During the WOT shellfish survey I joined in May we caught sandeel in coastal water from Scheveningen up to the area of the Wadden Islands. During another fish survey I joined on the Western Scheldt we also caught considerable amounts of sandeel, which gave the impression that sandeel is abundant all over the Dutch coastal water. Therefore, it surprises me that still so little is known about this fish and I think more effort should be made to better understand the role of sandeel in the ecosystem without a commercial incentive.

CONCLUSION

- The Voordelta is a transitional habitat suitable for both *Ammodytes sp.* and *Hyperoplus lanceolatus.* Although both *Ammodytes* species have a similar mode of life a preference of *A. marinus* for the deepest areas and of *A. tobianus* (in particular small <10cm) for the shallow parts was observed without signs of competition.
- *A. tobianus* appeared to fulfil its lifecycle in the Voordelta with recruitment every year.
- The absence of *A. marinus* recruitment in some years may indicate the population is dependent on larval drift from offshore breeding grounds beside the possibility of local reproduction.
- Sandeel in the Voordelta were younger (0-2 years old) than offshore (1-7 years old) suggesting migration of older fish to other areas in the North Sea or a shorter lifespan.
- In autumn (Sept. – Oct.) sandeel were found both buried in the sand and in the water column during the day suggesting a transitional period between activity and hibernation. This also suggests high vulnerability to bottom disturbing fisheries during this time of the year.
- There was high uncertainty in the age determinations because the otolith structures were often vague. Therefore, the PMR fish could contain older individuals as well.
- The data is considered not optimal for the purpose of this study since more than half of the fish caught in the benthos survey were damaged and the probable selectivity of the fish survey for the larger fish.

RECOMMENDATIONS

This section provides some recommendations for more complete and representative collection of sandeel data. An optimal sampling program specifically designed for sandeel should meet the following requirements:

- Because sandeel alternates between a mode of life buried in the seabed and swimming in the water column, the combination of two types of sampling techniques are recommended. To catch sandeel buried in the sand a bottom dredge can be used, scraping of the upper layer of the seabed. To catch sandeel in the water column, a pelagic fishing net can be used.
- Since a pelagic net only gives an idea of a small portion of the water column, acoustic echolocation technology can be used to get a more complete overview of fish swimming in the water column. Hereby, a school of fish is acoustically located after which the school can be precisely targeted with the net to determine species and length composition
- To make a good comparison between the portion of sandeel buried in the sand and swimming in the water column, the locations and the number of sampled locations need to be equal.
- To get a complete overview of sandeel activity during the year, it is suggested to sample at different times of the year, at least once in winter, in spring, in summer and during the fall.
- To get an overview of sandeel presence in Dutch coastal waters, sampling should occur at varying depth from as close inshore as possible to depths around 20 meters when possible.

Methods that can be used for age reading validation include the following:

- For 0 group fish, the first annulus can be verified by counting daily growth rings
- For 1+ group fish, theoretically a tagging study with tetracycline marking of the otoliths could be done. However, this method is not expected to be feasible for sandeel, due to low return rates of tagged fish.
- A marginal increment study, that is monitoring otolith growth and the deposition of opaque and translucent material on the edge of the otolith deposition throughout the year.

Finally it is recommended to also start collecting damaged fish from benthos surveys to avoid the loss of large amounts of data. The inferred SE/SG regression equation can then be used to estimate total length based on the size of the head. Thereby, the heads can be collected in a bag per sampling locations, but the heads should be frozen carefully next to each other to avoid clusters which may damage the heads when defrosting.

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ATTACHMENTS

1. Snout – eye (SE) and snout – gill (SG) correlation with total fish length

The following results describe the SE/SG relationship with total length as found with the 2015 PMR and 2016 IBTS data. In general, length showed the strongest correlation with SG distance (figure 32 and 33) which could be explained by the slightly larger distance than SE. Still, both species and measurements showed a strong, significant correlation with high R^2 values (figure 34 & 35). The regression equation for both *Ammodytes* species is presented in figure 36 & 37. Table 2 summarizes linear model coefficients.For total body length calculation, SG is advised on the basis of the highest R^2 values.

However, there were a few factors that might influence the SE/SG – total body length relationship. It was assumed that the relation between length and head measurements is similar for fish collected in different surveys. However, some differences in slope and/or intercept were found (figure 38). The analysis showed a significant influence of the species, age and survey (table 3).

The survey effect seemed to be caused by the smallest fish, where the data tended to be less centralised along the regression line, suggesting a more sigmoidal curve. Thus, the head size of small fish is apparently proportioned differently with total body length in comparison to larger fish. This might result in systematically overestimation of total body length for the smaller fish. Figure 39 displays the difference in regression lines between small fish (mainly 0 – year class) and larger fish. However, no clear conclusions can be drawn considering the skewed distribution of lengths between surveys. More data is needed to support this potential effect. Furthermore, the SE/SG data is checked for differences between males and females but no structural difference between the sexes was found (table 4 and 5).

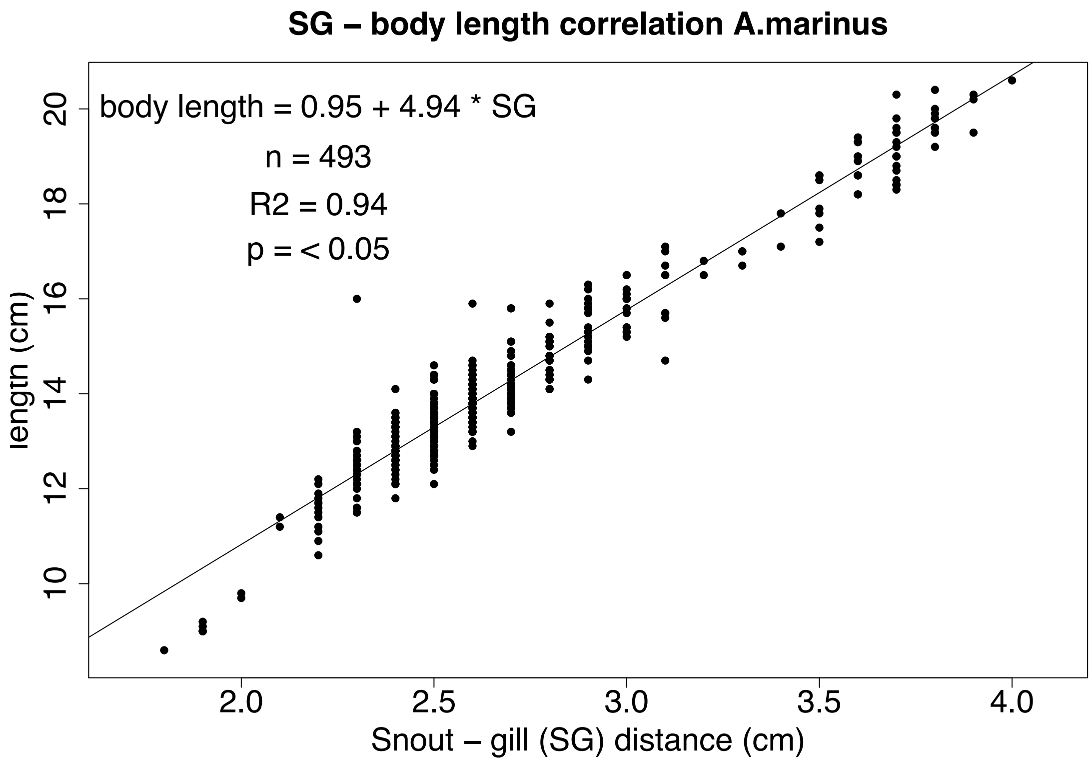

Figure 32: Snout – gill (SG) distance of *A. marinus* in relation to total body length.

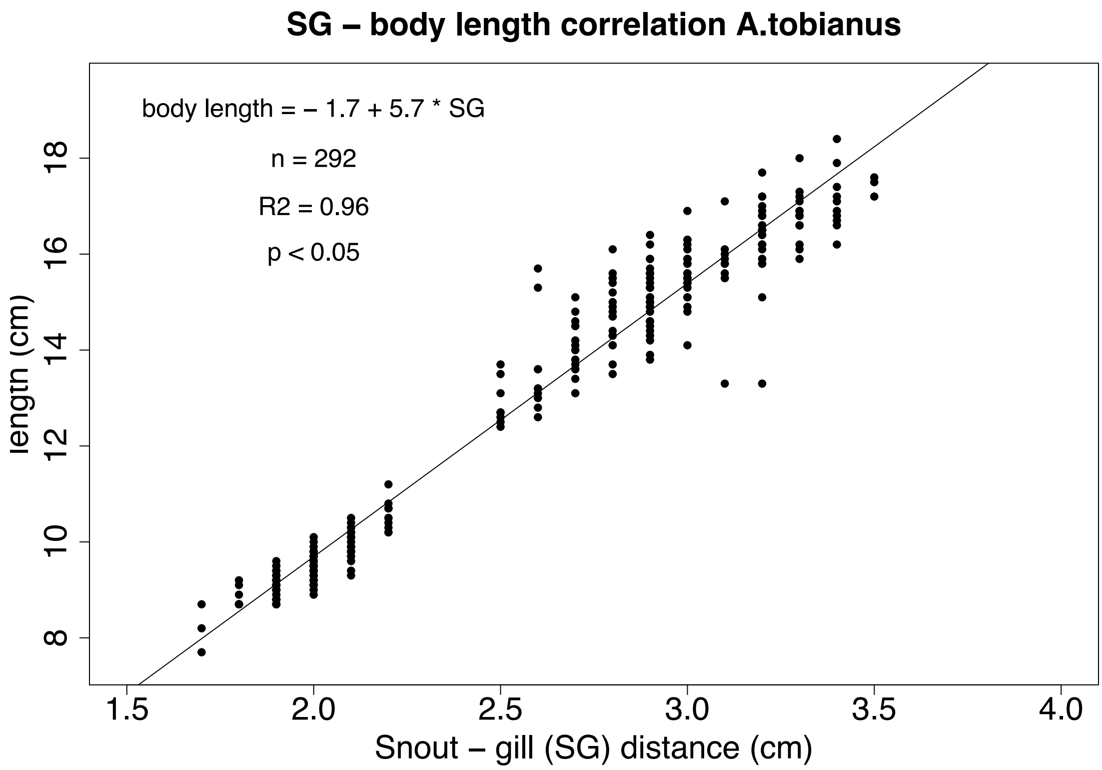

Figure 33: Snout – gill (SG) distance of *A. tobianus* in relation to total body length.

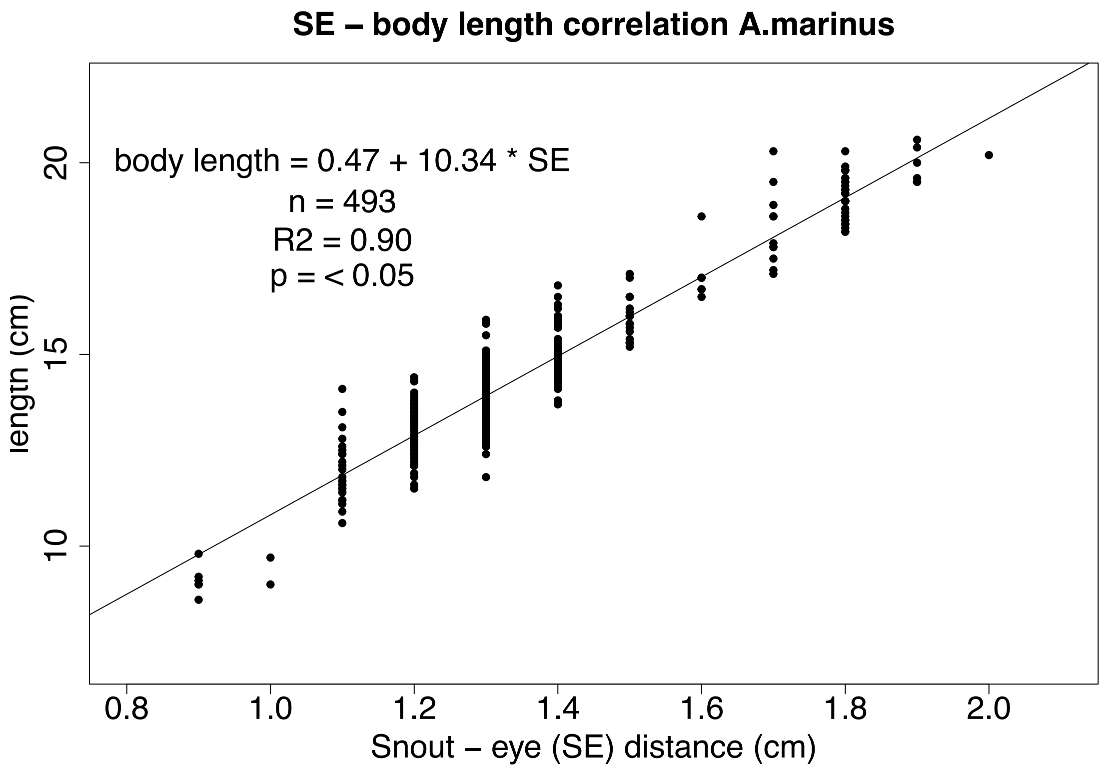

Figure 34: Snout – eye (SE) distance of *A. marinus* in relation to total body length.

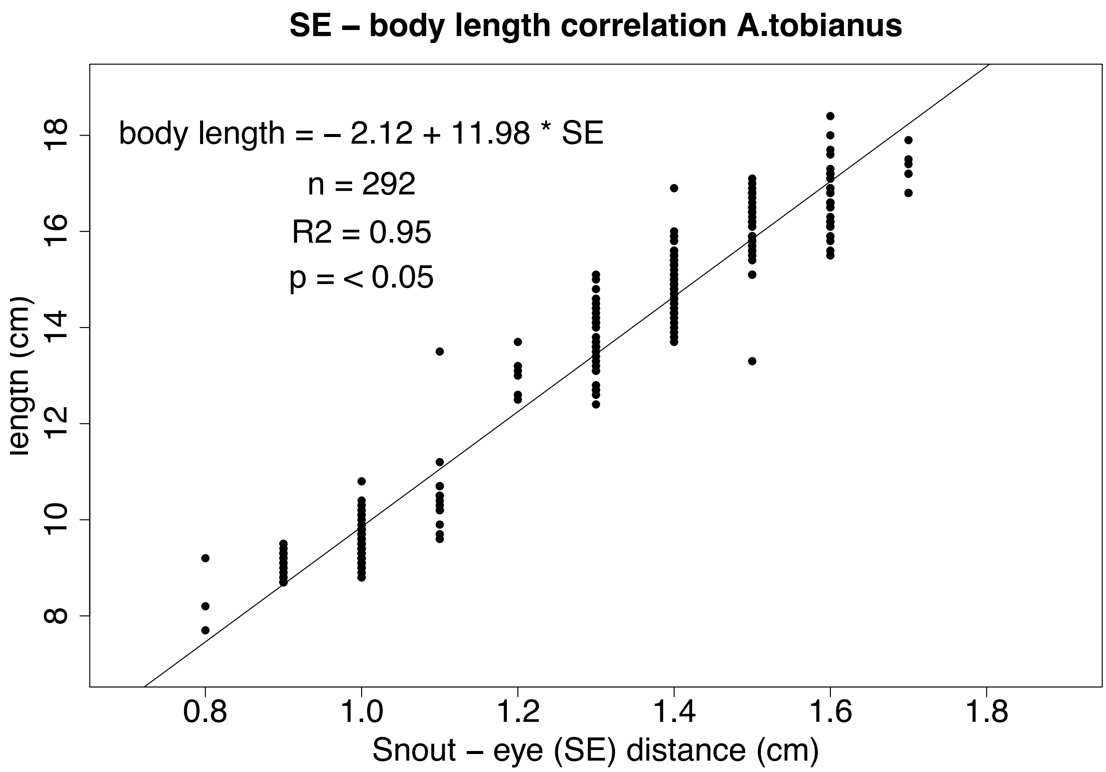

Figure 35: Snout – eye (SE) distance of *A. tobianus* in relation to total body length.

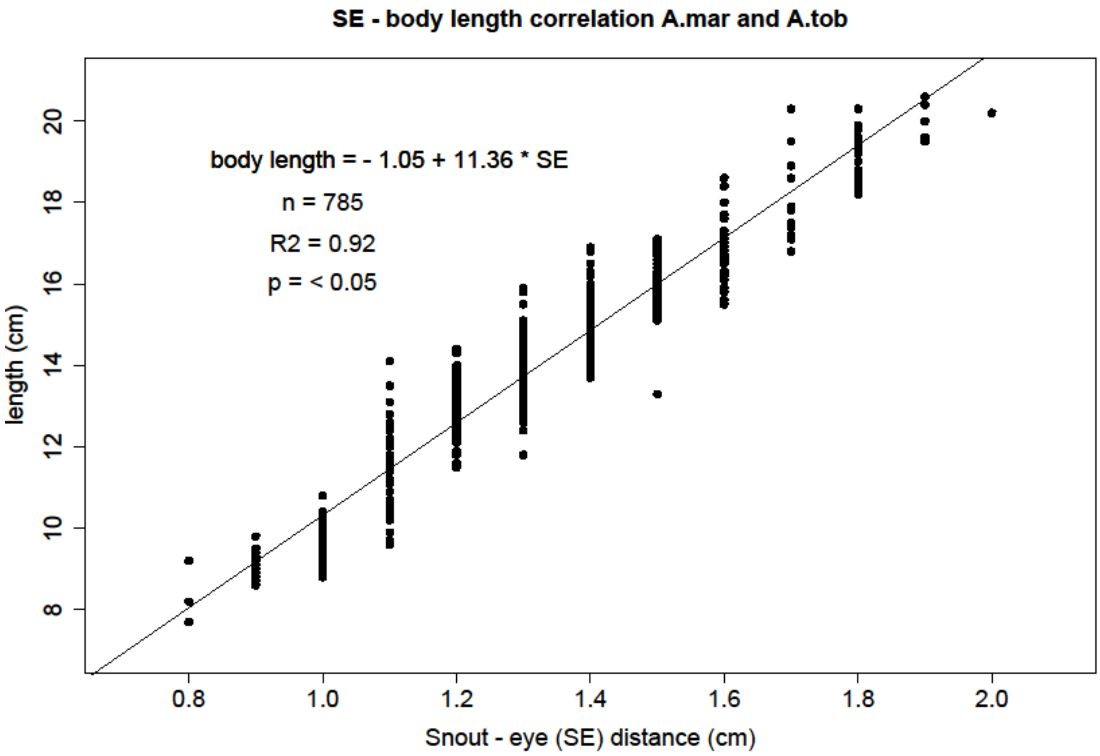

Figure 36: Snout – eye (SE) distance of both *Ammodytes* species in relation to total body length.

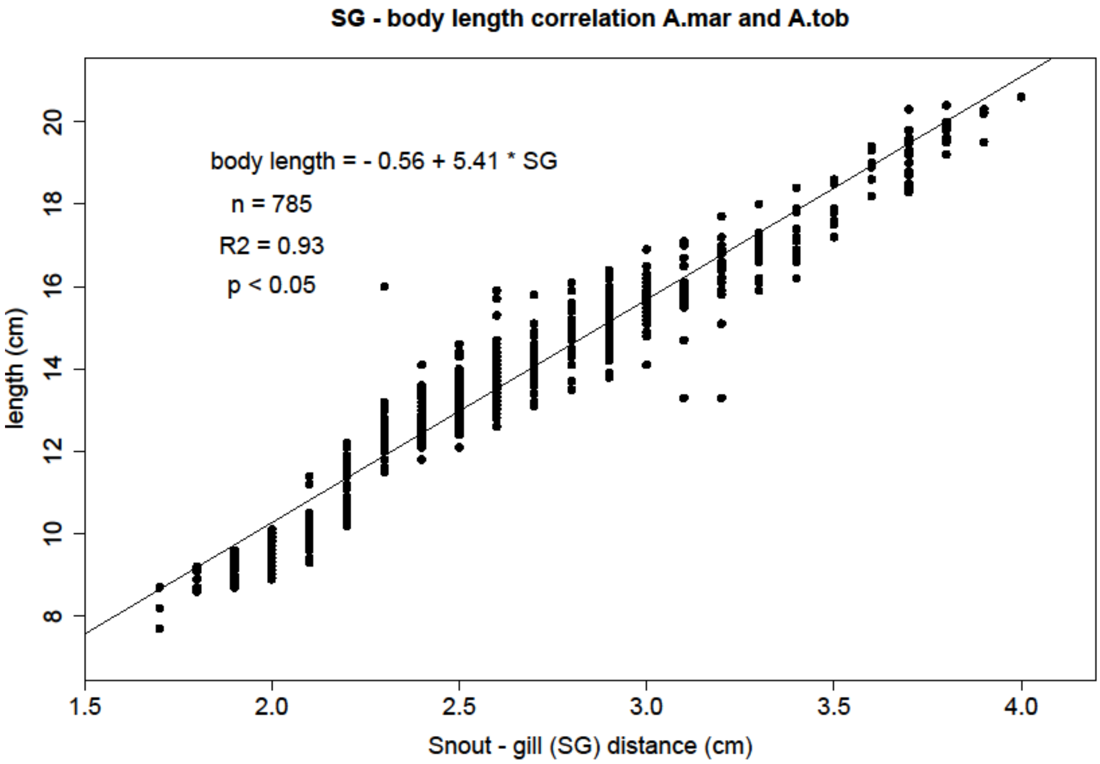

Figure 37: Snout – gill (SG) distance of both *Ammodytes* species in relation to total body length.

Table 2: linear modelling results of total body length as a function of the head measurements.

Species	Parameter	Estimate	Std. error	p - value
A.marinus	SE	10.3438	0.1545	0
A.marinus	SG	4.9396	0.0576	0
A.tobianus	SE	11.9762	0.1612	0
A.tobianus	SG	5.6971	0.0684	0
A.tob. & A.mar.	SE	11.3582	0.1190	0
A.tob. & A.mar.	SG	5.4124	0.0516	0

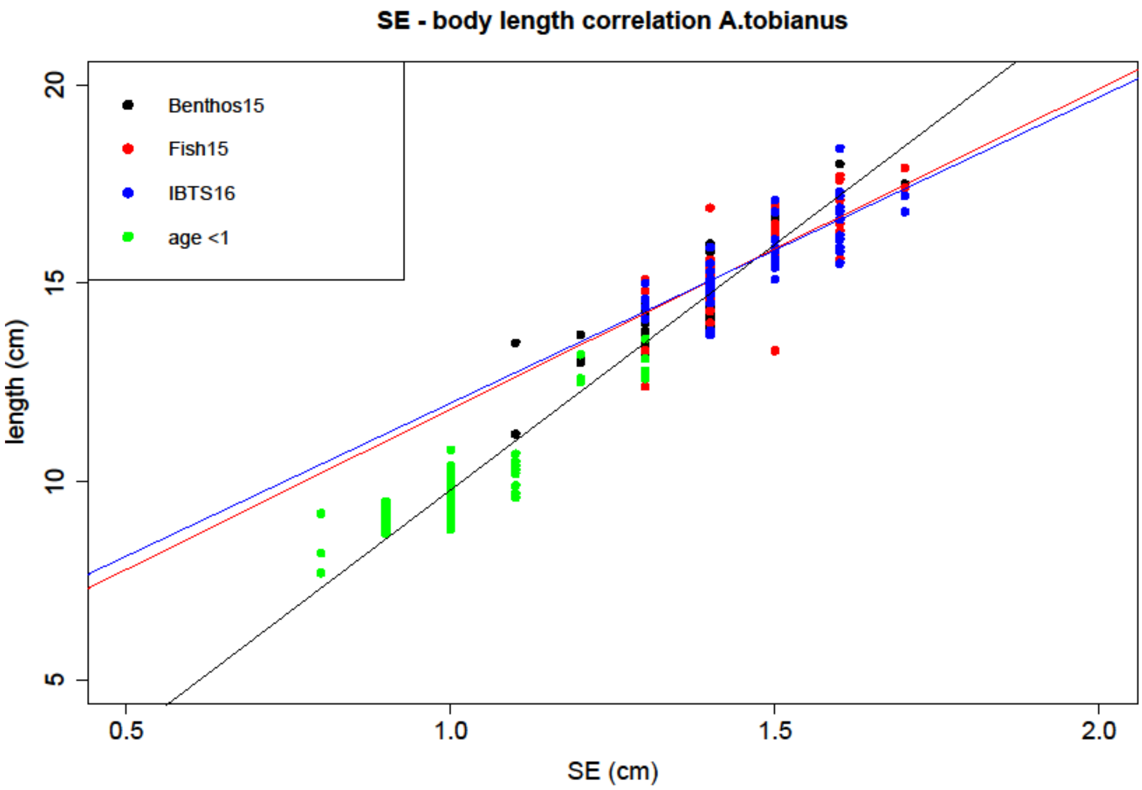

Figure 38: Snout – eye (SE) distance of *A. tobianus* in relation to total body length, showing a comparison of this relationship for data from different surveys. 0 – year class fish are indicated in green.

Table 3: Analysis of Variance (anova) results testing the influences of the following factors on the head measuremets of *A. marinus* and *A. tobianus*.

Factor	Parameter	Df	Sum. Sq.	F value	P - value

Species	SE	1	0.9	45.0	0
Age	SE	1	18.2	904.8	0
Survey	SE	2	2.0	1.0	0
Species	SG	1	2.4	27.3	0
Age	SG	1	86.6	983.9	0
Survey	SG	2	6.6	37.3	0

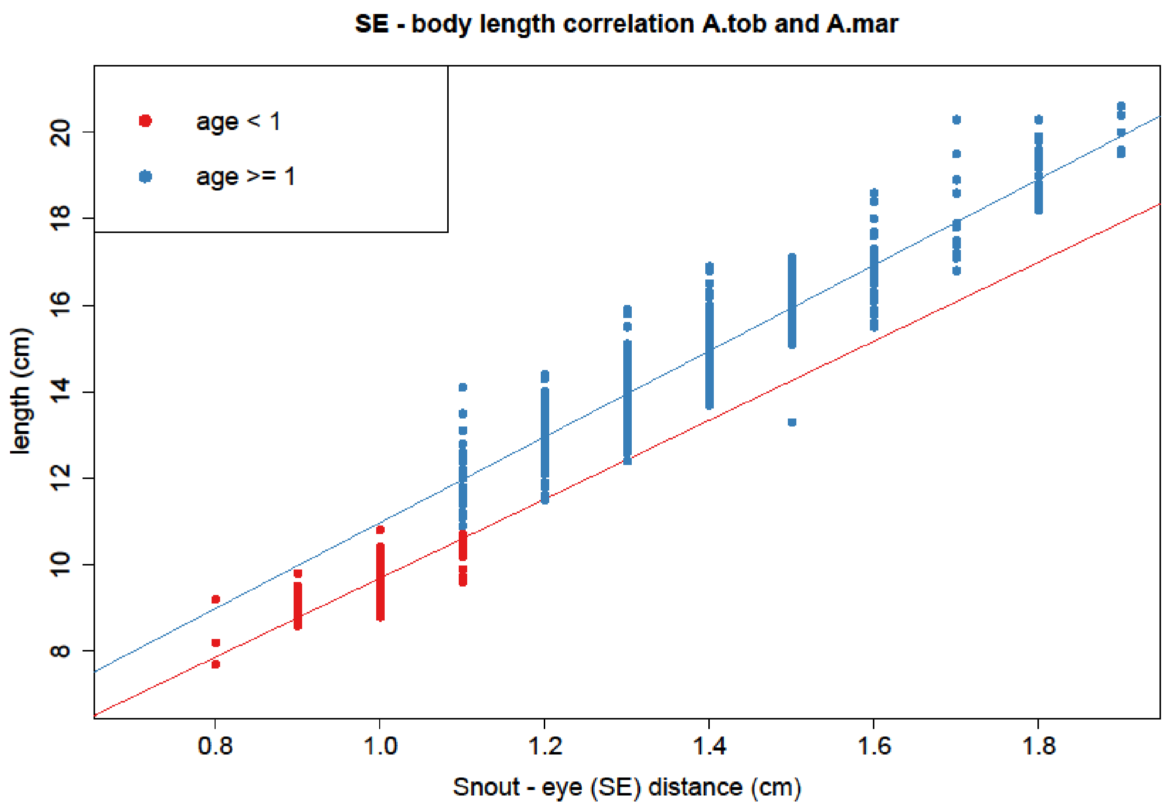

Figure 39: Snout – eye (SE) distance of both *Ammodytes* species in relation to total body length, indicating the difference between relations for small fish and larger fish.

Table 4: MeanSE length (cm) of male and female *A. tobianus* of the PMR benthos survey 2015 for each year class.

Year class	Sex (1: male / 2: female)	Mean SE length (cm)
0	1	1.02
0	2	0.98
1	1	1.39

1	2	1.41

Table 5: Mean SE length (cm) of male and female *A. marinus* of the PMR benthos survey 2015 for each year class.

Year class	Sex (1: male / 2: female)	Mean SE length (cm)
0	1	0.95
0	2	0.90
1	1	1.26
1	2	1.26
2	1	1.28
2	2	1.26

2. Otolith analysis

The following paragraph provides a brief description of the results concerning otolith reading. Figure 40 shows a selection of microscopic images of sandeel otoliths, whereby the estimated age is indicated.

The structures in sandeel otoliths were sometimes quite vague and distinguishing between real and “false” growth rings could be a challenge. Otoliths of *A. tobianus* showed high structural diversity, most likely as a result of two different spawning seasons (figure 40, picture 3, 4, 5 and 6). Consequently, sandeel were given different ages by different readers. Images of a selection of doubtful otoliths were sent to Danish experts to ask for their age determinations, and *A. marinus* was generally estimated of higher age than previously aged at Imares. However, no feedback was received regarding *A. tobianus* since this species was not of interest to them.

To eliminate uncertainty, cross sections of IBTS otoliths were made and stained with a neutral red dye. The red dye attaches to a protein present in the growth ring structure, revealing the age of the fish more clearly. However, the effectiveness of this method may vary between species and locations. The stained sections showed variable results. In some cases, the stained sections were very illustrative, showing higher ages than previously thought (figure 41). In other cases, uncertainty still remained unfortunately. Though, best results were achieved for *A. tobianus*, which also showed highest structural diversity in the otoliths. In general, stained cross sections are not considered better, but they can be clarifying regarding vague growth rings and cores.

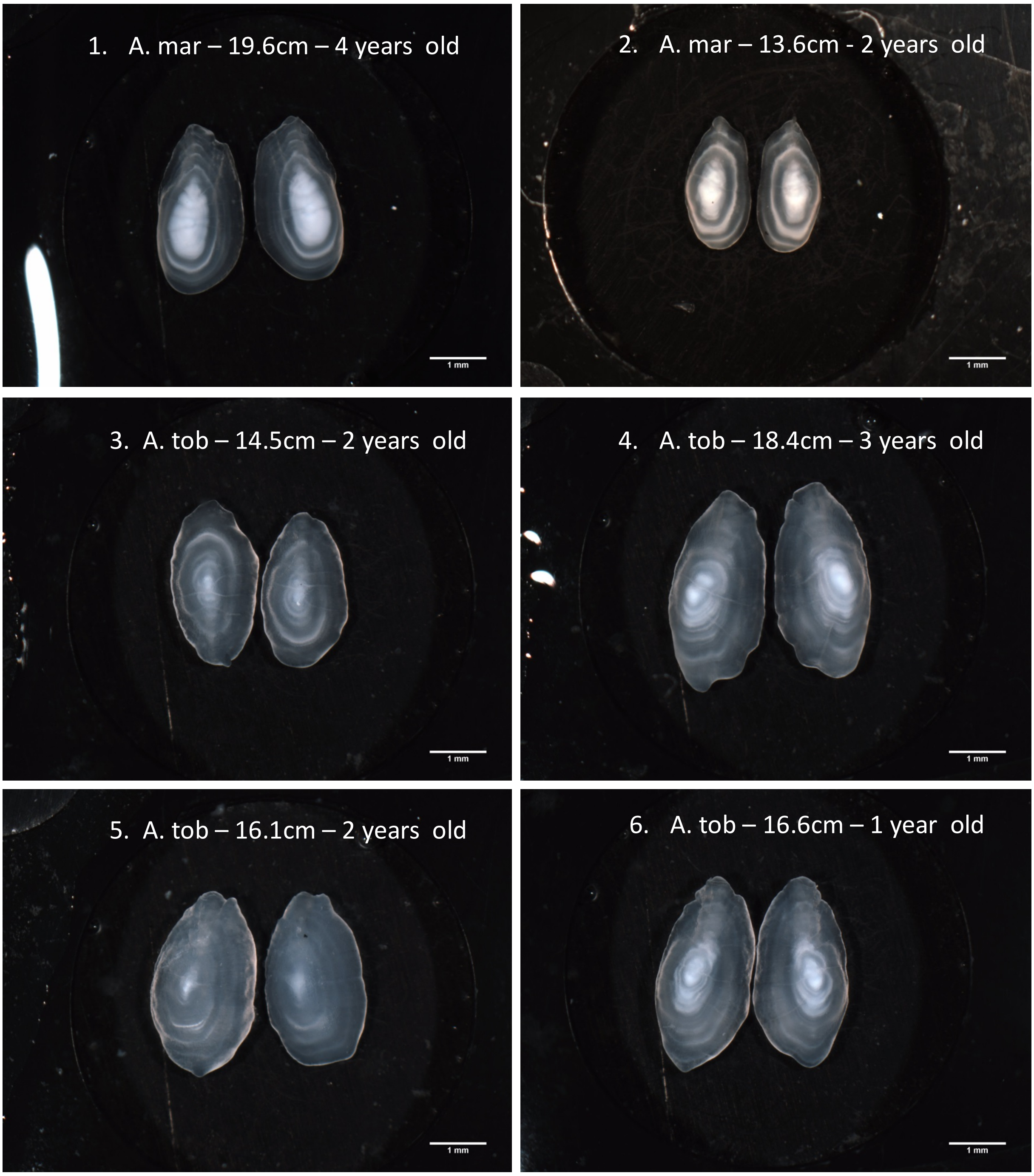

Figure 40: A selection of sandeel otolith pictures. Species, fish length and estimated age are indicated.

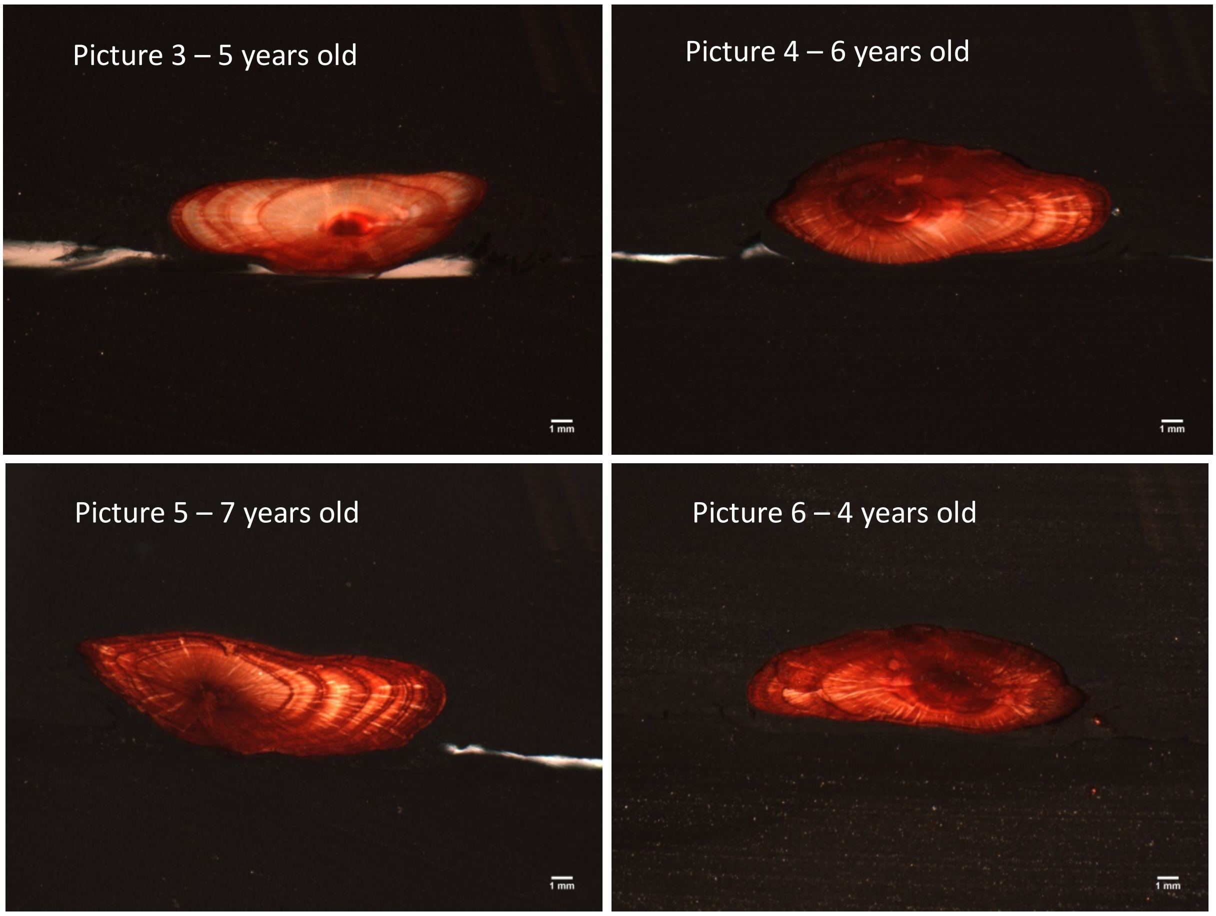

Figure 41: Image selection of neutral red stained sandeel otoliths. Picture numbers correspond to the numbers in figure 40 and the new age estimation after staining is indicated.