Developments in benthos and fish in gullies in an area closed for human use in the Wadden Sea

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Abstract
Glorius, S.T., I.Y.M. Tulp, A. Meijboom, L.J. Bolle and C. Chen (2018). *Developments in benthos and fish in gullies located in an area closed for human use in the Wadden Sea; 2002–2016*. Statutory Research Tasks Unit for Nature & the Environment, WUR. WOt-technical report 129 / Wageningen Marine Research report C092/18. 86 p.; 70 Fig.; 4 Tab.; 44 Ref; 7 Annexes.

In the eastern Dutch Wadden Sea an area was closed for anthropogenic bottom-disturbing activities in 2005. The 'natural' development of the fauna in gullies located within this area was monitored and compared with the development in gullies outside the closed area. Emphasis was put on sampling the benthic fauna (every autumn). Eleven years after closure (2016) the fish population was sampled again and additional benthic samples were taken, the sea floor was mapped and the fishing pressures were calculated. Preliminary results show that throughout the investigated period the open gullies were subject to moderate shrimp fishing pressures and that the closed gullies were not fished. Closure of the gullies has not yet led to the formation of biogenic structures on the seafloor, but has led to an increase in the species richness of small benthic fauna and to subtle changes in benthic species composition. Due to the limited availability of data it was not possible to detect statistically significant differences in the fish population.

Key words: Rottum reference site, closed area, Wadden Sea, benthos, fish, BACI, species richness

Referaat
Glorius, S.T., I.Y.M. Tulp, A. Meijboom, L.J. Bolle and C. Chen (2018). *Ecologische ontwikkeling binnen een voor menselijke activiteiten gesloten gebied in de Nederlandse Waddenzee, 2002-2016*. Wettelijke Onderzoekstaken Natuur & Milieu, WOt-technical report 129 / Wageningen Marine Research report C092/18. 86 p.; 70 Fig.; 4 Tab.; 44 Ref; 7 Annexes.

Sinds november 2005 is een klein deel van de Nederlandse Waddenzee gesloten voor (potentieel) schadelijke menselijke activiteiten. Het gebied ligt ten zuiden van Rottumerplaat en Rottumeroog en beslaat zo'n 7400 hectare. Doel van de sluiting is om de ongestoorde ontwikkeling van de natuur in de Waddenzee te kunnen volgen. Dit rapport beschrijft de tussentijdse resultaten, 11 jaar naar sluiting. In het monitor-programma is de nadruk gelegd op veranderingen in de bodemfauna die jaarlijks en vanaf 2002 in het najaar bemonsterd worden. In 2016 zijn aanvullende bemonsteringen uitgevoerd. In dat jaar is de vis-gemeenschap herbemonsterd, zijn additionele bodemmonsters genomen gericht op het bemonsteren van de wat grotere organismen, zijn de karakteristieken van het bodemoppervlak in kaart gebracht en is de visserij-druk in het gebied berekend. Voorlopige resultaten laten zien dat in de open geulen garnalenvisserij heeft plaatsgevonden gedurende de hier bestudeerde periode en dat in de gesloten geulen geen visserij heeft plaatsgevonden na 2005. Sluiting van de geulen heeft nog niet geleid tot vestiging van biogene structuren. Wel is de soortenrijkdom toegenomen en hebben er zich subtiele veranderingen in bodemdiersamenstelling voorgedaan. Vanwege de beperkte hoeveelheid gegevens was het niet mogelijk om uitspraken te doen over veranderingen in de vispopulatie.

Trefwoorden: bodemdieren, vissen, benthos, geulen, Waddenzee, referentiegebied Rottum, gesloten gebied

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Preface

The project 'Reference area Rottum' has been carried out within the framework of the statutory research tasks within the theme Nature Information Infrastructure, coordinated by the Ministry of Agriculture, Nature and Food Quality. Closure of the 'Reference area Rottum' allowed for an undisturbed development of nature in the Wadden Sea. Insights from studying the development of such area(s) can help to set targets for nature conservation and development, and to generate scenarios that can be used to underpin policy and management.

This investigation would not be possible without the assistance of the crew and vessels of the Wadden unit of the Ministry of Agriculture, Nature and Food Quality. Special thanks goes to my colleagues Karin Troost, Loes Bolle and Ingrid Tulp that made the additional monitoring effort in 2016 considering benthos and fish possible.

Sander Glorius

Contents

Summary

In the Netherlands an area located in the eastern Dutch Wadden Sea was closed for anthropogenic bottom-disturbing activities in November 2005, when agreements in a policy decision on shellfish fisheries adopted in 2004 (*Beleidsbesluit Schelpdiervisserij* 2005–2020) and in an official order published in the Government Gazette in 2005 (Staatscourant nr. 224, 17 November 2005) came into effect. The closed area is located south of the islands of Rottumeroog and Rottumerplaat (together with Zuiderduintjes known as the Rottum area). It covers 7,400 ha, which is approximately 3% of the total Dutch Wadden Sea, and includes a complete coastal inlet system with gullies, mudflats and drainage channels.

Monitoring programme
The closure of the area allowed the fauna and flora in this area of the Wadden Sea to develop undisturbed and a sampling scheme was set up to monitor this. The main aim of the monitoring programme is to compare the 'natural' development of gullies in an area that is largely closed for human activities with the development in gullies where these activities are still allowed. Emphasis was put on monitoring the small benthic fauna (>1 mm) sampled in two gullies located inside the closed area (Boschwad and Schild) and two gullies outside the area (Spruit and ZO-Lauwers). Every autumn since the start of the monitoring programme in 2002, 15 to 24 Van Veen grab sediment samples were taken in each gully to determine the benthic species composition. This sampling regime enabled a 'Before-After, Control-Impact' (BACI) analysis to be made (Smith *et al.,* 1993; Underwood, 1993 and 1994).

The demersal fish population in the gullies was sampled in the years before closure but not on a regular basis in the years after. The fish population in the closed gully was first sampled again ten years after closure (2016), while in the open gullies additional samples were taken whenever possible within the Demersal Fish Survey (DFS) programme conducted by Wageningen Marine Research (WMR). This sampling regime did not permit a formal BACI analysis to be made.

In 2016 dredge samples targeting larger sized benthic species (>5mm) were taken in addition to the Van Veen grab samples. Also, a sonar survey was conducted to map the relief and structure of the seabed over larger areas to increase the chance of detecting biogenic structures. The largest anthropogenic activity in the area is the shrimp fishery and in 2016 an effort was made to calculate the fishing intensity in the area over the research period using data recorded using the Vessel Monitoring System (VMS).

In the absence of induced disturbance of the seabed (by shrimp fishing) we expected that the benthic fauna in the closed gullies might be able to develop into a more species rich, abundant community with a larger proportion of larger (and older) specimens and an increase of the abundance of structure-forming sessile epifauna species. We also expected a more species rich and abundant fish population and an increase in the mean length of fish species in the closed gullies.

Results
Throughout the investigated period (2002–2016) the open gullies (Spruit and ZO-Lauwers) were subject to moderate shrimp fishing pressures and the closed gullies were not. Naturally induced disturbance of the seafloor (caused by storms and water currents) was high in both of the open gullies and in the closed gully 'Schild'. Although the closure of Schild and Boschwad has led to reduced fishing intensities, this has not yet led to the formation of biogenic structures on the seafloor. However, it has led to an increase in species richness of small benthic fauna and to subtle changes in benthic species composition. Molluscs have become more dominant in the benthic community, as has the tube building sand mason worm (*Lanice conchilega*), which has become established in the closed gullies in relatively high densities. Differences are especially pronounced in the more sheltered areas, i.e. in the end sections of Boschwad and Schild.

Currently it is not known whether or not the observed differences in the benthic communities will continue to increase in the future. For example, the tube building annelid species *Lanice conchilega,* encountered in the closed gullies, might facilitate new settlement that can lead to a more permanent and dominant presence of this species, which in turn might lead to a further increase in species richness and presence of shellfish. The presence of cockles, observed in Boschwad, might aid the establishment of mussel spat, which can attach themselves to the cockle shells. On the other hand, it is also possible that distinctive species will disappear in the future as a result of natural mortality, erosion and/or absence of recruitment, and that open and closed gullies will become more similar over time. It is expected that, in contrast to the more turbulent environment of Schild, conditions at Boschwad are favourable for the development and settlement of species that are sensitive to seabed disturbance.

Due to the limited availability of data it was not possible to detect statistically significant differences in the fish population between the open and the closed gullies. It was observed, but not statistically proven, that the trend in fish densities in the open gullies followed the trend observed in the surrounding area (DFS area 619) and that density and richness in the closed gully in 2016 was not markedly different from the situation before closure (2002–2005).

1 Introduction

The Wadden Sea is a large scale subtidal and intertidal ecosystem. The system provides for a high production of biomass supporting high numbers of fish, shellfish and birds. In 2009 the Wadden Sea became a designated Natura 2000 area and embedded in the World Heritage list. Nevertheless, the Wadden Sea is used intensively for fishing-, mining- and tourism activities. In 1991 Denmark, Germany and the Netherlands agreed to establish closed areas in the Wadden Sea were all disturbing human activities were banned (Esbjerg Declaration §33.3). This would allow for an undisturbed development of nature in the Wadden Sea. Insights from studying the development of such area(s) can help to set targets for nature conservation and development, and to generate scenarios that can be used to underpin policy and management. Germany and Denmark established closed areas before the trilateral conference held in 2001.

1.1 Closed area

In The Netherlands a closed area around the islands of Rottum was established in 2005, Figure 1.

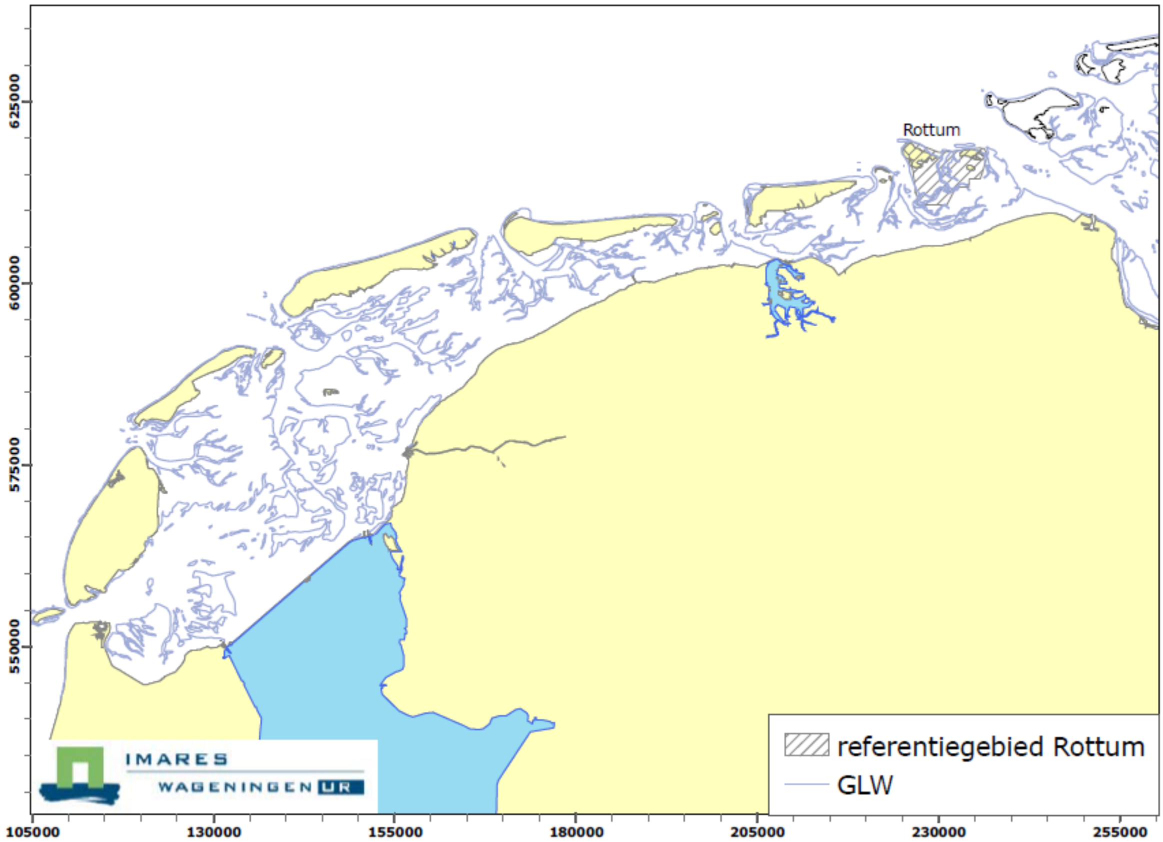


Figure 1*. The Dutch Wadden Sea with the location of the closed area "closed area Rottum".*

In the area all bottom-disturbing activities are banned, originally noted in the 'Structuurnota Zee- en Kustvisserij' (1993) and later in 'Beleidsbesluit Schelpdiervisserij' (spring 2004). The area covers 7400 ha, which is approximately 3% of the total Dutch Wadden Sea. In November of the year 2005 the closure of the Rottum area came into effect. The originally proposed boundaries of the closed area were slightly adjusted so that it would be part of the area already closed for shrimp fisheries (Staatscourant no. 224, November 17th 2005). At the same time the southern part of the island Rottumerplaat was excluded from the closed area.

1.2 Description closed area Rottum

The Dutch closed area is located in the eastern Dutch Wadden Sea located south of the islands of Rottumeroog and Rottumerplaat (together with Zuiderduintjes also known as the area Rottum, Figure 1 & 2). A complete coastal inlet system is located within the closed area as well as a gully system, mudflats and drainage channels (Figure 2). The gully system (ca. 500 ha and ca. 0.5% of the total Dutch subtidal Wadden Sea) consists of two main gullies, Boschwad and Schild. Both gullies are shallow (Boschwad around 2 meter and Schild around 4 meter) and dynamic, continuously changing their course. Deep gullies are absent within the area. There is considerable sedimentation within the area (Lavaleije & Dankers, 1993), and shell debris is present in the gullies (Fey-Hofstede *et al.,* 2015).

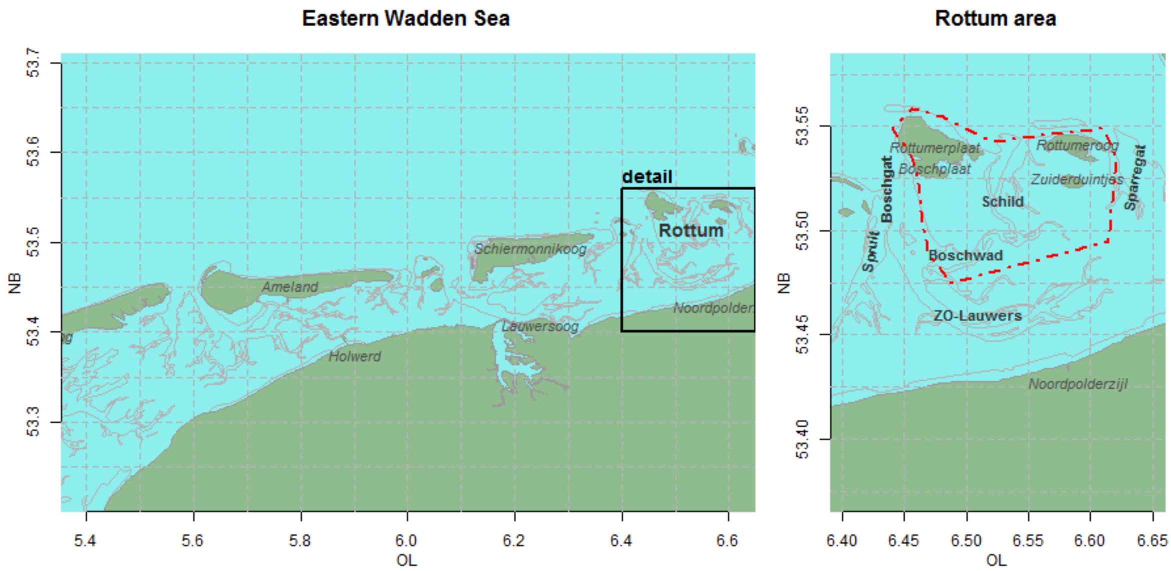


Figure 2. *Maps showing the sampled gullies in 2016. Left the eastern Wadden Sea with the Rottum area and the gully Boschwad east of the island Ameland. Right the Rottum area with the gullies Boschwad, Schild (within the closed area) and the gullies Spruit, Zuidoost (ZO)-Lauwers and Sparregat (outside the closed area).*

1.3 Gullies open to human activities

An important reason to assign reference site Rottum was to be able to monitor the natural development in an area were human (seabed) disturbance is reduced to a minimum in order to compare its development with areas were (seabed) disturbing activities are allowed. Gullies open for human activities, followed over time for reasons of comparison, should be (as much as possible) similar with respect to biotic and abiotic conditions to the closed gullies located within the closed area Rottum. It is to be expected that gullies in the near vicinity of the closed gullies are more similar in (a)biotic composition then gullies located further away. Gullies in the near vicinity of gullies in the closed area Rottum are: Zuidoost-Lauwers, located south of Rottumerplaat, Spruit, located west of Rottumerplaat, and Sparregat, located east of Rottumeroog.

2 Human activities and possible effects

Fishing and recreation are the most important (historic) human activities in the area and are described here.

2.1 (Historic) fishery in the area

Before the establishment of the closed area, several types of seabed disturbing fisheries occurred such as mechanical- and manual collection of cockles and mussel(seed)- and shrimp fishery.

Cockles
For centuries cockles were gathered by hand, using a rake with a net attached (Ens *et al.*, 2004). Since the 1950's, cockles were harvested mechanically and throughout the Wadden Sea. Also within the closed area Rottum cockles were collected until 1993 when all forms of shellfish fishery in the Rottum area became restricted under the Sea and Coastal Fisheries Policy (SN93). There is no quantitative information about the intensity of the cockle fishery over the period 1950-1993.

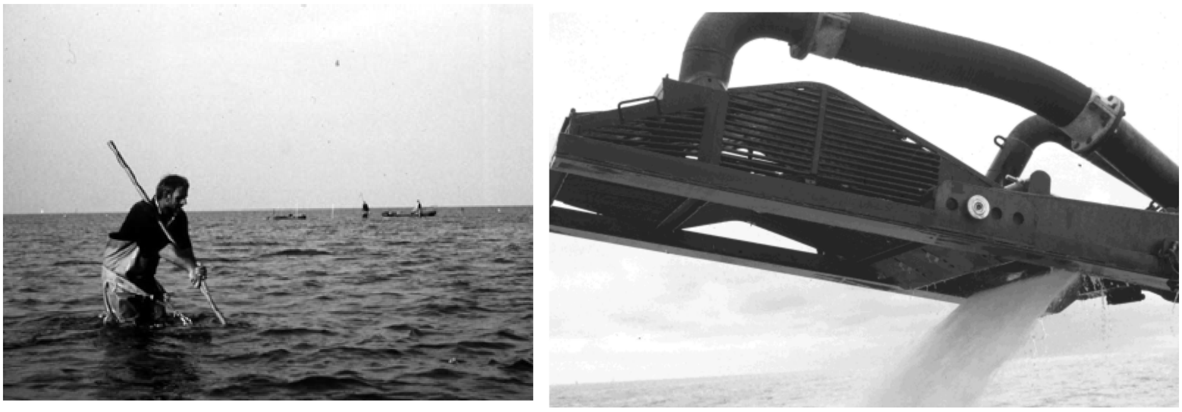


Figure 3*. Left; fishermen gathering cockles by hand-raking. Right; close-up picture of a suction dredge (photo taken by Jaap de Vlas). Both pictures taken from Ens et al., 2004.*

Mussel fishery
Since 1950's mussel fisheries occurred in the western Wadden Sea. It took a decade before mussel fishery expanded to the eastern part of the Dutch Wadden Sea. When a mussel parasite occurred in the eastern part of the Dutch Wadden Sea mussels from this part of the Wadden Sea were seen as a threat for the mussel culture plots and they were not fished upon as a consequence. Although it is clear that mussel beds were fished there is little to none information about the quantities of mussels extracted from the tidal flats in the closed area Rottum. In 1985 and 1989 mussel beds were exploited in the area (Dankers *et al*., 2003).

Shrimp fishery
Shrimp fishery is the most intensive form of fisheries in the Wadden Sea (Jongbloed *et al*., 2014). Shrimp fishery takes place throughout the year and the western Wadden Sea is most intensively fished. This type of fisheries takes place in the sublittoral- and the larger and deeper parts of the gullies. Shrimp trawlers drag a net with a beam equipped with a ground rope with 35-40 rubber bobbins over the sea bed. Since 1990's shrimp landings have increased considerably (Bear *et al*., 2017). Contact with the bottom is made by the trawl shoes and the rubber bobbins. Whether the net, especially when filled, makes contact with the seafloor is not clear, but likely.

There is little quantitative information available about the shrimp fisheries before 2004 in- and around the closed area. Using Vessel Monitoring through Satellite (VMS) data from 2004 onwards it can be observed that in 2005 both (later closed) gullies Boschwad and Schild and open gullies ZO-Lauwers and Spruit were fished. After the establishment of the reference site Rottum in November 2005 the closed gullies were not and the open gullies were fished. See Section 5.1 and Annex 1 for a more detailed description of the fishing activities.

2.2 Recreation

A limited and fixed number of guided touristic tours over the mudflat (to Rottumeroog) is permitted. Damage to the mud flat is expected to be minimal for this activity. A yearly maximum of 68 trips with a vessel are allowed in the area to pick up or drop off people to assist a total of 43 guided touristic tours and 25 excursions from Forestry Commission (Provincie Groningen, 2017).

2.3 Human and natural disturbance of the seafloor habitat

Apart from seafloor disturbance caused by fishing gears, the seafloor is also affected by natural disturbance caused by storms. Van Denderen (2015) investigated the effects of beam trawl- (a gear with a higher impact on the seafloor compared with a shrimp trawl) and natural disturbance on the composition and functioning of benthic communities in the North Sea and found similar effects of both types of disturbance; a decline in long-lived, hard-bodied and suspension feeding organisms. (Van Denderen, 2015). To assess the impact of the disturbance of the seafloor to benthic- and fish communities, it is furthermore important to distinguish direct effects, removal, damaging or killing of organism for example, and indirect (and sometimes also more long term) effects through food web interactions (Glorius *et al*., 2015; Jongbloed *et al*., 2014; Van Denderen, 2015; Tulp, 2009).

2.3.1 Shrimp fishery

Compared to the number of studies on the effect of beam trawls, far less studies on the effect of shrimp fisheries (which is relevant here) on the benthic community have been conducted. Especially the long term (and indirect) effects of shrimp fishery on the seafloor remains largely unknown (Jongbloed *et al*., 2014, Glorius *et al*., 2015 and Tulp, 2009. Compared to bottom trawl fisheries targeting flatfish, the floor contact of a shrimp trawl is of a different nature. A flatfish beam trawl is a heavy gear that is equipped with tickler chains that are pulled through the upper layer of the sediment, thereby physically disturbing the sediment and damaging or killing organisms living in and on top of the sediment (Kaiser *et al*., 2002). A shrimp trawl is lighter and has no tickler chains. At the front of the net a ground rope with 35-40 rubber bobbins keeps the shrimp beam trawl in contact with the seafloor. Contact with the bottom is made by the trawl shoes and the rubber bobbins. Whether also the net, especially when filled, makes contact with the seafloor is not clear. The effects of, fishing induced, disturbances are also depending on the frequency and speed of the fishing vessels, sediment type, water depth and natural dynamic of the area as well as the occurring flora and fauna (Gillet, 2008).

2.3.2 Sediment structure and turbidity

As possible effects of seabed disturbance is depending on many factors, there is no consensus in the literature. Abrasion of the seafloor by shrimp trawl might affect its physical structure (Gillet, 2008). Structuring organisms (such as fields of *Lanice sp.* for example) and boulders might be removed and originally occurring sand ripples, pits and bumps might be levelled off. Structuring organisms tend to stabilise the seafloor preventing suspension of sediments. When removed, resuspension of sediments by passage of a shrimp gear becomes more likely. Sediment resuspension depends on the penetration depth of the (in this case beam trawl) gear (EU project BENTHIS - Rijnsdorp *et al*., 2017). Compared to the beam trawl we expect that passage of a shrimp gear also causes resuspension of sediment but to a lower extent as the penetration depth is lower. Swirling up of sediment particles leads to more turbid water, and might uncover buried species while, after settlement of the particles, buries immobile species elsewhere (Gillet, 2008). High turbidity decreases the light availability for photosynthesis which might lead to a decrease in algal growth and indirectly growth of faunal species (Ierland & Van der Veer, 1982). Mussels respond to increasing suspended solids concentration by initially adjusting their morphology (altering the size of the gill and palp) and eventually a decrease in food assimilation (Essink *et al*., 1989 & 1990 and Widdows *et al.,* 1979). Frequent disturbance might lead to an increase of sediment grain size (Gillet, 2008), a more rapid recycling and availability of nutrients (that in turn enhances photosynthesis and algal growth) and higher availability of detritus. If seabed disturbance (beam trawl) increases or decreases the food availability for fish and benthos is altered and population impacts depend on the regulation of the benthic community (top-down or bottom-up) and prey preference of fish (Van Denderen, 2015).

2.3.3 Benthos

The (direct) effects of a shrimp fishing gear on the benthic community is still obscure (Tulp, 2009; Jongbloed & Tamis, 2011; Glorius *et al*., 2015). The primary effect of shrimp fisheries is the removal of shrimp. As shrimp forms a food source for some fish species (juvenile whiting and cod) and is a predator of shellfish spat such as cockles and mussels (Beukema & Dekker, 2014, Jongbloed, 2015) there might be some indirect effects. Spatfall success of bivalves might be increased due to a decrease in predation pressure by shrimp for example. There is controversy about this relationship, as shrimps are small (and not yet fished) when predation on shellfish spat occurs and the relation between shrimp fishery and the next generation of shrimp is also unclear (Jongbloed, 2015 and Beukema & Dekker, 2014).

There are several mechanisms by which the passage of a shrimp gear might affect the benthic community. Indirect effects (such as burial or reduced growth) will be described in Section 2.3.2. The gear itself may directly impact benthic species by damaging, killing and/or removal of species that are living in and on top of the sediment (Kaiser *et al*., 2002). It is expected (but largely not proven yet) that (structure forming) sessile epifaunal organisms are most prone to damage by shrimp fisheries (Gillet, 2008; Glorius *et al*., 2015). Examples of such communities and species that occur in the Wadden Sea are: Pacific oysterreefs, *Sabellaria sp.* reefs (German part of Wadden Sea), mussel beds, seagrass meadows, *Sertularia sp.* reefs (White weed) and fields of *Lanice sp.*, anemones and hydrozoa. Vorberg concluded that the impact of trawling over Sabellaria reefs is limited to the trawl shoes (Vorberg, 2000). Also an impact on *Lanice sp.* by the trawl shoes was observed but no damage was observed from the bobbins (Berghann and Vorberg, 1998). The reduction of White weed *Sertularia cupressina* beds, once common in the Wadden Sea (Lavaleije and Dankers, 1993), has also been related to shrimp fisheries although this is subject of debate. Berghahn and Vorberg (1998) argue that the shrimp fishery is unlikely to have affected these beds as *S. cupressina* is known to be a very flexible structure which is pressed flat against the sea bottom in contact with the trawl. Dankers & Baptist (2010) however show that there is no basis for this conclusion (Glorius *et al*., 2015).

2.3.4 Fish

A suite of flatfish, other demersal fish and pelagic fish species reach the Wadden Sea as postlarvae and spend their juvenile phase here (marine juveniles) (Van der Veer *et al.,* 2000; Elliott *et al.,* 2007). Other species inhabit the region on route to either marine or fresh water spawning sites (diadromous species) or during certain times of the year (marine seasonal migrants) or only occasionally (marine adventitious species) (Elliott *et al.,* 2007). In addition to temporary visitors, many species spend (almost) their entire life in shallow waters (estuarine residents) (Elliott en Hemingway, 2002).

There are several mechanisms by which shrimp gear may affect the fish community. The gear itself may directly impact fish by damaging or killing fish. Bycatch can at certain times of the year be substantial (Van der Hammen *et al.,* 2015), and although the bycaught fish are returned to the sea after capture, a considerable proportion may get damaged, bruised or killed (Berghahn en Purps, 1998). This effect may also differ for fish of different size. Therefore mechanisms caused by shrimp fishing that may alter the fish community could include: reduction of total fish abundance, reduction in abundance of certain species, change in number of species, and change in size structure. Also indirect effects through changes in sediment as the result of bottom trawling affecting the habitat for fish is possible.

2.4 Recovery

The closed area ‘Rottum’ has been, just as the whole Wadden Sea, subject to anthropogenic activities (collection of mussels, cockles and shrimp among others) for decades. Therefore it cannot be considered a pristine environment at the start of this monitor program and the closure of the area. As a consequence, it is not possible to study the impact of anthropogenic activities but only the recovery of the system when it is released from those activities (Fey *et al*., 2013). Some of the once occurring biogenic structures have disappeared and the Wadden Sea system has changed (Van Duren *et al*., 2009). Recolonization of once disappeared species and biogenic structures is not self-evident, and may take a considerable amount of time or might even be impossible to occur.

3 Research questions

An important reason to assign the closed site was to be able to monitor the natural development in an area were human (seabed) disturbance is reduced to a minimum in order to compare its development with areas were (seabed) disturbing activities are allowed.

The focus of this research is on benthos and fish in the subtidal parts of the Rottum area. The following main research question is formulated:

> *How does a Wadden Sea ecosystem develop in which human disturbance is reduced to a minimum in comparison to areas open to human disturbance?*

Shrimp fishery
Considering the shrimp fisheries, we evaluate to what extent the open gullies in the Rottum area were fished since 2005 and if there is any trend in fishery intensity in the open gullies.

Benthic fauna
In this report, we asses if the benthic communities in the gullies located within the closed area Rottum develop different from the communities in the gullies open for human activities. If disturbance of the sediment has negative effects on the development of the benthic fauna, we expect to find differences between the open and closed gullies. We expect a higher species diversity, a higher total density of benthic fauna, a higher contribution of structure forming sessile epifauna species and a larger proportion of larger (and older) specimens in the closed gullies as compared to the open gullies.

Considering the benthic communities, we evaluate more specifically whether:
1. There is a difference in the benthic community composition between open and closed gullies and if this difference changed over time.
2. If the total benthic density, species richness and diversity are higher in the closed gullies when compared to the open gullies.
3. Densities of (structure forming) sessile epifauna species, such as the tube worm *Lanice conchilega*, and mollusc species such as mussel *Mytilus edulis* and oysters *Crassostrea gigas* are higher in the closed gullies compared to the open gullies.
4. Species can grow older (and larger) in the closed gullies compared to the open gullies.

Fish fauna
Depending on the time fish spend in the Wadden Sea and during which life stages, species may respond differently to disturbance (by e.g. shrimp fishing). Marine juvenile species only spend a relatively short period in the Wadden Sea, while resident species complete their life cycle within the Wadden Sea. If fishing causes direct mortality in fish, then we expect the mean length of fish species to increase in the closed gullies.

In this report we evaluate whether:
1. Developments in total fish abundance differs between the open and closed gullies.
2. Developments in fish abundance for marine juveniles and estuarine residents differs between the open and closed gullies.
3. Developments in fish abundance per species differs between the open and closed gullies.
4. Developments in number of fish species differs between the open and closed gullies.
5. Developments in mean length per fish species differs between the open and closed area.

4 Methods

4.1 General

The main aim of the monitor program is to compare the 'natural' development of gullies in an area that is (largely) closed for human activities and to compare it with the development in gullies where these activities are still allowed. The monitor program started in 2002, three years before closure of the area in 2005. In that period (2002-2005), monitoring consisted of sampling with a Van Veen grab targeting the (smaller sized) benthic fauna, and sampling with a trawl net targeting the demersal fish fauna. Besides the two gullies located within the Rottum area (Boschwad and Schild), gullies open for human activities were sampled as well (Spruit and ZO-Lauwers) with the same gears. Samples were taken every autumn. The samples taken in all gullies prior to the closure of the site in 2005 reflect the baseline.

In later years (>2005 to present), emphasis was put on monitoring the (small sized) benthic fauna in both the closed and open gullies (every autumn). The demersal fish community was not monitored within the program after 2005, although additional samples in the area were taken as much as possible within the Demersal Fish Survey (DFS) program conducted by Wageningen Marine Research (WMR). Only in 2016 the demersal fish community in the open gullies was sampled again. Also additional benthic samples were taken with a suction dredge targeting the larger sized benthic species and the structure of the seabed was mapped with sonar. In this chapter, the sampling methods and the statistical analyses are described.

4.2 Fishing intensity

Data recorded by the Vessel Monitoring System (VMS) were used to track and monitor the activities of fishing vessels and can, in combination with logbooks (containing information on fishing trips, days and gear), be used to calculate the fishing intensity. The standard method is described in detail in Hintzen *et al*. (2012). In short, speed profiles were made per fishing type to distinguish different activities of the fishing vessels (fishing, sailing etc.). Between two VMS recordings (pings), additional pings were constructed by interpolation of the data to obtain a finer spatial resolution. By combining ship speed, gear width and time interval between pings, the swept area was calculated for each ping. A spatial grid was created (area of 0.235 km^2) and in each grid cell, the swept area was summed per year in order to calculate the fishing intensity. The quality of the VMS data improved over time as in 2006 logbook data of shrimp fishery became available and the time interval between VMS pings decreased in 2014 resulting in a more reliable spatial resolution.

4.3 Benthic fauna

The benthic community was sampled with two devices in 2016, a Van Veen grab sampler and a suction dredge. Although there is overlap in sampled species between the two devices, the van Veen grab is more suitable for sampling the smaller sized and deeper buried (up to 25 cm) species and the suction dredge is more suitable for sampling of the larger sized and less deeply buried species.

4.3.1 Smaller sized benthic fauna

With a Van Veen grab sampler sediment samples with a surface area of 0.18 m^2 were taken up to 25 cm deep into the sediment. The sediment samples were sieved over a sieve with a mesh of 1mm and species were identified in the laboratory.

Number of samples per year and gully

Since 2002, each year in autumn samples were taken in each gully with a Van Veen grab sampler and the assistance of the vessel Ms. Harder and its crew (Table 1). Next to the two closed gullies Boschwad and Schild (located within the closed site), two open gullies Spruit and (Zuidoost) ZO-Lauwers were sampled. The number of samples were equally distributed over areas without and with beds of shell debris, that occurred there in 2002. Samples were taken both before (2002-2005) and after (>2005) the establishment of the closed area Rottum. See Table 1 for the number of samples taken per year in each gully.

Table 1. *Number of Van Veen Grab samplers taken per gully and year. Samples taken before closure of the area are printed in italic font.*

Year	Closed gullies		Open gullies	
	Boschwad	Schild	Spruit	ZO-Lauwers
2002	*6*	*11*	*0*	*17*
2003	*14*	*24*	*20*	*23*
2005	*14*	*24*	*20*	*23*
2006	14	24	21	22
2007	14	24	18	23
2008	14	24	20	24
2009	14	25	21	22
2010	14	24	21	21
2011	14	24	21	23
2012	14	24	21	23
2013	14	24	21	23
2014	14	24	21	23
2015	14	24	21	23
2016	14	24	21	23

Applied method in the field

The vessel Ms. Harder (owned by Department of Waterways and Public Works) and its crew assisted in the sampling. Once the vessel was manoeuvred into position, the Van Veen grab, operated by the crew of the Ms. Harder using the vessel crane, was lowered to the seafloor. The position was recorded and the water depth was noted. On the seafloor a sediment sample with a surface area of approximately 0.18 m^2 and 25 cm deep was taken. Once recovered on deck, the sample was emptied above a sieve (1 mm mesh) and general information about the sample (such as sediment type and amount of shell debris) was noted. On board the sample was sieved using local seawater to remove sand and other particles. The remaining material (fauna, shells, stones and other particles) was stored in a polyethylene container and preserved with a 6-10 % buffered formaldehyde in seawater solution. Samples were stored prior to identification and counting of the fauna in the laboratory of Wageningen Marine Research (WMR).

4.3.2 Laboratory analysis

In the laboratory, shell, stones and other particles were separated from fauna. Shell debris was weighted. The separated fauna was examined and identified by making use of a stereomicroscope. Standard taxonomic keys and references were used to identify each taxon. The sampled organisms were identified to the lowest taxonomic level possible (WoRMS Editorial Board 2016). The number of individuals of each taxon was determined. Specimens that could not easily be identified were kept aside for further examination. Juveniles whose species-specific features were not sufficiently developed and damaged species, were identified to a higher taxonomic level. In the years prior closure of the area, only larger sized species were identified. From 2006 onwards procedures were altered and all individuals were identified and counted. Examples of the benthic species were taken up in the Taxonomic Reference Collection that has been maintained for several years at WMR as part of their

Quality Assurance procedures. Species densities and weights of the shell debris were stored in a database that is maintained by WMR.

4.3.3 Data analysis

The data were analysed in two ways 1) univariate analysis of community parameters and 2) multivariate analyses in which the development of individual species within the community is followed

Univariate analysis
The Van Veen grab sampling schema allows for a 'Before-After, Control-Impact' (BACI) analysis (Smith *et al*., 1993 & Underwood, 1993 and 1994) as both open and closed gullies are sampled both before and after designation of the closed site at Rottum. In this type of analysis, key is to investigate the development over time between the control gullies (Spruit and Zuidoost-Lauwers) and closed gullies (Boschwad and Schild) indicating an effect of the closure. Several types of effects are possible and some of them are shown in Figure 4.

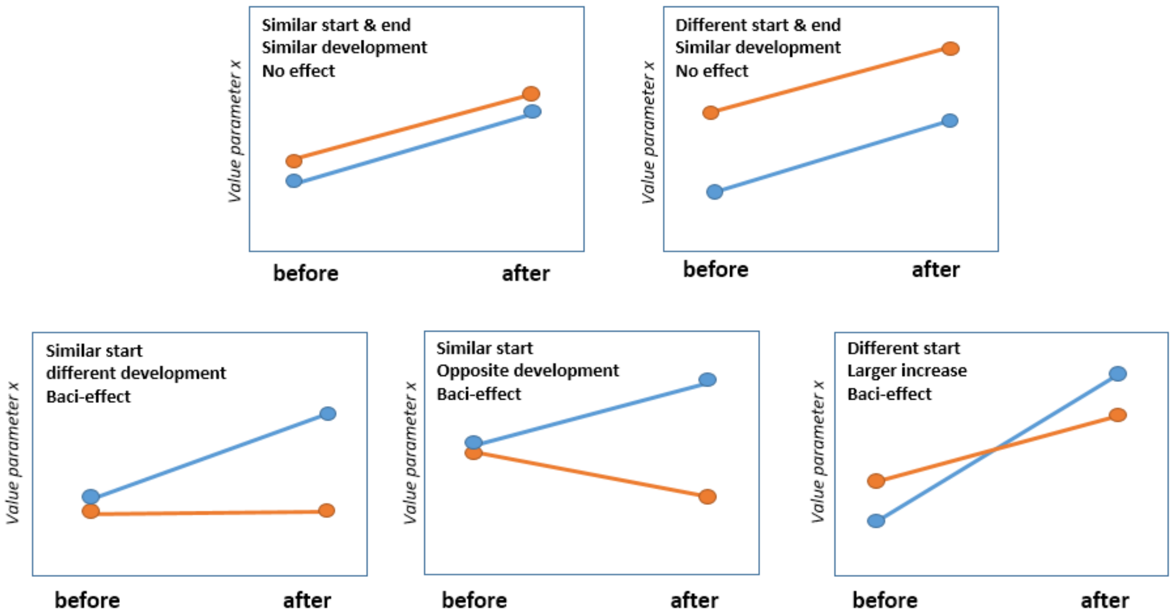


Figure 4. *Schematic representation of different possible outcomes of a Before After Control Impact (BACI) design. Blue dots impact site, orange dots control sites. Top row; no effect, bottom row showing different situations with an effect of the treatment (closure to human disturbance)*

The advantage of this set-up is that differences between the gullies at the start is accounted for as well as an autonomous development (as occurring in the control / open gullies Spruit and ZO-Lauwers). BACI designs are especially suitable to detect large and permanent changes in the mean, but are less suitable to gradual- and/or temporary changes and changes in variability (Schwarz, 2015).

As gradual changes are difficult to detect in a BACI design, the dataset was split in order to be able to check for a possible different development over both a short- and long(er) time period. In case of the short time period the 'before' data set consisted of the years 2002 till 2005 and the 'after' dataset of the years 2006 till 2011. In case of the long(er) time period the 'before' data set consisted of the years 2002 till 2005 and the 'after' dataset consisted of the sampling years 2012 till 2016.

Variables and data preparations
The following variables were investigated within the BACI framework; sample species richness, -density, -diversity (Shannon), -evenness (Pielous'), shell debris and estimated species pool (Chao). The Chao equation (Chao, 1987) was applied to calculate the expected species pool (= species discovered in the samples + expected number of undiscovered species) in a set of samples, here defined per gully and year.

Before calculating these variables, the following data preparation steps were conducted. Different hydrozoa, bryozoan and barnacle species were each merged to their (higher) taxonomic level as these were, over the course of the program, not always identified in the same detail.

Species richness represents the number of different taxa found in each sample. Not all taxa could be identified to the species level (as for example juvenile species were encountered which did not fully develop species specific features needed for identification). As a simple summation of taxa can cause bias, aggregations were made. Not fully identified taxa were treated as follows; when no other species or taxa were present at higher levels of identification in a particular sample, the taxa was counted and contributed to species richness. However, when other species or taxa were present at a higher level of identification it was not included in the calculation of richness as species might be counted twice in that case.

The species diversity index was calculated with the use of Shannon-Wiener Index (equation 1). This index measures the order (or disorder) within a sample taking both the evenness and the number of species into account. The number increases by an increasing number of species but is reduced when species evenness is low.

Shannon-Wiener Index:

$$H = -\sum P_i(\ln P_i)$$

Equation 1.

H= species diversity
Pi = share of species compared to total amount of species

Evenness was calculated using Pielous' evenness index, see equation 2. This is a measure of how (dis)similar the abundance is distributed over the different taxa. The index is expressed in a number between '0' and '1'. A low evenness indicates that the individuals in a sample is numerically dominated by a single taxa, while the evenness value is maximal when all individuals are equally spread over the different taxa in a sample.

Evenness:

$$E = \frac{H}{\ln(S)}$$

Equation 2.

E = Evenness
H = Species diversity
S = Number of species

Statistical models
Linear mixed effect models were applied to test if change (development) of the variables differ between the open and closed gullies. Model formulation followed Schwarz (2015) 'BACI with multiple sites; multiple years before/after'. In the models bot *fixed* effect factors (the ones you are interested in) and *random* effect factors are included. The random factors are variables that you expect to explain some of the variability and that you are not interested in, but would like to compensate for. Random factors (intercept) incorporated in the model were gully nested in year as it is expected that both gully and year has its effect on the parameter(s) under investigation but are not relevant in examining the effect of designation of the closed area Rottum. The fixed part of the model consisted of period (before and after designation of the closed area Rottum), and treatment (the open gullies Spruit & ZO-Lauwers and the closed gullies Boschwad & Schild). When the interaction between the fixed factors (period x treatment) is significant, this would imply an effect of the treatment (closure of the area Rottum).

In the linear mixed model a different variation structure was allowed for in the period before and after 2006, as more taxonomic groups were considered after 2006. Presence of spatial autocorrelation was checked by spatially plotting the residuals and constructing variograms. To account for spatial autocorrelation the following correlation structures were tested; exponential, gaussian, lineair, rational quadratic and spherical. Using the Akaike Information Criterion (AIC) the best model was selected. Residuals of the best model were plotted against fitted values, period, treatment, gully and year for validation purposes. Densities were square root transformed prior analysis to arrive at normal distribution of the data.

Analyses were performed in R (R Core Team 2016) using the functions available in the following libraries: vegan (Oksanen *et al.*, 2017) for calculation of diversity indices and nlme package (Pinheiro *et al*., 2018) for the linear mixed model.

Multivariate analysis
The development of the benthic community composition over the years per gully was studied using the ordination technique constrained correspondence analysis (CCA). In a multivariate dataset there are as many dimensions as there are species and in ordination techniques these dimensions are reduced in a such a way that they explain as much variation in the species sample matrix as possible. By reducing the dimensions the most important trends in the data are shown and can be explored. In a CCA the correlation between species scores and samples scores (as linear combinations of environmental variables) is maximized. The environmental variables shell debris and water depth were used as predictor variables. Species that were present in less than 10 samples (1%) were disregarded prior analysis. Analyses were performed in R (R Core Team 2016) using the functions available in the library Vegan (Oksanen *et al*., 2017).

4.3.4 Large size benthic fauna

For the large sized benthic fauna a suction dredge was used, taking samples with a relatively large surface area. Dredge samples were only taken in 2016 (June). The sampled area varies depending on the length of the sampling track but is generally around 30 m^2. The sediment penetration depth is 7 cm. The samples were sieved over a sieve with a mesh of 5 mm and species were identified, counted and weighted in the field. This technique is especially suitable to sample the larger sized and shallow buried part of the benthic community. Especially species that occur in aggregations are better sampled as these are more easily missed with a Van Veen grab sampling only 0.18 m^2.

Number of samples gully
Next to the gullies Boschwad and Schild, located within the closed area Rottum, the gullies Spruit, Zuidoost-Lauwers and Sparregat were sampled that are open for human activities. Each gully was divided in five segments; start- middle-, and end section as well as between start and middle and between middle and end section. In each segment three dredge samples were taken in longitudinal direction when possible. In each segment a sample was taken at the deepest (center) part of the gully and one at each side of the gully. When the gully was too shallow or the slope of the side to steep a sample perpendicular to the flow direction of the gully was taken.

Applied method in the field
The vessel YE42 and its crew operated the suction dredge and assisted the sampling. First a cross section of the gully was made in order to determine the sampling locations (center and at the two sides of the gully). The sampling tracks were logged and stored in the navigation software MaxSea, and used to measure the exact track length (aimed to be 150 m). The collected sample was pumped on deck and sieved over a mesh of 5 mm. Species were identified and from the shellfish and crab species their size or age was determined and individuals were counted and weighted (fresh weight). Starfish, worms, and fish were identified and the individuals were counted as well. Results were immediately stored in a Microsoft Access file and, once back at the office, checked and stored in a database maintained by WMR.

4.3.5 Data analysis

The number of different species and total density and biomass were calculated. Differences in species richness, density and biomass between the gully segments (start/opening, middle-, end section, and between start and middle section and between middle and end sections of the gully) and position (centre or both sides of the gully) were tested using (generalized) linear mixed effect models with gully as random effect. Density and biomass were 4th root transformed to normalise the data and were modelled using a Gaussian distribution. Species richness was not transformed and the Poisson distribution was used in the models. When significant (at the 0.05 level) effects were encountered, a Tukey post hoc tests was performed in order to examine the within group differences. (Generalized) linear mixed effect models were also applied to investigate the difference in richness, density and biomass between gullies. When location or position within a gully was of significance, these factors were incorporated in the models as a random effect. Boxplots were constructed for visual inspection of the data. Analyses were performed in R (R Core Team, 2016) using the functions available in the following libraries: lme4 (Bates *et al.,* 2015) for generalised linear mixed models and nlme package (Pinheiro *et al.,* 2018) for the linear mixed models.

The development of the benthic community composition between and within the gullies was studied using the ordination technique detrended correspondence analysis (DCA). DCA is an indirect gradient technique utilizing only the species – samples matrix and maximizing the correspondence between species- and samples scores. Species that were present in less than 4% of the samples were disregarded prior analysis. Analyses were performed in R (R Core Team, 2016) using the functions available in the following library: vegan (Oksanen *et al*., 2017).

4.4 Seafloor mapping

Commissioned by WMR, Deep BV (DEEP) conducted the sonar survey in 2016 using side scan sonar and multi-beam. With these techniques heterogenic surfaces, formed by for example fields of *Lanice sp.* and sublittoral mussel beds, of the seafloor can be detected. Compared with the Van Veen grab- and suction dredge samples a larger area can be covered. The gullies Boschwad, Schild, ZO-Lauwers and Spruit were scanned by DEEP.

4.4.1 Sonar

The survey vessel VOLANS, owned by DEEP, was used for the fieldwork for both the side scan sonar and multi beam measurements. Side scan sonar is an acoustic technique. The apparatus (Klein 3900) that is towed behind the vessel, a sound beam (narrow in horizontal plane and wide in vertical plane) is emitted (500 kHz or 900 kHz) and the reflected sound is recorded by transducers that convert the acoustic signal in an electric signal.

The survey vessel was equipped with an R2Sonic 2024 echo sounder (400 kHz) for multi beam recordings. This device measures 256 beams of 0.5^{o} and variable angle of 10 – 160^{o}. The 'backscatter' function in the software was applied as well, comparing the intensity of the outgoing signals with the received (reflected) intensity, providing information about the characteristics of the seafloor.

As individual recordings had a very narrow bandwidth, many tracks were needed to obtain overlapping images and it was difficult to make a mosaic covering the hole surface area of the gullies. Despite extra effort (an extra field campaign) it was not possible to scan the entire surface area of the gullies and especially in ZO-Lauwers only a small part of the entire gully could be scanned.

4.4.2 Data analysis

By merging the individual recordings of each track, a mosaic was generated showing the intensities of the signals or a large surface area. High intensities are associated with boulders or coarse sediments, low intensities with fine sand. All measurements and data manipulations and interpretations were conducted by DEEP and described in more detail in Lange (2016).

4.5 Fish

We evaluate developments of the fish community in the closed and open gullies and the surrounding area (DFS area 619) based on several parameters describing the fish fauna: total abundance, abundance of marine juveniles, abundance of resident species and mean length. Fish abundance is highly variable and factors that are known to be of influence include water depth, tide, temperature and water visibility. In addition, natural variation in fish abundance from year to year is very large. In the analyses we try to take these factors into account as much as possible to distinguish between these sources of variation and variation caused by the treatment (open versus closed to fishing).

4.5.1 Trawl sampling

The monitoring program was carried out in combination with the annual Demersal Fish Survey (DFS), running since 1970 and carried out throughout the entire Wadden Sea in autumn (Oct/Sept). Prior to closure for human use, Schild (later closed) and Spruit (open) were sampled in the years 2002, 2003 and 2005. Even though there was no official assignment, the open area Spruit and later on Boschgat (see reason below) was sampled within the DFS survey throughout the whole research period (2002 till 2016). Because of sedimentation, sampling in Spruit was no longer possible and from 2012 onwards a third gully, Boschgat was used as a reference instead. The closed gully Schild was resampled once, ten years after closure in 2016.As an extra reference, the whole tidal basin where the area is situated is used (area 619 of the DFS). The DFS area 619 covers the tidal basin that is delimited in the west roughly half way the island Schiermonnikoog (at the border were tidal currents meet) and to the east to the island Rottumeroog. See Table 2 for an overview of the gullies and DFS 619 area sampled in the different years. This setup does not allow a formal Before After Control Impact (BACI) analysis because of the discontinuous time series and the change in closed area. Therefore possibilities for a formal statistical evaluation are very limited.

Table 2. *Overview of sampling years in the different gullies.*

Gully	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Schild	x	x		x	closed for fisheries										x
Spruit	x	x	x	x	x	x	x	x	x	x					
Boschgat											x	x	x	x	x
DFS 619	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

In all gullies 12 hauls were taken. Sampling was stratified to depth and tide. In every gully six hauls were carried out during ebb and six during flood tide at three different water depths. Every haul was carried out in duplo, but the trawl path was chosen so that we did not follow the exact same path. Haul duration was 7.5 min (instead of the usual 15 during the DFS survey).

Sampling was carried out with a 3 m-beam trawl, rigged with one tickler chain, a bobbin rope, and a fine-meshed cod-end (20 mm). Fishing in DFS area 619 was restricted to the tidal channels and gullies deeper than 2 m because of the draught of the research vessel. The combination of low fishing speed (2–3 knots) and fine mesh-size results in a positive selection of small fish both among and within fish species. The gear used is suitable for demersal species, but suboptimal for pelagic species such as herring and sprat.

All hauls are carried out moving along the tidal current. Fish are sorted, counted and measured to the cm below. The mean abundance per species for area 619 was calculated weighed by surface area for every depth stratum. For every haul, the position, date, time of day, depth, water visibility (secchi depth) and surface water temperature were recorded.

4.5.2 Data analyses

In the results section we first present the plain data in boxplots comparing the T0 (2002 till 2005) and T1 (2016) situation in the DFS survey for area 619 as well as the T0 (2002 till 2005) in the open gully Spruit, the T1 (2016) in the open gully Boschgat and the T0 (2002 till 2005) and T1 (2016) in the closed gully Schild. Thereafter we present the results of the statistical analyses in which we use different statistical models to correct for variables such as water depth, water visibility and tidal phase.

Abundance

Some fish species were grouped by their genus; sandeel sp., gobies sp. and pipefish sp.. For species-specific analyses the five most common species occurring in DFS area 619 were selected (Table 3): plaice, five-bearded rockling, sole, gobies sp. and flounder. Among these two are marine juvenile (plaice, sole) and three resident species (five-bearded rockling, flounder, gobies sp).

The total fish abundance (numbers/10.000 m^2 (nha) and weight/10.000 m^2 (wha)), as well as water depth and water visibility were 4th-root transformed to obtain a better linear relationship and a homogeneous residual distribution. All calculations and plots are based on the transformed variables.

Correlations between explaining variables (water depth, water visibility, temperature, tidal phase (ebb or flow) and year) were checked before analysis. If two or more variables were highly correlated, one of them was removed.

Temperature was strongly correlated to year and water visibility. Therefore, temperature was not selected as a covariate in the model for year indices estimation. Low correlation was observed between depth and water visibility in all four areas. Therefore, year, depth and water visibility were selected as independent covariates in estimating total abundance in DFS, Spruit (open) and Schild (closed since 2006), while for Boschgat water visibility was excluded. The total fish abundance in Spruit (open) and Schild (closed since 2006) seem to be less susceptible to water depth and water visibility, likely because of less variation.

We calculated year indices, after taking into account the variation caused by the explanatory variables. An area-specific model was fitted for every response variable (total fish abundance, biomass, abundance marine juvenile and resident species, abundance/presence of the selected species, number of species and mean length) in every area (DFS619, Schild, Spruit and Boschgat)

The optimal model was independently selected for each area according to minimum AIC (Akaike information criteria). The following models were tested:

response ~ factor(year) + depth (1)
response ~ factor(year) + water_visibility (2)
response ~ factor(year) + water_visibility + depth (3)
response ~ factor(year) + depth + factor(tide) (4)
response ~ factor(year) + water_visibility + depth + factor(tide) (5)

***Table 3**. Frequency of occurrence (% of hauls in which the species was found) in the hauls in DFS area 619 used to select the most common species.*

species	dutch name	english name	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
		n hauls	12	10	9	9	11	10	10	11	10	9	10	10	10	9	10	8	9	8	8	8	8	10	8	7	9	9	9	10	10	10	10	9	9	8	9	8	8	9	8	6	7	10	10	11	11	10
Pleuronectes platessa	schol	plaice	100	90	89	100	91	100	100	91	100	100	100	100	100	89	100	100	100	88	100	75	100	100	100	100	100	100	100	90	100	90	100	56	78	100	89	75	100	100	63	100	86	100	100	91	100	90
Ciliata mustela	5-dr meun	5-b rockling	50	70	100	67	100	100	60	36	80	44	80	80	60	100	90	63	33	100	75	88	50	100	50	86	89	56	89	70	90	100	100	100	89	100	100	38	100	67	100	67	100	100	100	82	100	70
Solea solea	tong	sole	75	70	67	67	0	80	80	36	100	89	90	80	70	67	90	88	89	100	88	50	13	100	75	14	44	67	100	60	90	60	100	56	78	38	89	38	63	78	100	67	86	90	100	55	82	80
Merlangius merlangus	wijting	whiting	58	50	44	33	82	100	100	73	100	89	100	100	50	100	100	75	56	88	88	88	100	90	75	100	78	89	44	60	70	100	90	33	22	25	0	0	88	56	50	100	57	40	100	45	82	40
Pomatoschistus sp.	grondel sp.	gobies sp.	100	10	44	67	73	100	80	82	20	0	30	90	20	56	30	25	0	13	50	25	50	100	88	100	67	78	100	70	100	100	90	89	78	88	89	50	100	78	88	83	86	90	90	55	73	90
Platichthys flesus	bot	flounder	33	30	89	22	64	60	100	45	60	78	60	30	30	67	40	63	56	25	75	63	50	80	50	71	100	78	33	60	90	90	100	67	78	75	56	38	75	100	100	67	86	40	60	45	91	80
Clupea harengus	haring	herring	17	20	22	0	64	0	40	55	40	44	70	90	90	89	90	75	89	75	88	88	50	90	75	100	100	100	67	90	80	30	80	56	44	88	33	50	88	100	50	17	57	50	50	91	73	50
Limanda limanda	schar	dab	100	90	89	100	100	100	90	82	100	89	100	90	80	100	90	63	100	38	100	100	100	100	63	71	67	89	44	10	0	0	90	22	11	13	11	13	100	33	13	33	14	20	90	27	9	0
Syngnathus sp.	zeenaald sp.	pipefish sp.	50	10	44	22	36	80	40	18	60	22	40	60	60	56	60	0	22	25	13	88	75	70	100	100	56	89	89	40	80	90	100	78	67	88	67	63	50	78	88	83	14	70	60	0	64	40
Agonus cataphractus	harnasmannetje	hooknose	33	40	33	44	55	100	50	45	90	33	50	30	30	78	40	38	44	25	50	25	75	90	38	86	100	44	78	30	40	70	70	33	78	63	22	25	75	44	75	83	86	80	90	45	91	0
Liparis liparis	slakdolf	sea snail	33	30	56	33	82	50	10	18	80	56	70	90	50	100	80	63	56	63	75	38	0	60	63	29	0	67	0	40	70	80	100	22	11	75	67	13	63	56	75	83	86	80	100	18	73	40
Osmerus eperlanus	spiering	smelt	33	0	67	0	55	80	40	18	10	22	70	80	50	44	20	63	89	50	50	25	25	10	25	43	67	33	56	60	50	50	70	33	78	50	56	50	50	67	63	100	100	50	20	82	27	30
Zoarces viviparus	puitaal	eelpout	25	10	78	44	45	30	60	18	40	22	50	60	90	100	80	88	67	88	50	75	50	100	50	29	22	33	33	20	20	20	10	0	0	0	11	13	13	11	25	33	71	60	70	55	45	30
Sprattus sprattus	sprot	sprat	8	0	11	22	82	40	100	73	60	33	80	80	60	78	20	63	67	50	25	0	0	30	25	57	11	56	56	30	60	40	20	22	0	50	11	25	13	44	50	33	71	20	0	27	0	0
Myoxocephalus scorpius	zeedonderpad	bullrout	17	10	56	22	9	30	10	18	40	33	30	40	80	89	90	63	44	25	50	25	0	20	0	43	22	100	67	10	0	70	40	11	33	75	22	0	50	44	38	17	57	50	20	27	64	20
Gadus morhua	kabeljauw	cod	92	0	11	0	27	0	100	45	100	44	100	30	20	100	30	50	44	25	38	0	13	60	38	86	56	67	33	50	0	30	90	11	11	13	11	0	50	0	25	17	0	0	10	9	0	0
Ammodytes sp.	zandspiering sp.	sandeel sp.	0	0	0	11	0	20	20	9	20	11	10	0	20	0	0	38	11	0	25	25	0	0	0	0	22	33	33	20	0	60	30	44	11	38	11	0	38	33	0	0	0	30	40	9	36	20
Chelidonichthys lucerna	rode poon	tub gurnard	0	0	0	0	0	30	20	0	0	0	0	50	20	11	10	0	33	13	0	25	0	0	13	0	0	0	0	10	40	30	0	0	0	0	0	0	38	22	25	67	0	20	90	55	9	30
Anguilla anguilla	aal	eel	0	0	11	0	0	0	20	0	0	0	0	40	60	11	30	0	0	0	0	13	0	10	0	0	11	22	11	0	0	10	20	0	0	13	0	0	0	0	0	0	0	0	0	0	0	0
Pholis gunnellus	botervis	butterfish	0	20	33	11	18	0	0	0	10	0	20	10	20	22	20	0	11	13	13	0	13	10	0	0	11	0	0	0	0	0	0	0	0	13	11	0	0	0	0	0	0	10	0	0	0	0
Trisopterus luscus	steenbolk	bib	0	0	0	0	0	0	10	0	40	11	30	0	0	0	50	13	0	13	0	13	38	10	0	0	11	11	0	0	0	0	10	11	0	13	0	0	0	11	0	0	0	0	0	0	0	0
Alosa fallax	fint	twaite shad	0	10	22	0	0	10	0	27	10	0	0	0	40	0	0	0	11	0	0	13	0	0	0	14	0	0	0	0	10	0	0	0	11	0	0	50	0	0	0	0	14	10	20	0	0	0
Scophthalmus rhombus	griet	brill	0	0	0	0	9	10	0	0	10	0	0	0	0	0	0	0	0	0	13	0	0	0	13	0	0	22	11	0	0	10	20	0	0	0	0	0	0	22	0	33	0	10	10	27	0	0
Scophthalmus maximus	tarbot	turbot	0	0	0	0	0	0	0	0	0	0	0	10	0	0	10	0	0	0	0	0	0	0	0	0	0	33	0	0	0	0	0	0	11	0	11	0	0	0	13	17	0	0	10	9	0	30
Echiichthys vipera	kleine pieterman	lesser weever	0	0	0	11	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	22	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0
Eutrigla gurnardus	grauwe poon	grey gurnard	8	0	0	11	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gasterosteus aculeatus	3-d stekelbaars	3-sp stickleback	0	0	0	0	0	0	0	0	0	0	0	0	10	0	10	0	0	0	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trachurus trachurus	horsmakreel	horse mackerel	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	11	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	0
Dicentrarchus labrax	diklipharder	th. grey mullet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	25	0	0	0	0	0	0	0	0	0	0	0	0
Hyperoplus lanceolatus	smelt	greater sandeel	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	25	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arnoglossus laterna	schurftvis	scaldfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	10
Belone belone	geep	garfish	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Callionymus lyra	pitvis	dragonet	0	0	11	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lampetra fluviatilis	rivierprik	river lamprey	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Microstomus kitt	tongschar	lemon sole	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0
Petromyzon marinus	zeeprik	sea lamprey	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Enchelyopus cimbrius	4-dr meun	4-b rockling	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Entelurus aequoraeus	adderzeenaald	snake pipefish	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pollachius pollachius	pollak	pollock	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Before analysis we investigated the presence of the species by computing the proportion of zero hauls. For common species in all four areas, such as plaice and five-bearded rockling, we applied the same linear model as describe above. For species with a moderate proportion of zero hauls (sole, *gobies sp.,* flounder), we applied a delta-linear model, where a two-step modelling was involved. First, a glm model with binomial distribution was applied on the presence and absence of the species. Afterwards, a linear model was applied on the non-zero hauls. The year indices were calculated as the product of 1) probability of non-zero estimated per year from the first model and 2) the year coefficient from the second model. The confidence interval of the year indices were computed through a randomized test using a bootstrapping procedure. For more rare species (whiting, with many zero hauls), we applied a GLM only on presence/absence.

Number of species

Since species richness is defined as a count of species, it can be modelled as a Poisson distribution. Therefore, a GLM with Poisson response distribution and log-link was applied to fit richness with candidate predictors year, depth, water visibility and tide. The model was applied to DFS619, Boschgat (open), Spruit (open) and Schild (closed since 2006) separately. The optimal model was independently selected for each area according to minimum AIC (Akaike information criteria).

The final selected model can be symbolized as:

response ~ factor(year) (1)
response ~ factor(year) + depth (2)
response ~ factor(year) + water visibility (3)

Mean fish length

A mixed linear model was applied to fit length with candidate predictors year, depth, water visibility and tide. Haul was used as a random intercept in the model. The model was applied to DFS, Boschgat (open), Spruit (open) and Schild (closed since 2006) separately. The optimal model was independently selected for each area according to minimum AIC (Akaike information criteria). In all cases, we ended up with the same choices of the optimal model (1) as follows:

$$y_{ij} = \beta_{\text{year},k} \times year_{ij} + \beta_1 \times depth_{ij} + \beta_2 \times visibility_{ij} + a_i + \varepsilon_{ij}, \quad a_i \sim N(0, \sigma_a^2);\ \varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2), \qquad (1)$$

where y_{ij} indicates the length of fish j in the ith haul. Coefficients $\beta_{\text{year},k}$ refers to the coefficient of the correspondent year of the fish $(year_{ij} = k, k \in \text{year list in the data})$.

Note that in the length analysis, zero hauls do not provide length information and therefore the analysis is limited to the same five species (plaice, sole, gobies sp., five-bearded rockling, flounder).

Important: Because area-specific models were used for all analyses, the year indices plots can only be used to compare time trend between areas. It is not possible to use the year indicators to compare absolute values among areas.

5 Results

5.1 Fishing intensity

Based on *interpolated* VMS-data from 2006 onwards there were hardly any VMS-signals recorded in Boschwad and Schild (Figure 6) after closing the area. But also at the T0 (only data available of 2005) fishing intensity in the later closed areas Boschwad and Schild were lower than the open gullies Spruit and ZO-Lauwers. In the gullies Spruit and ZO-Lauwers the area around the sampling points (0.236 km^2) were on average fished once every year. Other gullies in the Dutch Wadden were fished more intensively, see Figure 5 showing fishing intensities in the year 2016 and the figures in Annex 1 for the years 2005 till 2016.

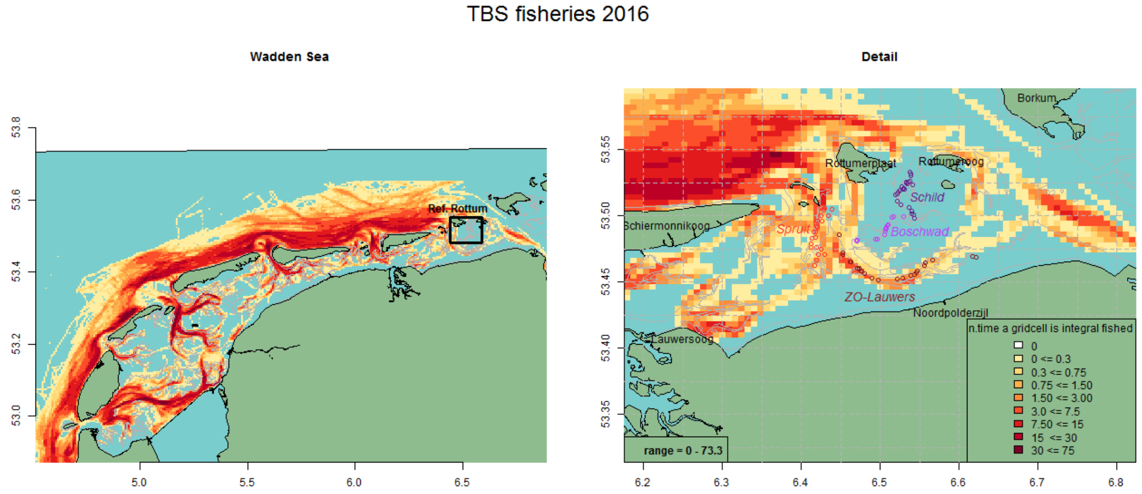


Figure 5. *Cummulated fishing intensity based on interpolated VMS-data of the year 2016 in the wadden sea (left) and detail of the Rottum area (right).*

There is considerable annual variation in fishing intensity. The fishing intensity in Spruit was lower in 2005-2008 compared with ZO-Lauwers in the same period. In 2011 and 2014 (Spruit and ZO-Lauwers) and in 2013 (ZO-Lauwers) fishing intensity was relative low. In recent years (2015 & 2016) intensities were high again. The fishing intensity in the open gullies was on average slightly higher at T2 (2012-2016, 0.230 km^2) as compared to T1 (2006-2011, 0.197 km^2).

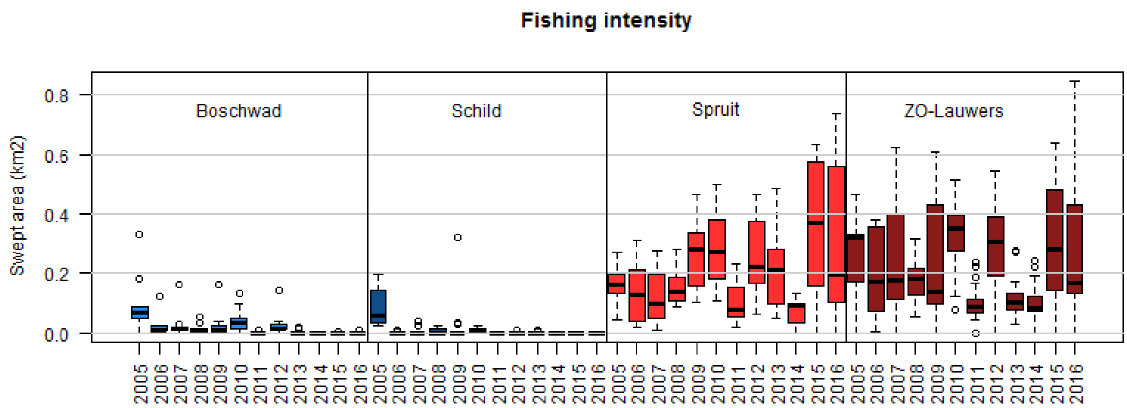


Figure 6*. Fishing intensity in an area (with a surface area of 0.236 km^2) around the Van Veen grab sampling locations in the four gullies for the year 2005 (before closure) and period after closure (2006 till 2016). Closed gullies (Boschwad and Schild) are depicted with blue bars, open gullies (Spruit and ZO-Lauwers) are depicted with red bars.*

Important note; *as additional pings were constructed by interpolation of a trajectory between two VMS-pings, it is possible that some, in this way artificially 'constructed', pings were located within the closed gullies. This is especially likely for Boschwad as ZO-Lauwers is curved below and around Boschwad (see Annex 1) making it possible that in the interpolation of two VMS pings, both located in ZO-lauwers, an interpolated trajectory of pings is constructed going through Boschwad. From 2014 onwards the time interval between VMS pings decreases, making the interpolation step more reliable. This can be seen in the figures displayed in Annex 1 as well.*

5.2 Sediment structure

In Figure 7 backscatter image of the gully Boschwad is shown and in Figure 8 the bathymetry images the gullies Boschwad, Schild, Spruit and ZO-Lauwers. In Annex 2 backscatter and bathymetry maps for the gullies Boschwad, Schild, ZO–Lauwers and Spruit can be found. The bathymetry maps show the height of the seabed while the backscatter image shows variations in hardness of the (top layer) of the seafloor. High backscatter intensities are associated with harder surfaces (indicated by red shaded areas) and low intensities with softer substrates (indicated by light grey areas). Biogenic structures, such as mussel- and oyster beds, result in a hard surface causing a high backscatter intensity but also hard materials such as gravel and shell debris. Such areas were found in the middle section of Boschwad, and in the outer sections of the meanders in the other gullies. No oysters and just a few mussels and some tube worms were found in the reference samples that were taken by Deep B.V. in these areas while shell debris was found frequently. It is therefore likely that these areas consisted mainly of beds consisting of empty shells. These areas largely overlap with areas containing large quantities of shell debris as detected in 2003 (Fey-Hofstede *et al.,* 2015).

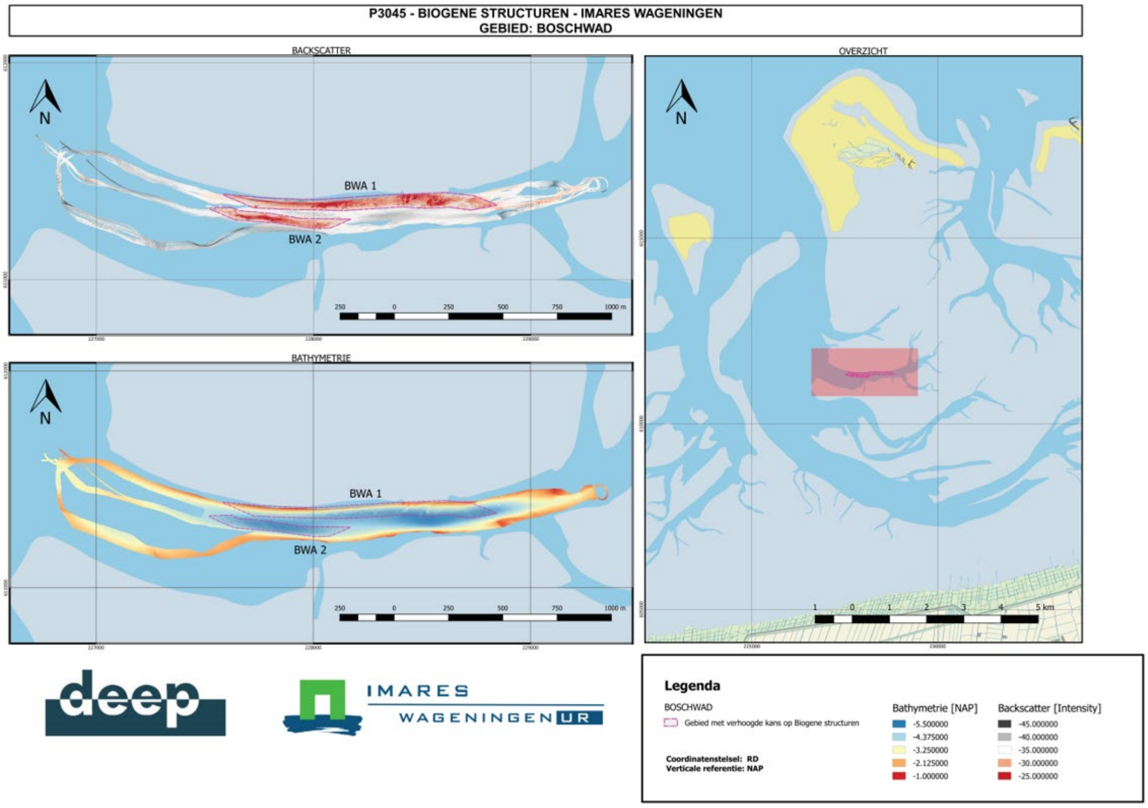


Figure 7. *Maps showing backscatter (top left) and bathymetry (bottom left) results of the closed gullie Boschwad.*

Bathymetry maps of the gullies show sand ripples, both in Schild and in Spruit at the start of the gully, see Figure 8. In ZO-Lauwers the start of the gully was not scanned but at the end section of the gully large variation in seafloor relief was detected. In Boschwad, sand ripples were much smaller and there was less variation in seafloor relief. High sand ripples can only develop in turbulent environments indicating the relative low (natural) disturbance due to wind and wave actions in Boschwad. This is consistent with the ecotope map made of the entire Dutch Wadden Sea in which Boschwad is classified as 'low dynamic' (Baptist *et al.*, 2016).

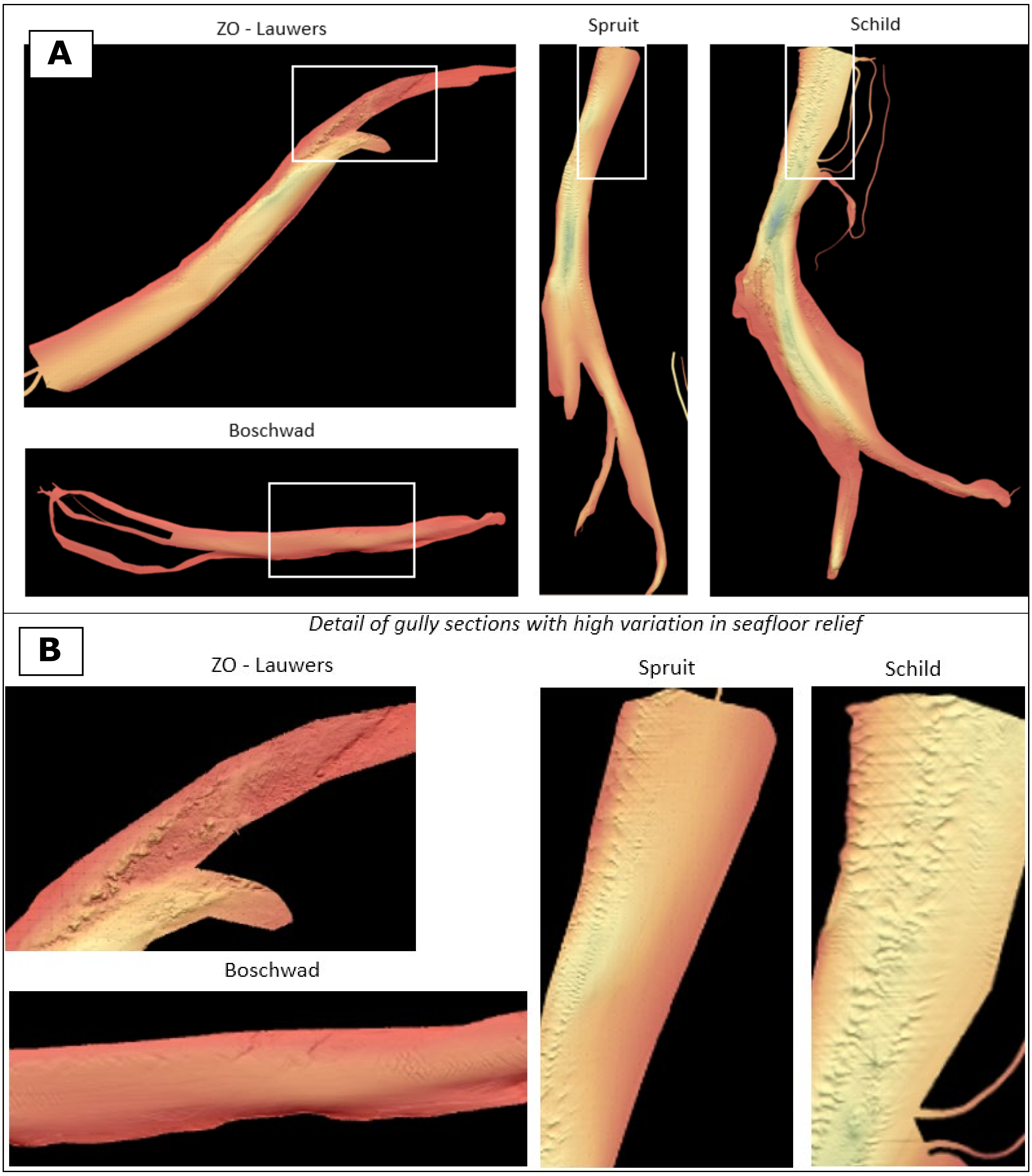


Figure 8*. Bathymetry maps of 2016 showing end section of ZO-Lauwers (open) and middle sections of Spruit (open), Boschwad (closed) and Schild (closed). In panel A bathymetry map of scanned areas in which the detailed sections shown in more detail in panel B are indicated by white rectangles. In Annex 2 more detailed maps are shown.*

A finer sediment structure and lower amount of shell debris in the van Veen grab samples illustrates the more sheltered environment in Boschwad, see Figure 9. Boschwad is also the shallowest gully of the four.

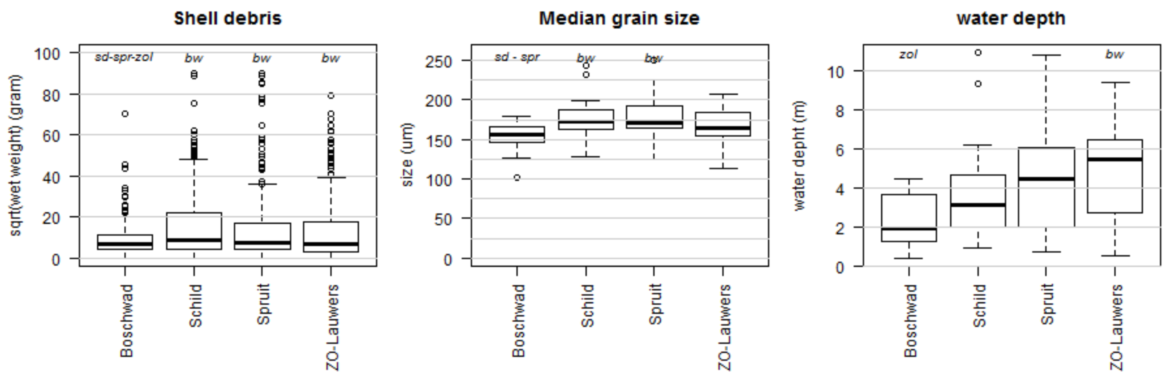


Figure 9. *Shell debris (left graph), median grain size (middle) and water depth (right) at the van Veen grab sample locations per gully.*

5.3 Benthos

5.3.1 Small benthos in Van Veen grab samples

In Figure 10 the development of shell debris, density, richness, species pool, diversity and evenness from T0 (2002, 2003 and 2005) to T1 (2006 until 2011) and from T0 to T2 (2012 until 2016) are shown.

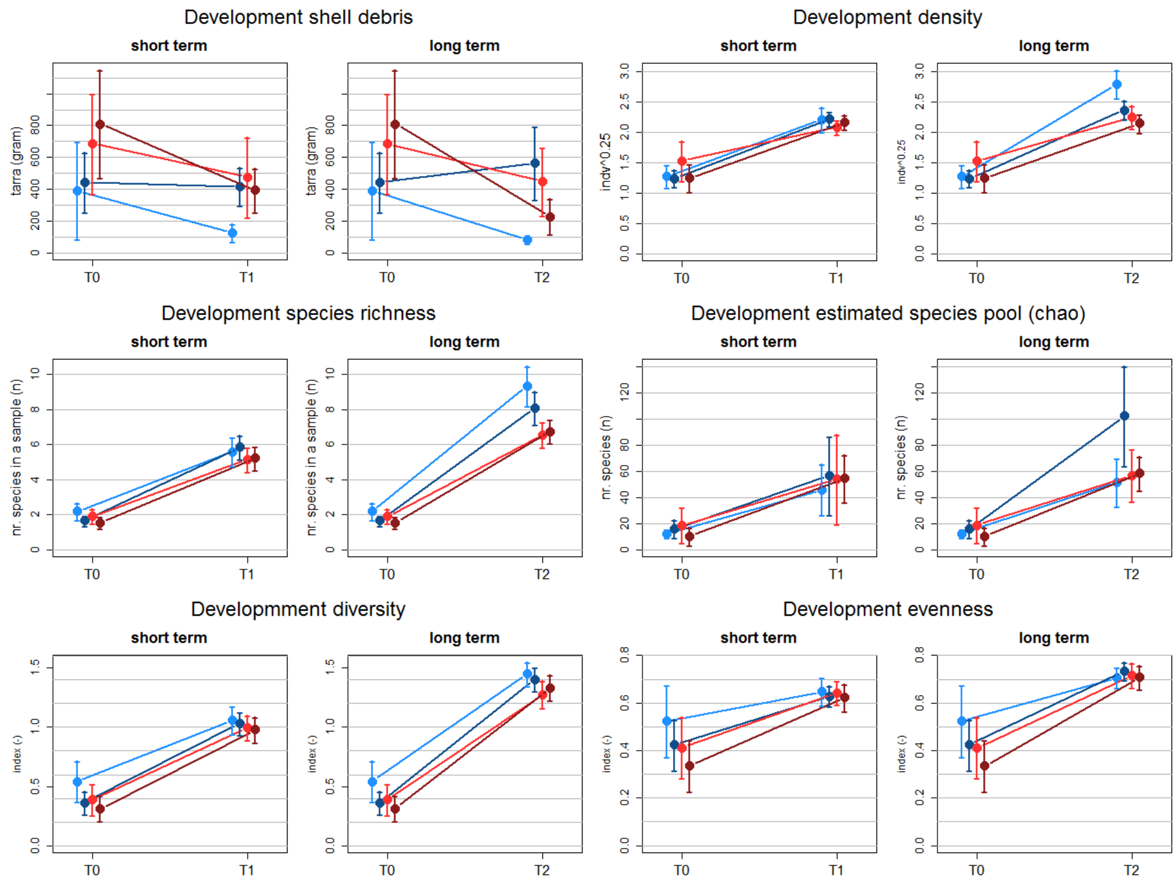


Figure 10. *Interaction plots showing the development of shell debris (top left), density (top right), sample species richness (middle left), estimated chao species pool (middle right), diversity (bottom left) and evenness (bottom right) in the van Veen grab samples for the four gullies Boschwad (closed, light blue), Schild (closed, dark blue), Spruit (open, light red), ZO-Lauwers (open, dark red). Error bars show the 95% confidence intervals.*

Shell debris in the Van Veen grab samples decreases over time for all gullies except for Schild. In Schild the average amount of shell debris stayed roughly the same in T1 while it increased in T2, see Figure 10. The Van Veen grab samples taken in Boschwad contained the lowest amount of shell debris on average. The development of shell debris in Boschwad is comparable with the open gullies and despite the different development observed in Schild, the overall development in shell debris is not significantly different between the open and closed gullies over the two periods (T0-T1 and T0-T2, Table 4).

Because after 2006 also smaller sized organisms (>1 mm) were identified and counted as well, species density, richness, species pool, diversity and evenness were higher in T1 & T2 compared with T0. Of the investigated parameters, only species richness (number of species in each sample) showed a significant different trend between the open and closed gullies, see Table 4. In the closed gullies the number of different species in a Van Veen grab samples increased more compared to the open gullies (difference=1.9) when compared with the open gullies going from T0 to T2, see Table 4, Figure 10 and Figure 11. As on average 7.5 species were encountered in one Van Veen grab at T2, this is a substantial difference.

The change in species richness from T0 to T1 and from T0 to T2 per sample location is shown in Figure 11. The circle size is relative to the change. Changes were overall larger between T0 and T2 than between T0 and T1. The largest increase in species richness was found at the eastside and shallower section of Boschwad and at the middle section of Schild. In ZO-Lauwers the largest changes were found at the end of the gully in the east while in Spruit the biggest change was seen at the entrance of the gully at the north side.

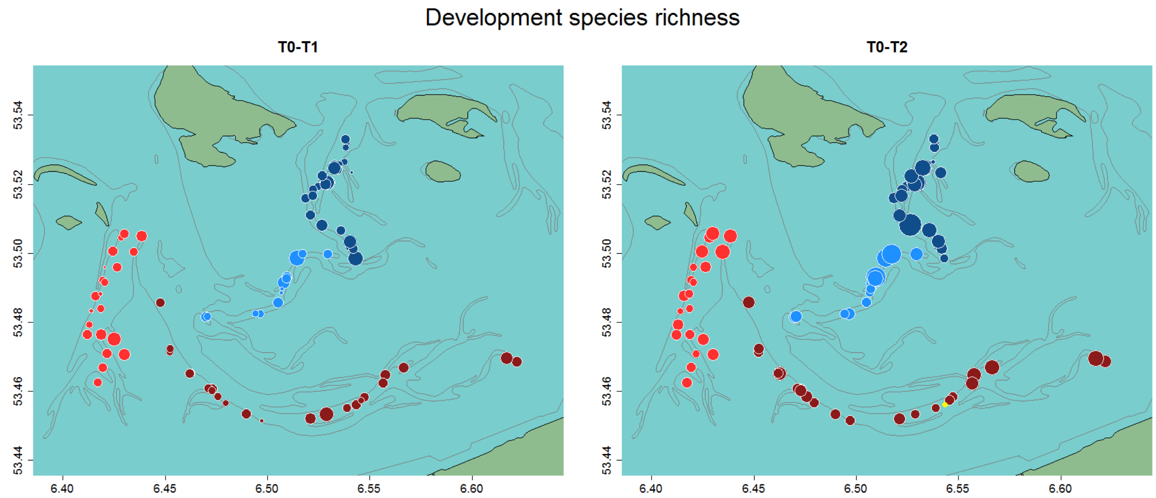


Figure 11. *Development of species richness from T0 to T1 and from T0 to T2. The size of the circles corresponds to the in- or decrease. Circles encircled by a yellow line (one sampling location in ZO-Lauwers) indicate a decrease in species richness. Light red circles represents samples taken in Spruit (open), dark red in ZO-Lauwers (open), light blue in Boschwad (closed) and dark blue in Schild (closed).*

Table 4. *Results linear mixed models for development (T0-T1 & T0-T2) of shell debris (grams), density (ind.^0.25/sample), richness (n), species pool (n), diversity (-) and evenness (-). In column 'difference' the mean difference in development (change in open minus change in closed) gullies is shown*

Parameter	T0 – T1			T0 – T2		
	T value	P value	difference	T value	P value	difference
Shell debris	-1.024	0.316	168.1	-1.917	0.069	316
Density[1]	-0.330	0.744	0.224	-1.794	0.087	0.515
Richness	-0.411	0.684	0.35	-2.612	***0.016****	1.9
Species pool	0.502	0.620	-2.45	-1.27	0.218	19.8
Diversity[1]	0.689	0.497	-0.045	-0.028	0.977	0.020
Eveness[1]	1.388	0.178	-0.094	1.244	0.227	-0.091

[1]Excluding Peringia ulvae.

The mean development of the species pool was not significantly different between the open and closed gullies from T0 – T2. The estimated species pool (Choa equation) at T2 was notable higher for Schild compared to the other gullies, while Boschwad had a lower than average species pool at T2. This is confirmed by the species accumulation graphs of each year (see Annex 3), that does not show a clear different development between open and closed gullies.

Apart from the community indices as described above, also the development of individual species within the community was analysed using multivariate statistics. The CCA diagram in Figure 12 shows the development of the benthic community over time for the four gullies. The variation in benthic community assemblage that could be explained by water depth and shell debris (combined accounted for 2.3% of the inertia (variation)), is compensated for and the inertia that is left is shown in the diagram. The first- and second axis together explain 17.7% of the inertia in species composition. The third axis (not shown) explains also a considerable amount of inertia (7.8%).

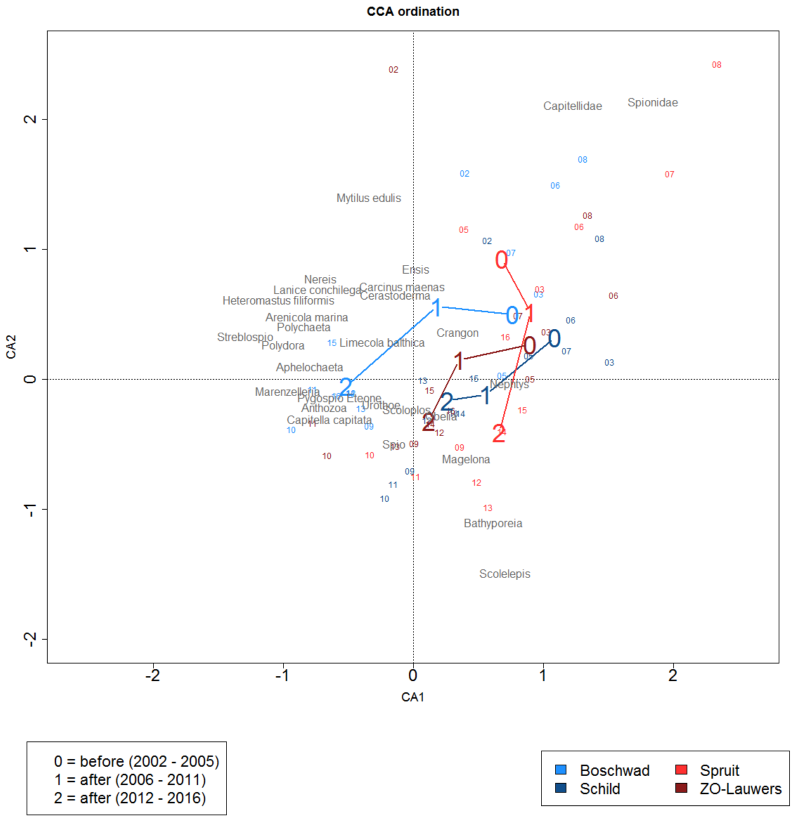


Figure 12. *CCA diagram result showing gully-year and gully-period centroids as well as the top 30 species with highest scores. Rare species, present in less than 3% of the samples were excluded. The first axis explains 9.0% and the second 8.6% of the variance. . Peringia ulvae was excluded as densities were occasionally so high that they dominated the analysis.*

The T0, T1 and T2 centroids move from the top right- to the bottom left side of the diagram showing the change in benthic community over time. At T1 smaller species (annelids for example) become important in the communities which is a result of a methodological difference; only after 2005 smaller species were identified and counted, see Section 4.3.2. Noticeable is also the position of Capitellidae and Spionidae in the diagram (families within the phyla annelid). These taxa are important from 2006 until 2008 while species within these families (such as *Capitella capitela, Marenzelleria sp., Polydora sp.*) become important after 2009. This is also a result of methodological difference; in more recent years species were identified to a higher taxonomical detail. A CCA ordination were Capitellidae and Spionidae taxa were aggregated resulted in a similar pattern as depicted in Figure 12, so differences between gullies were unaffected by this difference in taxonomical detail.

Regarding the species composition, all four gullies develop in a different way. Boschwad moves a bit further away to the left hand side and Spruit moves more in downward direction, along the second axis. Especially Boschwad moves towards the side of the diagram were several mollusc species are located, such as *Cerastoderma sp (cockle), Mytilus edulis* (blue mussel), *Ensis sp.* (jack knife clam) *(T1)* and *Limecola balthica* (baltic tellin). Also annelid species are apparently important members of the benthic community of Boschwad and seem to be of less importance in the other gullies, these include both complete buried species such as *Nereis sp.*, *Heteromastus filiformis*,*Capitella capitata* and but also *Lanice Conchilega* which partly erects out of the seafloor. Next to the annelids, Anthozoa (sea anemones) is located near the T2 centroid of Boschwad.

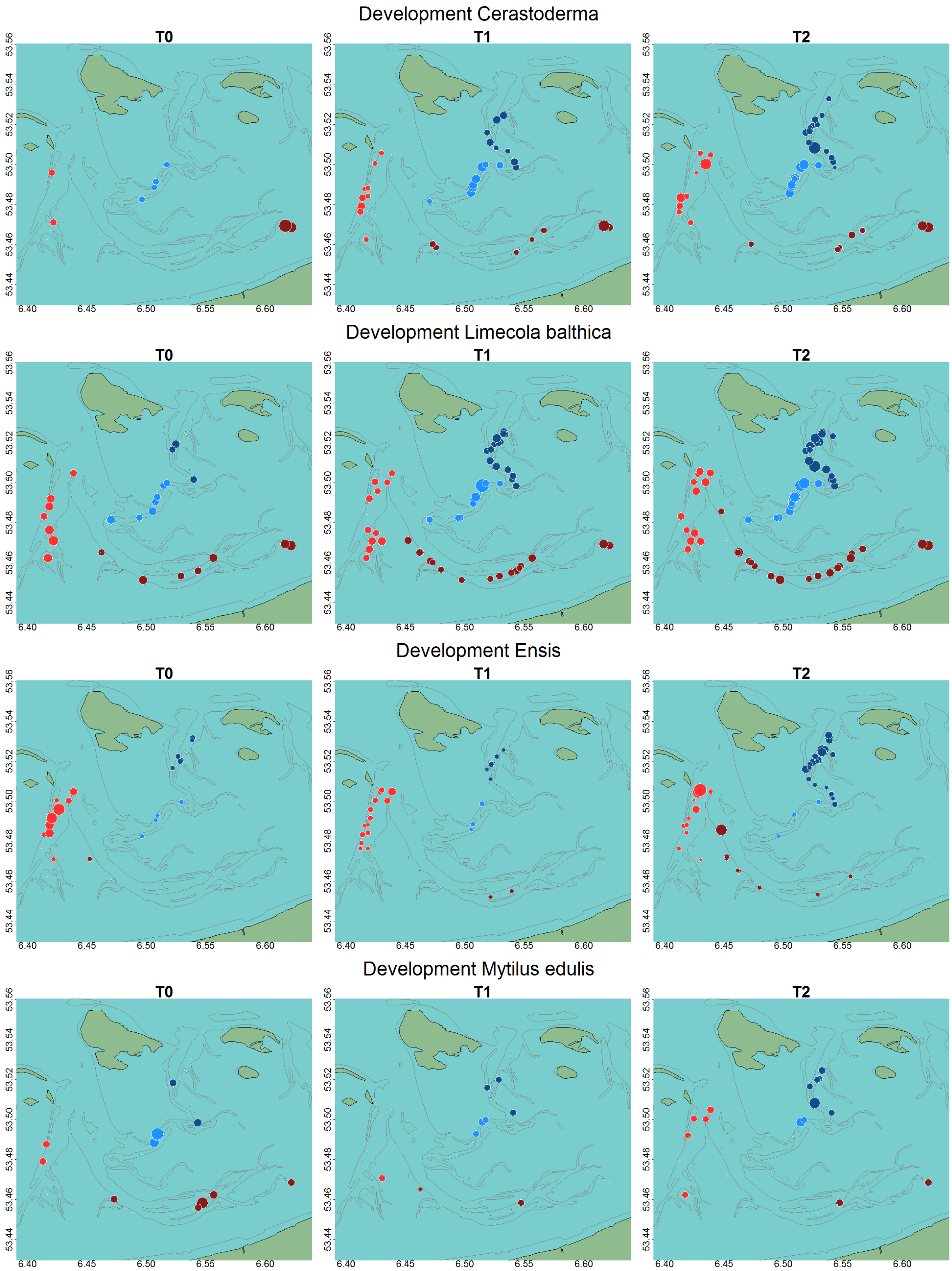
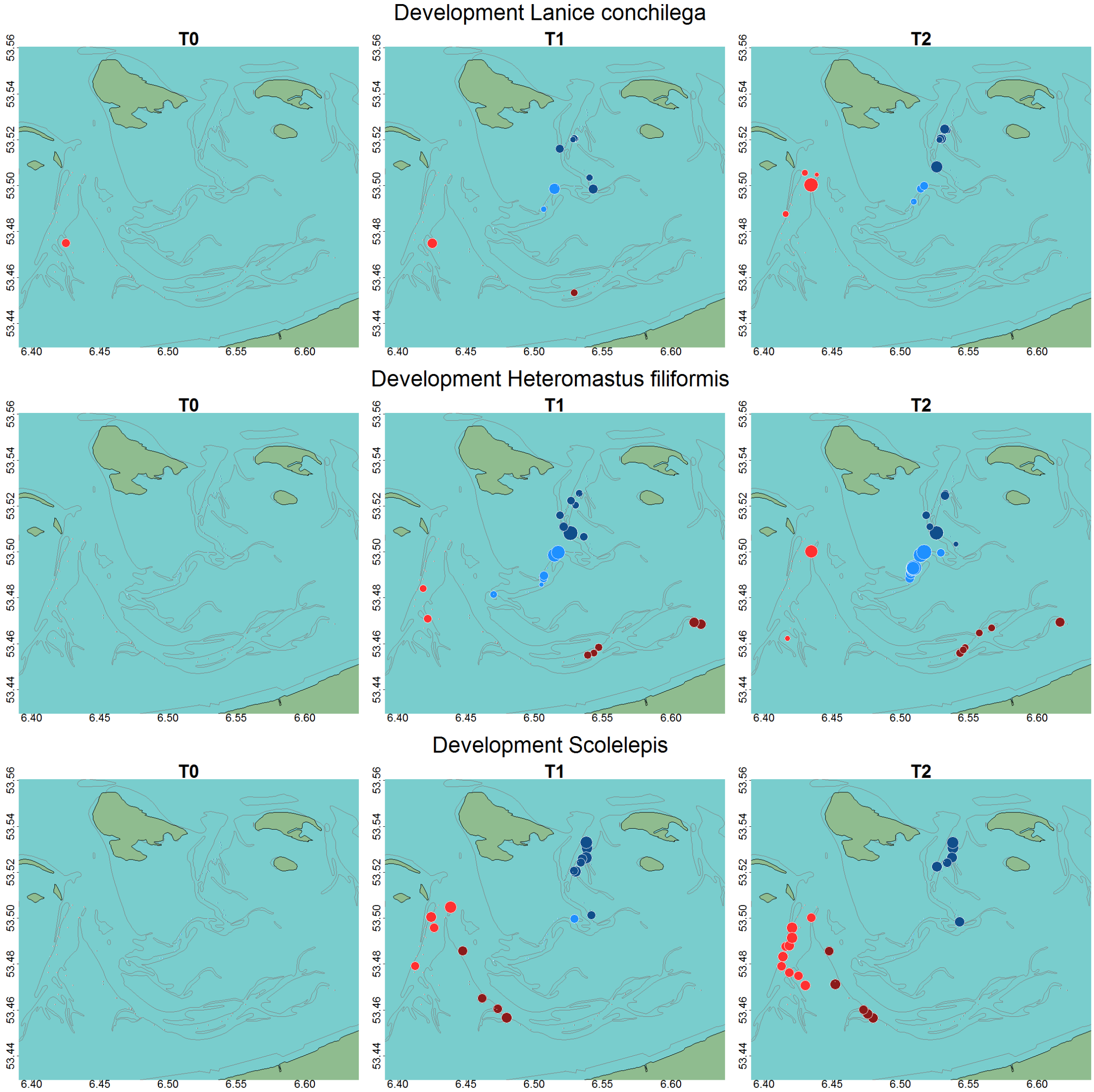


Figure 13*. Mean densities (double root transformed) and spatial distribution at T0, T1 and T2 of important mollusc species as identified in the CCA analyses.*

The T2 centroids of the closed gullies Spruit and ZO-Lauwers and Schild are positioned towards the bottom of the diagram, away from the mollusc species. Here several annelid species (*Spio sp., Magelona sp., Scolelepis sp.* and *Nephtys sp.*), arthropods (*Bathyporeia sp., Urothoe sp.*) and hydrozoans are positioned in the diagram and are important distinctive members of the communities in the open gullies.

Cerastoderma edule (cockles), *Limecola balthica* (baltic tellin), *Mytilus edulis* (mussel) and *Ensis sp.* (razor clam) were encountered in all gullies and at all time periods (Figure 13 and Annex 5). The frequency species were encountered (= number of samples containing the species), densities and spatial distribution differed per species, gully and time period. *Ensis sp.* concentrates at the entrance of the gullies while *Cerastoderma edule* were more often and in higher densities found in samples taken at the end of the gullies. Especially at T2 gullies differed. *Cerastoderma edule* were encountered relative often and in high densities in both of the closed gullies. *Ensis sp.* was often encountered in Schild, and at the entrance sections of ZO-Lauwers and Spruit, while nearly absent in Boschwad. See also Annex 5 were densities per phylum and gully are shown and Annex 6 with barplots showing species density and occurrence per gully. In 2013 (Boschwad) and 2013 and 2014 (Schild) one of the Van Veen grab sample contained dozens of individuals but in general mussels were not encountered in the samples and densities were generally low. It seems unlikely that there are currently (large) sublittoral mussel beds present in the area.



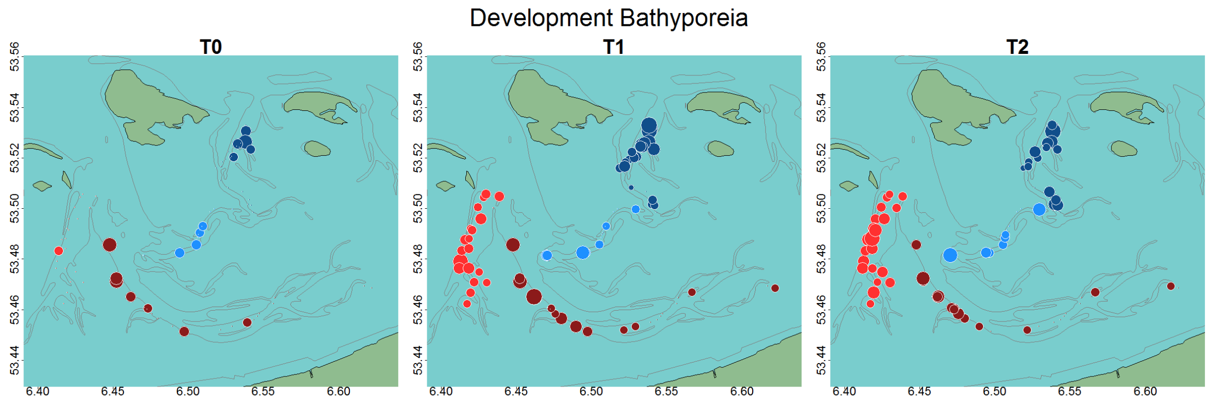


Figure 14. *Mean densities (double root transformed) and spatial distribution at T0, T1 and T2 of important Annelid and Arthropod species as identified in the CCA analyses.*

In Figure 14 taxa are shown that were most important defining the difference in the benthic communities between the gullies at T2 (located at the top left to bottom right diagonal in CCA diagram). *Lanice conchilega* was encountered more often in the samples taken in Boschwad and Schild (16 samples contained *Lanice sp.* in T2 period) with densities up to 375 individuals per m^2. In the open gullies *Lanice conchilega* were less frequently encountered (4 samples contained *Lanice sp.* in T2 period) and were only found in the entrance of Spruit. In one of those samples densities were high (664 ind./m^2). *Heteromastus filiformus* densities were relative high (at the end sections) of the closed gullies. *Scolelepis sp.* on the other hand, were found in all gullies except for Boschwad at T2.

5.3.2 Large benthos in suction dredge samples

In the suction dredge most of the encountered species belong to the phyla mollusca (10 species), followed by annelids and arthropods (each 6 species). Two cnidarian species were encountered, Hydrozoa (hydroidpolips) and Actiniaria (anemones) and one Echinodermata: *Asterias rubens* (starfish). Many samples contained the molluscs *Ensis sp.* (jack knife clam), *Cerastoderma edule* (cockle) and *Limecola (Macoma) balthica* (baltic tellin), see Figure 15. *Carcinus maenas* (crab) and *Crangon crangon* (shrimp) were the most frequently occurring (arthropods) species. From the annelids, *Nepthys sp.* was present in most of the samples while *Lanice conchilega* was found in the highest densities. In Annex 7 barplots showing species density and occurrence per gully can be found.

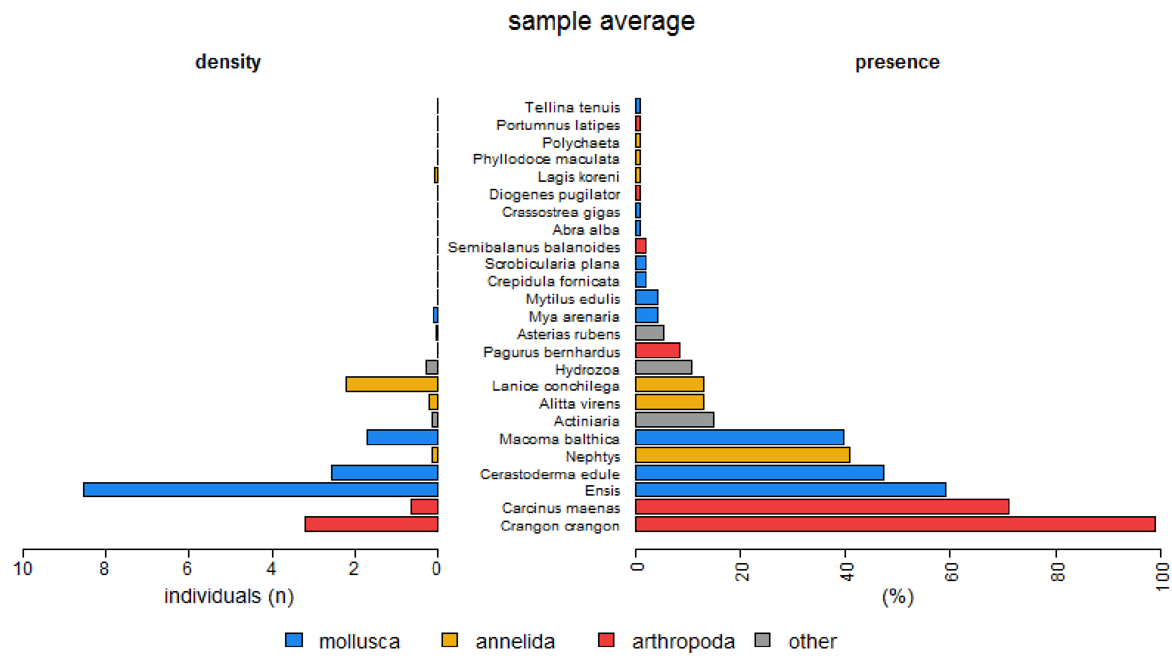


***Figure 15**. Sample mean density and frequency of occurance (presence in samples) for species found with the suction dredge in 2016.*

The number of different species (richness), number of individuals (density) and total biomass in the sample are depiceted per gully and sampling location in Figure 16. Within each gully five locations were sampled; at the entrance of the gully (position 1), in the middle (position 3), and at the end (position 5), a point in between the entrance and middle (position 2) and location in between the middle and the end of the gully (position 4).

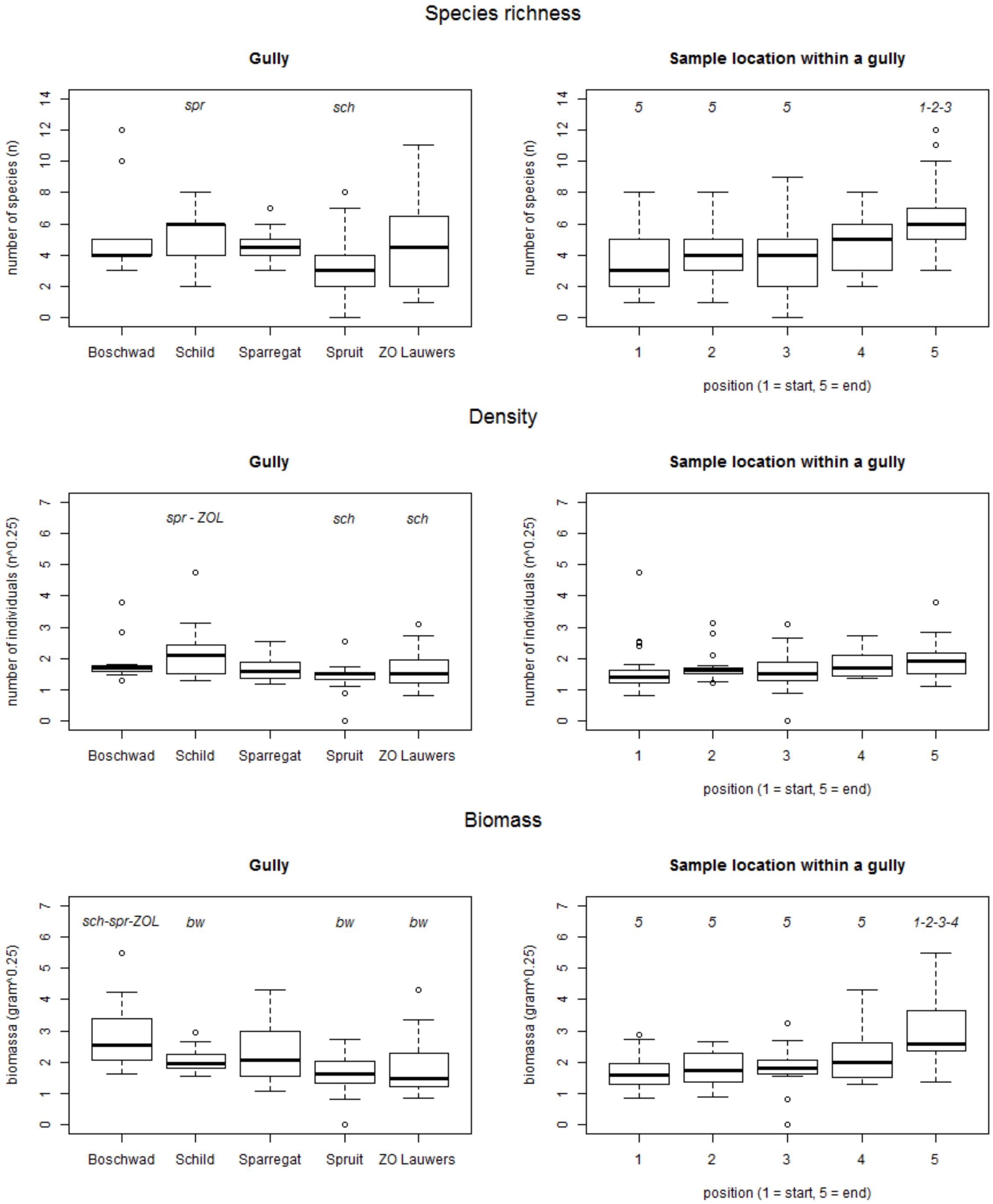


Figure 16. *Boxplots showing species richness (top), 4th root transformed density (middle) and 4th root transformed biomass (bottom) of the suction dredge samples taken in 2016 for the five gullies (left) and position within a gully (right). Significant differences (at the 0.05 level) between gullies is indicated in the figures by abbriviations of the gullies (boschwad=bw, schild=sch, sparregat = spa, spruit= spr, ZO-Lauwers=zol) and position of the gully.*

No significant differences in species densities were found between the different sampling locations within a gully. In the samples taken at the end of the gully (position 5) both biomass and species richness was significantly higher. On average the number of species was almost twice as high at position 5 (6.6 species) compared to position 1 (3.6 species) and the biomass (in grams) was more than ten times as high. As throughout the gully sections densities were equal while biomass was higher at the end of the gullies, the community at the end of the gullies must contain more larger (heaver) individuals.

Some differences between the gullies were found. Species richness was higher in Schild as compared to Spruit and densities were higher in Schild compared to both Spruit and ZO-Lauwers.

In Figure 17 the ordination diagram is shown. Centroids of the open gullies are positioned closer to each other compared to the centroids of the closed gullies which radiate out, each in a different direction. This indicates that there are differences in the communities of the closed gullies Schild and Boschwad.

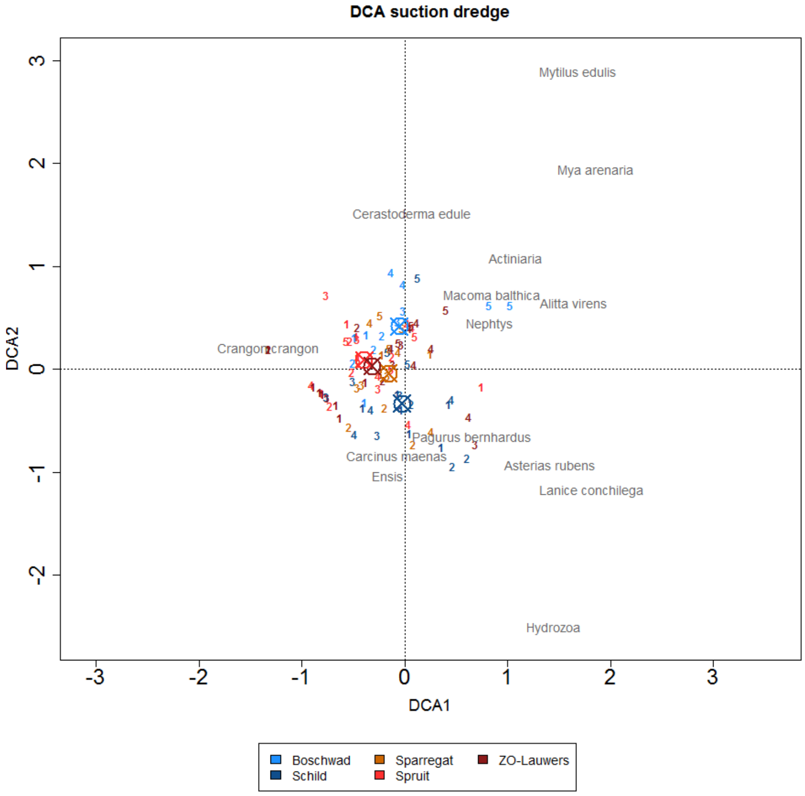


Figure 17. DCA diagram showing the benthic community as sampled with the suction dredge in 2016. Species abundance data were square root transformed and rare species that were present in less than 4% of the samples were not included in the analysis. The sampling positions within a gully are depicted by the numbers one until five and gully centroids are depicted with circles containing a cross.

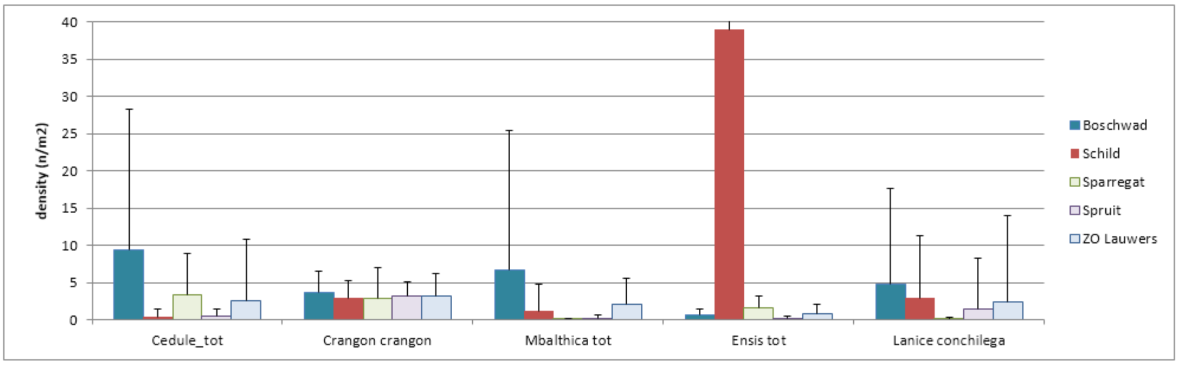


Figure 18. *Densities of five most occuring species in suction dredge; Cerastoderma edule (Cedule_tot), Crangon crangon, Macoma balthica (Mbalthica_tot), Ensis sp. and Lanice conchilega as encountered in suction dredge samples taken in 2016. Error bars represent one standard deviation.*

In Boschwad, the benthic community in the samples taken at locations towards the end of the gully (positions 4 & 5), are particular distinct while samples taken at the entrance of the gully are more similar to the species community found in Spruit, ZO-Lauwers and Sparregat, see Figure 17. At the end of Boschwad some mollusc species were encountered in high densities such as *Cerastoderma edule* (cockles) and Limecola (*Macoma) balthica* (baltic tellin), see Figure 17, Figure 18 and Annex 4. Cockle densities were significantly higher in Boschwad compared to Schild, Spruit and ZO-Lauwers. The community composition at Schild is characterised by abundant jack knife clam (*Ensis sp.*) with significant higher densities compared to the other gullies. No significant differences were found between the gullies for *Crancon crangon* (shrimp), *Macoma balthica* and *Lanice conchilega.*

We analysed the proportion of the different age classes because we expected that closure of a gully may affect the age structure of molluscs. In the gullies with high *Cerastoderma edule* densities (Boschwad, Sparregat and ZO-Lauwers) the age structure was different. In Boschwad and Spruit the population consisted mainly of >2 year old individuals (>96%) while in the ZO-Lauwers the one- (17%) and two year old (24%) classes are important as well, see Figure 19. The population of Limecola (*Macoma) balthica* in Boschwad consisted for a relative large part of larger sized (>15 mm) individuals while in Schild and ZO-Lauwers most individuals were small sized (<15 mm). In the other gullies very few individuals were found.

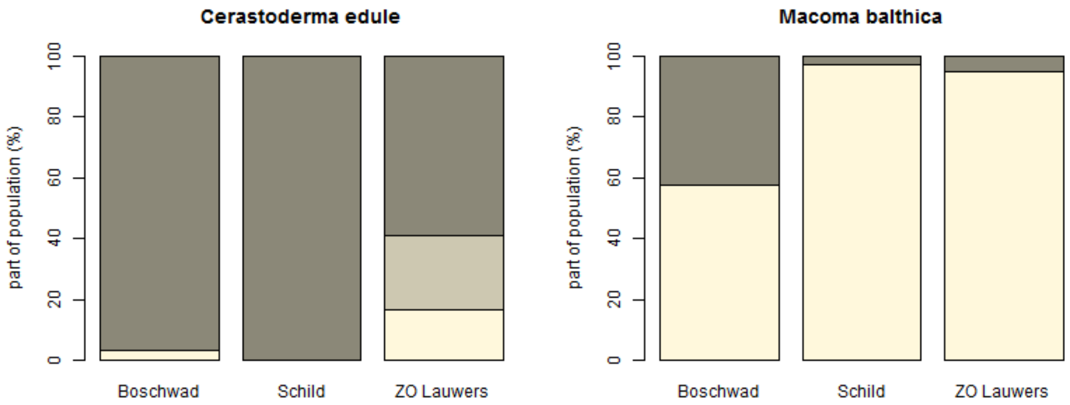


Figure 19. *Population structure of Cerastoderma edule (left) and Macoma balthica (right) in the gullies that contained high densities (Boschwad and Schild-closed and ZO Lauwers-open). Cerastoderma edule are divided by age; yellow - one year old, light grey - 2 years old and dark grey >2 years old. Macoma balthica are devided by length; light yellow small sized (<15mm) and dark grey large sized (>15mm).*

Particular high densities of *Ensis sp.* were found in Schild that consisted for a large part (72%) of small sized (<16 mm) individuals indicating a spatfall of *Ensis sp.* in the area, see Figure 20. In the other gullies the larger sized (>16 mm) individuals were dominant (between 76 – 89 %).

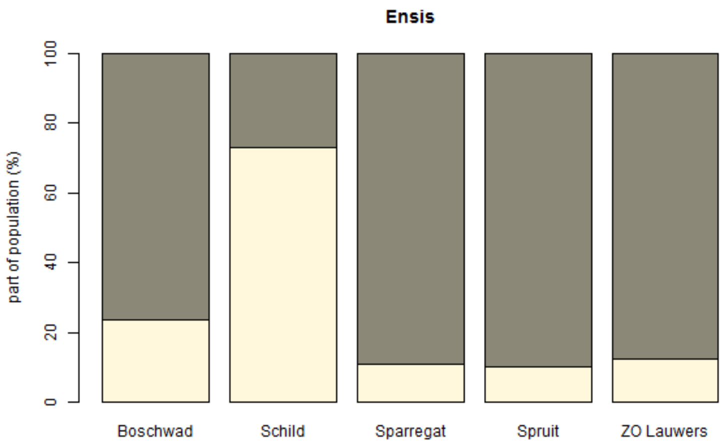


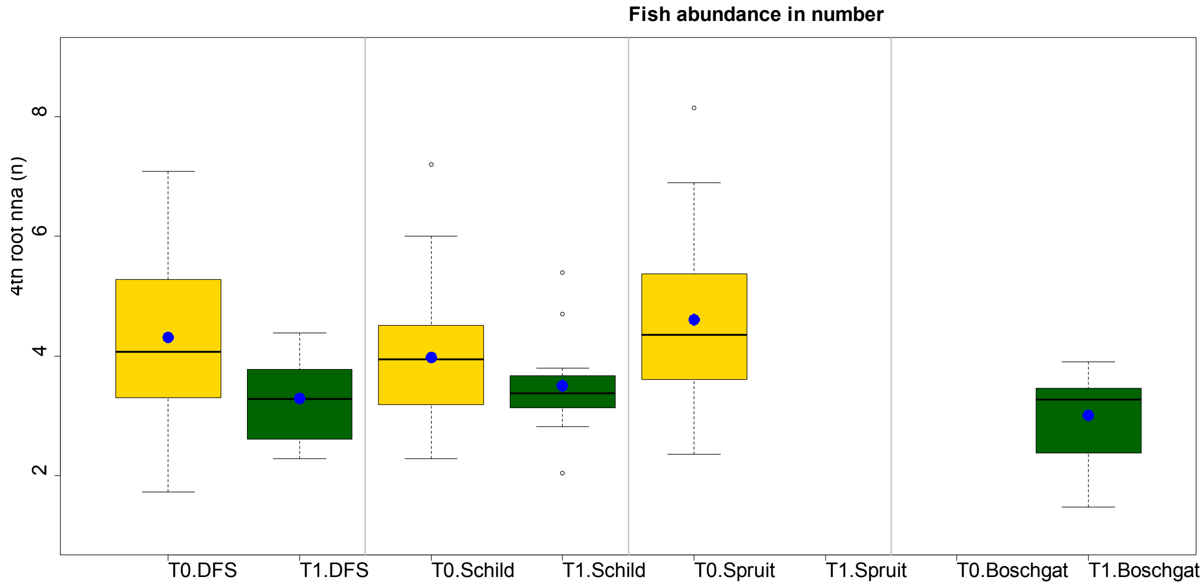
Figure 20*. Population structure of Ensis sp. individuals were dived by size; yellow – small sized (< 16mm), light grey – larger sized (>16 mm).*

5.4 Fish

5.4.1 Total fish abundance and abundance of marine juveniles and residents

The DFS enables to provide a longer time frame than only the development within the specific gullies around Rottum. Throughout all analyses (all figures in this and following paragraphs), it is clear that the confidence intervals are smallest for the DFS, which is related to a larger sample size. The DFS provides the general trend in the entire area, and can be used for interpretation of the results from the sampling in the open (Spruit / Boschgat) and closed gully (Schild)

Total abundance and abundance of marine juveniles was generally higher in T0 and T1 in Spruit and Boschgat than in Schild and DFS619 (Figure 21 and Figure 24). Abundance of estuarine residents was similar in all areas (Figure 24). In Spruit and Boschgat abundance in biomass was higher in T0 and T1 than in Schild and DFS619 (Figure 21).



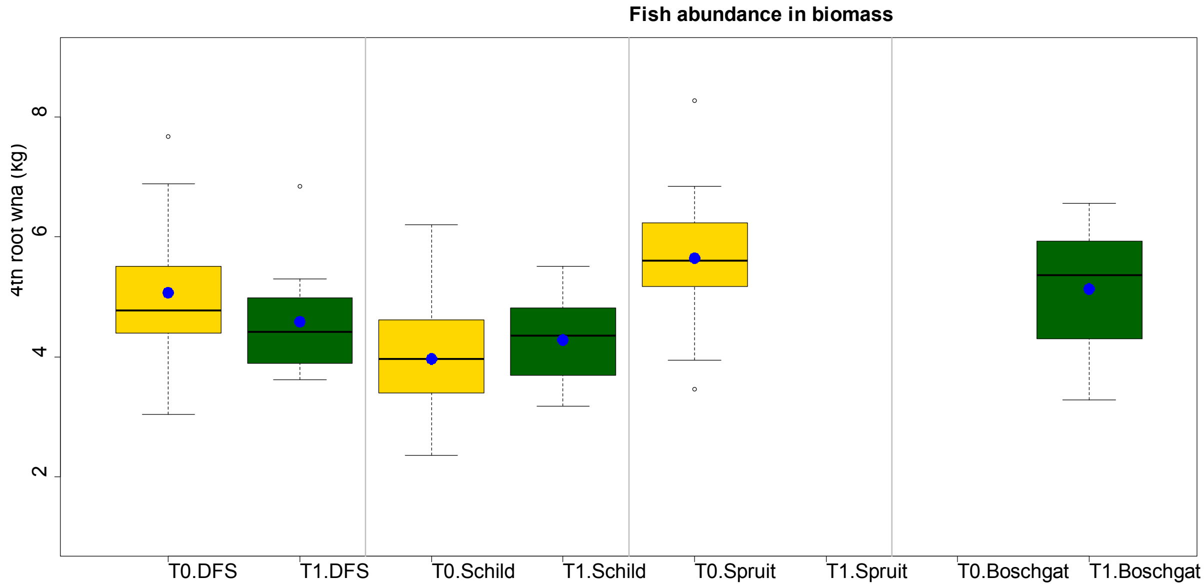


Figure 21. *Boxplots of total fish abundance (n/ha 4th root transformed) and fish biomass in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

Overall, the total abundance and the abundance of estuarine residents (Figure 22, Figure 23 and Figure 25) showed a similar trend for DFS: slowly decreasing since the early 80s (apart from a large increase at the beginning of 1990), with a stable trend from 2000 onwards. Both open areas (Spruit and Boschgat) showed good agreement with the yearly fluctuations of DFS. The large confidence bands in all areas imply that yearly fluctuations are random. With only very few and discontinued follow up years in Schild, it is not possible to draw conclusions on its time trend apart from the observation that 2016 is not markedly different with the situation at T0.

The abundance in biomass showed very little change since the early 1980s in DFS619 (Figure 23).

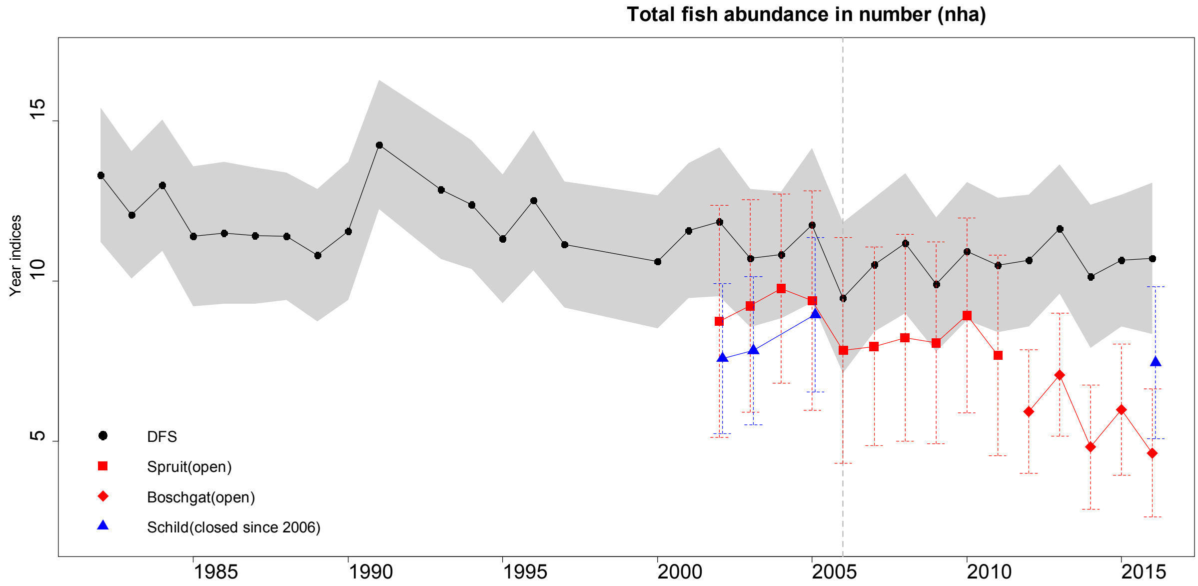


***Figure 22**. Estimated year indices and their 95% confidence intervals for total fish abundance in number for DFS (model 3), Spruit (model 3), Boschgat (model 4) and Schild (model 4). Because area-specific models were used, the year indices plots can only be used to compare time trend between areas, rather than directly using the absolute value as an indicator of response and compare them among areas.*

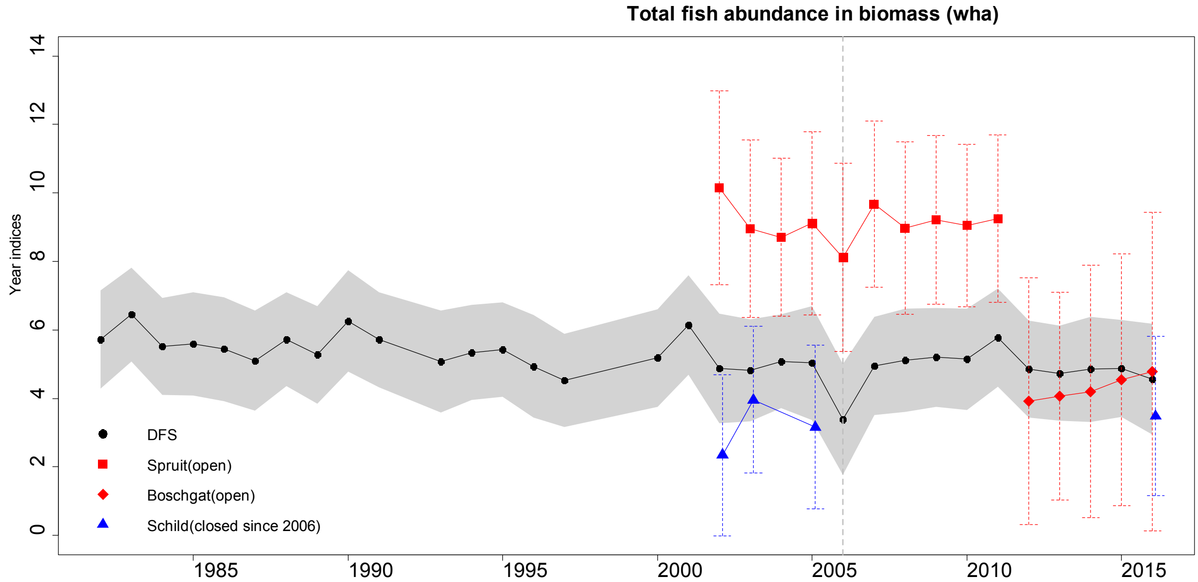


***Figure 23.** Estimated year indices and their 95% confidence intervals for total fish abundance in biomass for DFS (model 3), Spruit (model 3), Boschgat (model 4) and Schild (model 4). Because area-specific models were used, the year indices plots can only be used to compare time trend between areas, rather than directly using the absolute value as an indicator of response and compare them among areas.*

In DFS619, the abundance of marine juveniles showed a slowly decreasing trend since the early 80s, and the year indices became flat since 2000 (Figure 25). Also here the development in Spruit and Boschgat was in good agreement with that in DFS619, while the data points in Schild were too few and discontinuous to allow any conclusions apart from the observation that 2016 is not markedly different with the situation at T0.

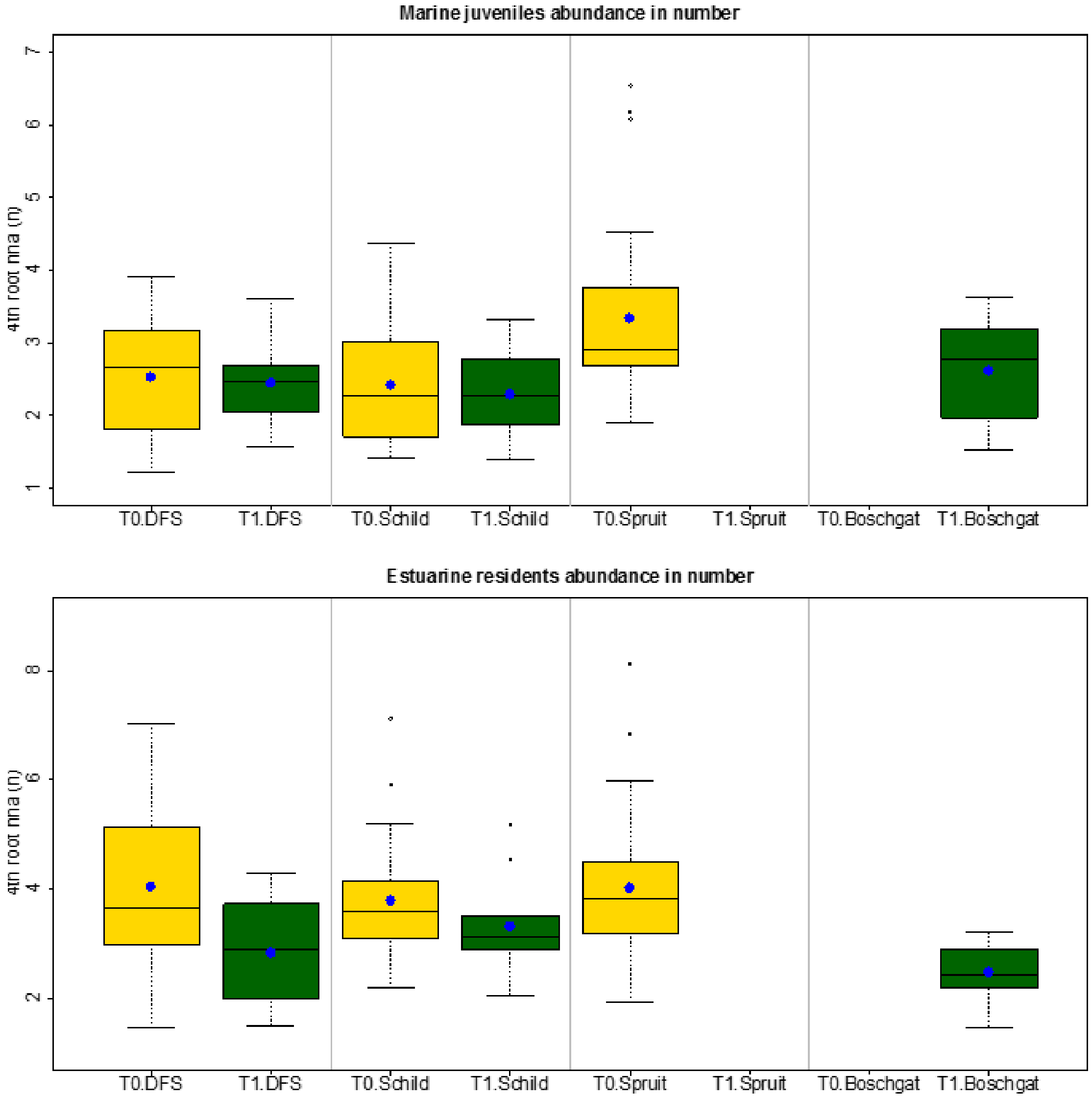


Figure 24. *Boxplots of abundance (n/ha 4th root transformed) of marine juveniles and estuarine residents in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

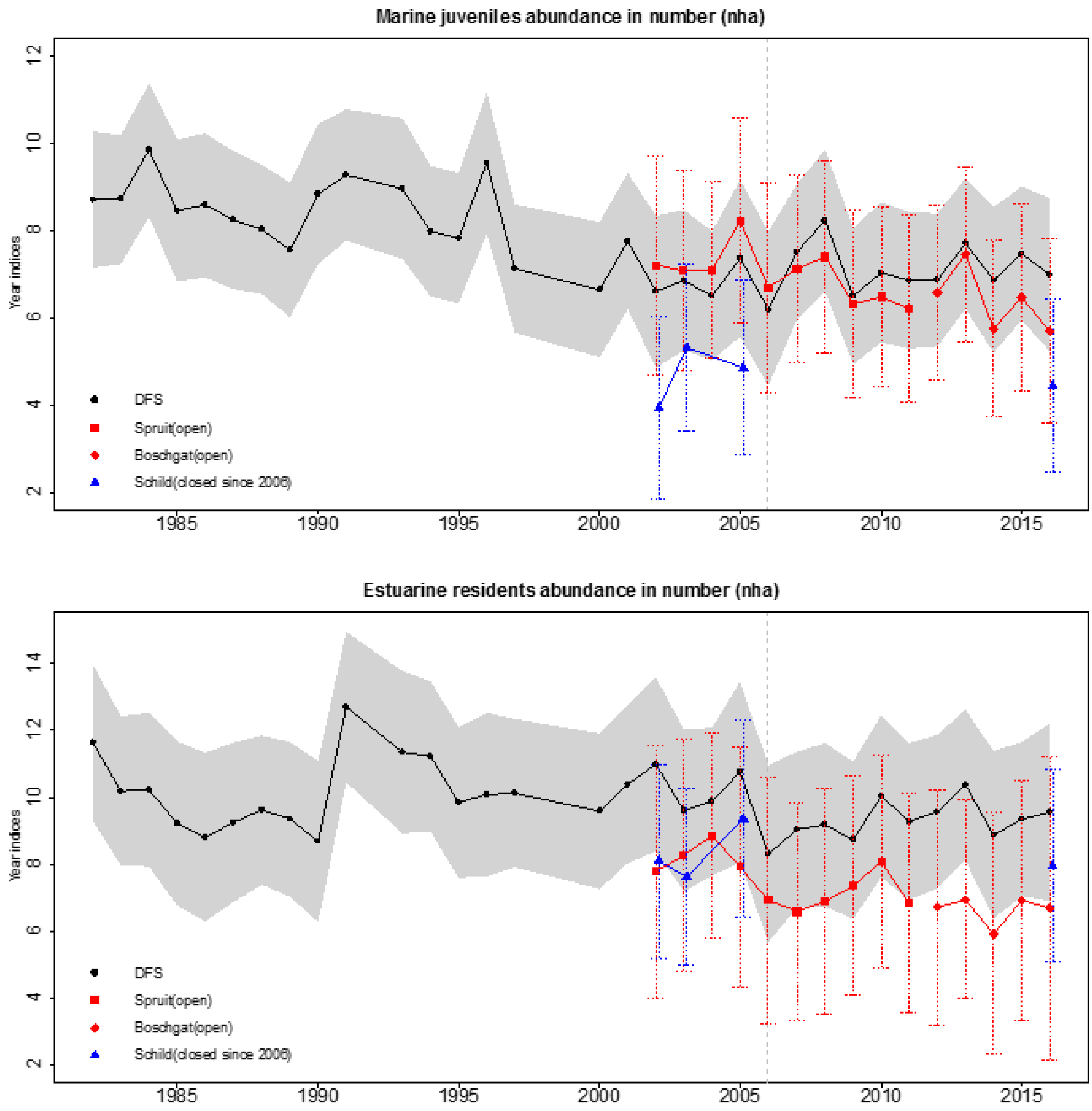


Figure 25. *Estimated year indices of abundance in number and their 95% confidence intervals for marine juvenile species: for DFS (model 3), Spruit (model 3), Boschgat (model 3) and Schild (model 3) and for resident species: DFS (model 3), Spruit (model 3), Boschgat (model 4) and Schild (model 4). Because area-specific models were used, the year indices plots can only be used to compare time trend between areas, rather than directly using the absolute value as an indicator of response and compare them among areas.*

5.4.2 Abundance per species

In general abundance of all species tended to be higher in Spruit compared to all other areas (Figure 26, Figure 28, Figure 30, Figure 32 and Figure 34).

Also in the developments of individual species (Figure 27, Figure 29, Figure 31, Figure 33 and Figure 35), the data points in Schild were too few and discontinuous to allow any conclusions. However none of the species showed a markedly different abundance in Schild T1 (2016) compared with the T0 (2002 – 2005). The development in the open areas (Spruit and Boschgat) of plaice and five-bearded rockling closely follow the development in the DFS area (Figure 27 and Figure 31).

Species characterised by more zero observations included sole, gobies sp. and flounder. The two-step models resulted in trends as given in Figure 29, Figure 33 and Figure 35. These species generally follow the same development in the open areas compared to the whole DFS area. E.g. peaks observed in sole in 2005 and 2013 and in flounder in 2005, 2008 and 2010 are seen in the open areas as well as the DFS area. At the beginning of the series, sole showed very strong fluctuations, where certain years with peaks are followed by a few years of decline. This pattern seems to be weakened in the more recent period.

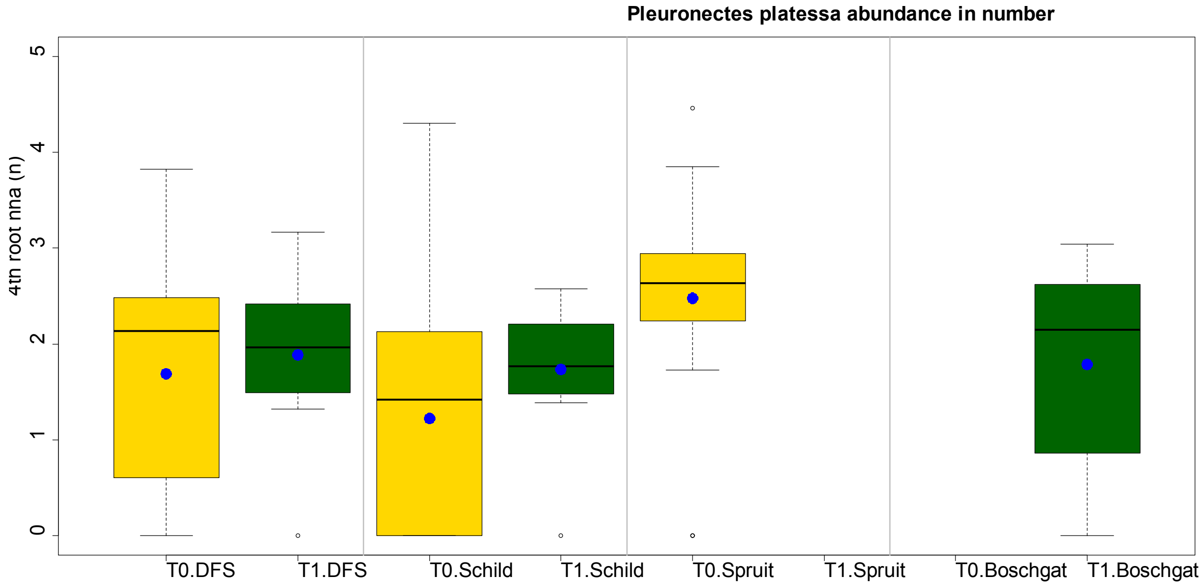


Figure 26*. Boxplots of abundance (n/ha 4th root transformed) of plaice and estuarine residents in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

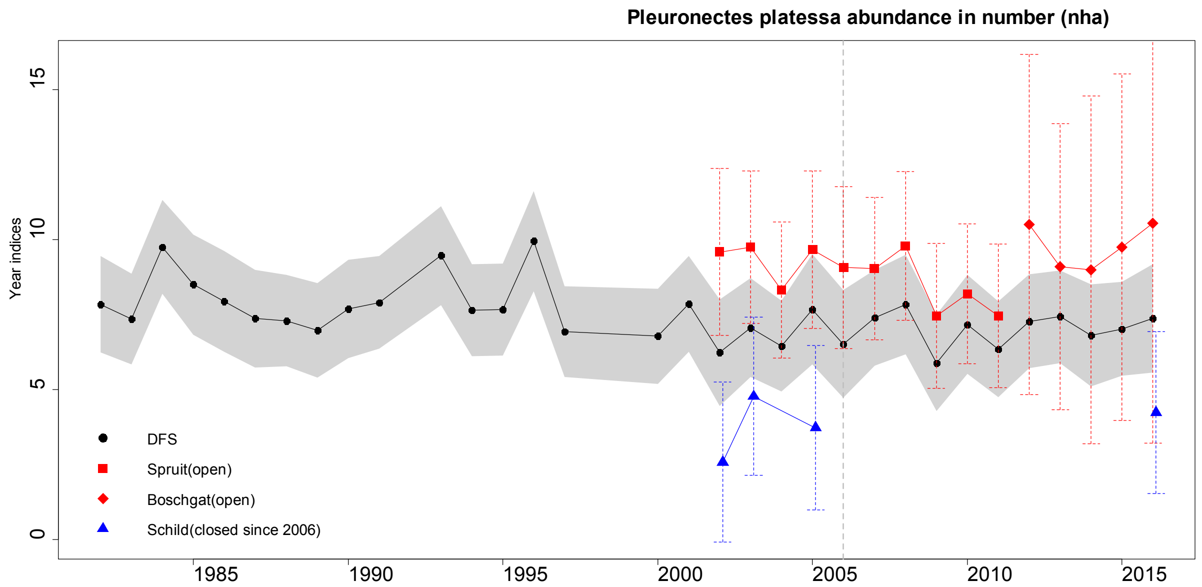


Figure 27*. Estimated year indices and their 95% confidence intervals for Pleuronectes platessa (plaice) abundance in number for DFS (model 3), Spruit (model 3), Boschgat (model 3) and Schild (model 1).*

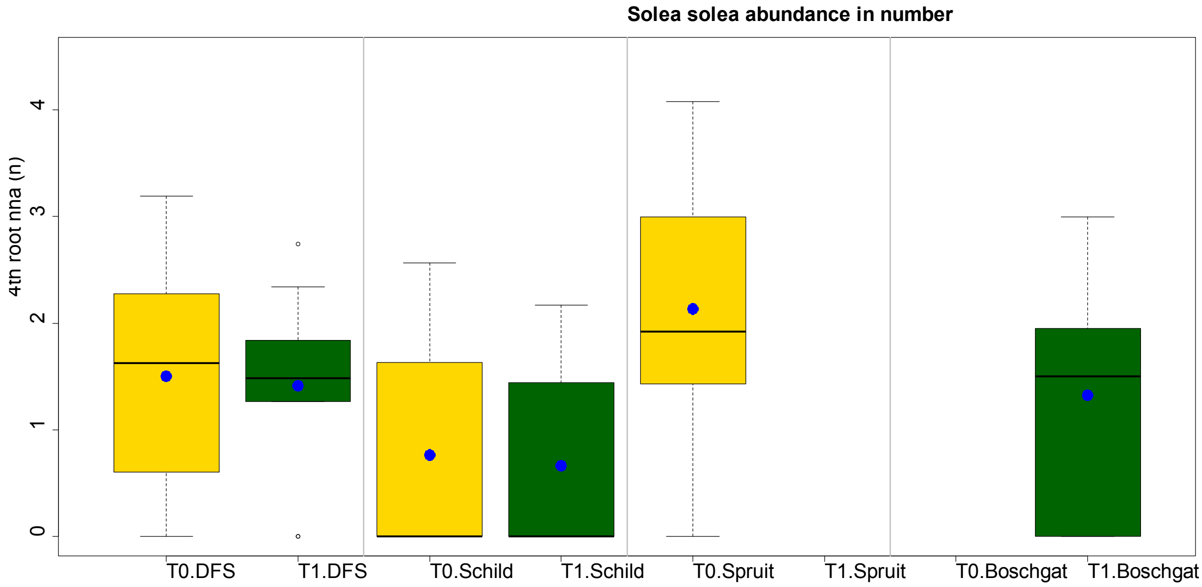


__Figure 28.__ Boxplots of abundance (n/ha 4th root transformed) of Solea solea (sole) in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).

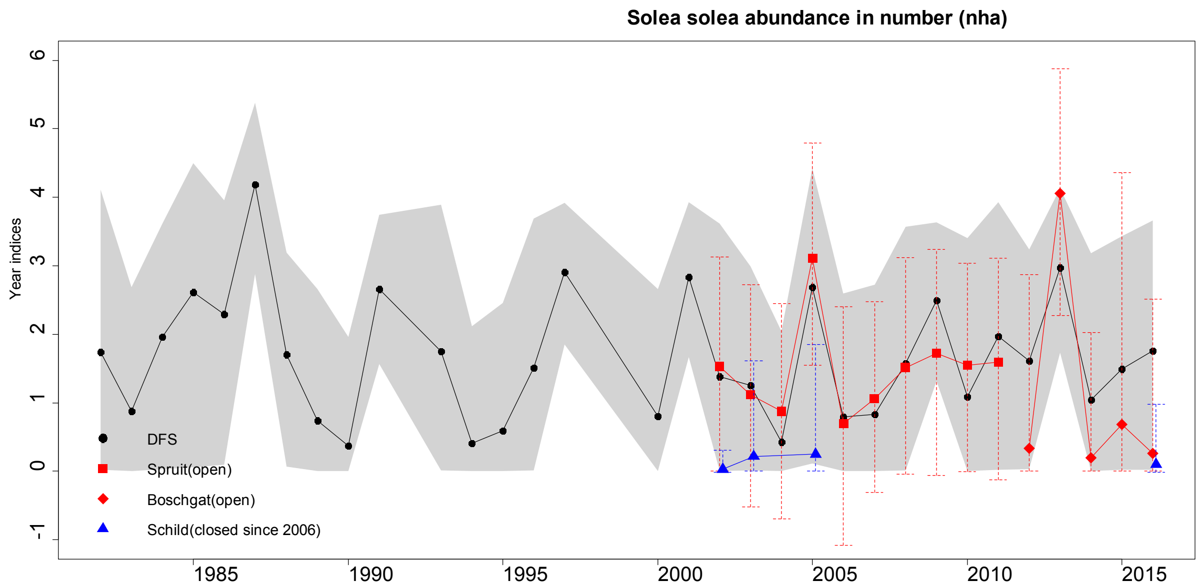


__Figure 29.__ Estimated year indices and its 95% confidence intervals for Solea solea (sole) abundance in number for DFS (part 1: model 3, part 2: model 3), Spruit (part 1: model 3; part2: model 1), Boschgat (part1: model 1; part2: model 4) and Schild (part1: model 1; part2: model 4).

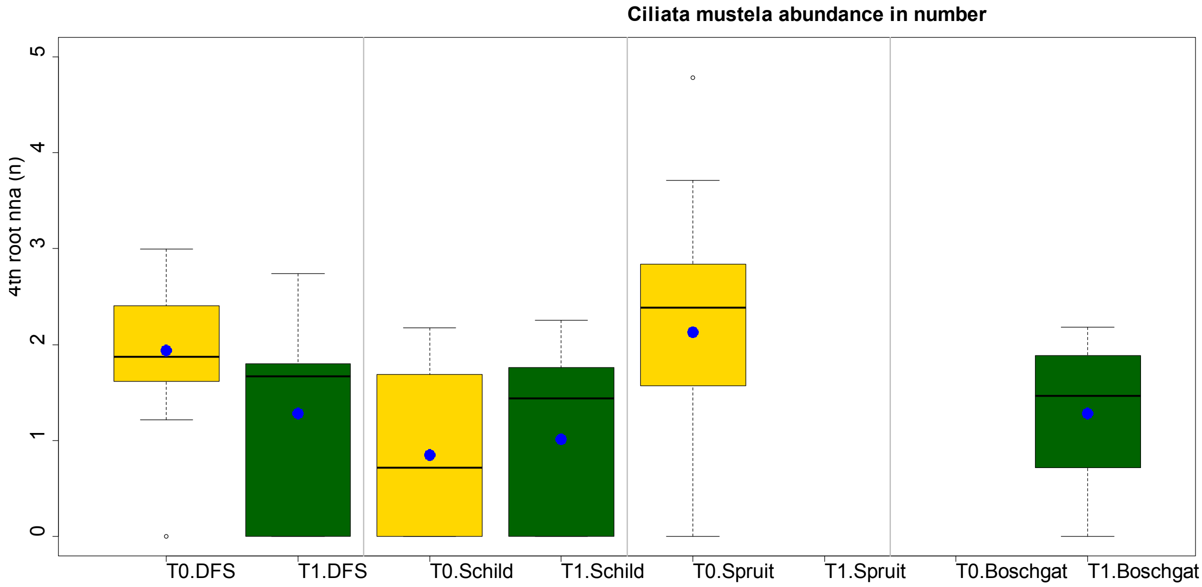


***Figure 30**. Boxplots of abundance (n/ha 4th root transformed) of Ciliata mustela (five-bearded rockling) in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

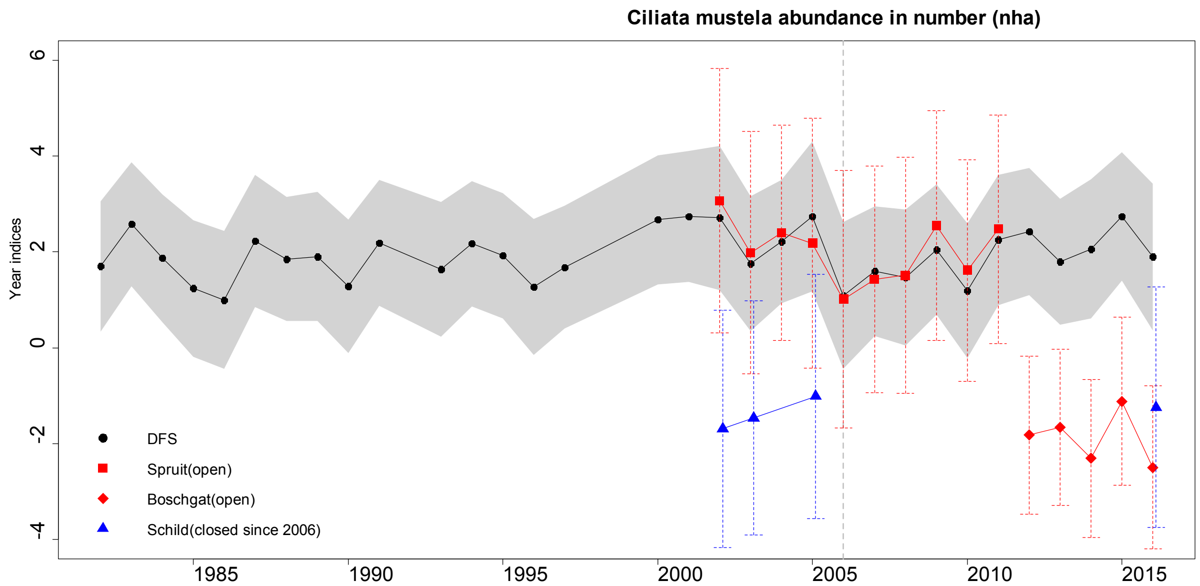


***Figure 31.** Estimated year indices and its 95% confidence intervals for Ciliata mustela (five-bearded rockling) abundance in number for DFS (model 3), Spruit (model 3), Boschgat (model 1) and Schild (model 1).*

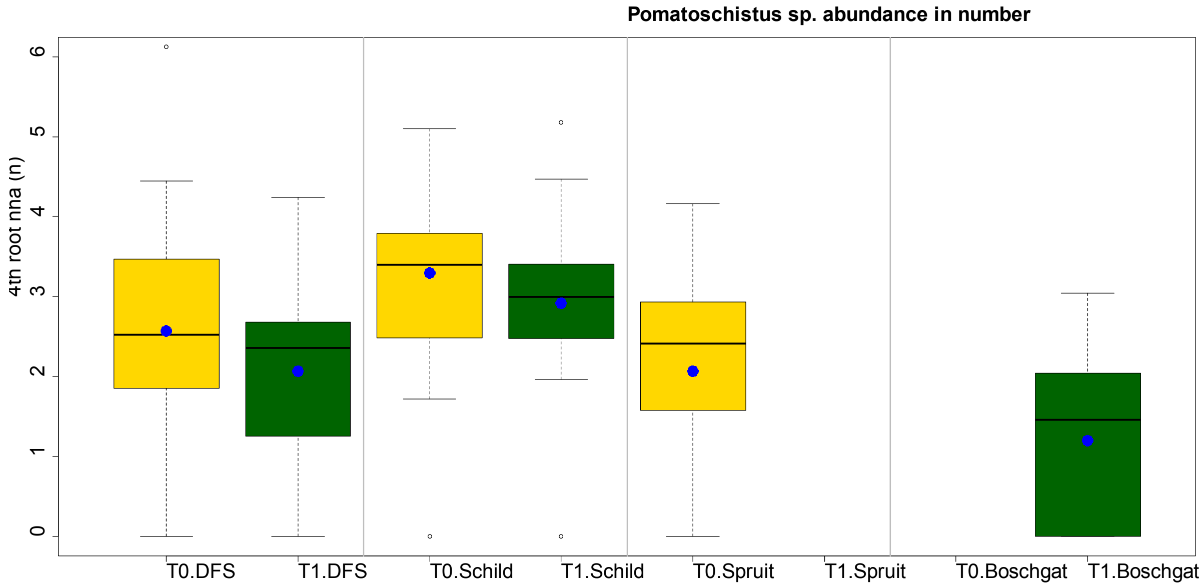


Figure 32. *Boxplots of abundance (n/ha 4th root transformed) of Pomatoschistus sp. (gobies sp.) in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

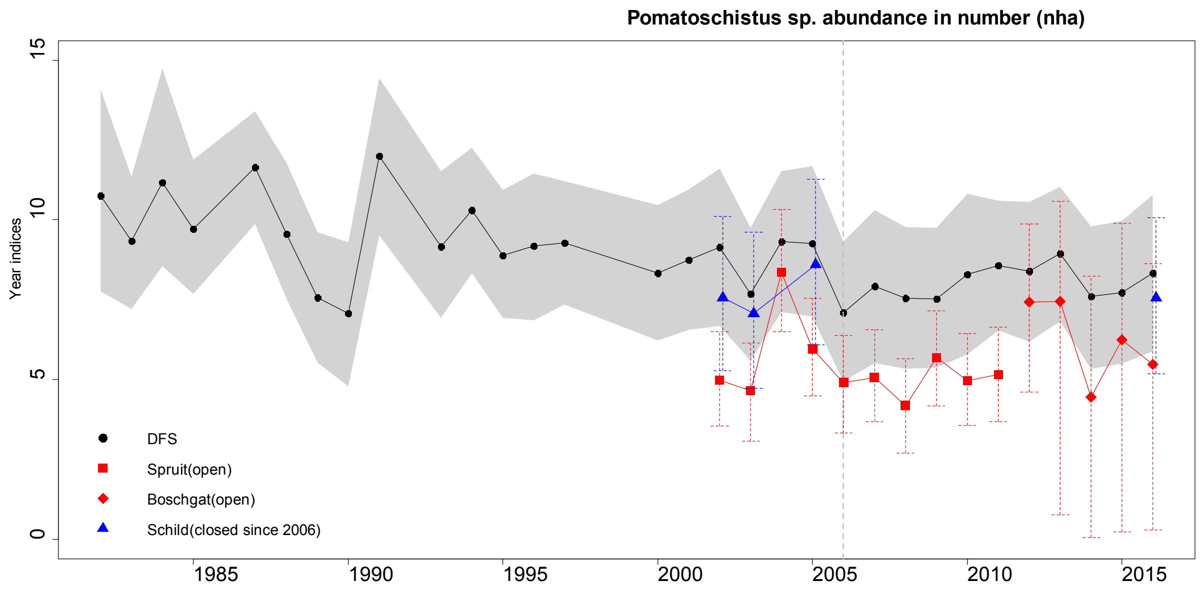


Figure 33*. Estimated year indices and its 95% confidence intervals for Pomatoschistus sp. (gobies sp.) abundance in number for DFS (part 1: model 3, part 2: model 3), Spruit (part 1: model 1; part2: model 1), Boschgat (part1: model 1; part2: model 1) and Schild (part1: model 1; part2: model 1).*

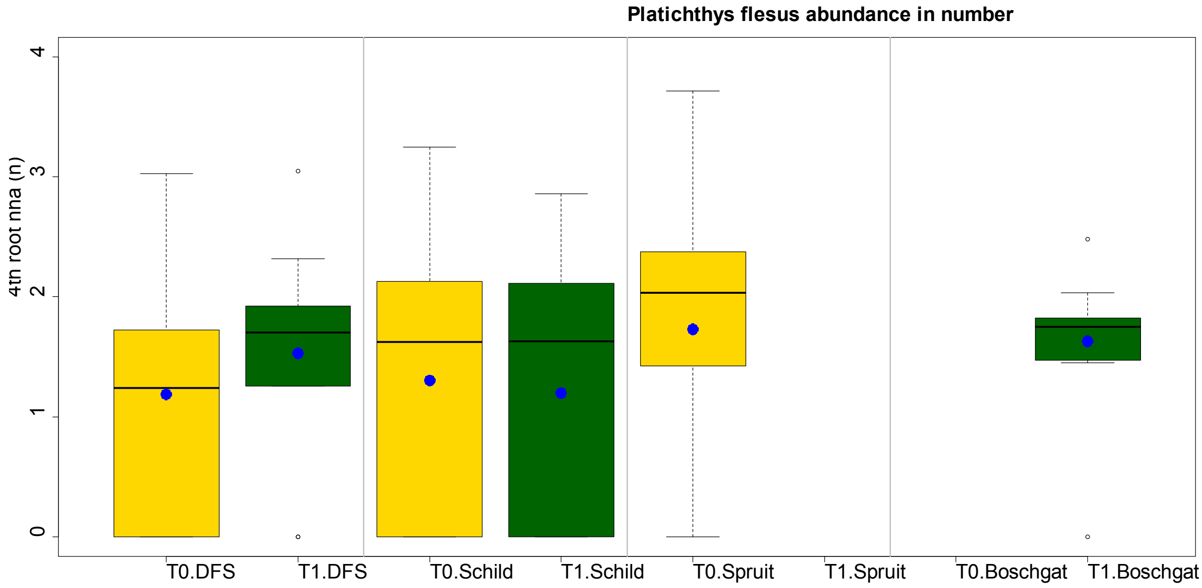


Figure 34. *Boxplots of abundance (n/ha 4th root transformed) of Platichthys flesus (flounder) in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

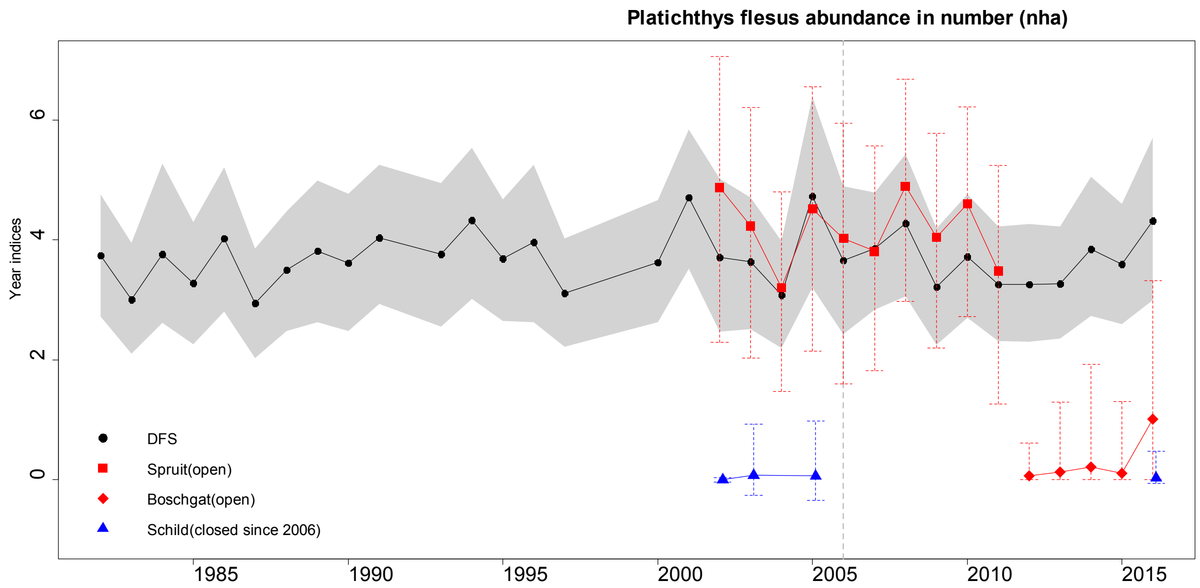


Figure 35. *Estimated year indices and its 95% confidence intervals for Platichthys flesus (flounder) abundance in number for DFS (part 1: model 2, part 2: model 2), Spruit (part 1: model 2; part2: model 2), Boschgat (part1: model 1; part2: model 1) and Schild (part1: model 4; part2: model 4).*

5.4.3 Number of species

In general the number of species per sample in Schild is much lower (5) than in the open areas and the DFS area (8-10, Figure 36). The number of species showed little variation between years and followed the same trend in the open areas Spruit and Boschgat as compared to the DFS area (Figure 37). This includes the dip in 2006. The observations in the closed area were too discontinuous to draw a conclusion. The confidence intervals for Boschgat are much larger than those for Schild and Spruit which means that the number of species found in the samples varied more in Boschgat when compared to the other gullies.

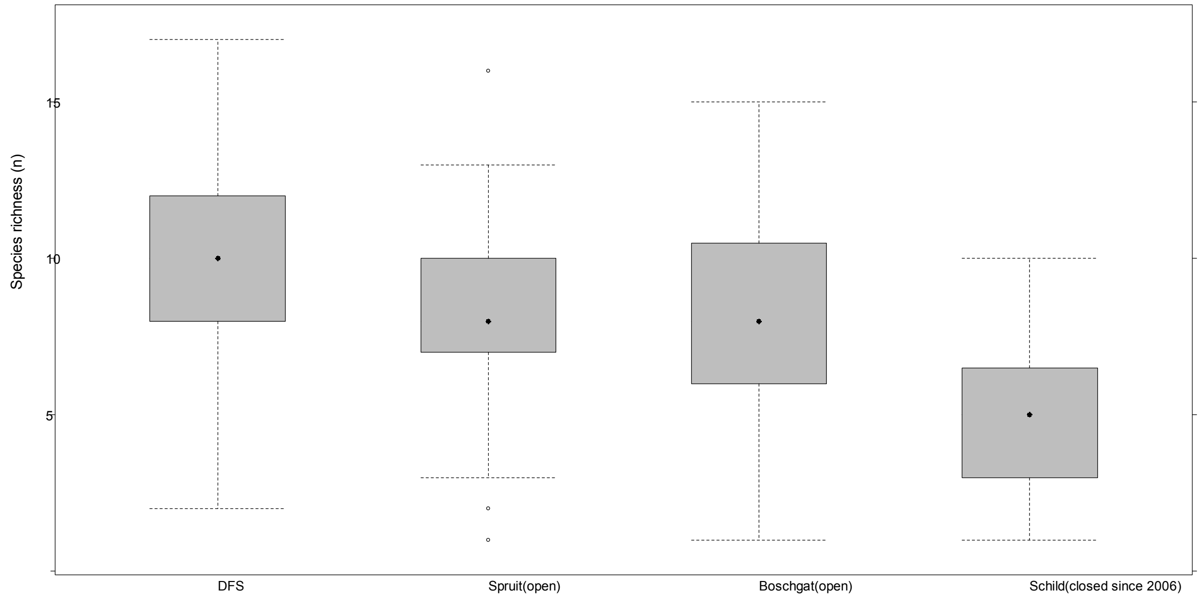


Figure 36. *Boxplots of species richness for the four areas (all available years are included).*

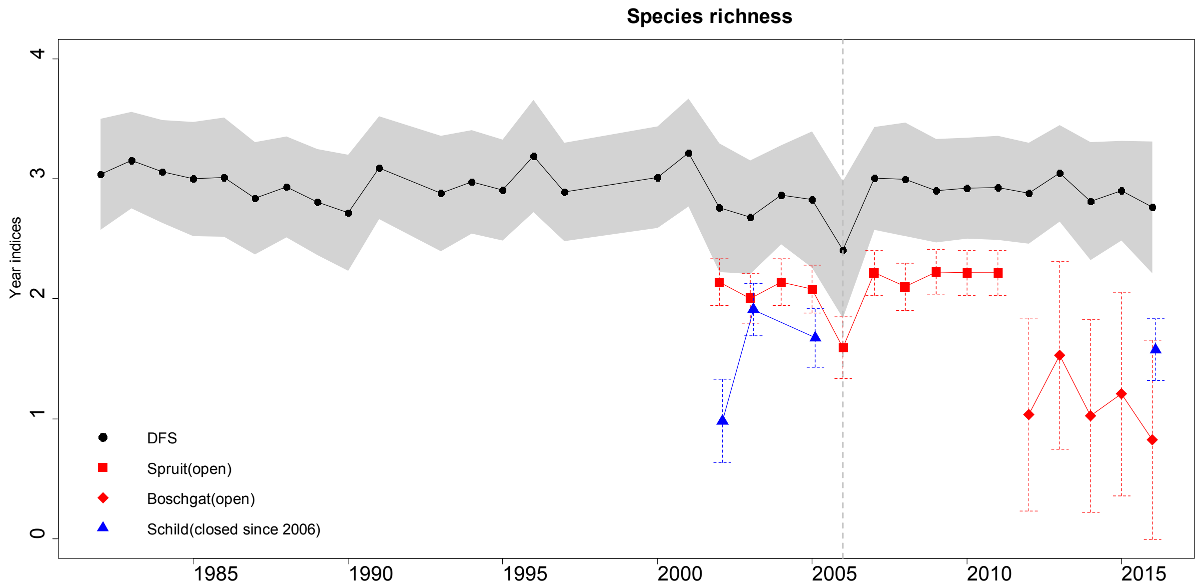


Figure 37*. Estimated year indices and their 95% confidence intervals for species richness for DFS (model 3), Spruit (model 1), Boschgat (model 2) and Schild (model 1).*

5.4.4 Mean length

Mean length (particular at T0) of plaice was lower in Schild compared to the other areas (Figure 38). The other four species were similar in size in all areas (Figure 40, Figure 42, Figure 44 and Figure 46).

Most species do not show a trend in mean size (Figure 41, Figure 43, Figure 45 and Figure 47). An exception is flounder, showing a slight decrease in the DFS area. Gobies show overall increasing trend of length in the DFS area. The estimated year indices of both Spruit and Boschgat follow the same trend as DFS, although the confidence intervals are large. Due to the low number of observations it is not possible to evaluate the developments in the closed area Schild. For most of the here investigated species, the mean values seem to be at the same level as before the closure. The mean length of plaice increased from T0 to T1 in Schild to similar values as observed in the other areas.

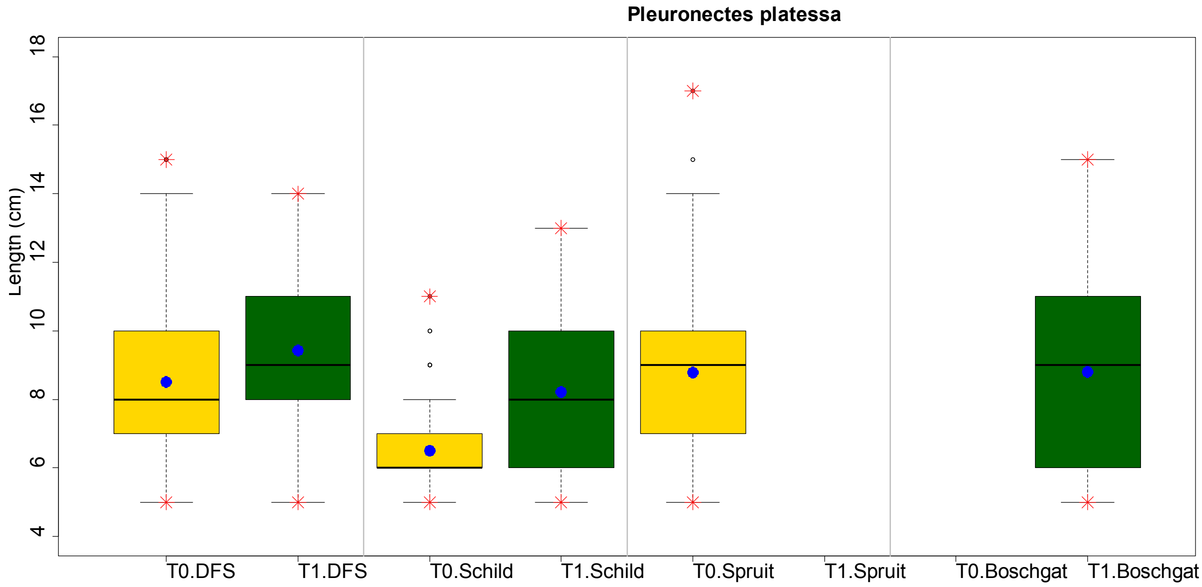


Figure 38. *Boxplot of mean length of Pleuronectes platessa (plaice) in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

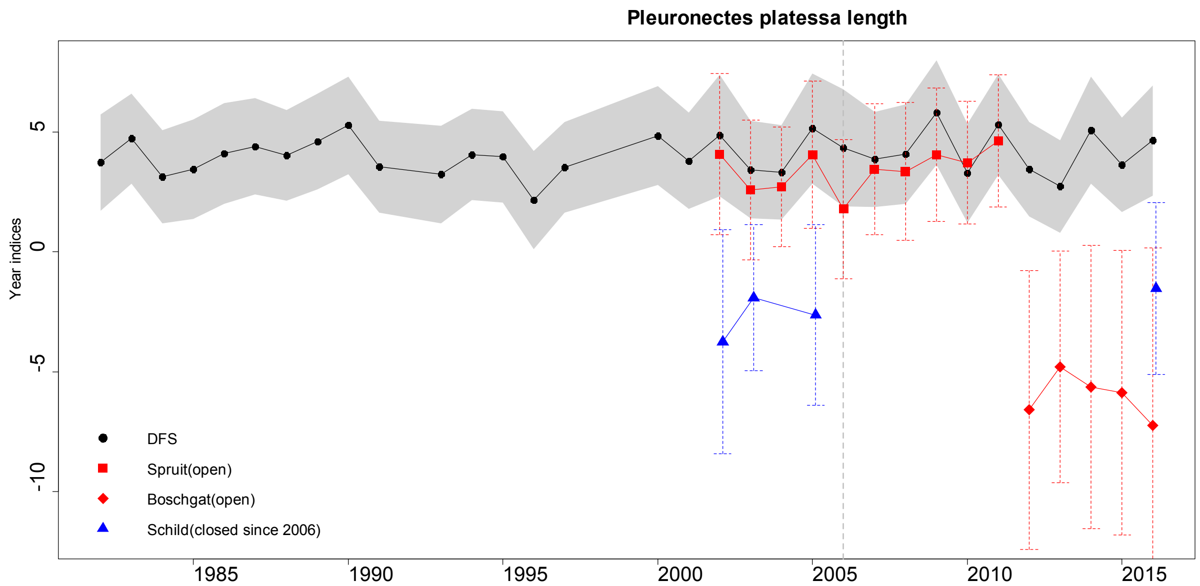


Figure 39. *Estimated year indices and its 95% confidence intervals for Pleuronectes platessa (plaice)*

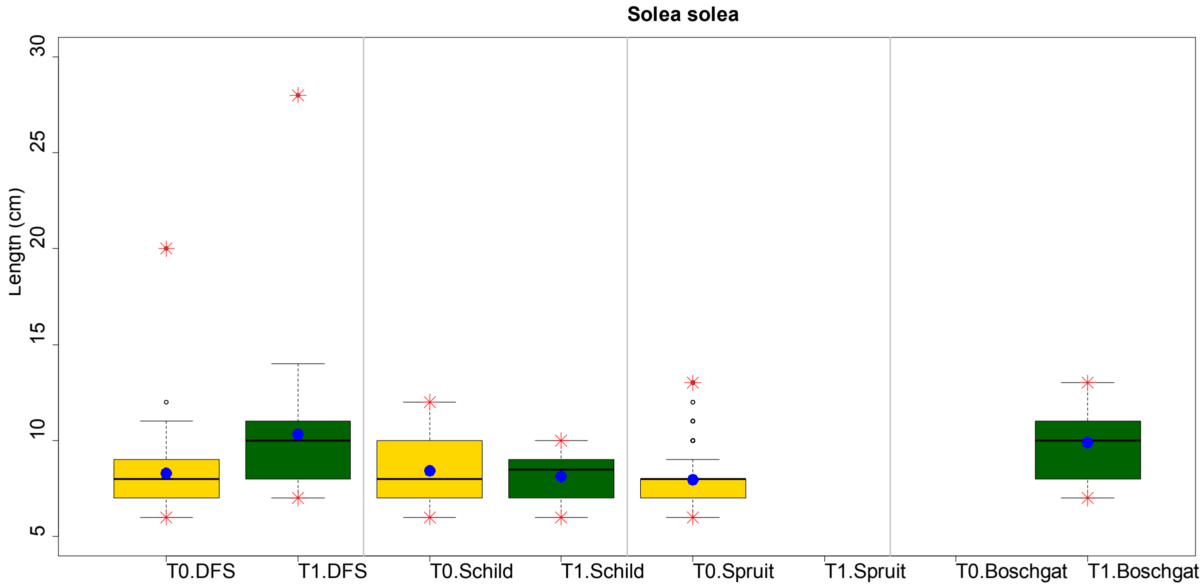


Figure 40. *Boxplot of mean length of Solea solea (sole) in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

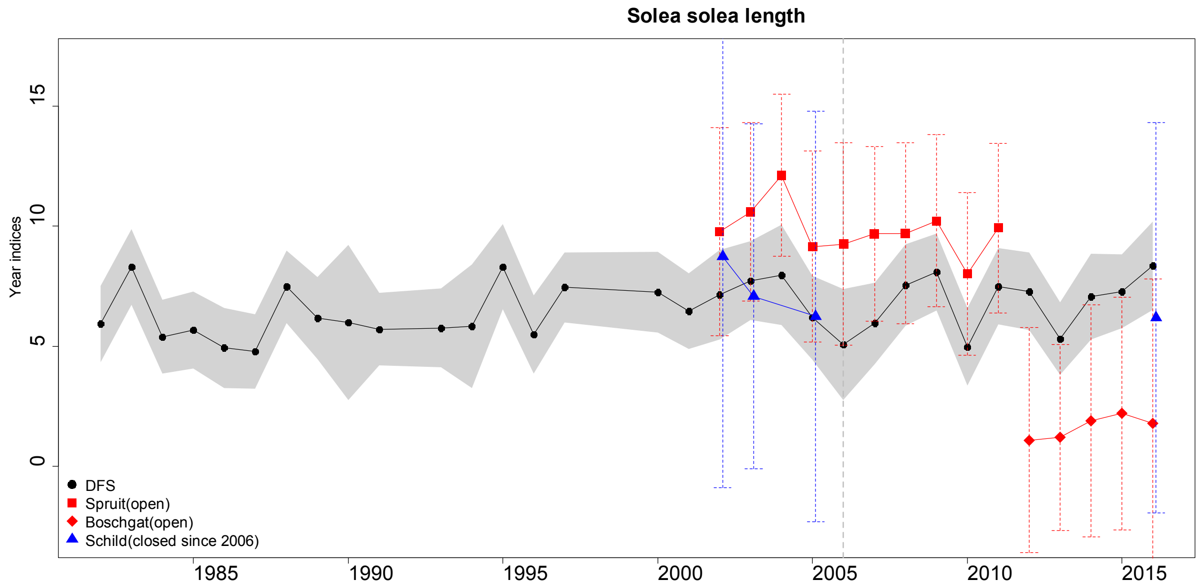


***Figure 41**. Estimated year indices and their 95% confidence intervals for Solea solea (sole) length for DFS (model 1), Spruit (model 1), Boschgat (model 1) and Schild (model 1).*

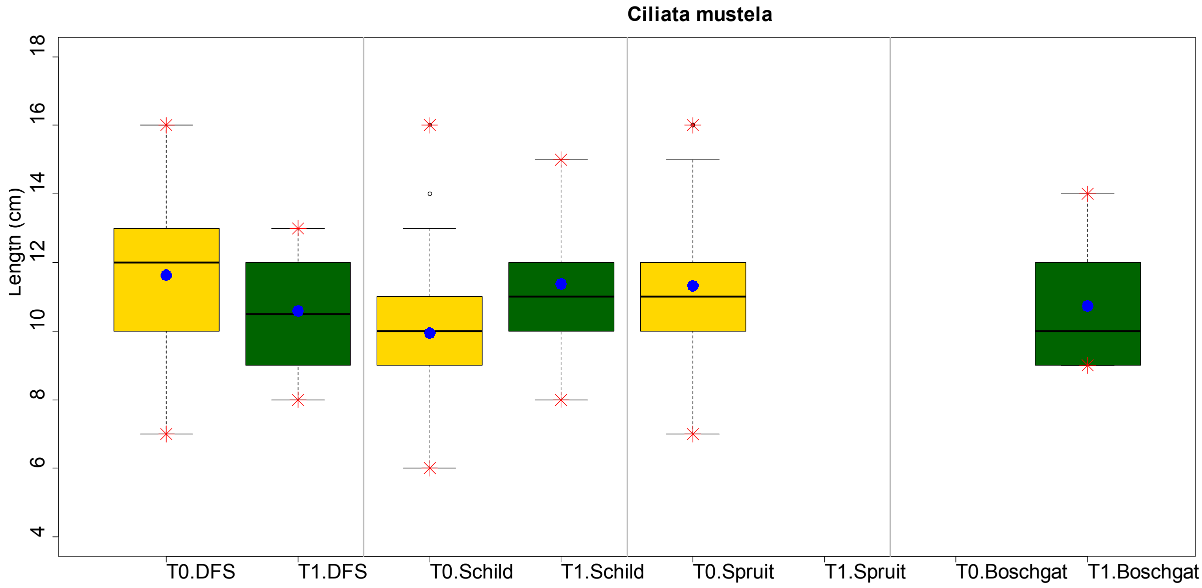


***Figure 42**. Boxplot of mean length of Ciliata mustela (five-bearded rockling) in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

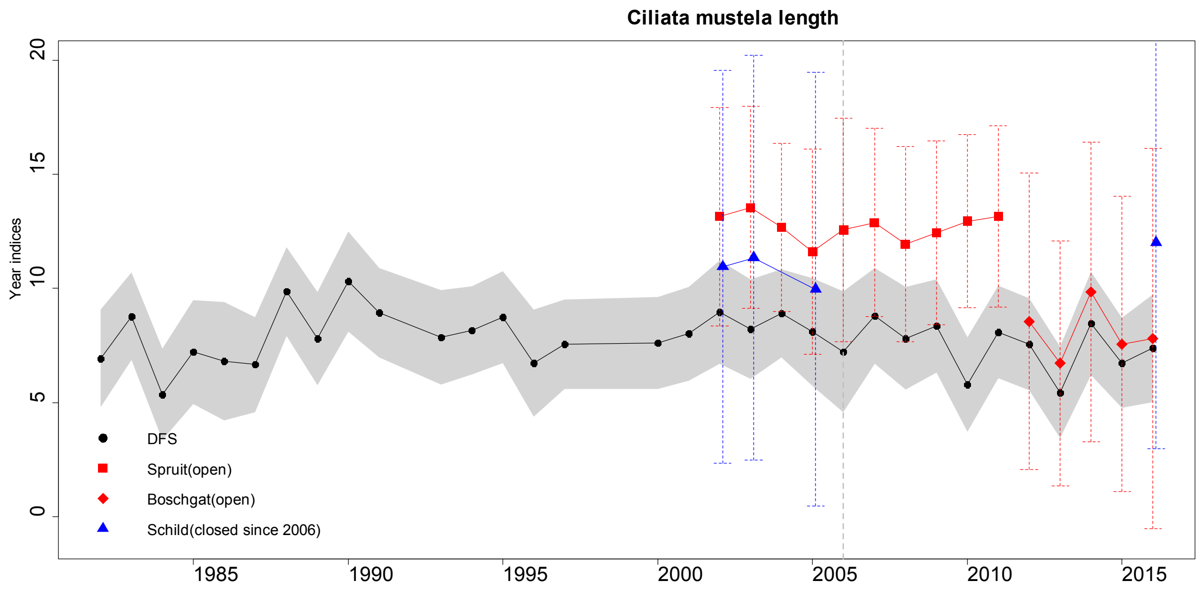


***Figure 43**. Estimated year indices and their 95% confidence intervals for Ciliata mustela (five-bearded rockling) length for DFS (model 1), Spruit (model 1), Boschgat (model 1) and Schild (model 1).*

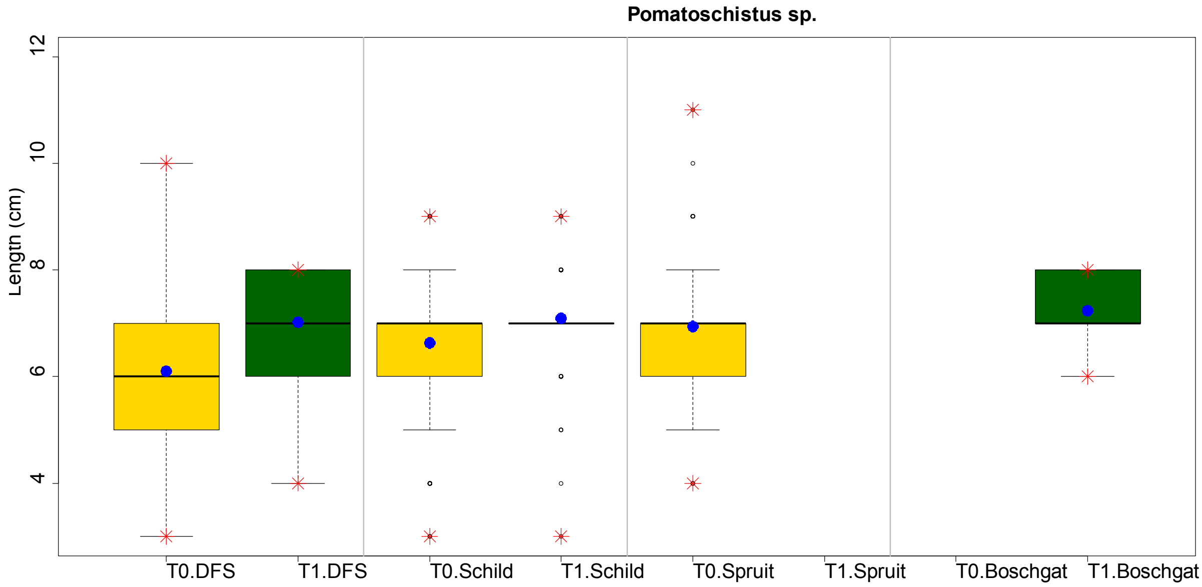


Figure 44. *Boxplot of mean length of Pomatoschistus sp. (gobies sp.) in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

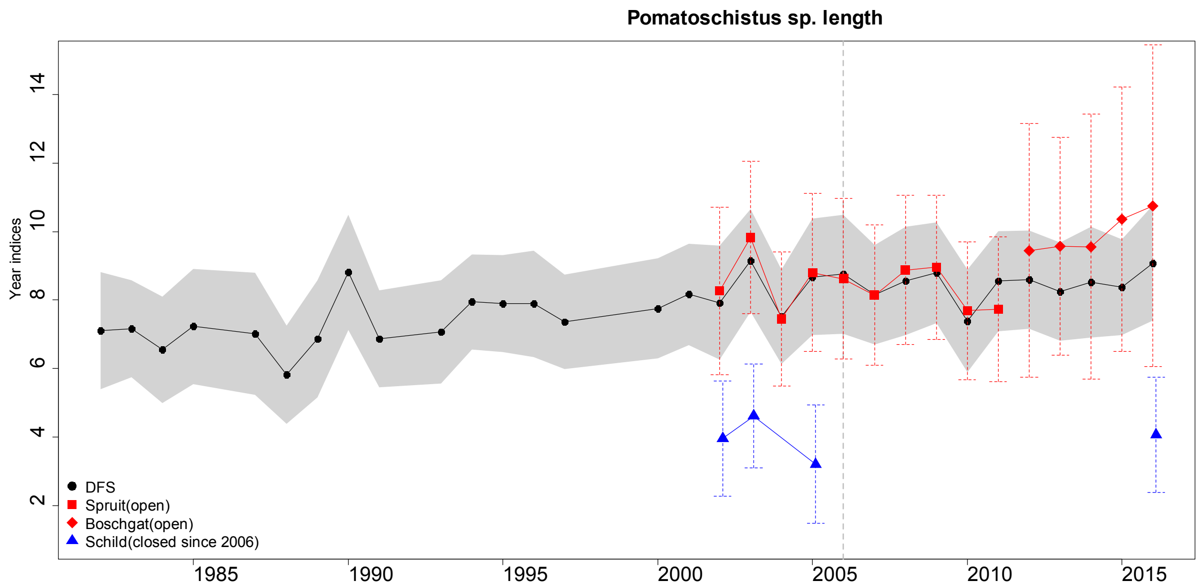


Figure 45. *Estimated year indices and their 95% confidence intervals for Pomatoschistus sp. (gobies sp.) length for DFS (model 1), Spruit (model 1), Boschgat (model 1) and Schild (model 1).*

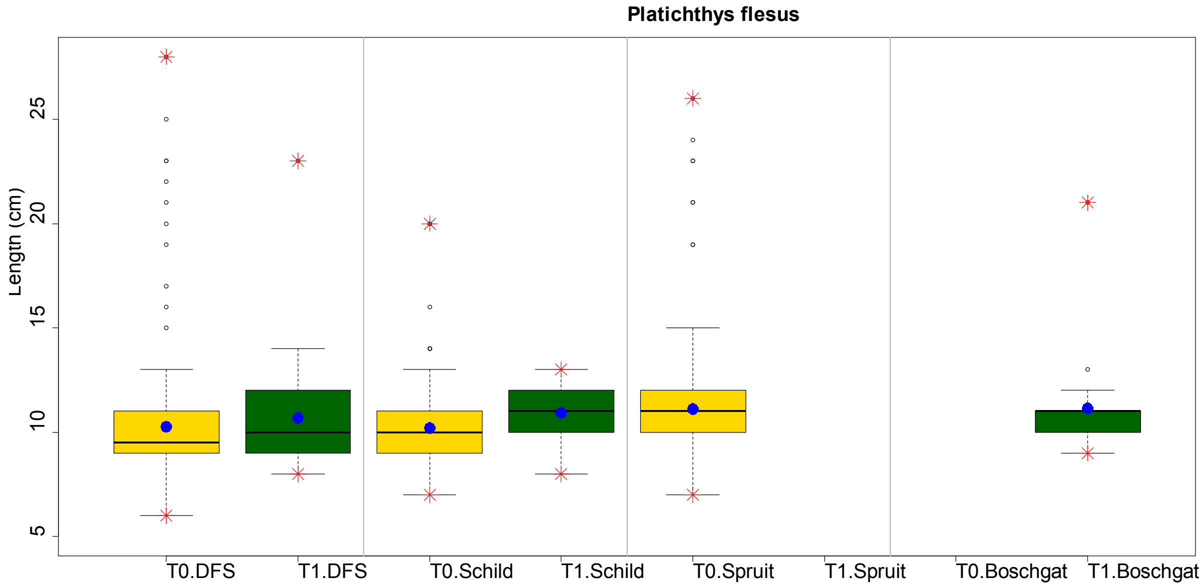


***Figure 46**. Boxplot of mean length of Platichthys flesus (flounder) in the DFS (T0; 2002-2005 and T1; 2016), the closed gully Schild (T0; 2002, 2003 & 2005 and T1; 2016) and the open gullies Spruit (T0; 2002-2005) and Boschgat (T1; 2016).*

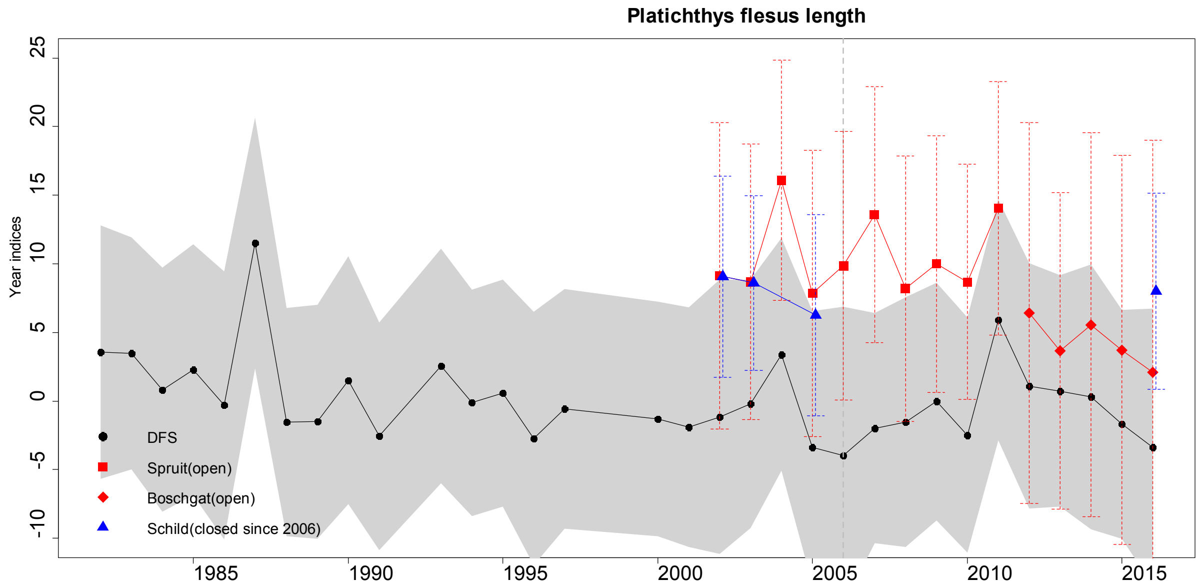


***Figure 47.** Estimated year indices and their 95% confidence intervals for Platichthys flesus (flounder) length for DFS (model 1), Spruit (model 1), Boschgat (model 1) and Schild (model 1).*

6 Discussion

6.1 Fishery and natural disturbance of the seabed

Interpolated Vessel Monitoring System (VMS) data shows that fishing intensity in and around the Rottum area is not particular high when compared to the whole Dutch Wadden Sea. Also, before closure of the gullies Boschwad and Schild, fishing intensity was relative low compared with the open gullies ZO-Lauwers and Spruit. The closure of the closed area Rottum in 2005 seems to be complied; the closed gullies were not fished after 2005 by the commercial fishing industry. This means that, considering fishing, there was a gradient ('treatment'), between the open and closed gullies, but the gradient (difference) was not particular high. There was considerable variation in fishing intensity from year to year in the open gullies but no clear in-or decreasing trend in fishing pressure over the years. There was no clear difference in fishing pressure between Spruit and ZO-Lauwers either.

Several studies indicate that natural induced disturbances (caused by wind and wave action) might influence the benthic community in the same way disturbance by (beam) trawl fishing does (Van Denderen, 2015). Natural induced disturbance of the seafloor can be an important factor determining the benthic species composition in a similar way as induced disturbance by passing of a bottom trawl net. High sand ripples generally only develop in dynamic environments and inspection of the bathymetry maps made in 2016 can give clues about the natural disturbance in the gullies. Based on the height of the sand ripples, natural disturbance in Schild is equally high compared to the open gullies Spruit and ZO-Lauwers. Boschwad is more sheltered from influences of the North Sea due to its location behind the island of Rottum and sand ripples are indeed much smaller indicating the area is less exposed. Therefore, while the absence of fishing in Boschwad leads to a reduced bottom disturbance allowing for a distinct development of the benthic community, this is not per definition the case for Schild as there is still considerable natural disturbance present.

6.2 Benthos

Seafloor structure

The sonar survey did not reveal areas with biogenic structures in Boschwad, Schild, ZO-Lauwers and Spruit. In each of the gullies, distinct areas were found that consisted of shell debris. The samples (Van Veen grab- and suction dredge samples) confirm that there are no (extended) areas with biogenic structures. Besides one occasion, no oysters (*Crassostrea gigas*) were found in the samples. Mussels were encountered both with the Van Veen grab and the suction dredge, but in just a few samples and in low densities, not indicating presence of sublittoral mussel beds. Compared to mussels, the tube worm *Lanice conchilega* were encountered in the samples more frequent and especially in the closed gullies densities were relative high (up to 375 ind./m^2). This does not yet qualify as a reef according to the definition of Rabout, that require 500 ind./m^2 (Rabout *et al*., 2009). Tubes of *Lanice sp.* reduce flow velocities and it is believed that tentacles and tubes of *Lanice sp.* that erect from the seafloor forms a suitable substrate for shellfish larvae to attach to. Positive relations have been found between species richness and *Lanice sp.* and several mollusc species such as *Donax vittatus*, *Ensis* sp., *Spisula* sp. and *Abra albra* (Rabout *et al*., 2007, Callaway *et al*., 2010 and Callaway, 2006).

Benthic community

The development of the benthic community is examined by investigating the development of several univariate parameters and by considering the benthic community as a whole in multivariate analyses. Univariate parameters that were tested were- shell debris, density, richness, species pool, diversity and evenness. As concluded before (Fey-Hofstede *et al*., 2015) variation between samples and years was large which is a common feature in benthic populations. Variation in recruitment success, habitat preferences, aggregation of species and natural- and anthropogenic disturbance all contribute to this variation. As a result, statistically significant differences can only be detected if differences in mean values are large.

For all the here investigated parameters (shell debris, density, richness, species pool and diversity) except evenness the longer term increase was larger in the closed gullies compared to the open gullies but only the increase in species richness was statistically significant.

The multivariate analysis of species encountered in the Van Veen grab showed that the benthic community in Boschwad developed differently from the other gullies and a different community composition in 2016 was supported by the results of the suction dredge. *Limecola* (*Macoma) balthica* and *Cerastoderma edule* were characteristic mollusc species of the community in Boschwad. Based on dredge samples of 2016, *Cerastoderma edule* densities were significant higher compared to the other gullies. The population structures were also different with more larger sized *Macoma balthica* and older (*Cerastoderma edule*). Also the presence of many annelid species, including some tube building species, characterized the benthic community in this gully. These differences might be explained by closure of the area and - or, the more sheltered environmental conditions of Boschwad. The *Crangon crangon* (shrimp) densities was in 2016 not different from the other gullies and the occurrence of *Ensis sp.* was markedly low in Boschwad.

Some (very) subtle differences in the community composition of Schild were found in recent years. *Lanice conchilega* and *Ensis sp.* were more often encountered and *Ensis sp.* densities were (based on dredge samples taken in 2016) significant higher compared with the other gullies. The *Crangon crangon* (shrimp) densities was in 2016 not different from the other gullies. Based on the sand ripples observed on the sonar image and its exposure towards the North Sea in Schild the natural disturbance caused by wave and wind action is probably high. This might explain why the benthic community is not that different from the open gullies as natural- and anthropogenic disturbance might influence the benthic community in a similar way. Strong seabed disturbances leads to a coarser sediment structure that is beneficial for the (perpendicular and deep) burying *Ensis sp.* that might explain the high density of this species in Schild. Tolerance of *Ensis sp.* to (anthropogenic) disturbance was found in several recent studies (Glorius e*t al*., 2015; Tulp *et al*., 2018).

6.3 Fish

The sampling design for fish consisted of only a few years of observations, and in only one closed and one open area. The closed area was sampled three times before closure and once after closure. Additionally the open area sampled in the T0 was a different one from the T1 situation. This is clearly not a good basis for a sound BACI analysis which require multiple samples taken at multiple times before and after closure in several (and the same) open and closed sites. Therefore this setup cannot be used to statistically evaluate the effects of closing of Schild from human impacts.

The information from the surrounding DFS619 area shows that year to year variability in fish densities can be large. The comparison of the trend in DFS619 with Spruit and later on Boschgat (both open) shows that developments within the same tidal basin closely follow the same pattern.

Already at T0 the open and closed gully show considerable differences in fish densities with higher densities in Spruit (open area) than in Schild (closed area). Such a difference was not apparent in mean fish length (for any of the species). Schild also contained less species per haul than Spruit. Therefore we can conclude that before closure Schild was an area with a poorer fish fauna than Spruit, and that improvements from closure could not be detected because of the limited set up of the monitoring program.

For the same reasons, we cannot draw conclusions on the difference in developments between the open and closed area regarding the development of total fish abundance, species-specific fish abundance, number of species, or mean length. It can be observed, but not statistically proven, that 2016 is not markedly different from T0.

7 Conclusions

Throughout the investigated period, (2002 – 2016), open gullies (Spruit & ZO-Lauwers) have been subject to (moderate) shrimp fisheries. Natural induced disturbance of the seafloor was high for both of the open gullies as well as the closed gully Schild. Closure of Schild and Boschwad has led to reduced fishing intensities, but this has not yet led to formation of biogenic structures on the seafloor. However, it has led to an increase in species richness of small benthic fauna as well as to subtle changes in benthic species composition. Molluscs have become more dominant in the benthic community and also higher densities of *Lanice sp.* have established in the closed gullies. Differences are especially pronounced in the more sheltered areas, i.e. in the end sections of Boschwad and Schild.

Currently it is not known if the observed differences in the benthic communities will continue to increase in the future. For example the tube building annelid species *Lanice conchilega,* encountered in the closed gullies, might enhance new settlement that can lead to a more permanent and dominant presence of this species which in turn might lead to a further increase in species richness and presence of shellfish. The presence of cockles, observed in Boschwad, might aid the establishment of mussel spat that can anchor itself to the cockleshells. On the other hand, it is also possible that distinctive species disappear in the future by natural mortality, erosion and/or absence of recruitment and that open and closed gullies become more similar over time. It is expected that, opposite to the more turbulent environment of Schild, conditions at Boschwad are favourable for the development and settlement of species that are sensitive to seabed disturbance.

With the limited availability of data it was not possible to statistically detect difference in the fish population between the open and the closed gullies.

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Justification

Wageningen Marine Research

ReportC092/18 – WOt-technical report 129 (2018)
Project Number: 431.81000.06

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research.

Approved: Robbert Jak
Researcher

Signature:

Date: 4 December 2018

Approved: Jakob Asjes
Manager integration

Signature:

Date: 4 December 2018

Annex 1 Fishery

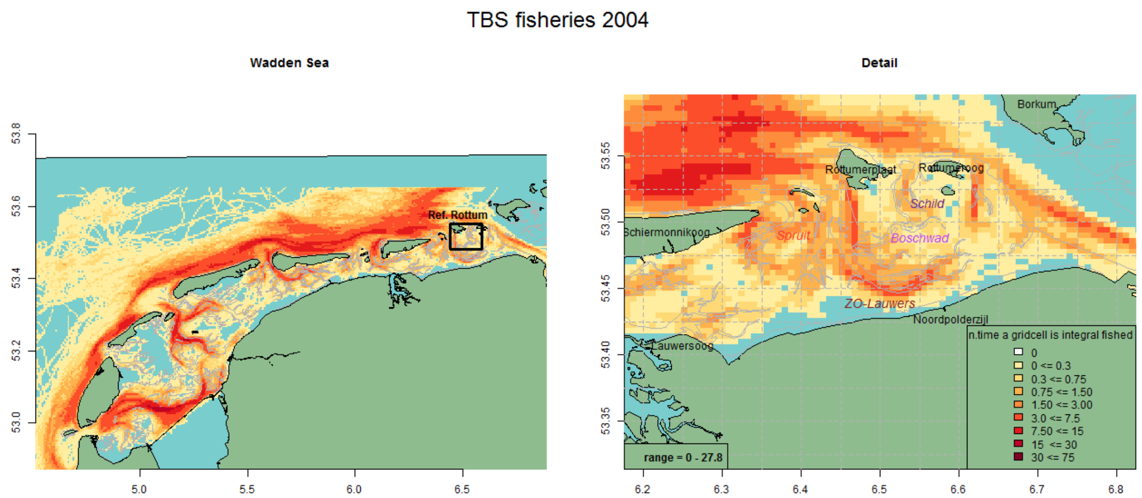


Figure 48. *Cummulated fishing intensity based on interpolated VMS-data of the year 2004 in the wadden sea (left) and Rottum (rigth) area.*

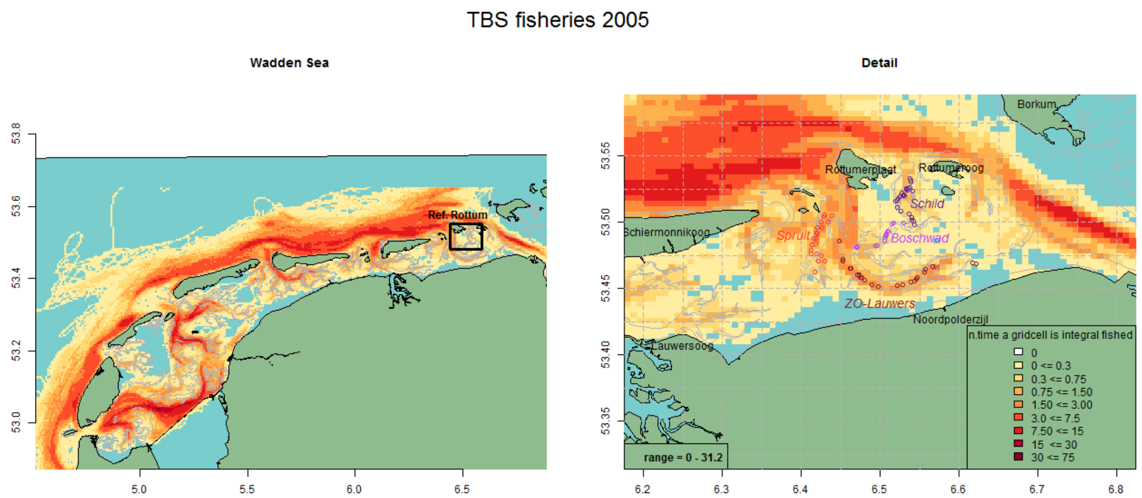


Figure 49. *Cummulated fishing intensity based on interpolated VMS-data of the year 2005 in the wadden sea (left) and Rottum (rigth) area.*

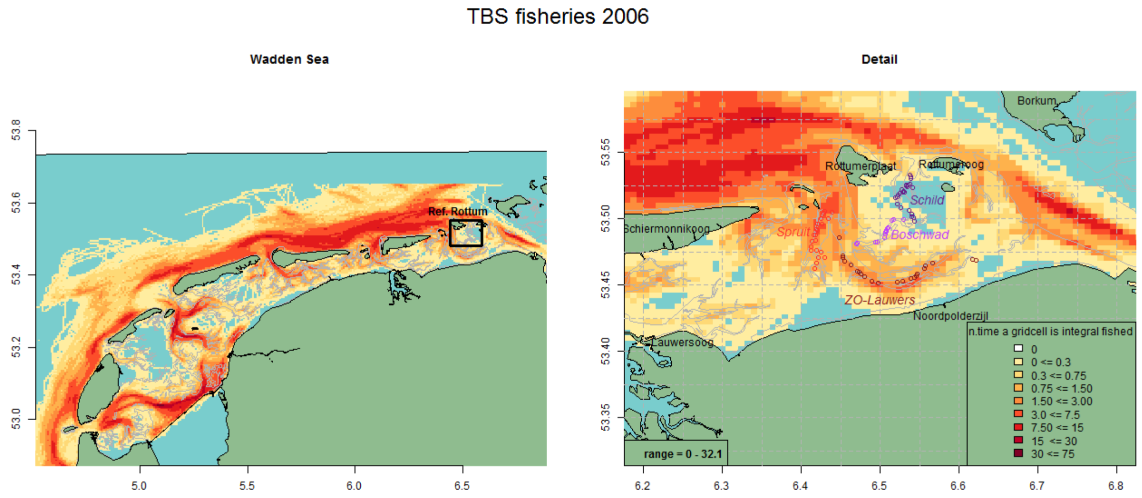


Figure 50. *Cummulated fishing intensity based on interpolated VMS-data of the year 2006 in the wadden sea (left) and Rottum (rigth) area.*

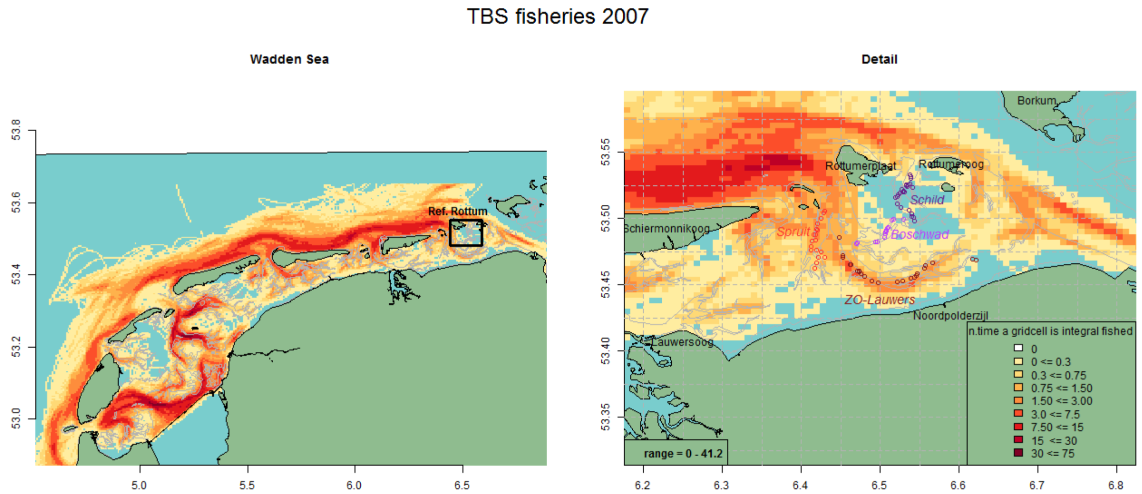


Figure 51. *Cummulated fishing intensity based on interpolated VMS-data of the year 2007 in the wadden sea (left) and Rottum (rigth) area.*

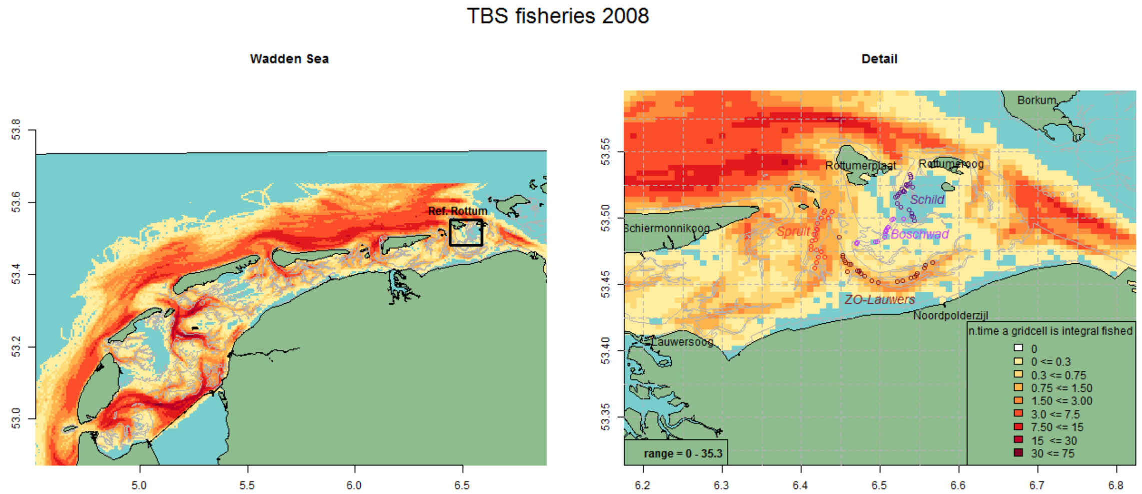


Figure 52. Cummulated fishing intensity based on interpolated VMS-data of the year 2008 in the *wadden sea (left) and Rottum (rigth) area.*

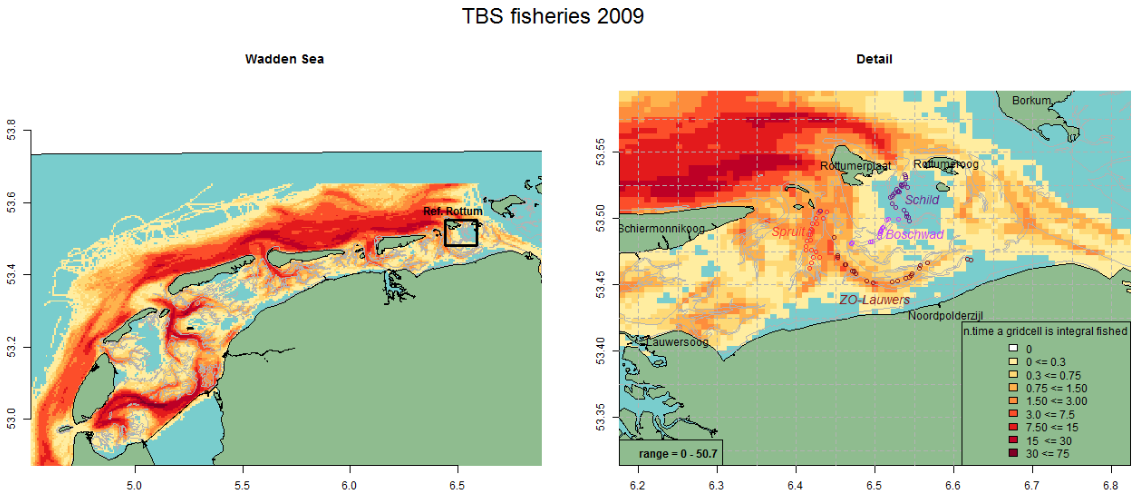


Figure 53. *Cummulated fishing intensity based on interpolated VMS-data of the year 2009 in the wadden sea (left) and Rottum (rigth) area.*

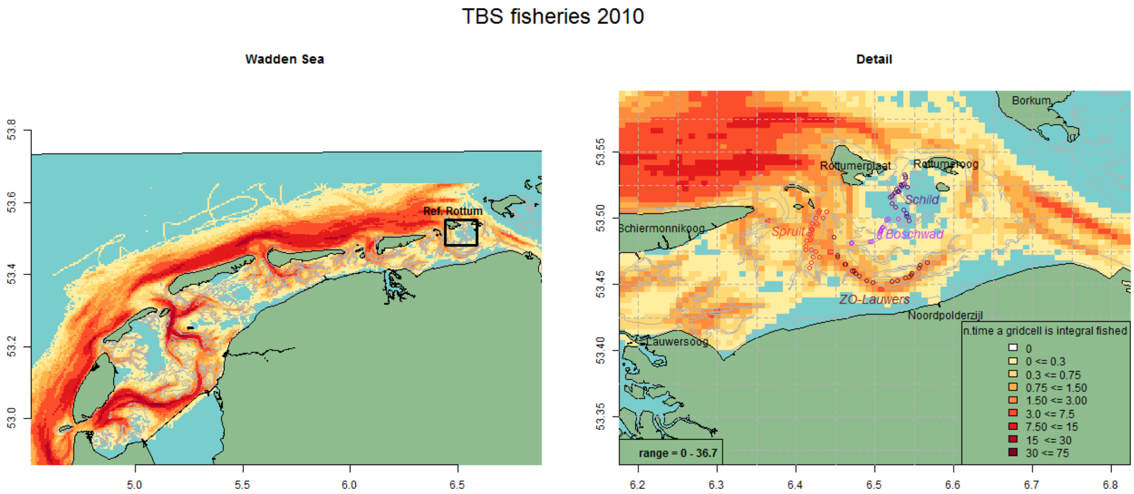


Figure 54*. Cummulated fishing intensity based on interpolated VMS-data of the year 2010 in the wadden sea (left) and Rottum (rigth) area.*

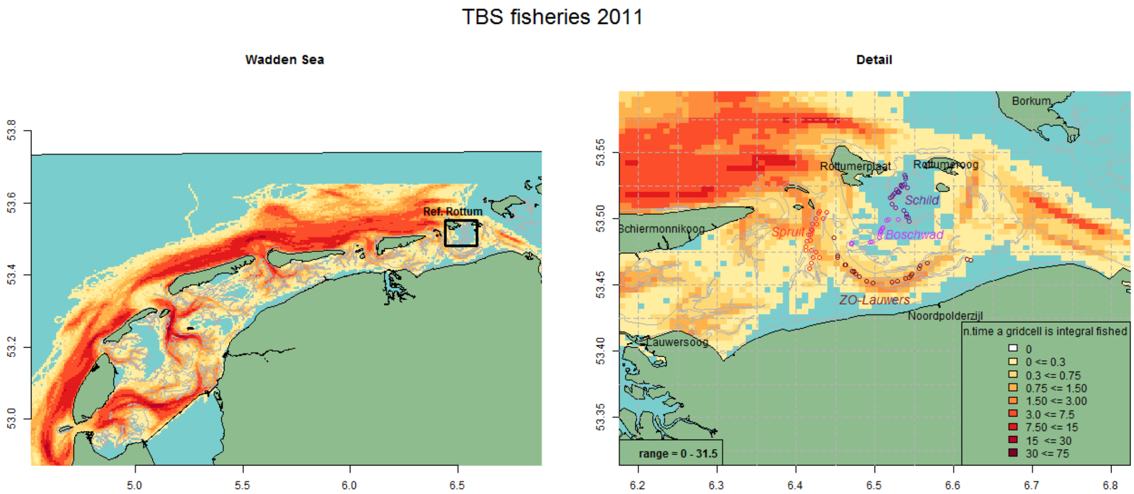


Figure 55*. Cummulated fishing intensity based on interpolated VMS-data of the year 2011 in the wadden sea (left) and Rottum (rigth) area.*

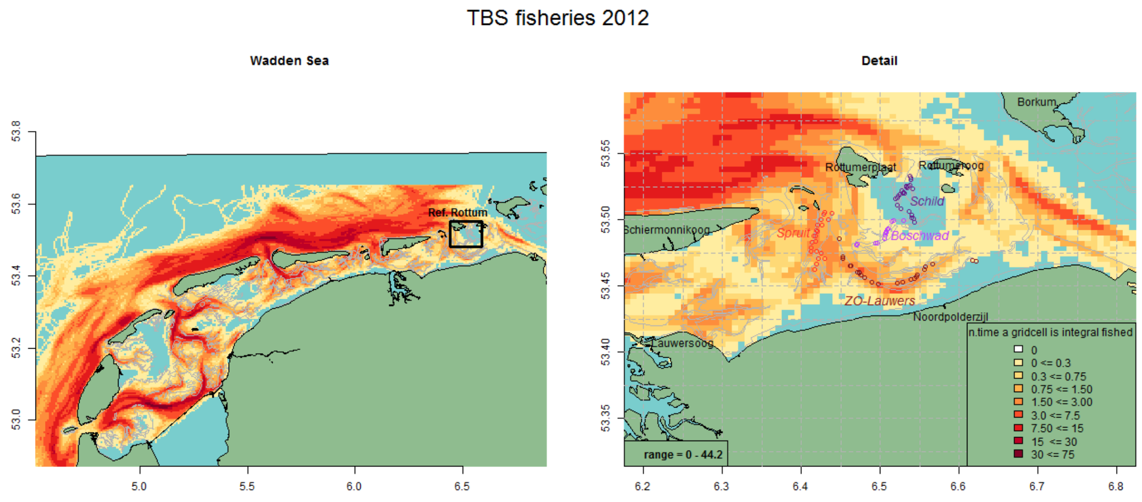


***Figure 56**. Cummulated fishing intensity based on interpolated VMS-data of the year 2012 in the wadden sea (left) and Rottum (rigth) area.*

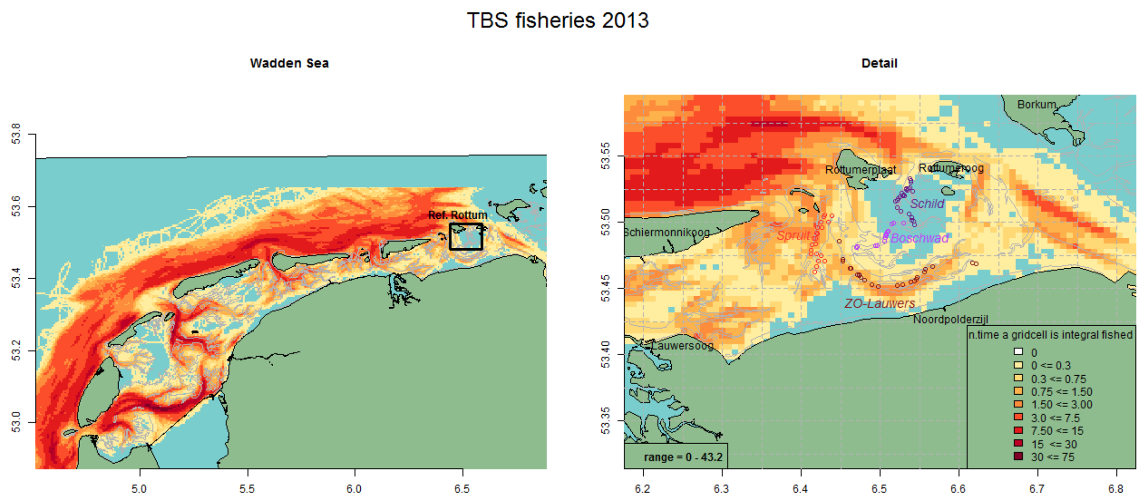


***Figure 57**. Cummulated fishing intensity based on interpolated VMS-data of the year 2013 in the wadden sea (left) and Rottum (rigth) area.*

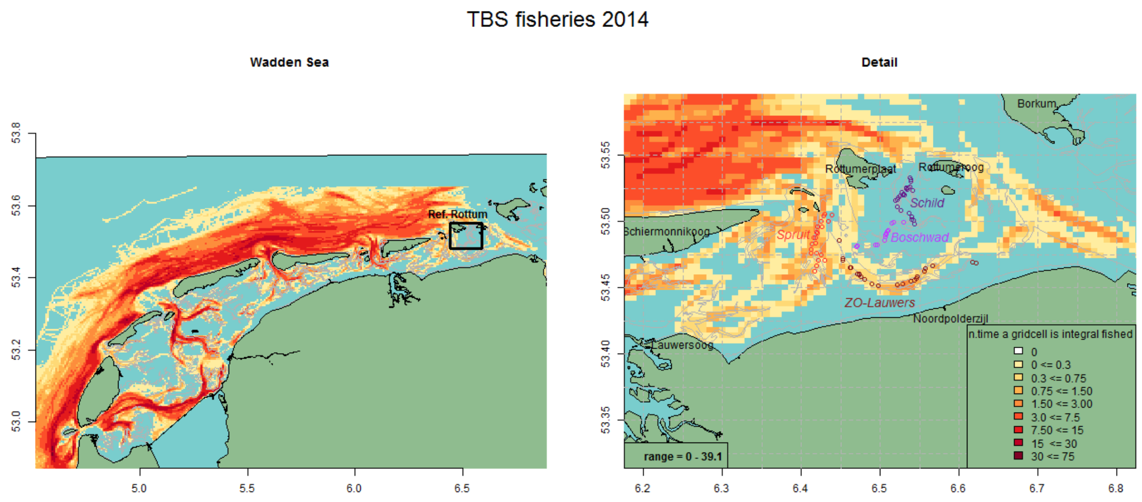


***Figure 58**. Cummulated fishing intensity based on interpolated VMS-data of the year 2014 in the wadden sea (left) and Rottum (rigth) area.*

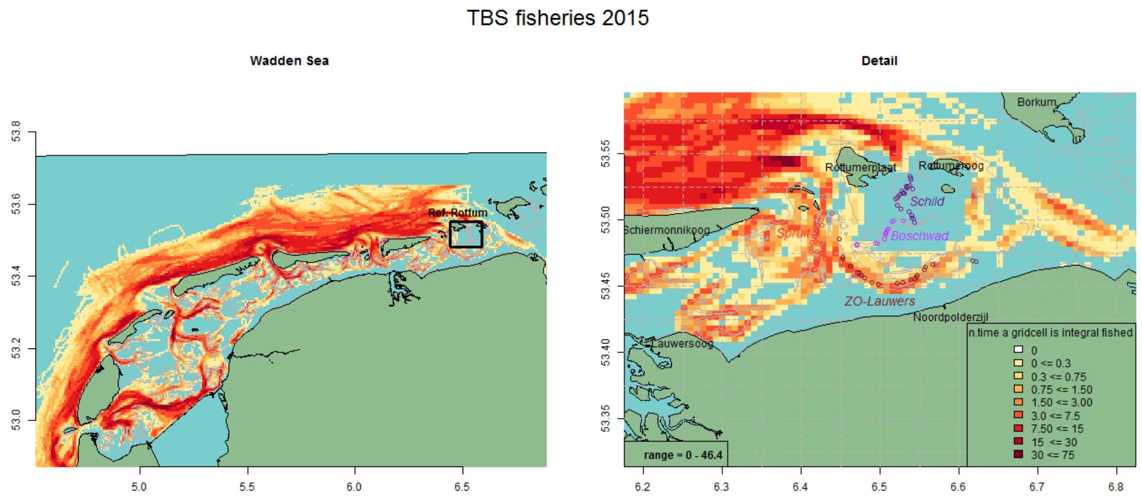


Figure 59. *Cummulated fishing intensity based on interpolated VMS-data of the year 2015 in the wadden sea (left) and Rottum (rigth) area.*

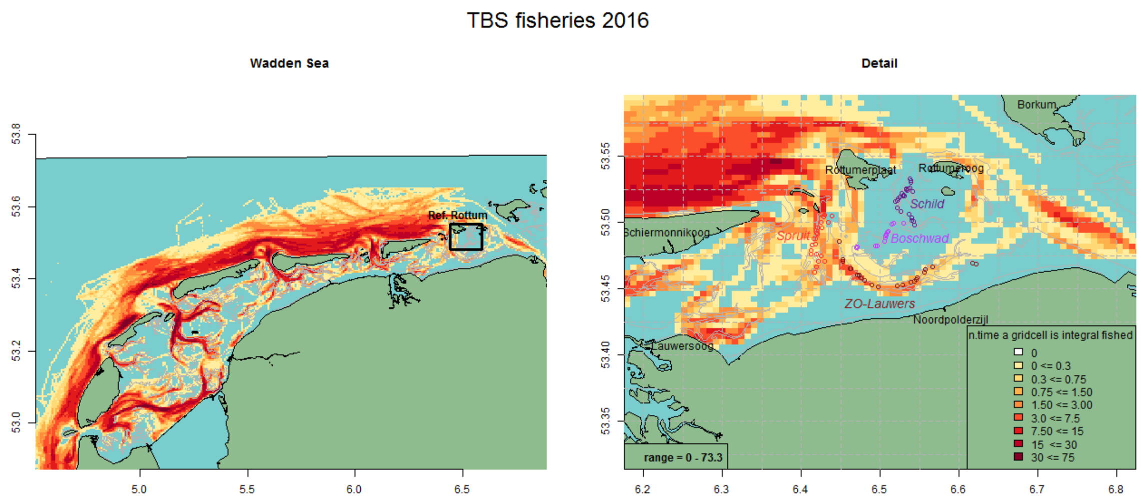
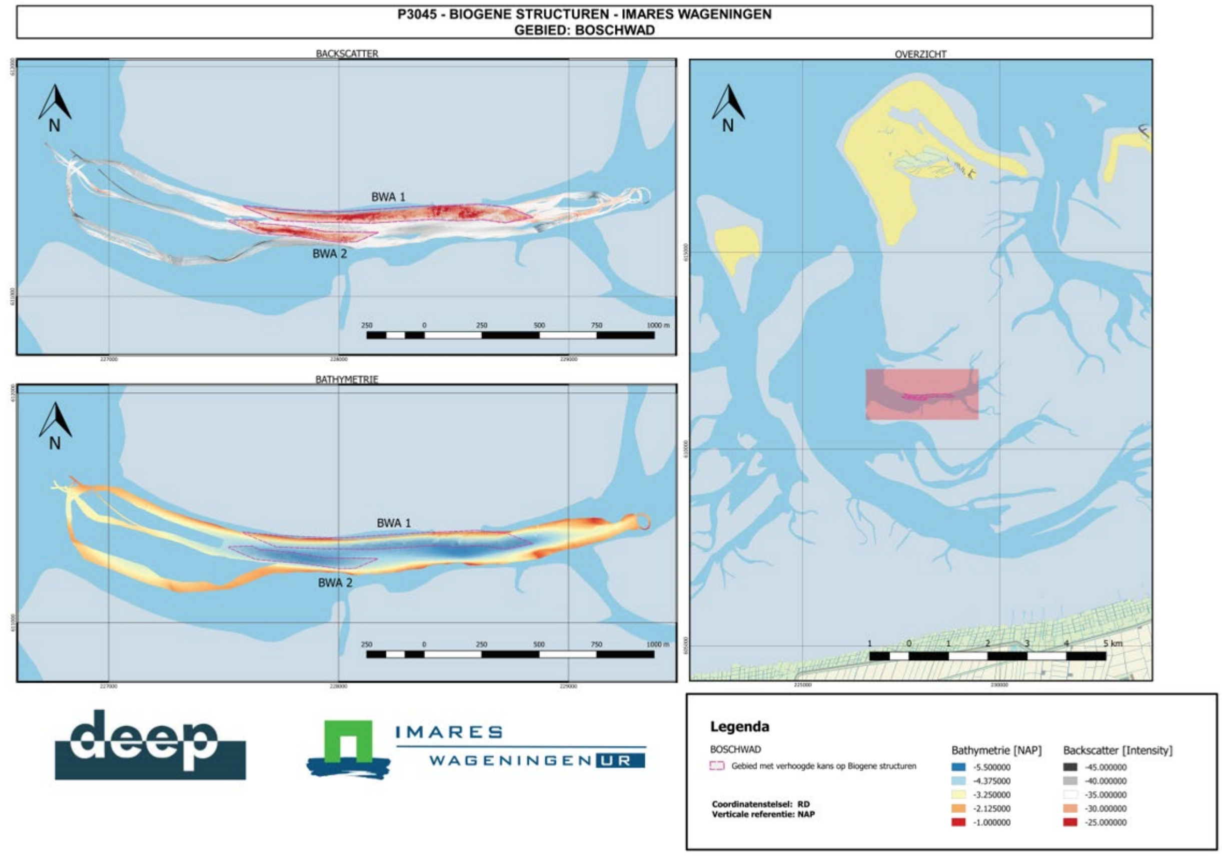
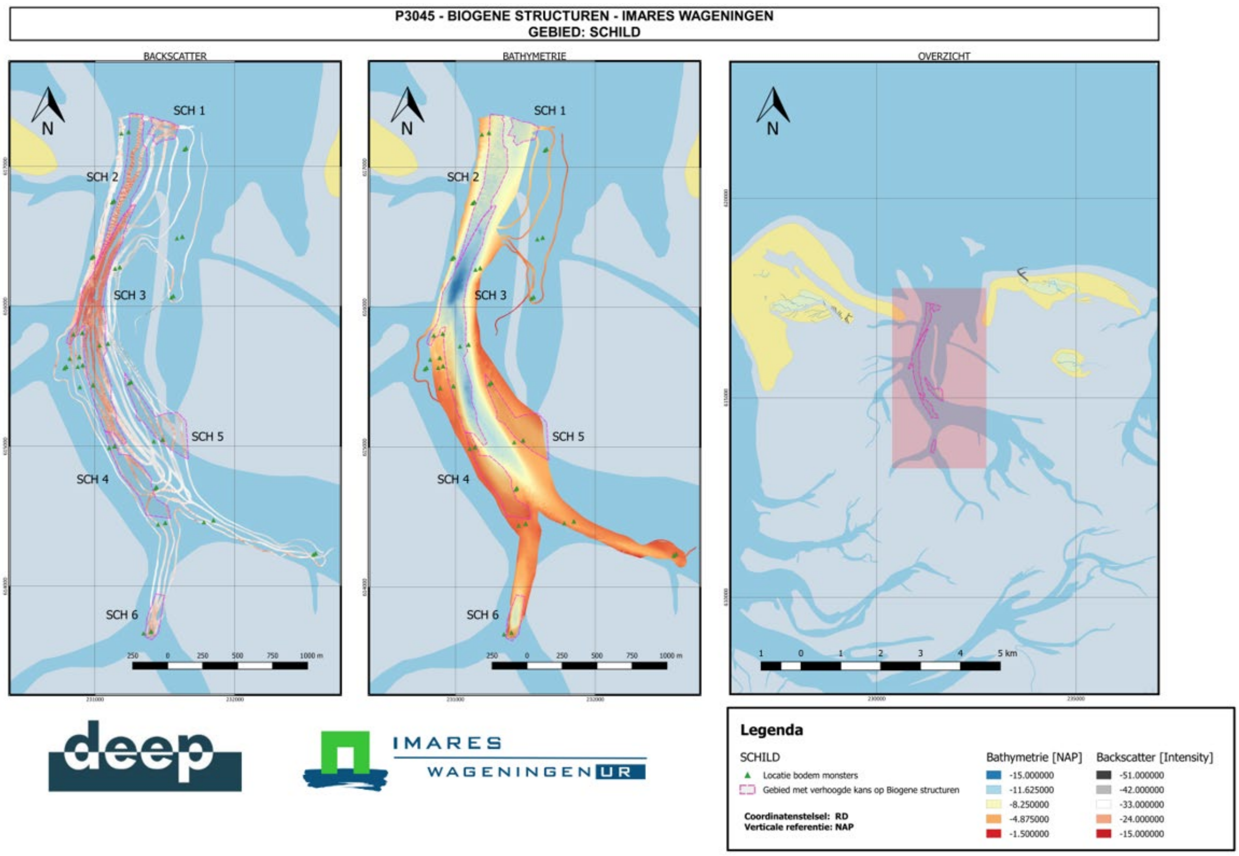
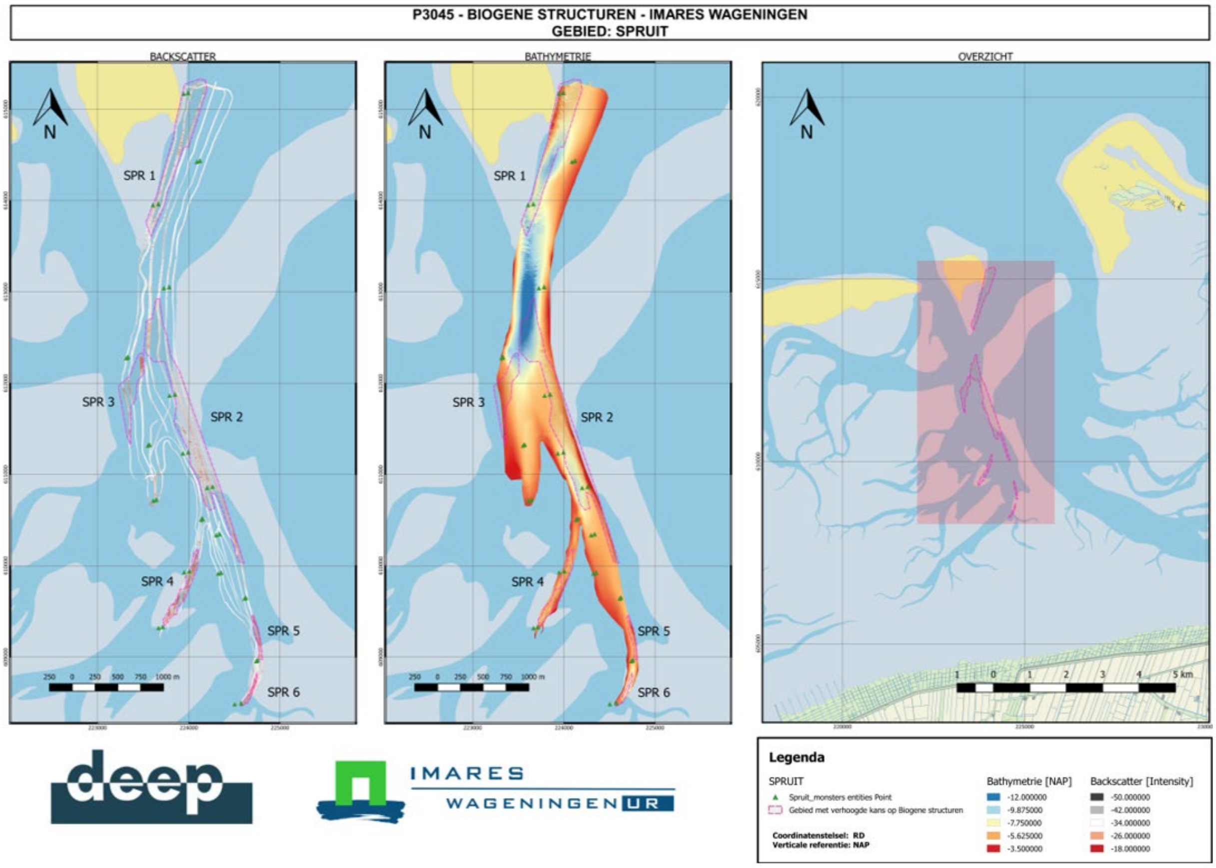


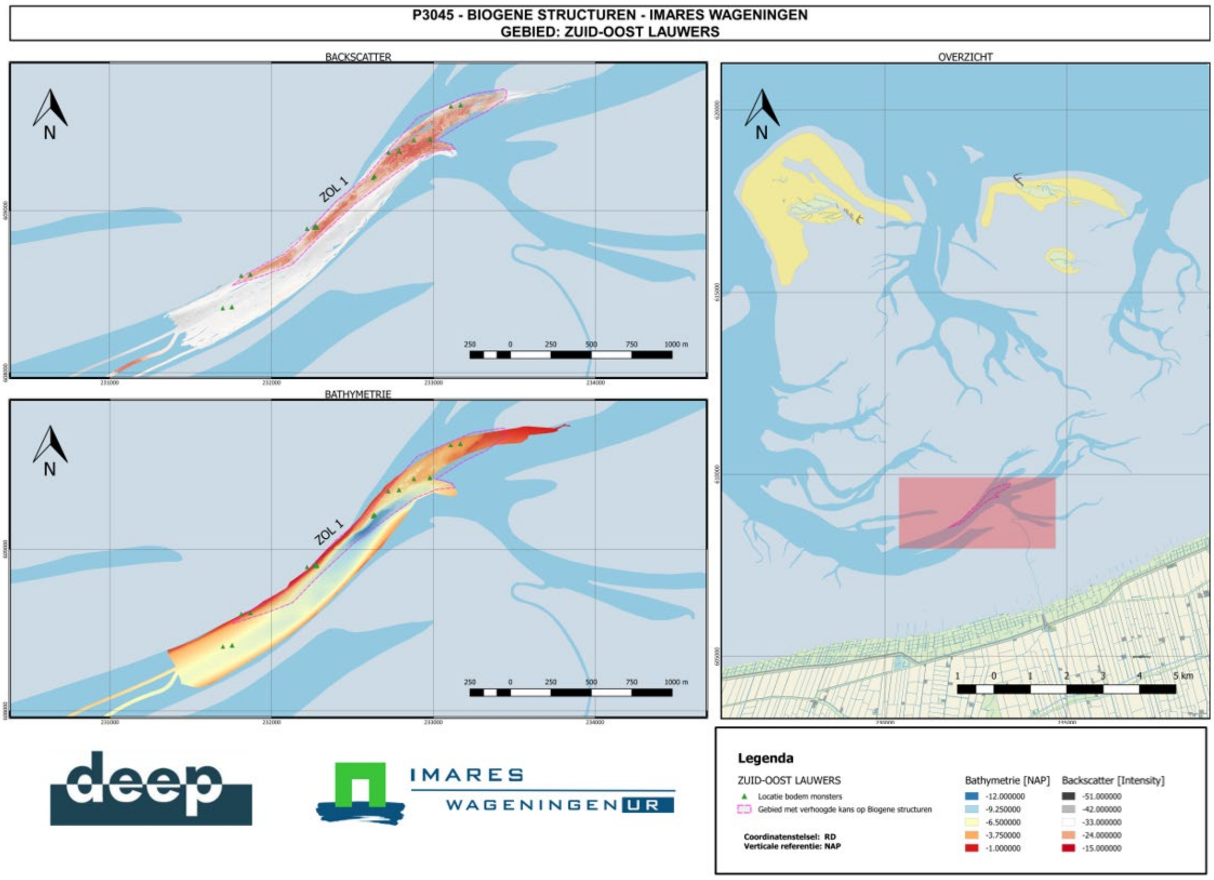
Figure 60. *Cummulated fishing intensity based on interpolated VMS-data of the year 2016 in the wadden sea (left) and Rottum (rigth) area*

Annex 2 Backscatter and bathymetry






P3045 - BIOGENE STRUCTUREN - IMARES WAGENINGEN
GEBIED: SPRUIT
BACKSCATTER
BATHYMETRIE
OVERZICHT
N
SPR 1
SPR 2
SPR 3
SPR 4
SPR 5
SPR 6
250 0 250 500 750 1000 m
1 0 1 2 3 4 5 km
deep
IMARES
WAGENINGEN UR
Legenda
SPRUIT
Spruit_monsters entities Point
Gebied met verhoogde kans op Biogene structuren
Coordinatenstelsel: RD
Verticale referentie: NAP
Bathymetrie [NAP]
-12.000000
-9.875000
-7.750000
-5.625000
-3.500000
Backscatter [Intensity]
-50.000000
-42.000000
-34.000000
-26.000000
-18.000000


P3045 - BIOGENE STRUCTUREN - IMARES WAGENINGEN
GEBIED: ZUID-OOST LAUWERS
BACKSCATTER
BATHYMETRIE
OVERZICHT
N
ZOL 1
250 0 250 500 750 1000 m
1 0 1 2 3 4 5 km
deep
IMARES
WAGENINGEN UR
Legenda
ZUID-OOST LAUWERS
Locatie bodem monsters
Gebied met verhoogde kans op Biogene structuren
Coordinatenstelsel: RD
Verticale referentie: NAP
Bathymetrie [NAP]
-12.000000
-9.250000
-6.500000
-3.750000
-1.000000
Backscatter [Intensity]
-51.000000
-42.000000
-33.000000
-24.000000
-15.000000

Annex 3 Species accumulation graphs

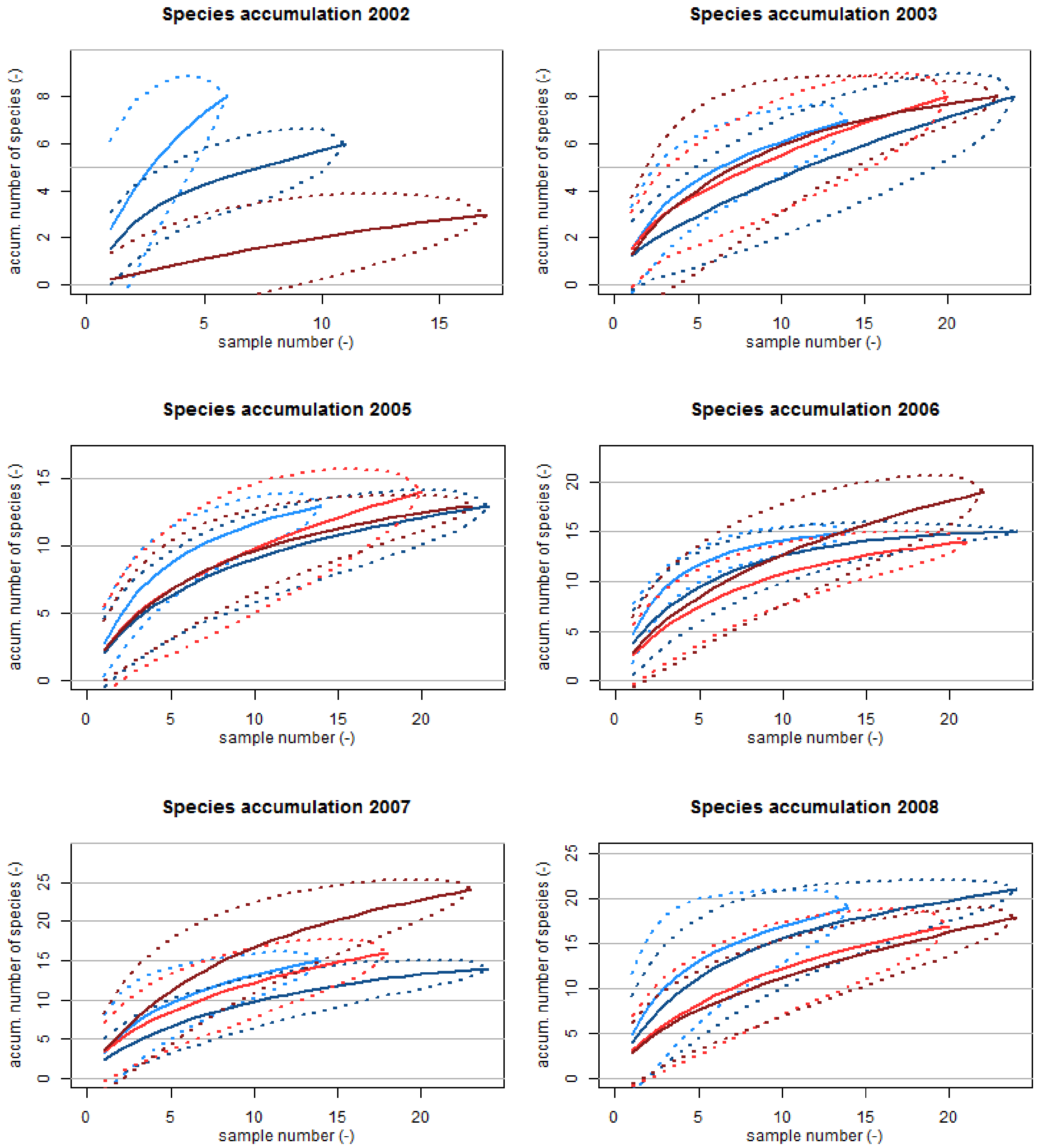


Figure 61*. Species accumulation graphs of Boschwad (light blue), Schild (dark blue), Spruit (light red) and ZO-Lauwers (dark red) for the years 2002 until 2008.*

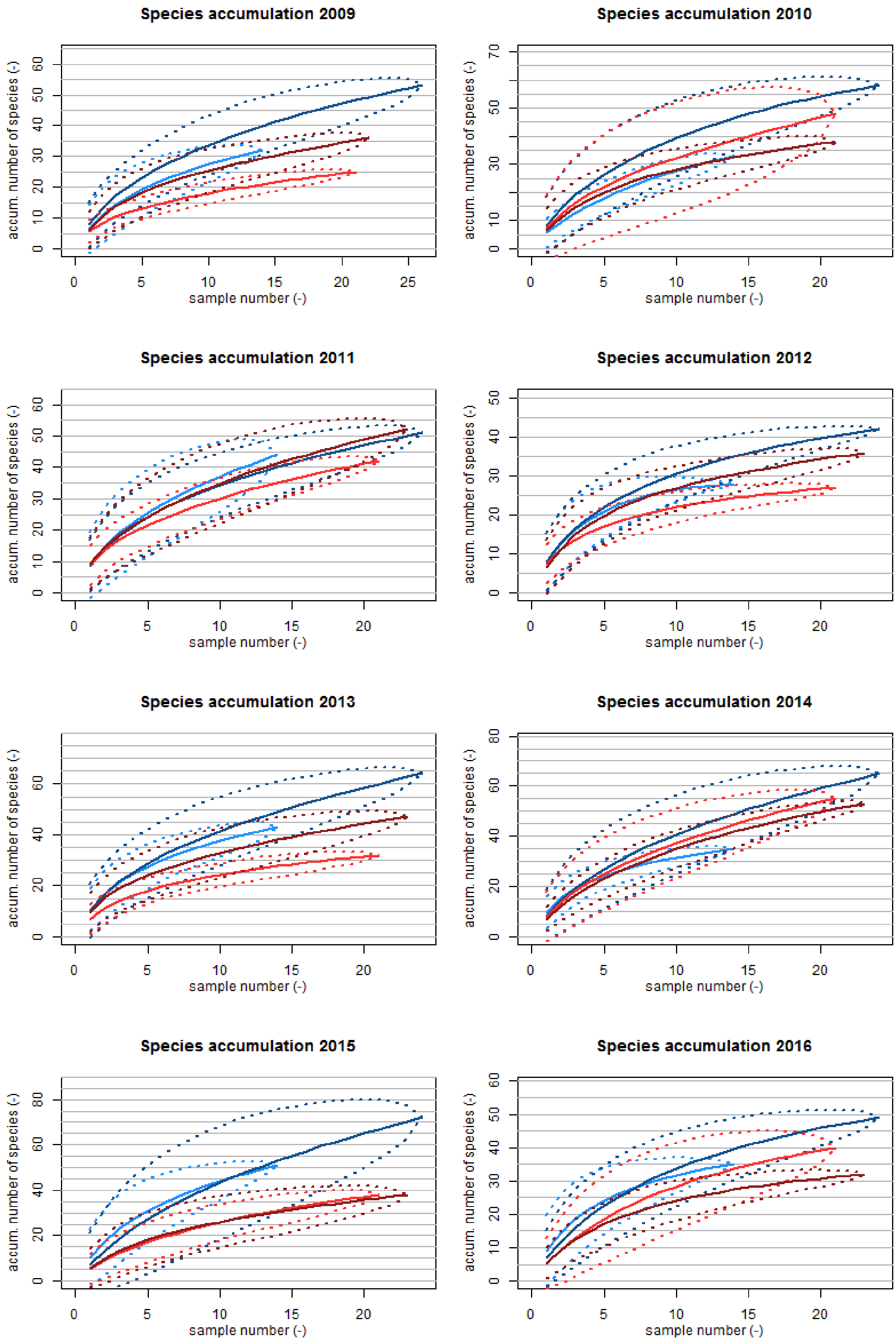


Figure 62. *Species accumulation graphs of Boschwad (light blue), Schild (dark blue), Spruit (light red) and ZO-Lauwers (dark red) for the years 2009 until 2016.*

Annex 4 CCA Van Veen grab samples

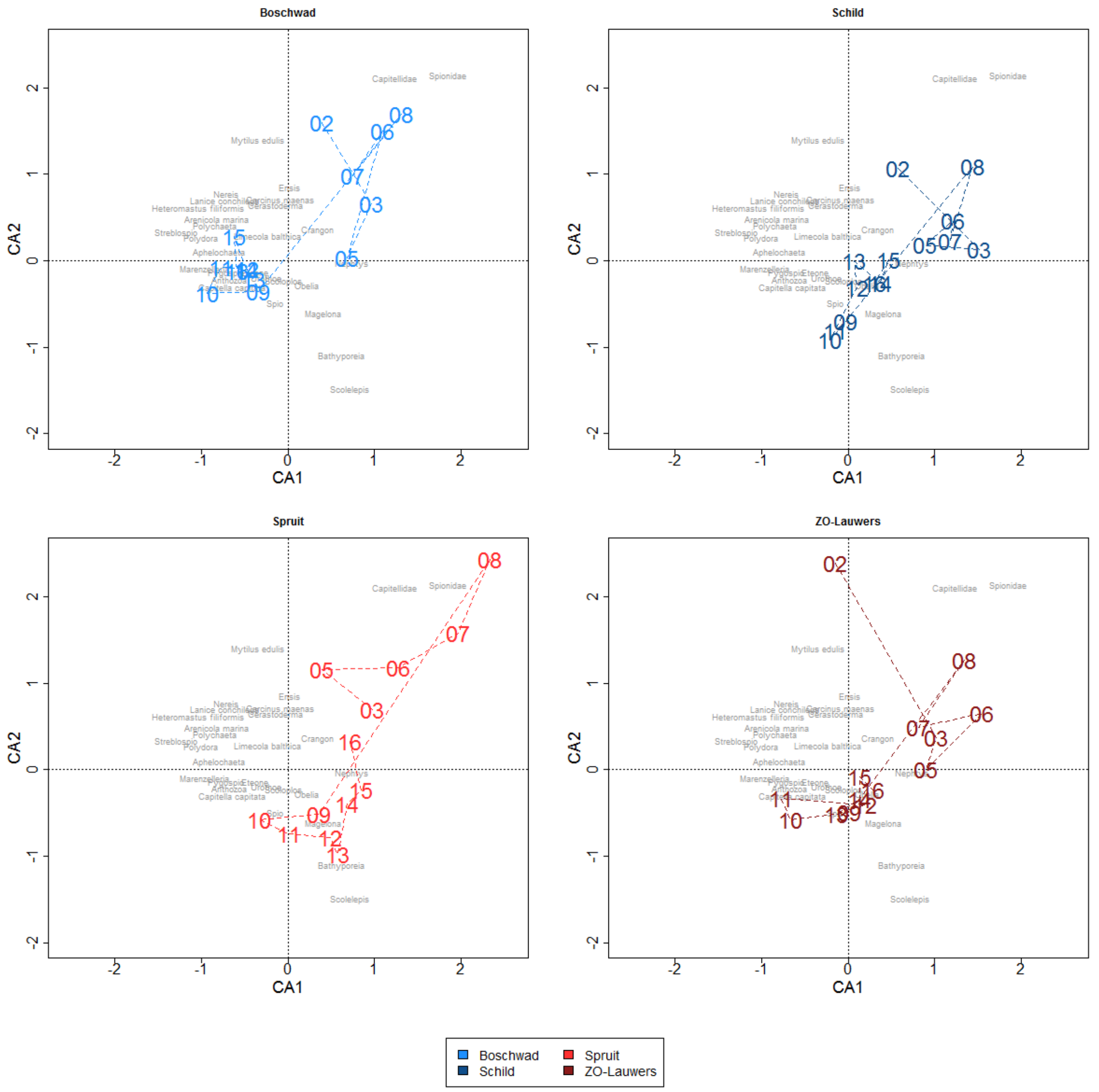


Figure 63. *CCA biplot per gully. Sampling years are depicted in red showin only the last two numbers of the millennium (20xx) and subsequential years are connected with a dashed line.*

Annex 5 Development phyla Van Veen

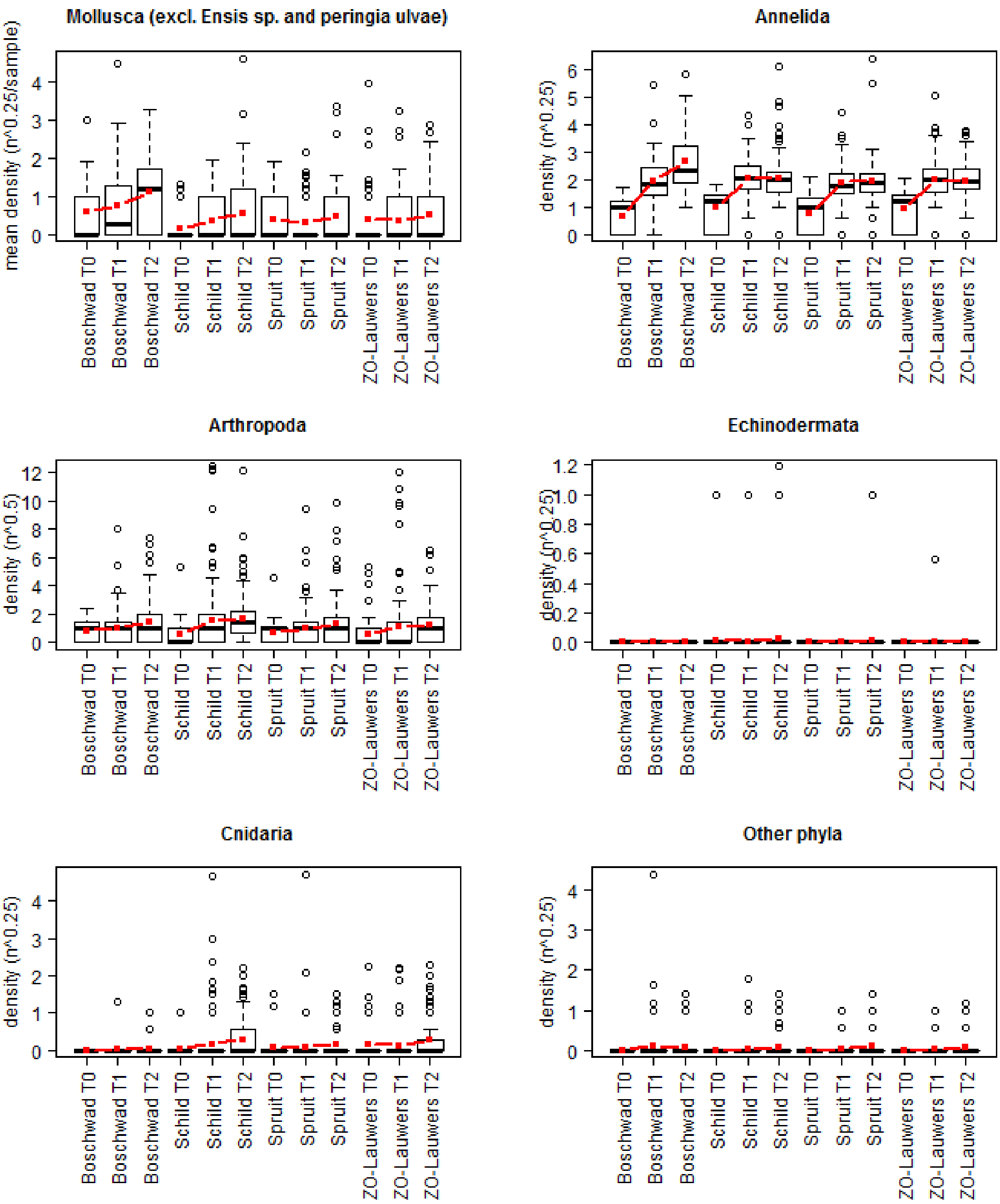


Figure 64. *Development of species density per phylum, sampling period (T0 –T1 and T2) and gully as encountered in the Van Veen grab samples.*

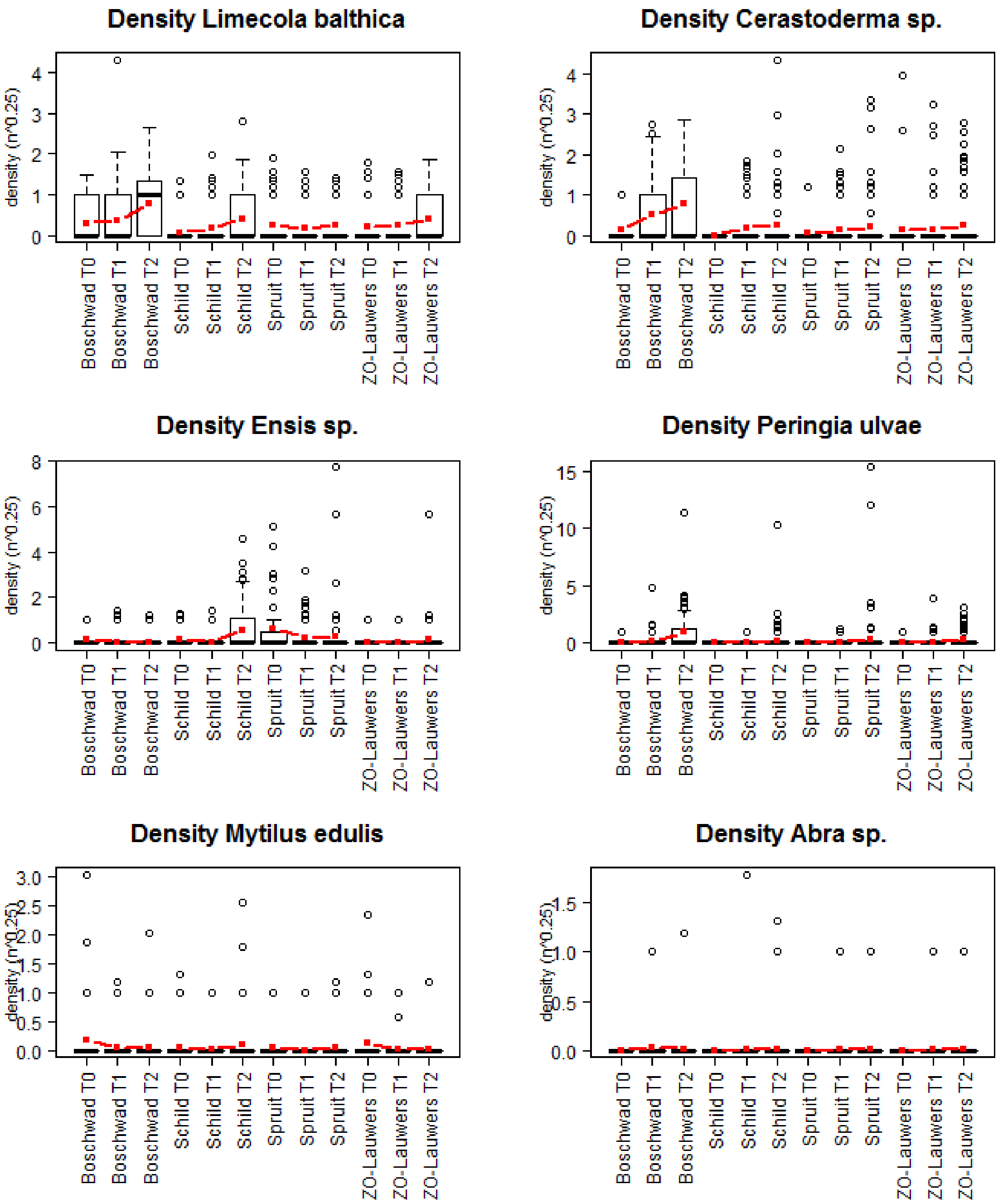


***Figure 65**. Development of the top six most important mollusc species (based on occurrences) per sampling period (T0 –T1 and T2) and gully as encountered in the Van Veen grab samples.*

Annex 6 Species composition v Veen grab samples

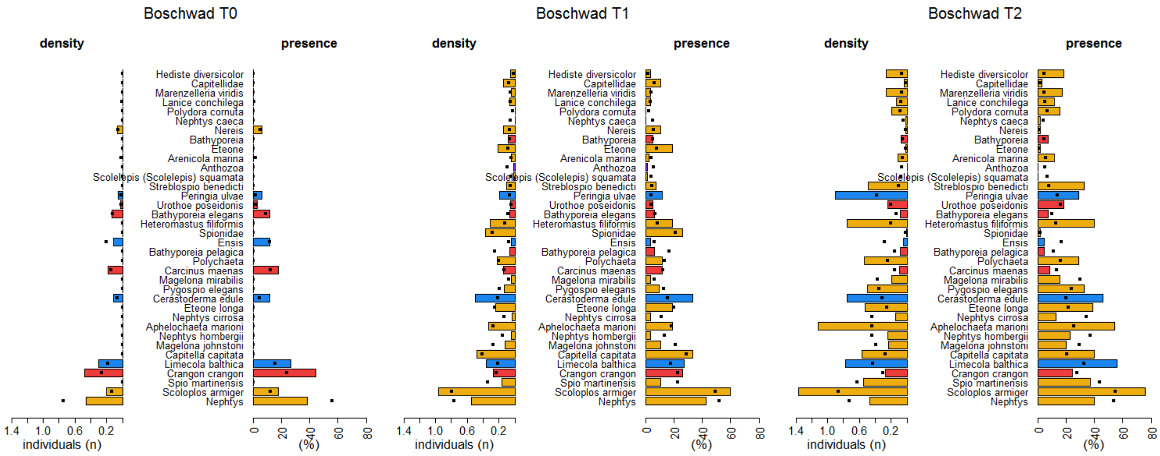


Figure 66. *Mean species densities (n) and occurrence in the Van Veen grab samples (%) taken in Boschwad at T0 (left), T1 (middle) and T2 (right) for all species that occur in at least 5% of the samples. Arthropod species (red bars), Mollusc species (blue bars), Annelida species (yellow bars) and species that belong to other phyla in grey bars. Black dots indicate the mean density and occurrence for all gullies.*

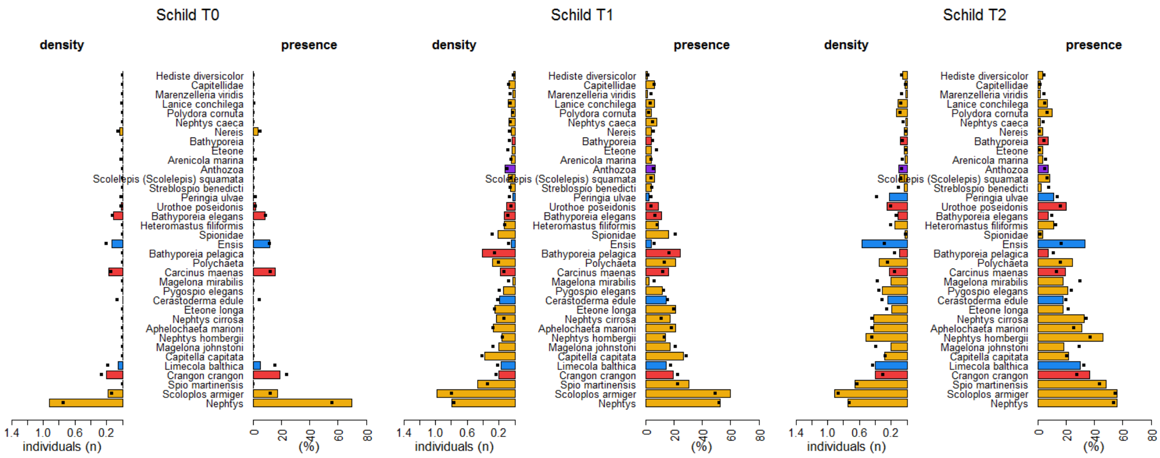


Figure 67. *Mean species densities (n) and occurrence in the Van Veen grab samples (%) taken in Schild at T0 (left), T1 (middle) and T2 (right) for all species that occur in at least 5% of the samples. Arthropod species (red bars), Mollusc species (blue bars), Annelida species (yellow bars) and species that belong to other phyla in grey bars. Black dots indicate the mean density and occurrence for all gullies.*

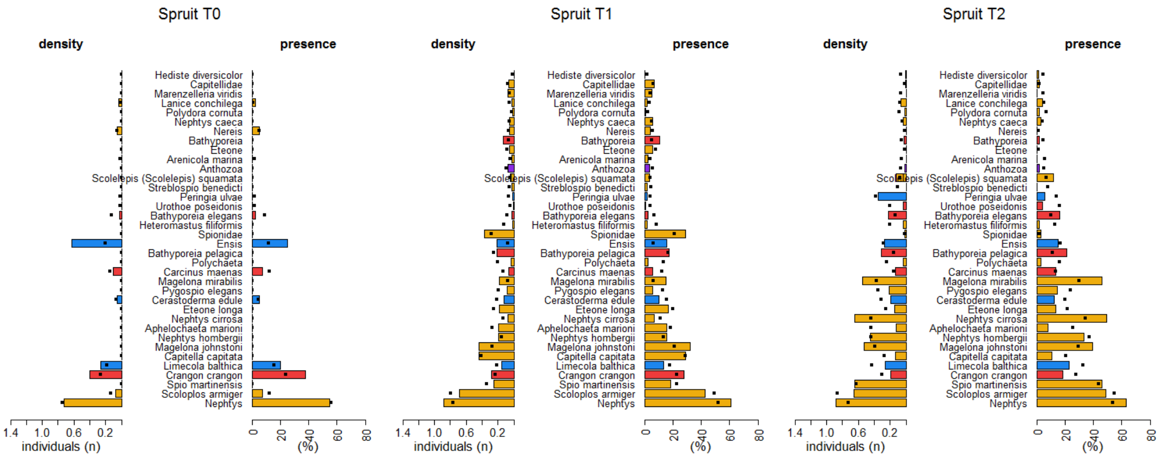


Figure 68. *Mean species densities (n) and occurrence in the Van Veen grab samples (%) taken in Spruit at T0 (left), T1 (middle) and T2 (right) for all species that occur in at least 5% of the samples. Arthropod species (red bars), Mollusc species (blue bars), Annelida species (yellow bars) and species that belong to other phyla in grey bars. Black dots indicate the mean density and occurrence for all gullies.*

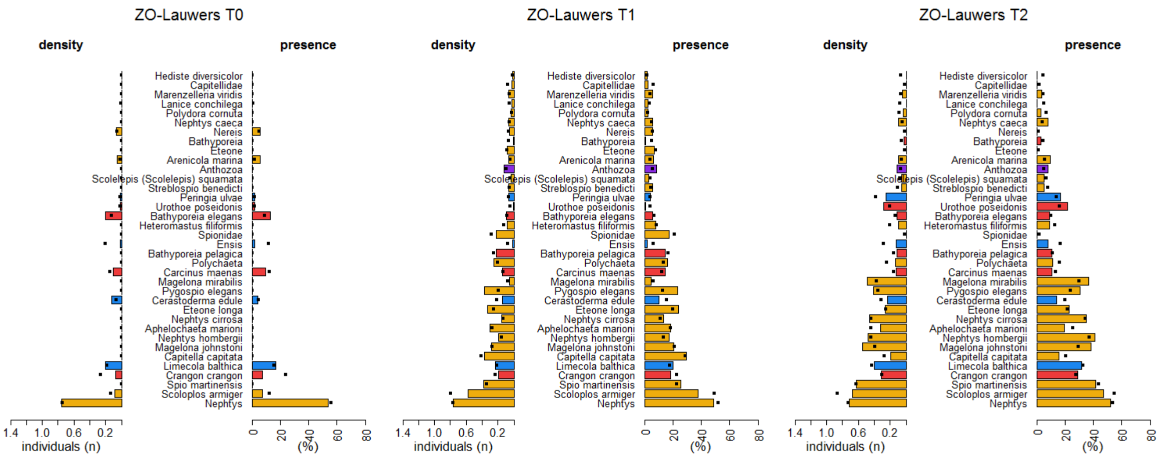


Figure 69*. Mean species densities (n) and occurrence in the Van Veen grab samples (%) taken in ZO-Lauwers at T0 (left), T1 (middle) and T2 (right) for all species that occur in at least 5% of the samples. Arthropod species (red bars), Mollusc species (blue bars), Annelida species (yellow bars) and species that belong to other phyla in grey bars. Black dots indicate the mean density and occurrence for all gullies.*

Annex 7 Species composition suction dredge samples

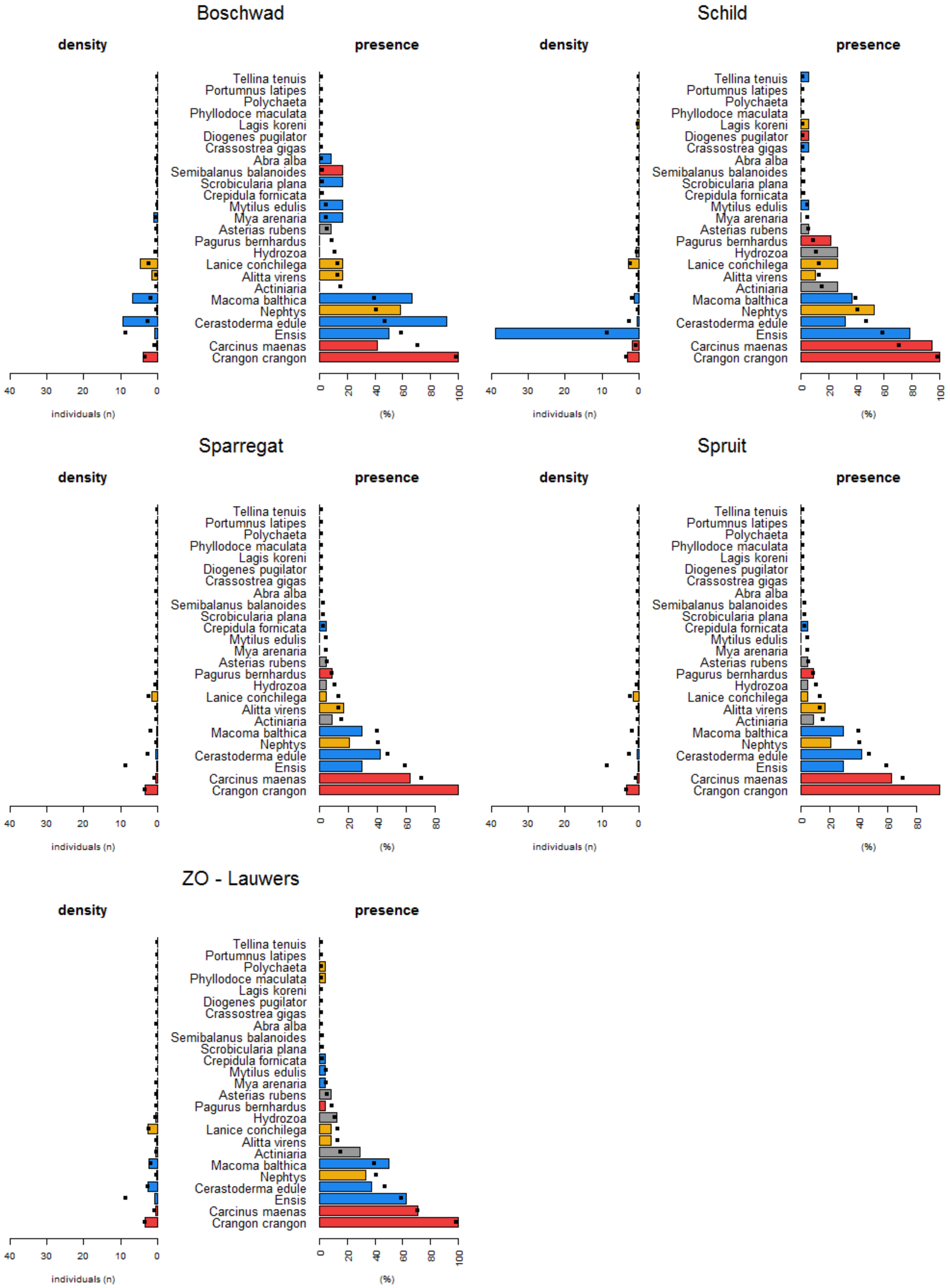


Figure 70. *Mean (sample) species density and occurrence in the suction dredge samples of 2016 per gully. Arthropod species (red bars), Mollusc species (blue bars), Annelida species (yellow bars) and species that belong to other phyla in grey bars. Black dots indicate overall mean values.*

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