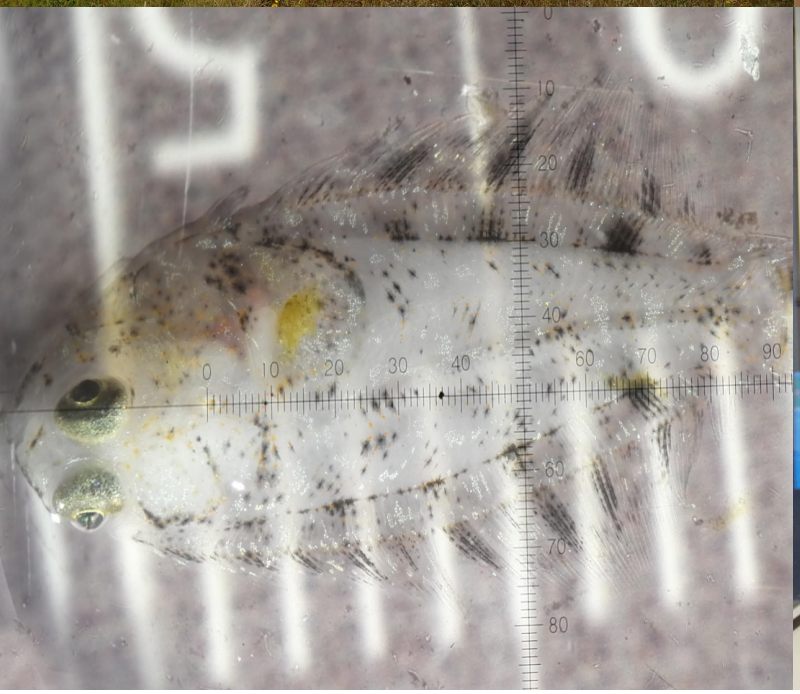

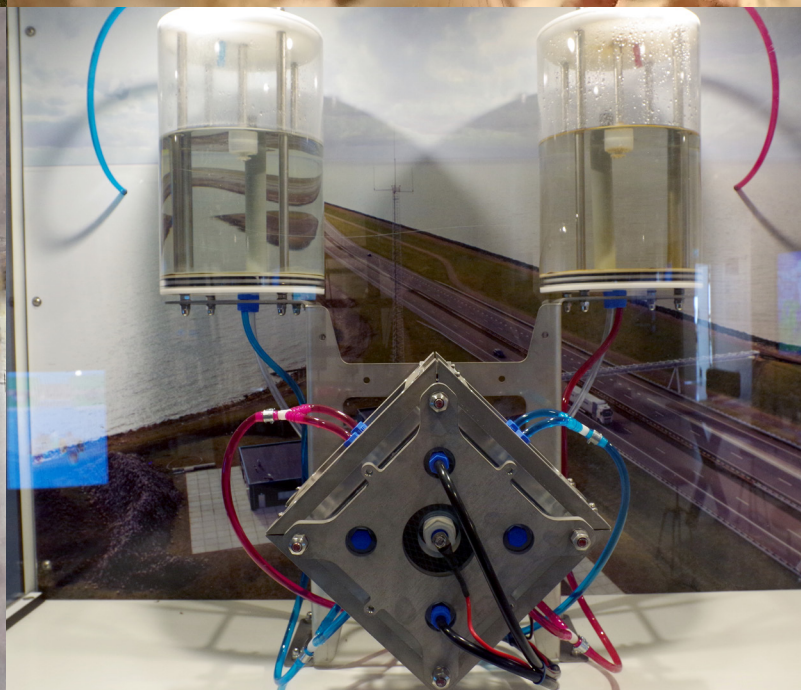

All Creatures Great and Small

Environmental Impact Study of Blue Energy, RED pilot Breezanddijk. Deliverables 2.4 & 2.5 of project OOBE (Onderzoek Omgevingseffecten Blue Energy).

L. van Walraven & Z. Jager

NIOZ Royal Netherlands Institute for Sea Research

The experimental Blue Energy facility of REDstack BV on the Afsluitdijk, Breezanddijk, with the NIOZ laboratory container used for analysis of samples on site. Photo: L. van Walraven

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L. van Walraven & Z. Jager

A joint publication together with ZiltWater Advies

Please cite as:
Van Walraven, L. & Jager, Z. (2020). All Creatures Great and Small - Environmental Impact Study of Blue Energy, RED pilot Breezanddijk. Deliverables 2.4 & 2.5 of project OOBE (Onderzoek Omgevingseffecten Blue Energy). NIOZ Report 2020-05. NIOZ Royal Netherlands Institute for Sea Research: Texel. 99 pp. https://doi.org/10.25850/nioz/7b.b.3

All Creatures Great and Small - Environmental Impact Study of Blue Energy, RED pilot Breezanddijk

Deliverable 2.4 & 2.5 of the OOBE-project (Onderzoek Omgevingseffecten Blue Energy - Effecten van inname systeem op organismen en omgekeerd)

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Colophon

Report number	NIOZ report 2020-05
Published by:	NIOZ Royal Netherlands Institute for Sea Research
Date:	02-05-2020
Authors:	Lodewijk van Walraven, Zwanette Jager
Title:	All Creatures Great and Small - Environmental Impact Study of Blue Energy, RED pilot Breezanddijk. Deliverables 2.4 & 2.5 of project OOBE (Onderzoek Omgevingseffecten Blue Energy).
Project name:	OOBE: Onderzoek Omgevingseffecten Blue Energy.
Tasks:	
Analyses & Reporting	Lodewijk van Walraven, Zwanette Jager.
Lab work	Lodewijk van Walraven, Zwanette Jager, Dennis Mosk, Evaline van Weerlee, Danila Uvarov.
Field work	Lodewijk van Walraven, Zwanette Jager, Dennis Mosk, Evaline van Weerlee, Danila Uvarov, Ewout Adriaans. On location assisted by REDstack BV: Simon Grasman, Erik Tippersma, Menno Eisma and colleagues.
To be cited as:	Van Walraven, L. & Jager, Z. (2020). All Creatures Great and Small - Environmental Impact Study of Blue Energy, RED pilot Breezanddijk. Deliverables 2.4 & 2.5 of project OOBE (Onderzoek Omgevingseffecten Blue Energy). NIOZ Report 2020-05. NIOZ Royal Netherlands Institute for Sea Research: Texel. 99 pp. https://doi.org/10.25850/nioz/7b.b.3

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1 Summary

Blue Energy produces energy from the controlled mixing of fresh water and sea water with ion-selective membranes using the RED-technology (Reverse Electro Dialysis). Untreated surface water is used, which requires treatment by different filtration steps to obtain water that is clean enough to be used in the process of energy production. The substantial volumes and the high filtration grade needed, raised concerns about the environmental impact of the water use by Blue Energy on the ecosystems of the Wadden Sea and Lake IJssel. This led to the initiation of the environmental impact study 'Onderzoek Omgevingseffecten van Blue Energy' OOBE (Grasman, 2016).

The OOBE-project aimed at studying and assessing the impact of the water use, and entailed different workpackages (WP): 1. inventory desk study, 2. monitoring of organisms, 3. shellfish pre-filtration, 4. impact assessment of upscaled installation, 5. communication and 6. coordination. This report presents the outcome of WP2, more specific the deliverables of WP2.4 and WP2.5 combined, wherein the possible impact of RED on (and by) aquatic organisms ('great and small') was investigated through monitoring and experiments at the Blue Energy testing facility at Breezanddijk from autumn 2016-2019. Different filtration steps were applied and compared in this report: micro-screen, rapid sand filter; shellfish prefiltration is reported in WP3.

Large plankton organisms were sampled with a Cintropur (filter) bypass in the installation, after passage of the protective pump casing, and before filtration on the microscreen. The macroplankton consisted of eggs and fish larve (in spring), crustacean larvae (crab and shrimp, in summer), larger copepods and worms (epitoke stage of Myrianida sp., larval stages of Spionid worms). The intake of macroplankton was compared with samples collected simultaneously with RV Stern outside the salt water intake in the Wadden Sea.

Seasonal patterns were recurring. Fish larvae of gunnel, plaice, flounder, sole, herring and gobies were found in the samples inside as well as in the Wadden Sea monitoring. Glass eel, Nilson's pipefish, three-spined stickleback and river lamprey were caught in the RV Stern samples but did not enter the Blue Energy installation. The protective pump casing thus prevented entrance of fish >30 mm. Perforated screens or wedgewire panels both performed rather well in keeping fish larvae, with hints of a slightly better performance of the wedgewire screens. Due to low larval concentrations, especially in 2019, the data are unfortunately inconclusive and more investigations are required.

For small aquatic organisms the possible impact of RED and vice versa was investigated for zooplankton that live their entire life in the water column (holoplankton) such as copepods, ciliates and rotifers, but also for organisms that only spend part of their life in the water column such as larvae of bivalves, crustaceans and fish. The occurrence of these organisms was monitored at several locations in the Blue Energy testing facility; in untreated feed water from Wadden Sea and lake IJssel, in effluent from the different filtration methods tested and in the brackish retour flow back to the Wadden Sea.

Community composition and seasonal patterns of Wadden Sea zooplankton at Breezanddijk were largely similar to those found in the 1970s when the last comprehensive survey of western Wadden Sea zooplankton was performed. The exception to this was a decrease in copepod biomass in summer and autumn. An important observation was that the inner western Wadden Sea the majority of the zooplankton biomass was contributed by meroplanktonic larvae of benthic organisms, with copepods only contributing to a minor part of the zooplankton biomass for most of the year except late spring.

Possible mortality of zooplankton by passage through the Blue Energy facility was investigated through *in situ* viability assessments as well as experiments where natural zooplankton communities were exposed to sudden salinity reductions of 1:1, 1:2 or 1:3 seawater:freshwater mixing ratio. At the current mixing ratio an estimated 72.4 % of zooplankton survived this osmotic shock, with mortality highest among smaller zooplankton such as rotifers and ciliates, and lowest for crustacean zooplankton. At higher mixing ratios the mortality of zooplankton increased. Mortality induced by factors other than osmotic shock were difficult to measure and varied between taxonomic groups. Using a conservative 50 % - 100% overall zooplankton mortality and an average generation time of one week, a scaled-up 100 MW Blue Energy power plant would cause an additional zooplankton mortality of 5 – 10% for the western Wadden Sea. This is a significant amount, but it is mitigated by the fact that the highest mortality is observed in the organisms with the smallest generation times (ciliates and rotifers).

The effectiveness of different filtration methods in retaining zooplankton varied. The drum sieve, used for most of the monitoring period, did not always retain all zooplankton smaller than the mesh size. Consequently, further downstream fouling by a large variety of macrofauna was observed, either settled on pipe surfaces (tunicates, hydroids, barnacles, ciliates) or living in sediment layers (bivalves, gastropods, worms). Shellfish filtration did not appear to influence zooplankton composition in the feedwater as expected. Rapid Sand Filtration effectively filtered out all but the smallest zooplankton organisms.

2 Acknowledgements

We would like to thank first and foremost Evaline van Weerlee and Dennis Mosk, who took and analysed most of the samples for WP2.4 (small organisms), on occasion assisted with the Cintropur sampling, and also helped with the sampling for WP2.5. Danila Uvarov is also thanked, for his help with sampling and the survival experiments. The REDstack operators Erik Tippersma and Menno Eisma are thanked for their assistance at Breezanddijk, and for making early starts of the Cintropur bypass which enabled the sampling of larger volumes per day. Chris Coutinho supplied REDstack data and invented a way to calculate the Delta indicator. Simon Grasman, with his technical overview, is thanked for all assistance, discussions and advice given during the project. Henk van der Veer and Peter Herman are thanked for their advice and comments on the research plans and on the finalisation of this report. And last, but not least, we like to thank Rik Siebers for his role in managing the OOBE project.

3 Introduction

Blue Energy produces energy from the controlled mixing of fresh water and sea water with ion-selective membranes using the RED-technology (Reverse Electro Dialysis). Untreated surface water is used, which requires treatment by different filtration steps to obtain water that is clean enough to be used in the process of energy production, involving a membrane technology that is vulnerable to fouling. Fouling can either be from clogging due to colloidal material, biological activity or any other substance that passes the pre-treatment and which hinders the production of energy.

The volumes of water used in a commercial RED-installation will be in the same order of magnitude as those of conventional power stations using once-through cooling. Due to the substantial volumes and the high filtration grade needed, concerns were expressed about the environmental impact of the water use by Blue Energy with the RED-technology on the ecosystems of the Wadden Sea and Lake IJssel (Leeuwarder Courant, 1 september 2012). This led to an exchange of ideas which resulted in the initiation of the applied research project and environmental impact study 'Onderzoek Omgevingseffecten van Blue Energy' OOBE (Grasman, 2016).

The OOBE impact study was carried out at a small-scale pilot installation, founded by REDstack in 2014, as this company has the aim of developing and selling the RED-technology as a sustainable energy source, the so-called 'Blue Energy'. This Blue Energy installation uses two water sources with differing salinity; Lake IJssel (fresh water) and the Wadden Sea (salt water), at this site (Breezanddijk) only separated by the closure dam (Afsluitdijk). Both feed water sources are brought together in cells in which the two water types are only separated by ion-selective membranes, which induces a controlled ion-transport in opposite directions for positively and negatively charged ions. This results in a current that can be transformed into electricity.

Membranes, anion and cation selective, are alternatingly piled in so-called stacks, with a narrow spacing of 100-500 µm between the membranes. Both sources of feed water are supposed to flow through those narrow inter-membrane spaces, which implies that the feed water has to receive treatment or filtration to remove its load of naturally occurring particles (clay, silt, suspended matter) and organisms (algae, diatoms, zooplankton). Both feed water sources (fresh and salt) are mixed at the end of the process and are returned into the marine surface water as a brackish discharge.

The operational water flows can be very large, as a rule of thumb one should consider 1 m^3/s of fresh water and 1 m^3/s of salt water to produce 1 MW of energy (at a mixing ration of 1:1). For a projected installation of 100 MW, this would imply water flows of 100 m^3/s both from fresh and salt origins, comparable in size to the water flows of the Thames (80 m^3/s) or Scheldt (120 m^3/s) estuaries (Goorden et al., 2017). Even if a future installation would be half the maximum projected capacity, it would still need considerable volumes (per second) of treated water. The pilot installation has a modest capacity, however, and uses a water flow up to 250 m^3/h (<0.07 m^3/s).

This type of water treatment, in combination with the large volumes, raises concerns on the impact on the ecosystems, and in particular for the Wadden Sea. The fresh water source would, if not used for Blue Energy, be discharged anyway to the Wadden Sea through the sluices of Kornwerderzand and Den Oever, leading to mortality of the discharged fresh water organisms. Addition of a Blue Energy installation does not change that situation. The main concern is therefore about the impact that withdrawal and treatment of large quantities of water may have on the Wadden Sea ecosystem through possible additional mortality of organisms.

The OOBE-project aims at studying and assessing the impact of the water use, and entailed different workpackages (WP): 1. inventory desk study, 2. monitoring of organisms, 3. shellfish pre-filtration, 4. impact assessment of upscaled installation, 5. communication and 6. coordination.

This report presents the outcome of WP2, more specific the deliverables of WP2.4 and WP2.5 combined, wherein the possible impact of RED on aquatic organisms was investigated through monitoring and experiments at the Blue Energy testing facility at Breezanddijk.

Possible impact of RED on aquatic organisms

The WP1-desk study described the potential environmental impact of the water intake, which involves main processes of impingement, entrainment and fouling by organisms from the intake water in the installation (Jager & Van Walraven, 2017).

Impingement is the process in which organisms are filtered from the water intake by screens and the filter residue is subsequently returned to the environment. During impingement, organisms can be exposed to mechanical damage, physical stress and possible suffocation. In the case of Blue Energy, additional osmotic stress can occur when fresh and marine water are combined. Survival after impingement is highly species-specific and is also determined by site-specific condition, such as the type of screens and the functioning of the return system, as well as environmental conditions and the behaviour and condition of the organisms. Several aspects, such as species composition and seasonal patterns in the occurrence and impingement of meroplankton ('large' organisms) and zooplankton ('small' organisms), were investigated in WP 2.4 of the Blue Energy environmental impact study.

Entrainment is the process in which organisms are not removed from the water intake but pass through the installation. In this way, they can be exposed to several types of stress: mechanical damage, damage by pressure differences, chemical stress, stress by temperature or osmotic differences, suffocation or predation. In a survey of industrial cooling impact studies, entrainment survival up to 90% was found to be possible, depending on species and circumstances. The most significant mortality inducing factors in industrial cooling, temperature increase (ΔT) and chemical stress, are expected to have a lower impact in RED systems. The temperature increase caused by the RED process is less than 0.17 °C. The ΔT in RED will thus depend mainly on the difference in water temperatures of the fresh- and marine water source. Chemical treatment is not used in RED. In the RED-process, the exposure to osmotic changes and mechanical damage are expected to be the most likely causes of damage and mortality. These aspects were investigated further in WP2.5 of the Blue Energy environmental impact study by monitoring of zooplankton mortality within the experimental facility and experimentally simulating the osmotic shock that occurs during the passage of the installation and to estimate the mortality rate that is caused by it. Different mixing ratios of fresh and salt water have been tested.

Research questions

The impact study focussed on the impingement and entrainment, the latter including fouling inside the installation (i.e. after the rotating drum filters) and the survival of small organisms. Since impingement and entrainment are dependent on the presence of organisms in the environment, the monitoring also paid attention to the background concentrations of organisms in the Wadden Sea by sampling from a research vessel near Breezanddijk.

The research questions were investigated by monitoring and studying the composition, abundance and seasonal patterns of zooplankton organisms impinged in the intake system of the REDstack installation at Breezanddijk from November 2016 to July 2019.

The main research questions, investigated in WP 2.4 and WP 2.5, are:

1. Which aquatic organisms are impinged and entrained in the Blue Energy pilot facility at Breezanddijk? And how does the impingement relate to the presence of organisms in the vicinity of the seawater intake (Stern samples).

This has been investigated for the following groups:

a. Fish and fish larvae
b. Macro-invertebrates and their larvae
c. Zooplankton

2. Does the addition of wedgewire panels (with 0.5-1 mm slits) to the outer pump casing of the seawater intake lead to an improved performance with regard to the impingement of macrofauna compared to the conventional intake screens with 3 mm round holes?

3. How do impingement and entrainment of zooplankton ('small' organisms) and sediment differ between different filtration techniques:

d. Rotating drumscreen (micro-screen)
e. Sand filter (RSF)
f. Shellfish pre-filtration

4. Which fraction of entrained zooplankton dies because of the osmotic shock experienced when fresh water and salt water are mixed?

a. At different temperatures (seasonal influence)
b. At different mixing ratios of fresh and salt water up to 1:3 salt:fresh

5. What is the influence of factors other than osmotic shock on mortality of zooplankton?

Impact assessment

The results of WP2.4 and WP2.5 should provide the knowledge that is needed to draw conclusions on the environmental impact of impingement and entrainment processes. These are assessed quantitatively in WP4 for an up-scaled installation.

Guidance

This report is a combination of WP2.4 (organisms at the intake) and WP2.5 (small organisms in the installation), as distinguished in the original project plan. However, at a later stage it was decided to join these into Deliverable D2.4/2.5 which is this report "All creatures great and small".

The description of the RED installation at Breezanddijk (Chapter 4) and Environmental parameters (Chapter 5) were combined for both work packages.

More detailed descriptions of the RED installation can be found in D2.2 by REDstack.

Chapters 6 and 7 deal with the intake of large organisms (WP2.4): the RV Stern sampling (Chapter 6) was needed to compare the intake of large organisms (Chapter 7) with the background concentrations present in the Wadden Sea. The large organisms were sampled by different methods than the small organisms. Chapter 8 deals with the small organisms' monitoring and survival experiments. Each Chapter contains its own Methods, Results, Discussion and Conclusions and occasional Recommendations.

4 The RED installation at Breezanddijk

The RED installation at Breezanddijk is the largest and most ambitious project of REDstack. The experimental facility was officially opened on the 26th of November, 2014 by H.M. King Willem Alexander of the Netherlands. At this testing facility, the RED-technology is applied and tested with untreated water. The aim is to investigate and optimize the performance of the RED installation with *in situ* fresh and salt water in order to enable upscaling of the RED technology and in the end develop a commercially sustainable energy production (www.redstack.nl).

At Breezanddijk, both water sources are amply available and only separated by a closure dam (Figure 1).

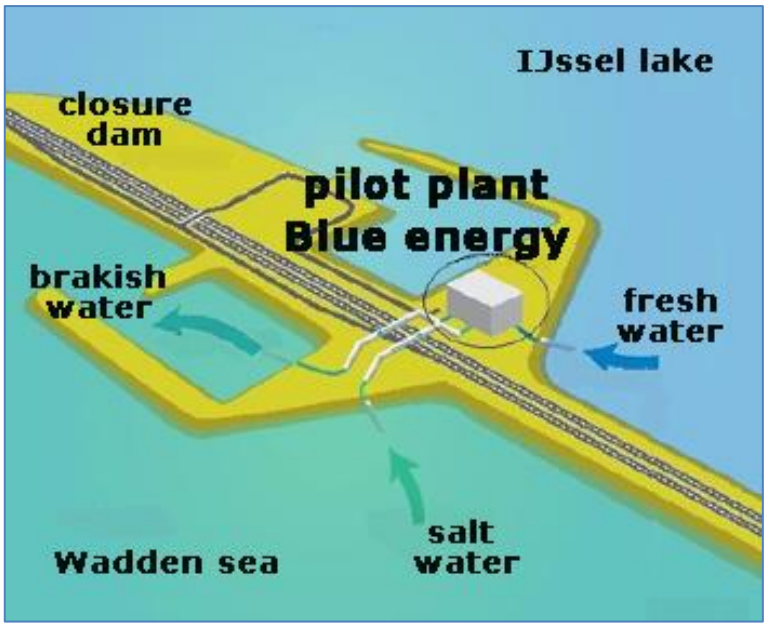


Figure 1. The Blue energy pilot plant at Breezanddijk (www.dutchwatersector.com).

4.1 Water intakes

Feed water for the RED power plant is drawn in from the Wadden Sea (seawater) and Lake IJssel (fresh water), see the schematized overview of the installation in Figure 2. The feed water sources (Wadden Sea and Lake IJssel) are shown at the left side of the figure, the different installation components (sieves, stacks, buffer tanks) inside the building are shown inside the blue transparent square at the right.

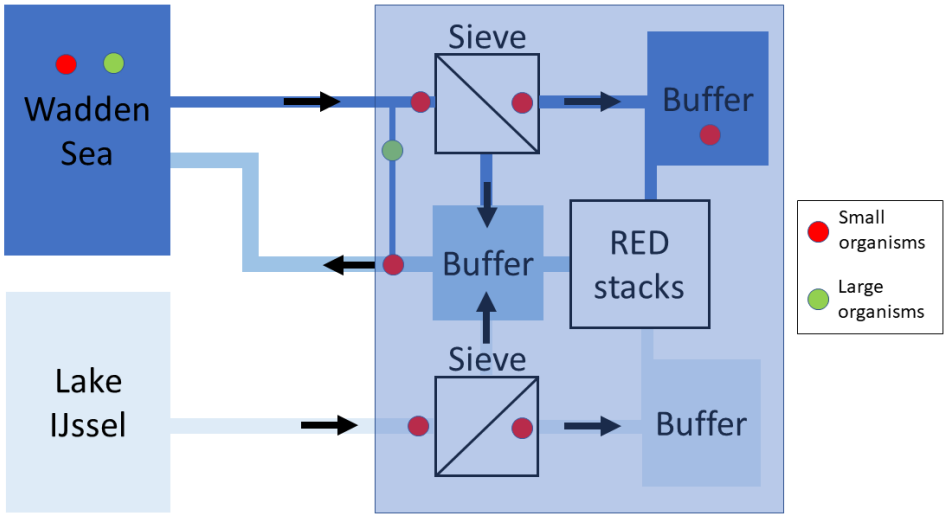
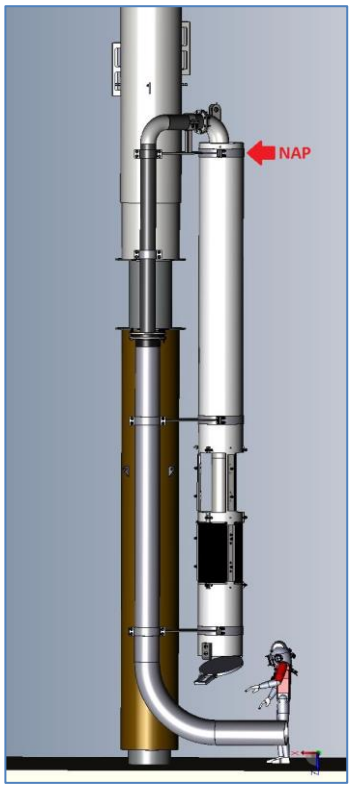


Figure 2. Simplified diagram showing location of the different sampling points for small (red) and large (green) organisms. Everything inside the installation building is included in the blue transparent square.

The salt water is pumped from the Wadden Sea at a location just outside the dams of the service harbour of Breezanddijk, the fresh water at a nearby location in the lake, both c. 75 m offshore. At the fresh water intake the water depth is c. 4 m relative to NAP (Amsterdam Ordnance Datum), for the seawater intake this is c. 9 m to NAP.

The water pump is installed in a casing that is attached to a fixed pole. This casing acts as a first filtration step and protects the pump from large debris (see picture). The water level inside the cover is measured with a pressure sensor.

See for detailed information D2.2.



4.2 Filtration steps

A pre-study of possible filtration methods concluded that filtration by a wedge wire in combination with a micro screen seemed the most obvious option at this site (Goorden et al., 2017). The first screening step is expected to keep out debris and large organisms, such as juvenile and adult fish as well as crustaceans (shrimps and crabs). Small organisms are not removed: organisms that are able to pass the pump cover are subsequently screened in the installation. After being drawn in by the pumps, with the protective casing of perforated or wedge wire screens, the feed water enters the installation and is further cleaned by using rotating fine meshed rotating drum sieves (Hubert micro-screens). Additional filtration systems were applied and tested, such as Rapid Sand Filtration (RSF) and biofiltration by shellfish (Wijsman & Smaal, 2017). All of these steps have been monitored in less or more intensity during 2016-2019.

4.2.1 Pump casing

On the fresh water side, the original pump casing is installed, made of a PVC pipe with a diameter of 630 mm which is equipped with filter slits of 3 by 100 mm over the full circumference of the pipe and over a height of 1.5 m. The casing is installed in such a way, that the filter slits are at a height of +1 to 2.5 m relative to the bottom of the lake. Previous to the start of the monitoring program, the installation was out of use between the end of July and the end of October, 2016. During that period, the (salt) water intake was adapted and a new pump casing had already been installed. This casing is also made of a PVC pipe with a diameter of 630 mm. But now it has six large rectangular openings, two times three of these equally spread over the circumference of the pipe. The pump casing is installed in such a way, that the intake openings are at a height +2.5 to 4.5 m relative to the sea floor. In these openings different kind of filter screens can be installed, during the course of the monitoring period two types have been installed.

At first these were screens made of perforated metal plates with small holes of 3 - 3.5 mm (Figure 3). This type of casing was in use between November 2016 (at the start of the monitoring) and October 2018.

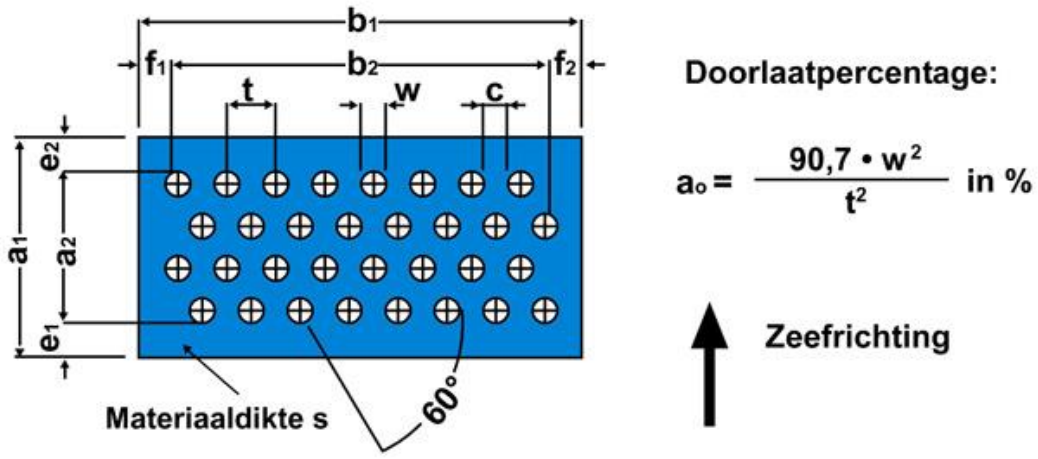


Figure 3. Perforated pump cover applied on the filter tube at the seawater intake.

Later these have screens have been replaced by screens made from wedge wires (as of October 4th, 2018 until the end of the monitoring mid 2019). Wedge wire panels have V-shape slots with narrow opening on the outside and wide opening inside the panel (Figure 4). This reduces clogging: large particles are kept outside the screen, whereas small particles pass through the narrow opening and enter the screen. The screen gives a relatively small resistance and delivers a high flow rate at a given screen size.

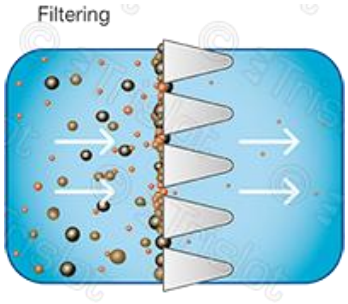
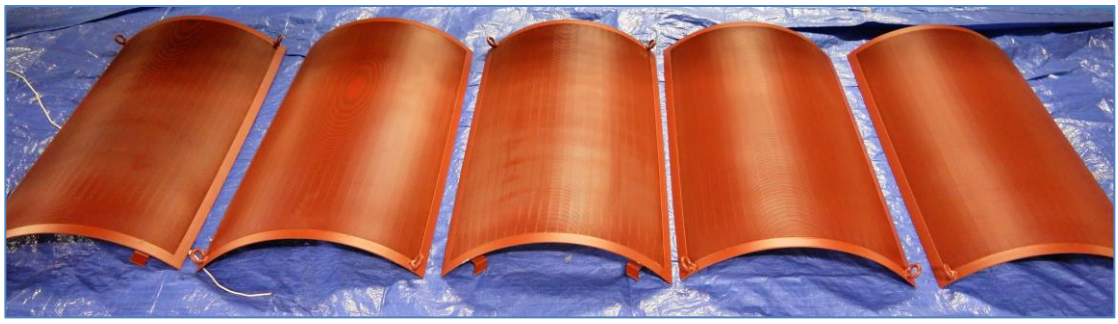


Figure 4. V-shaped surface profile of a wedge wire screen (www.trislot.be).

To limit the growth of barnacles and other organisms on the pump casing and screens of the sea water, all components are painted with Jotun Seaquantum S antifouling. The two sets of panels that surrounded the salt water pump had after-coating wire slit openings of 0.5 mm and 1 mm, respectively (see photo).



The intake velocity for perforated screens was 0.10 m/s, for the wedge wires this is 0.09 m/s, both at a flowrate of max. 250 m^3/h. However, during the entire monitoring period the most common flowrate was 100 m^3/h, lowering the intake velocity even further. The current velocity at the intake (pump casing) was thus always below the threshold of 0.3 m/s, which is considered BAT (Best Available Technology) and which is a condition in the water permit of RWS. The pumped water is transported via pipes (with an elevated crossing for the salt water pipe over the dike and the A7 highway).

4.2.2 Micro screen

The drum sieves are covered with a mesh cloth (initially 23 µm, later changed to 40 µm and in February 2019 returned to 20 µm). When the cloth of the drum sieve was replaced, attention was also paid to improving the sealing of the drum rotation axis and of the screen edges. The sieve allows only very small organisms to pass, while larger organisms are retained on the cloth. The drum sieves rotate with a rotation speed of 43-44 s for one complete rotation. The micro screen is cleaned by spraying pressurised filtered water from the outside, the spraying is instigated by detected water level differences between in- and outside of the drum sieve. Because clogging rate varies with varying organism and sediment density, drum sieve cleaning intervals are variable.

Within the drum sieve, the residue removed from the sieve is collected in a through, which discharges into the brackish buffer tank (B in Figure 3). The brackish buffer tank receives the effluent of both the fresh water and the salt water sieves and its contents are periodically discharged in the Breezanddijk harbour.

Details and a cross-section of the drum sieve can be found in the deliverable of WP 2.2.

4.2.3 Rapid Sand Filter (RSF)

An inventory of possible intake and filtration systems proposed Rapid Sand Filtration (RSF) as a possible filtration solution (Goorden et al. 2016). It was recommended to test

this system in a small-scale trial first. Therefore, a trial with an experimental RSF was initiated in November 2018, where a small part of the untreated Wadden Sea intake water was diverted to a test container, with three RSF filter columns in parallel. The filtered water, together with the water from a possible backwash, was returned to the untreated intake water pipe further downstream.

The RSF test container (supplied by Xylem) consisted of three filter columns (numbered: 500, 520 and 540). In the first test period (November - January) they were filled with three different kind of filter media: sand, gravel and dual media hydro-anthracite on top of sand, for columns 500, 520 and 540 respectively. Based on the filter performance, the dual media filter was selected for further testing. This involved testing the effect of different filtration velocities, with the filtration velocity being the flowrate of water treated per square meter of surface area of the filter. The total flow of these three filter columns together is ca. 1.5 m^3 per h. Either when the set point for maximum allowed head loss over the filter media or the set point for runtime of the filter is reached, whichever is reached first, a backwash is initiated to clean the filter media.

The backwash procedure of the RSF is described in detail in D2.2. At the beginning, the water column above the sand bed is drawn down, including part of the organisms that have accumulated above the filter bed. At the end of the backwash cycle, the backflush expands the filter bed which causes the filtered organisms to be released and carried away with the flush water. After this, the filter column settles and separates into the original layers. The flush water flows upwards and is released via the overflow into the backwash basin.

The contents of this collection basin (containing water from the overflow and the backwash) is redirected into the saltwater intake and at a later stage passes the sampling point for large organisms. The consequence was, that the samples of large organisms were occasionally receiving a partial influx of the overflow/backwash (as evidenced by the finding of hydro-anthracite grains in the samples). Although the water volume is small, organisms may have concentrated on the filter during the run time, which may be up to >24 hours. This may have influenced the sampling of large organisms, which is the reason why the RSF was briefly described in this report. Data were incomplete and did not allow analyses of the RSF-influence on large organisms. The interference occurred between 28 February and 8 May; after that date the RSF was decoupled and did not interfere with the sampling of large organisms anymore.

4.2.4 Shellfish filtration

In 2019 an experimental setup was tested, whereby blue mussels *Mytilus edulis* were used to pre-filter the Wadden Sea intake water. The water for the shell fish filtration was diverted from the main seawater intake before the sample point of large organisms. And since the water from the shell fish filtration is directly fed to the brackish water tank, there is no interference with the other monitoring programs.

Shellfish filtration was tested in two consecutive runs; the first in spring (March 15 - May 21), with a flow rate of 15 $m^3\ h^{-1}$, the second in summer (June 12 - September 11) with a flow rate of 5 - 15 $m^3\ h^{-1}$. The experimental biofiltration using mussels (*Mytilus edulis*) was the topic of WP3 of the OOBE-project and is reported separately in the Deliverables of WP3.

4.3 Sampling points selected for monitoring

The project plan WP2, distinguishes between the monitoring of organisms in the intake system (Task 2.4) and the monitoring of organisms in the installation (Task 2.5): organisms are monitored before (Task 2.4) or after (Task 2.5) passing the micromesh rotating drum sieve. A further distinction was made between small and large organisms,

which are monitored in different ways (see Methods large organisms, small organisms). Small organisms were monitored by the Royal Netherlands Institute for Sea Research (NIOZ), larger organisms by ZiltWater Advies (ZWA).

Throughout the RED installation, valves were installed (open circles “M” in Figure 5) where it is possible to connect a hose and take water samples. These sampling points are labelled V7XX with V71X used for fresh water, V72X for seawater, V73X for brackish water and V75X/V76X for the RED stack streets. The sampling points for fresh water and seawater drum sieve residue (V715 and V725) were modified by REDstack to allow easy discharge of filtered water. Additionally, it is possible to sample the effluent of the drum sieve directly after filtration by scooping water from the inside of the drum sieve.

The impinged filter residue tapped via sampling point V725 would be difficult to relate the sampled volume to the water flow of the intake, given the irregular flushing of the sieve. Therefore, at the start of the monitoring it was decided to use a by-pass from the seawater intake to the brackish water outflow for the sampling of larger organisms. For this bypass water a hose is connected to the untreated seawater sampling point (V722) and a Cintropur® canister filter with 1 mm mesh size. After filtration the water passes a flow meter, counting the total volume passing the flow meter, and is subsequently returned to the brackish water discharge pipe via a hose connected to sampling point V731 (see Figure 5, orange marking). Both the large and small organisms from salt water were collected at sample point V722, just before entering the drum sieve (see Figure 5). Small organisms were also sampled in the fresh water intake (a.o. V712).

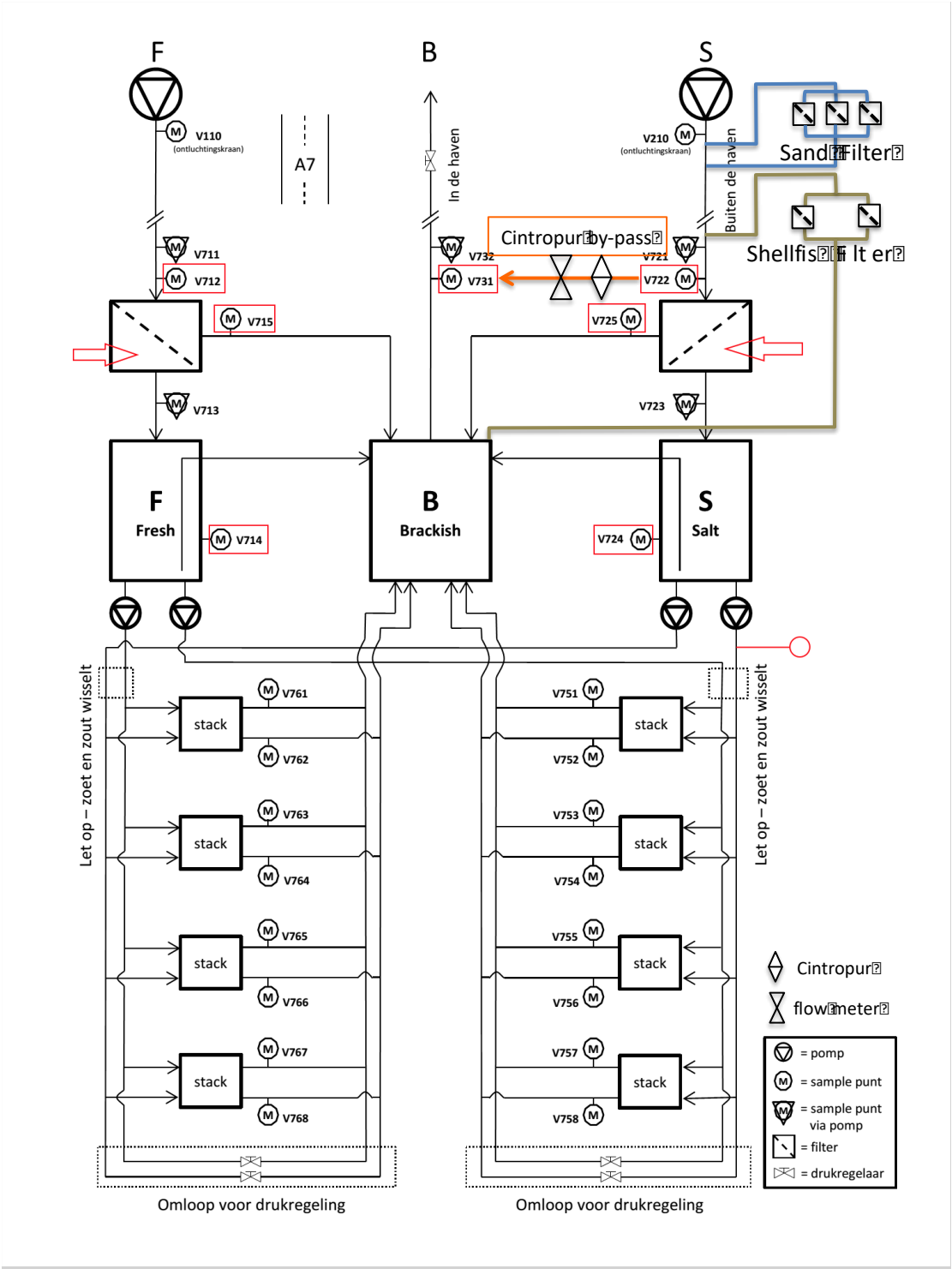


Figure 5. Schematic overview of the Blue Energy installation at Breezanddijk, with the sampling points considered in this study highlighted in red (small organisms) or orange (V722: small organisms and large organisms via Cintropur by-pass).

5 Environmental parameters

5.1 Methods

When the installation is operational, a number of data are automatically logged, with a frequency of 5 seconds, of the incoming and returning water flows and both stack streets. These are, a.o.: water flow, temperature, salinity, turbidity and conductivity. Total suspended solids, before and after the drum sieve, free chlorine before 2018 and Bromoform from 2018 onwards are measured by taking samples from the water on a weekly to monthly basis. The five measurement points (V711, V713, V721, V723 and V732), indicated by a special symbol in Figure 5, are each sampled in a cycle of 4 min flushing followed by 8 min of measurements of the turbidity; thus 12 min per cycle per water flow, five measurements per hour.

For this report, the hourly water temperature and conductivity measured at both stack streets were provided by Chris Coutinho and Simon Grasman of REDstack B.V. Conductivity was converted to salinity in gram of NaCl per litre. Periodically, the fresh and saline water flows are reversed, to prevent fouling, this is called a flow switch. After a flow switch it takes a certain amount of time for all of the previous water to be purged from the system, which can lead to deviations in the salinity measurements, especially when the flow is switched from seawater to fresh water. Because of this, measurements that were taken when a flow switch had occurred in the last hour are all excluded.

5.2 Results: water temperature

5.2.1 Seawater

Seawater temperature showed strong seasonal patterns (Figure 6). Maximum seawater temperature was 23.2 °C on 2018-07-24, minimum seawater temperature was 0 °C on 2017-02-15. During the summer of 2018, water temperatures were high and rose well above 20 °C. This was the warmest summer since 1706 according to KNMI, with also extremely low rainfall[3]. During the winter of 2018, the water temperatures dropped to zero. In March 2018 some data are missing, probably because of ice conditions. The temperature rise in spring was late in 2018 and lagged one month behind 2017 until mid-April. Seasonal temperatures in 2019 were comparable to previous years, with again a summer with exceptionally high temperatures [4].

[3] https://www.knmi.nl/nederland-nu/klimatologie/maand-en-seizoensoverzichten/2018/zomer
[4] https://www.knmi.nl/nederland-nu/klimatologie/maand-en-seizoensoverzichten/2019/zomer

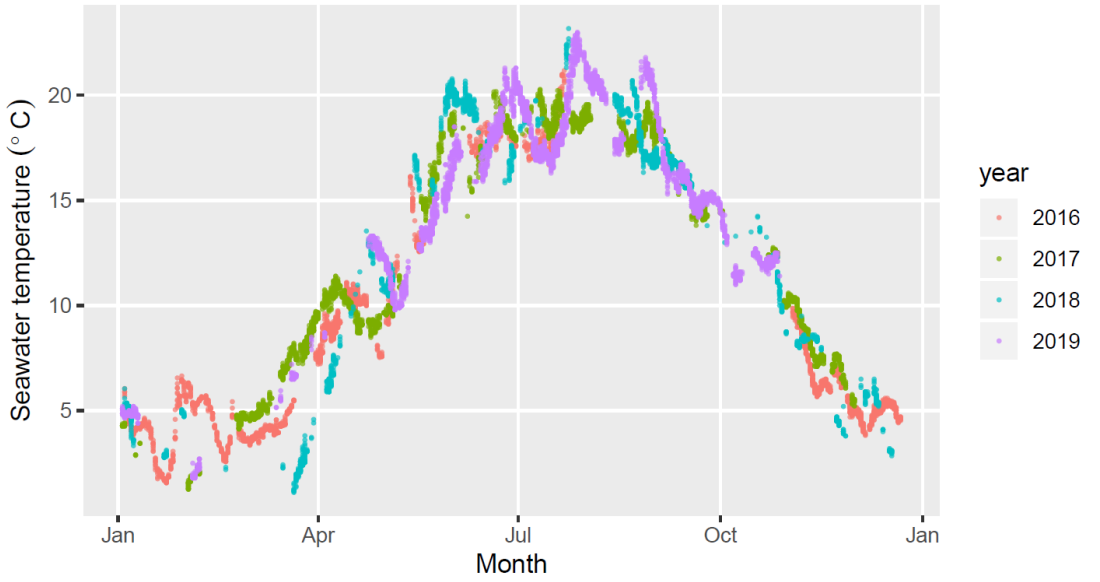


Figure 6. Seasonal pattern in seawater temperature in the Blue Energy installation at Breezanddijk. One measurement per hour, averaged for both stack streets.

5.2.2 Fresh water

The fresh water temperature showed a seasonal pattern that was to a large extent comparable to that of sea water (Figure 7). Details are presented in the next section. Maximum freshwater temperature was 25.2 °C on 2019-07-26, minimum fresh water temperature was 0 °C on 2017-02-14, 2017-02-15 and 2018-03-15.

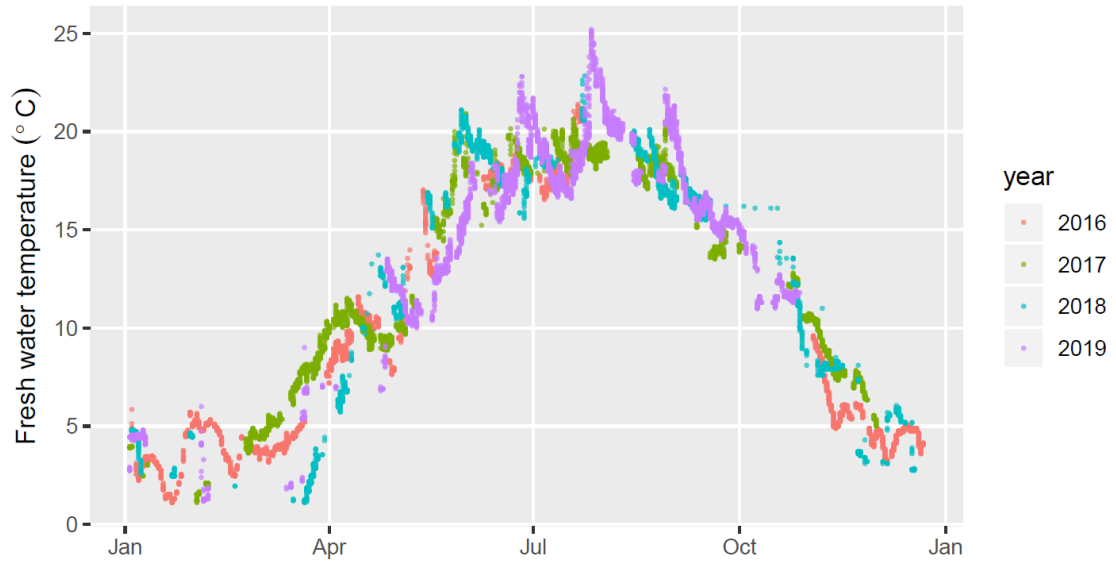


Figure 7. Temperature of the fresh water measured at REDstack Breezanddijk, hourly averages of both stack streets.

5.2.3 Temperature difference

In general, the sea water was warmer than the fresh water in winter, about equal in spring and autumn, and fluctuating in summer, with peaks of both positive and negative temperature differences (Figure 8). The temperature difference between fresh water and seawater in the installation was below 2 °C on all but two occasions, both occurring during the extremely warm 2019 summer with a maximum temperature difference of 3.9 °C. Assuming a 1/1 mixing of seawater and fresh water, the maximum instantaneous

temperature change Δt experienced by organisms is half of this: 2 °C on the two extremely warm occasions and below 1 °C on all other days. When seawater and fresh water are mixed in a ratio 1:2 or 1:3, the maximum instantaneous temperature change of seawater is 2.6 or 3 °C, respectively for the two extremely warm occasions and 1.3 or 1.5 °C for all other days. This is excluding a possible temperature change caused by the RED process itself, and assuming that the effect of the different heat capacity and density of fresh water and seawater is negligible.

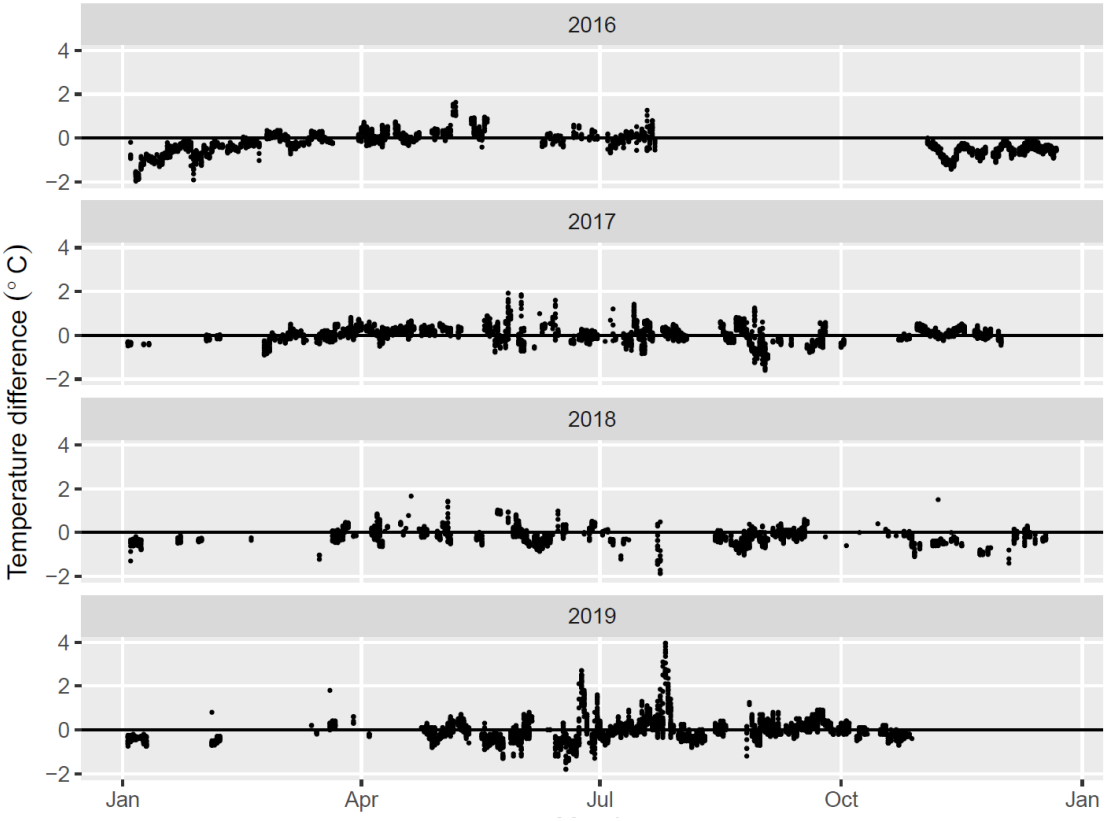


Figure 8. Difference between fresh water and seawater temperature (hourly averages of both stack streets) at REDstack Breezanddijk in 2016 - 2018. At positive values seawater was colder than the fresh water, at negative values seawater was warmer than fresh water.

5.3 Results: salinity

5.3.1 Seawater

Seawater salinity, like temperature, showed seasonal variations, but (unlike water temperature) sometimes high variations as well, even within one day (Figure 9). Highest salinity was 35.73 on 2018-08-14, lowest salinity was 10.63 on 2018-01-22. Lower values of salinity were measured, but these are likely taken after a flow switch from fresh water to seawater. Average salinity was 21.55 (S.E. 0.05). There were quite large interannual variations, especially in spring and summer. The largest difference was between July - September, with very high salinity in 2018, intermediate salinity in 2017 and low salinity in 2016. Summer 2018 salinity was at times more than twice as high as summer 2016 salinity, explained by exceptionally dry conditions, leading to no fresh water being discharged into the Wadden Sea from lake IJssel. This lasted well into autumn and winter, until January 2019.

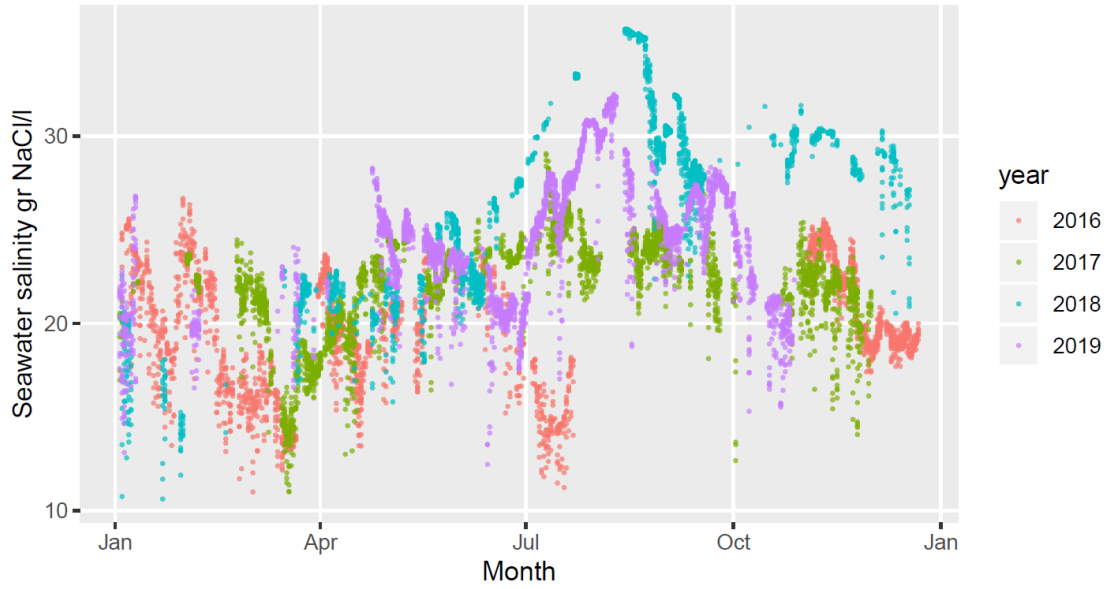


Figure 9. Seasonal pattern in seawater salinity in the Blue Energy installation at Breezanddijk. One measurement per hour, averaged for both stack streets.

5.3.2 Fresh water

Fresh water salinity was quite constant at low levels, except for peaks with outliers up to >2.0 gr NaCl/l in 2018 (August, December) and March 2019 (Figure 10). These outliers are likely the result of a flow switch where fresh- and seawater flows are reversed. Elevated salinity in summer – winter 2018 coincided with the above mentioned drought period, during which salinity of lake IJssel increased as well.

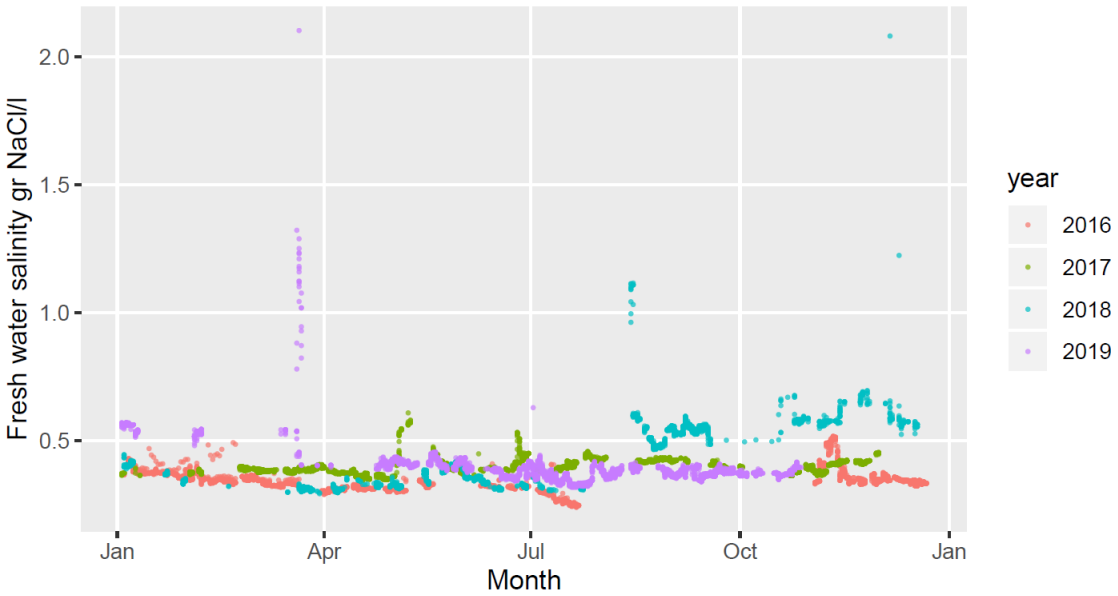


Figure 10. Salinity of the fresh water measured at REDstack Breezanddijk. Values are hourly averages of both stack streets.

5.3.3 Effluent salinity

Using the salinity measurements of seawater, we can estimate the salinity of the brackish effluent discharged by a Blue Energy power plant at 1:1 seawater/fresh water ratio as half of the seawater salinity assuming fresh water salinity is negligible (Figure

11). The average salinity is estimated at 10.77, with a minimum of 5.31, maximum of 17.87, and 95 % of the values lying between 7.46 and 13.74.

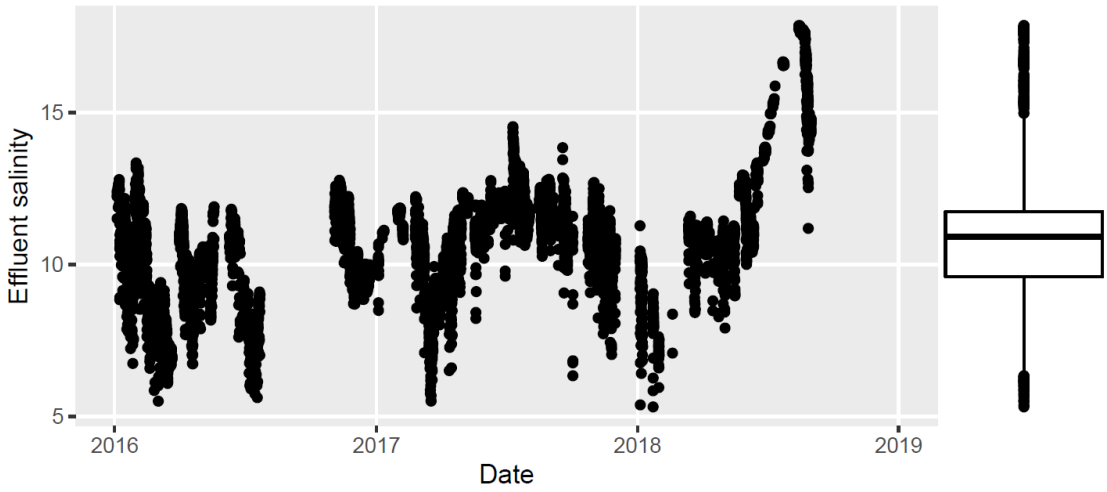


Figure 11. Estimated salinity of the brackish effluent discharged by the REDstack pilot plant at 1:1 fresh water/sea water dilution in 2016 - 2018.

6 RV Stern sampling

6.1 Methods

To investigate the species composition and density of fish, fish larvae and other zooplankton that could interact with the RED power plant at Breezanddijk, plankton samples were taken in spring 2017, 2018 and 2019 with the RV Stern near the seawater intake point of the RED power plant at Breezanddijk. Samples were taken with the ship at anchor, and every hour a set of samples was taken, consisting of three CTD casts, two Niskin bottle samples and one horizontal net tow.

6.1.1 CTD casts

Before each net tow and niskin sampling, and before returning to port, three consecutive CTD casts were performed using a Sontek Castaway handheld CTD device which measures conductivity, temperature and sound velocity (depth) at a sampling rate of 5 Hz during a 1 m/s free-falling downcast.

6.1.2 Niskin samples

After the CTD casts, two 8 liter Niskin bottle samples were taken at a depth of approximately 3 m, filtered over a 50 µm sieve and conserved on 4% formaldehyde for reference.

6.1.3 1 mm plankton net sampling

The sampling gear consisted of a plankton net with a 1 mm mesh size, a circular opening of 0.78 m^2, and a length of 5 m (CalCoFI net). With the ship at anchor, the net was deployed in a horizontal orientation approximately 3 m below the water surface for a duration of 5 - 15 minutes, depending on the strength of the tidal current. Water flow through the net was estimated using a Valeport flow meter mounted in the net opening. A minimum of three samples was taken on every sampling day. Sampling volumes ranged between 9 and 807 m^3. In total there were eight sampling days (Table 1). Bad weather conditions such as ice floes and landward winds prevented sampling in March in both 2017 and 2018.

The RV Stern large organisms sampling was on several occasions synchronised with the Cintropur samples at V722 in the installation, in order to allow comparisons (see 7.1).

Table 1. Date, tidal phase and sampling volume (m^3) of samples taken with the 1 mm CalCoFI net from RV Stern in the Wadden Sea near REDstack intake point Breezanddijk. Ebb is falling tide (↘), flood is rising tide (↗) , slack is turn of the tide (=).

Date	Time	Volume	Tide	Water level
2017-04-07	10:40	372.50	ebb	↘
	11:49	208.7	ebb	↘
	12:51	166.4	ebb	↘
	13:50	140.0	slack	=
2017-04-20	11:04	254.0	ebb	↘
	12:49	208.9	ebb	↘
	13:49	158.5	ebb	↘
2017-05-12	10:17	37.0	flood	↗
	10:40	111.3	flood	↗
	11:30	9.4	flood	↗
	13:00	358.7	ebb	↘
	13:55	807.3	ebb	↘
	15:07	320.6	ebb	↘
2018-04-06	09:50	340.6	flood	↗
	11:10	346.2	flood	↗
	12:15	116.7	flood	↗
2018-05-04	09:40	328.3	flood	↗
	10:35	258.4	flood	↗
	11:40	112.3	flood	↗
2019-03-01	09:44	239.1	ebb	↘
	10:45	119.8	slack	=
	11:45	155.9	flood	↗
	12:45	196.0	flood	↗
	13:45	506.4	flood	↗
	14:45	232.2	flood	↗
2019-04-11	09:26	250.1	flood	↗
	10:22	252.1	flood	↗
	11:20	170.6	flood	↗
	12:25	14.5	flood	↗
	14:21	42.9	ebb	↘
2019-04-26	09:20	76.7	flood	↗
	10:20	79.5	flood	↗
	11:20	260.8	flood	↗
	12:20	184.6	flood	↗
	13:20	179.6	flood	↗
	13:40	138.6	flood	↗
2019-05-23	09:00	327.4	flood	↗
	10:00	288.0	flood	↗
	11:00	185.3	flood	↗
	12:00	111.0	flood	↗
	13:00	52.6	slack	=

6.2 Results: RV Stern sampling

6.2.1 Environmental parameters

Vertical profiles of water temperature (Figure 12) and salinity (Figure 13) show that for the majority of samples taken the water column was well mixed, with no vertical gradient in temperature and salinity, although both parameters showed small temporal variation within each sampling day. On some days however, mainly on 12-05-2017, 26-04-2019 and 23-05-2019 there was a strong vertical gradient in salinity in some samples. These samples appeared to be taken mainly during or near the flood slack tide when current speed was low.

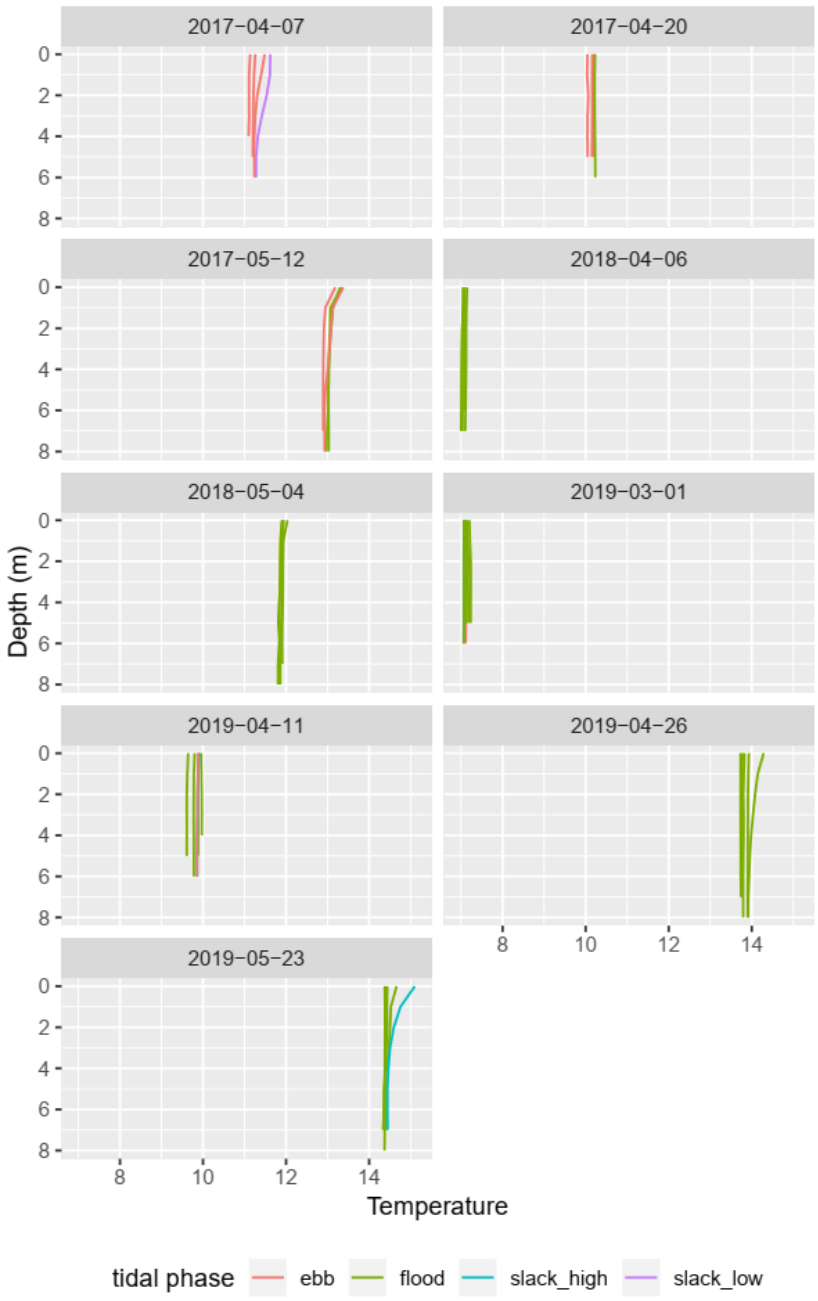


Figure 12. Vertical profiles of water temperature obtained using the Sontec Castaway CTD from RV Stern. Each line is an average of three downcasts.

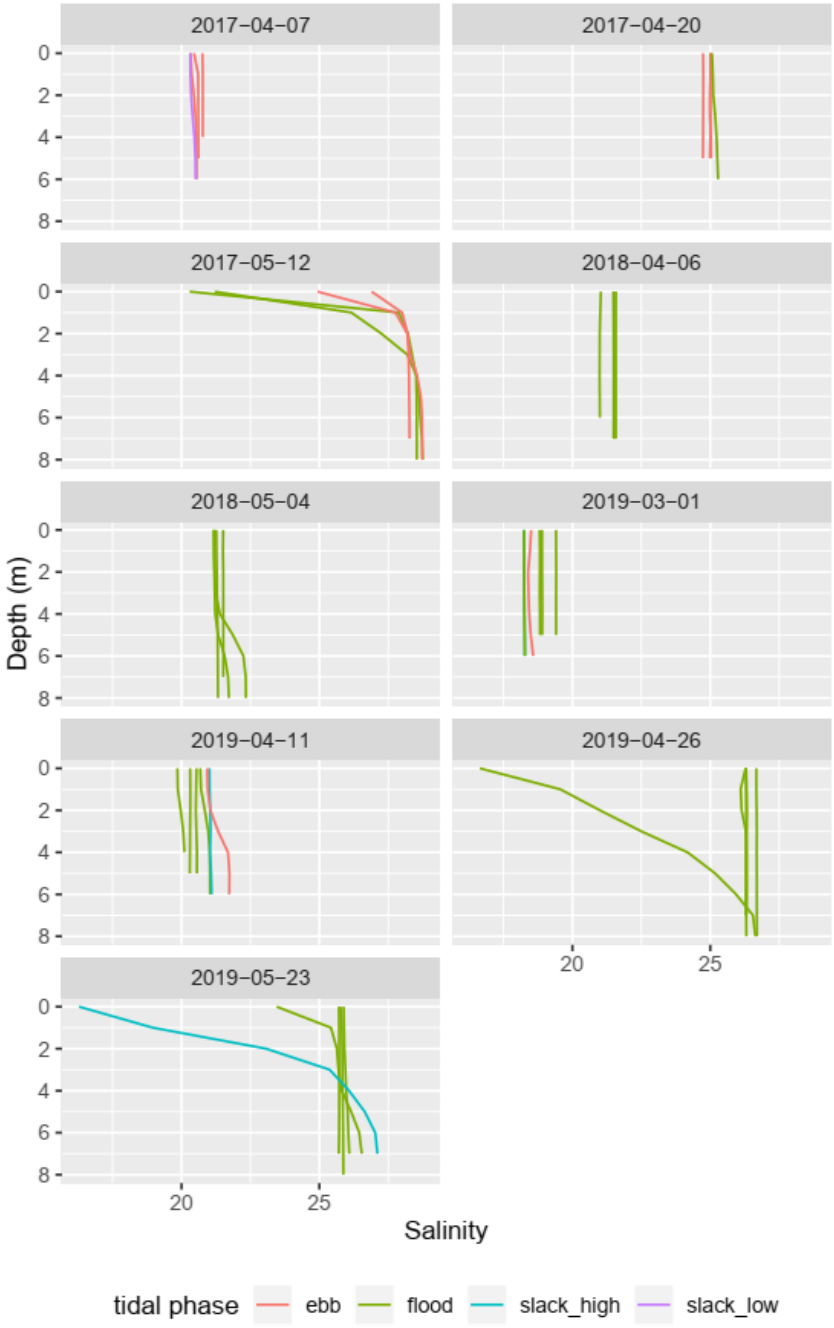


Figure 13. Vertical profiles of salinity obtained using the Sontec Castaway CTD from RV Stern. Each line is an average of three downcasts.

There was generally no thermocline over the vertical watercolumn. However, around HW there was a strong decline in surface salinity at some dates (12 May 2017, 26 April and 23 May 2019). This can be explained by fresh water that is discharged around LW at the nearby sluices of Den Oever (e.g. a volume of 400 m^3/s on 12 May 2017; data RWS), and that is carried in eastward direction towards Breezanddijk by the flood current.

The depression of salinity is likely dependent on the discharged water volumes. The vertical salinity gradient seems to be stronger at days with relatively high salinity (>25 PSU).

6.2.2 Fish larvae

Fish larvae density estimates were obtained on nine sampling days with NIOZ RV Stern; three in 2017, two in 2018 and four in 2019 (Table 2).

The most common fish larvae observed were of flounder *Platichthys flesus*, of gobies (Gobiidae) and herrings (Clupeidae). Larval gobies consisted mainly of the common goby *Pomatoschistus microps*. Clupeid larvae consisted mainly of herring *Clupea harengus*. The most common non-larval fish caught were pipefish (mainly Nilsson's pipefish *Syngnathus rostellatus*) and three-spined sticklebacks *Gasterosteus aculeatus*. Only two glass eels *Anguilla anguilla* and one river lamprey *Lampetra fluviatilis* were caught.

A striking observation was the almost complete absence of Clupeid larvae in 2019. Despite the larger sampling effort in 2019 compared to previous years, only very few clupeid larvae were found resulting in very low environmental concentrations.

Densities of fish larvae in RV Stern samples are discussed in more detail further on in the comparison with Cintropur 1 mm filter data (see Section 7.3.3).

Table 2. Mean density (n m^{-3}) of fish and fish larvae sampled by 1 mm ring trawl, RV Stern near REDstack Breezanddijk.

Year/Date	2017			2018		2019			
Group	April 7	April 20	May 12	April 6	May 4	March 1	April 11	April 26	May 23
Ammodytidae					0.003				
Anguilla anguilla	0.006			0.009					
Clupeidae	0.110	0.015	0.004	0.458	0.045			0.004	0.003
Gasterosteus aculeatus	0.017			0.003	0.011				
Gobiidae		0.719	0.132		0.062		0.018	0.023	0.147
Lampetra fluviatilis				0.003					
Pholis gunnellus	0.012					0.056			
Platichthys flesus	0.003	0.186	0.018		0.136		0.058	0.016	
Pleuronectes platessa				0.009		0.007	0.004		
Solea solea		0.005	0.031				0.069	0.030	0.008
Syngnathus sp.	0.017	0.042		0.003	0.040				

The length-frequency distribution of the fish collected in the RV Stern samples indicated species specific length spectra (Figure 14). The Clupeid larvae had modal length of about 20 mm (range 8-39 mm) and the postlarvae had a length between 40-45 mm.

The largest fish were the three-spined stickleback (*Gasterosteus aculeatus*) with a length range between 30-70 mm, and the pipefish (*Syngnathus sp.*) with length up to 130 mm. The flounder (*Platichthys flesus*) larvae had a modal length of 8 mm (range 5-11 mm), which is the size at which the flatfish metamorphosis occurrs in this species. Compared to flounder, the size range of larval plaice (*Pleuronectes platessa*) was slightly larger (9-15 mm), and of sole (*Solea solea*) smaller (3-10 mm).

The fish length was measured on formol-preserved fish, which may have undergone some shrinkage due to conservation.

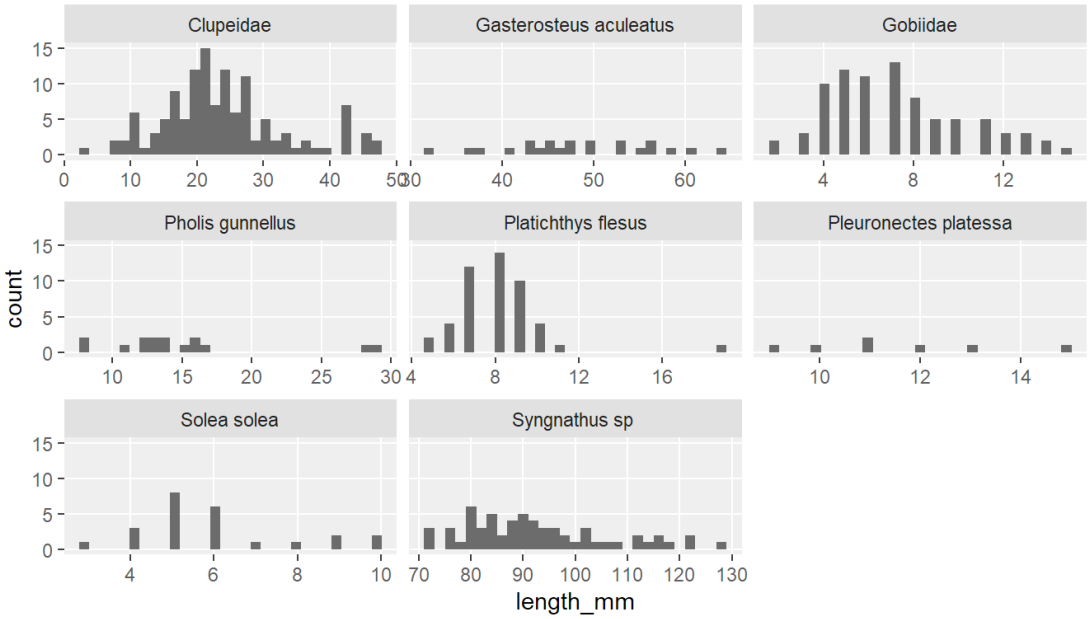


Figure 14. Histograms of fish size measured for the most common species encountered in RV Stern 1 mm net samples.

7 Large organisms

7.1 Methods

7.1.1 Cintropur filter

To collect sufficient numbers of large (meroplankton) organisms, which naturally occur in relatively low concentrations, the filtration of considerable (as large as possible) water volumes was required. Therefore, the feed water was filtered through a Cintropur N32 water filter (Figure 15), filter mesh size 1 mm (tailor-made by NIOZ), that was connected to sampling point V722 (see Figure 5). The Cintropur filter was placed in a by-pass in which a flow meter was built in after the Cintropur filter, in such way that the total volume of filtered water was logged. It was feasible to collect a total volume of 15-20 m^3 in one or more samples per day.

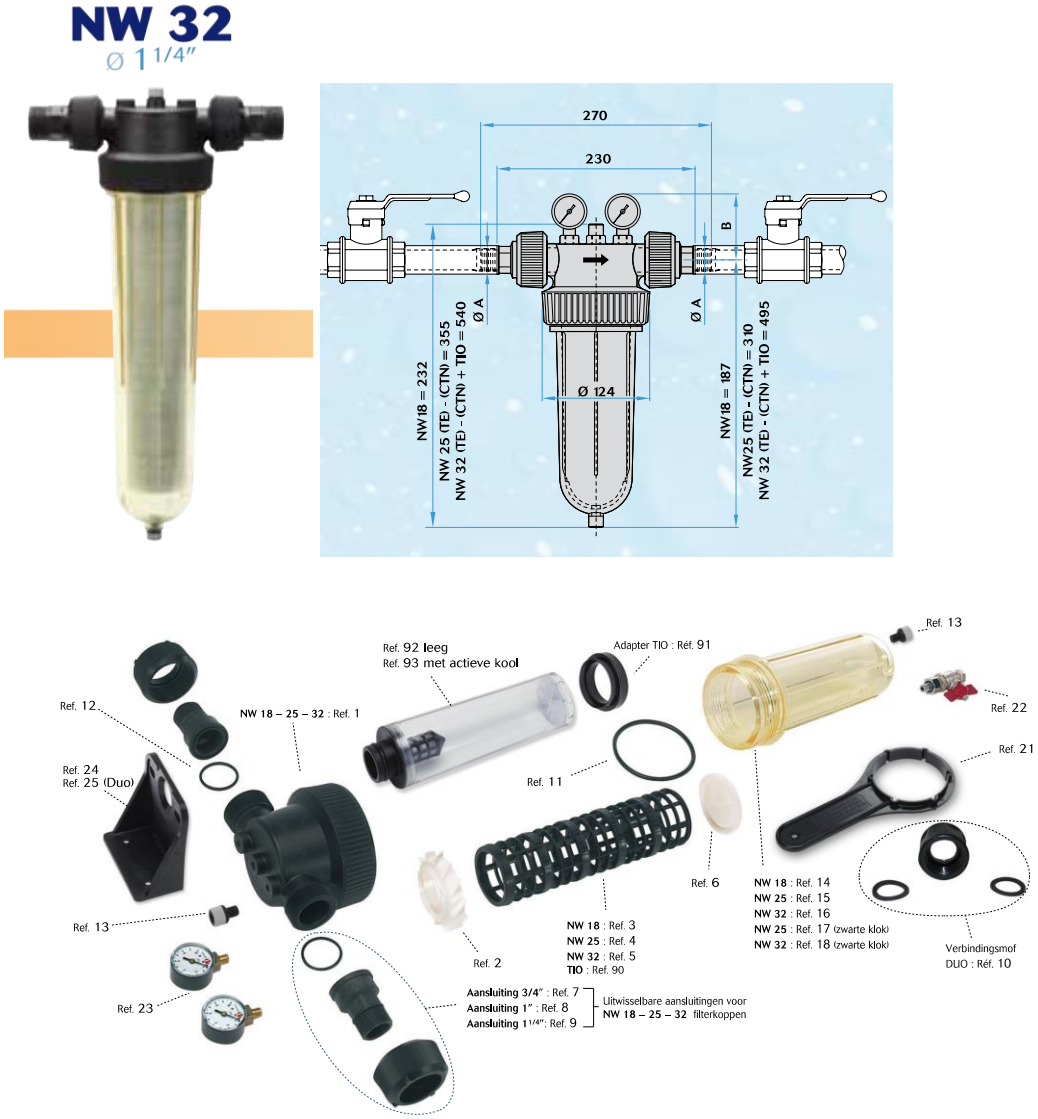


Figure 15. Cintropur filter, installation schedule and different filter elements.

7.1.2 Analysts

The sampling of larger organisms in the REDstack installation was facilitated by Erik Tippersma and Menno Eisma, operators at REDstack. The samples were collected and analysed by Dr. Zwanette Jager (ZiltWater Studies). Some determination issues were resolved with the aid of Dr. Lodewijk van Walraven (NIOZ). On a number of occasions, NIOZ analysts Evaline van Weerlee and Dennis Mosk assisted in collecting the Cintropur samples. Occasional formol samples were analysed at the NIOZ facilities on Texel. Data analyses were done by Zwanette Jager and Lodewijk van Walraven.

7.1.3 Sampling frequency

The monitoring started as soon as the revised seawater intake was in use per November 1st, 2016. The first sampling date was 17 November 2016 and the last was 4 June 2019. The sampling frequency of the larger organisms at the salt water intake was variable, on a weekly or two-weekly basis, and was dependent on the operations of the Blue Energy installation at Breezanddijk. The small organisms sampled by NIOZ were monitored with more or less the same frequency, but not necessarily on exactly the same date due to practical reasons of the limited working space in the laboratory (NIOZ-container) and the restricted availability of binoculars.

During one hour, a volume of c. 3 m^3 (3000 litre) was filtered by the Cintropur in the by-pass. Usually, two samples of several hours each were collected per sampling date. To collect the sample, the by-pass is closed, and the contents of the Cintropur is emptied in a 15 l-bucket after releasing the pressure. The filtered volume, as indicated by the flow-meter, was recorded for each sample. The Cintropur filter was flushed with filtrated seawater (200 µm filter), to avoid contamination of the sample.

In the laboratory, the filtrate was concentrated over a 500 µm round sieve (200 µm until February 17, 2017). The concentrated sample was poured into a modified Bogorov-Gollasch counting tray with 5 separate slits. The sample was counted with the aid of a binocular microscope with 8x - 40x magnification. The intact (living) organisms were counted. The larger organisms were not extremely motile. Only the copepods were moving such that the counting was difficult at times, but due to the modification of the counting chamber, the movements were limited. Some samples have been preserved in formol (with Bengal red colouring) and were counted later in the laboratory. In those samples, copepods have been counted reliably. Most copepods were of small size, and are counted in the NIOZ sampling of small organisms. The counting results were noted on a counting form and were later transferred to an Excel-file.

Between November 2016 and June 2019, a total of 184 valid Cintropur samples were collected and in total, more than 1500 m^3 of salt water was filtered (Table 3). The individual sample volumes ranged from 1451 to 47955 litres. Four samples with volumes of >35000 litres were taken overnight in 2019. The daily sample volume ranged from 4024 to 69455 litres (Figure 16). Samples taken were on occasion preserved on formol (mostly when parallel sampling with RV Stern, indicated with F in Table 3).

Table 3. Sampling occasions, number of samples and volume of the samples November 2016- June 2019. Night: samples that were collected overnight, C + RV Stern: samples taken simultaneously in- and outside of the BE-installation (Cintropur versus RV Stern).

Date	Week nr	Start Time	End Time	Nr of Samples	Volume (l)	Formol
2016				**n=14**	**87826**	
17-11-16	46	10:05	15:40	3	14041	
25-11-16	47	8:00	15:00	3	17051	
02-12-16	48	7:30	12:55	2	15700	
16-12-16	50	7:00	14:50	3	22430	
20-12-16	51	8:00	14:33	3	18604	
2017				**n=63**	**509171**	
06-01-17	1	7:00	13:25	2	18343	
03-02-17	5	7:30	15:00	3	21816	
17-02-17	7	7:20	15:15	3	23260	
24-02-17	8	8:00	15:10	3	20927	
17-03-17	11	7:30	15:05	3	22817	
24-03-17	12	7:45	13:55	3	18060	
31-03-17	13	7:30	14:30	2	21083	
07-04-17	14	7:10	14:33	4 C+RV Stern	20544	F
13-04-17	15	8:00	11:03	1	9169	F
20-04-17	16	7:05	15:00	4 C+RV Stern	19418	F
26-04-17	17	8:00	15:15	3	19435	F
05-05-17	18	7:08	14:25	3	22327	
19-05-17	20	7:20	14:45	2	22470	
24-05-17	21	10:00	14:00	1	12104	F
02-06-17	22	7:00	14:25	2	21760	
09-06-17	23	7:15	13:40	2	18180	
14-06-17	24	7:30	8:30	1	4024	F
26-06-17	26	7:05	13:35	2	18157	
19-07-17	29	10:03	13:15	1	8573	F
04-08-17	31	8:10	14:55	3	18834	
18-08-17	33	10:35	14:05	1	10265	
30-08-17	35	7:15	14:15	2	20361	
22-09-17	38	7:15	14:25	2	19900	
06-10-17	40	7:10	12:50	2	15825	
27-10-17	43	7:10	14:05	2	19395	
17-11-17	46	7:15	14:40	2	20981	
01-12-17	48	7:05	14:25	2	20629	
15-12-17	50	7:15	14:32	2	20514	
2018				**n=47**	**395948**	
16-03-18	11	7:15	14:42	3	20540	
21-03-18	12	7:18	14:45	3	20885	
29-03-18	13	7:20	15:00	2	20565	
11-04-18	15	7:10	14:15	2	19000	
18-04-18	16	7:30	14:15	2	18500	
25-04-18	17	7:10	14:55	3	20750	
02-05-18	18	8:05	15:15	3	20000	
17-05-18	20	7:04	12:30	2	15000	
23-05-18	21	9:06	14:27	2	15000	
30-05-18	22	7:02	15:15	3	20749	
06-06-18	23	7:15	14:50	3	20120	
13-06-18	24	7:10	14:45	3	20580	
27-06-18	26	7:15	13:50	2	17500	
22-08-18	34	7:08	14:00	2	18910	
29-08-18	35	7:15	14:22	2	19250	
05-09-18	36	7:05	14:00	2	19551	
12-09-18	37	7:08	14:30	2	21126	
18-10-18	44	7:10	14:15	2	26020	
31-10-18	44	7:10	15:00	2	21616	

Date	Week nr	Start Time	End Time	Nr of Samples	Volume (l)	Formol
16-11-18	46	7:10	14:28	2	20286	
2019				**n=60**	**534661**	
28-02-19	9	7:10	15:15	2	20560	
night	9	15:15	7:50	1	45595	F
01-03-19	9	7:50	15:50	7 C+RV Stern	18500	F
06-03-19	10	7:10	14:23	2	20250	
13-03-19	11	7:05	14:30	2	20199	
20-03-19	12	7:00	14:45	2	20450	
27-03-19	13	7:05	15:35	2	23705	
night	13	15:35	8:00	1	45750	
28-03-19	13	8:00	14:50	2	19050	
03-04-19	14	7:06	14:53	2	20100	
10-04-19	15	9:10	16:08	2	19191	
11-04-19	15	8:10	14:15	6 C+RV Stern	12784	
19-04-19	16	8:11	15:20	3	19967	
25-04-19	17	11:10	17:15	2	17400	
night	17	17:15	7:30	1	36030	F
26-04-19	17	7:30	14:52	6 C+RV Stern	17846	F
01-05-19	18	7:00	14:40	2	21870	
08-05-19	19	7:15	14:00	3	18500	
15-05-19	20	7:15	14:20	2	20030	
22-05-19	21	6:55	14:25	2	20901	
night	21	14:30	7:30	1	47955	F
23-05-19	21	7:35	14:22	6 C+RV Stern	17250	F
04-06-19	23	6:55	10:55	1	10778	

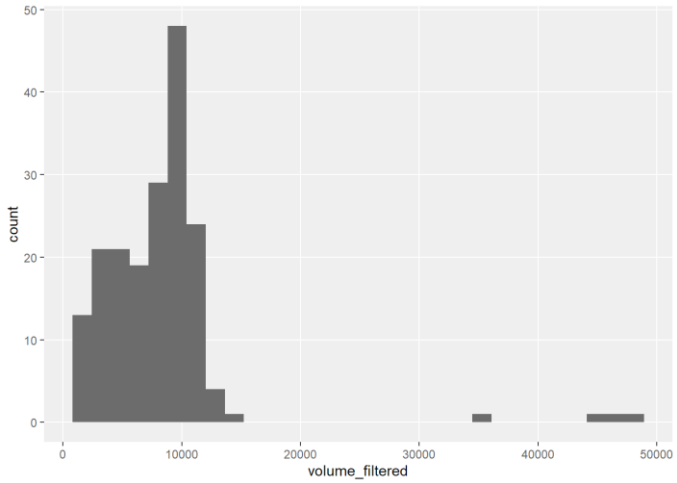


Figure 16. Histogram of Cintropur filtered volumes (count of litres).

7.2 Results: large organisms

In this section, the results of the Cintropur sampling of larger organisms, caught in the BE-installation (V722) from the Wadden Sea water before the rotating drum screens, are presented for selected taxonomic groups. Detailed information is presented in the separate Annex.

7.2.1 Data characteristics

The data histograms (Figure 17) indicate that the number of zero-observations was high and that most organisms occurred in low mean concentrations (up to 1 m^{-3} for fish eggs and fish larvae and up to 50 m^{-3} for other organism groups).

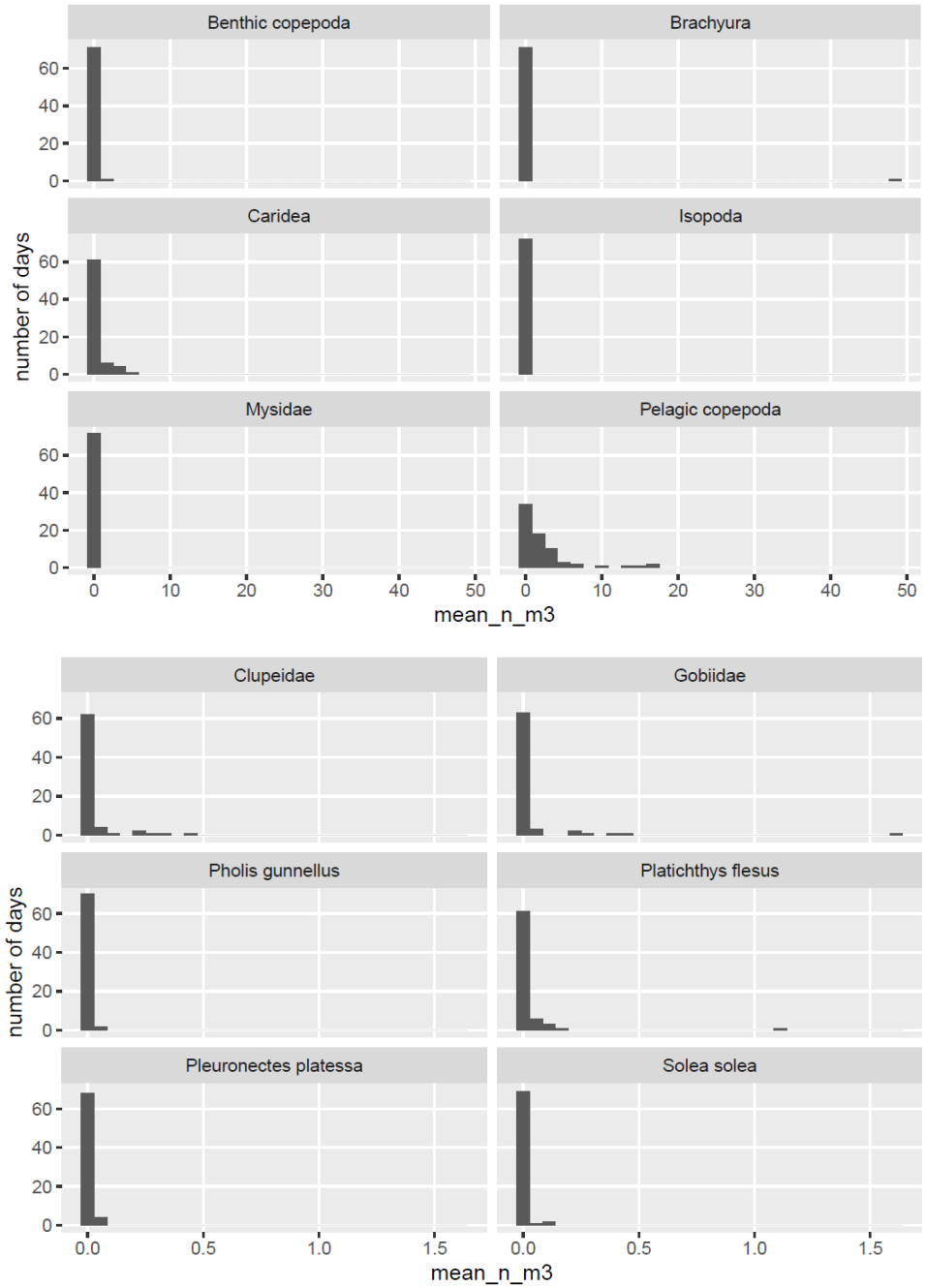


Figure 17. Histogram of mean density of Crustacea (top 6 panels) and fish larvae (bottom 6 panels) per sampling day in Cintropur filter samples collected at the seawater sampling point V722.

7.2.2 Seasonal patterns in species composition

The seasonal patterns are presented as species composition per sampling day for the Crustacea (Figure 18) and fish larvae (Figure 19).

Crustacea were present year-round (Figure 18). Among Crustacea, the pelagic copepods were relatively abundant in the winter and spring period. During the late spring and summer, Brachyura (crab larvae) and Caridea (shrimp larvae) took over, whereas Mysida were relatively more abundant in late summer and autumn (in 2017). The seasonal patterns look similar in the subsequent years, insofar sampled in those periods.

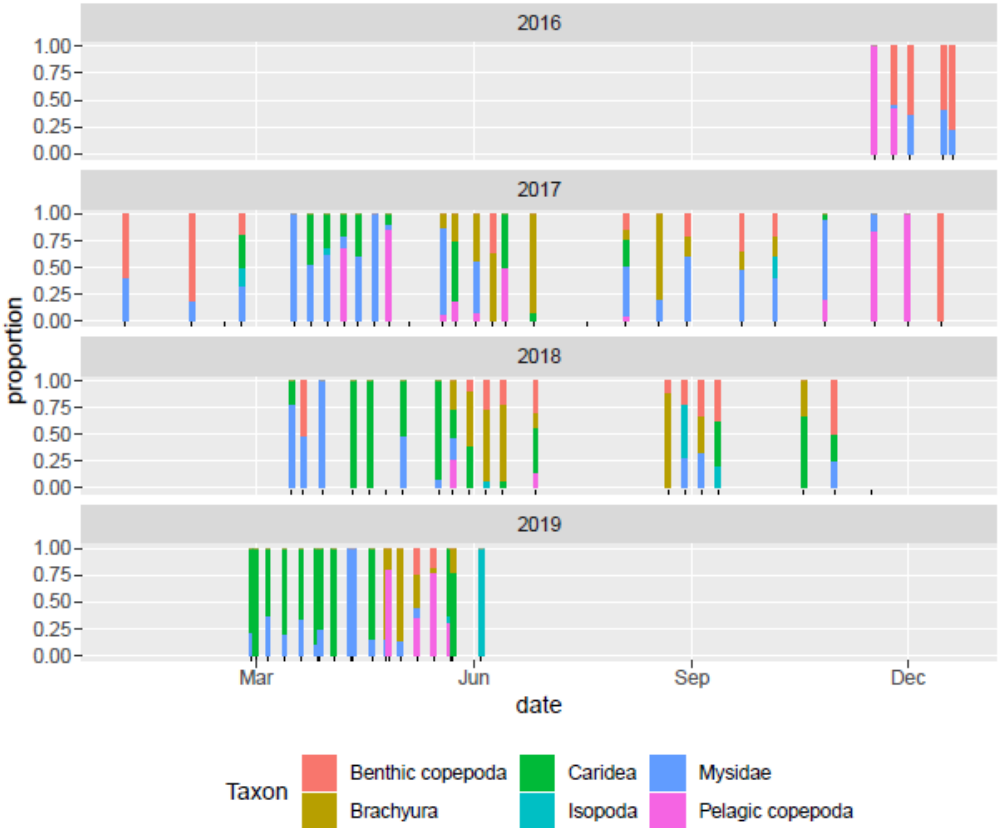


Figure 18. Species composition of crustacea per sampling day in Cintropur filter samples, collected at the seawater intake point V722.

The occurrence of fish larvae was restricted to the period between February and June and the seasonal pattern showed a consistent species sequence from year to year (Figure 19). The gunnel larvae (*Pholis gunnellus*) are the first to appear in February. They were missed in 2018 because of a late start of the monitoring (due to ice conditions). The next species, to occur in March, are plaice larvae (*P. platessa*) although these seem to be largely absent in 2019. Then the Clupeid larvae make their appearance (but not in 2019), more or less at the same time as the metamorphosing flounder larvae (*P. flesus*), followed by sole larvae (*S. solea*) and finally the Gobies, who are the last to enter the installation.

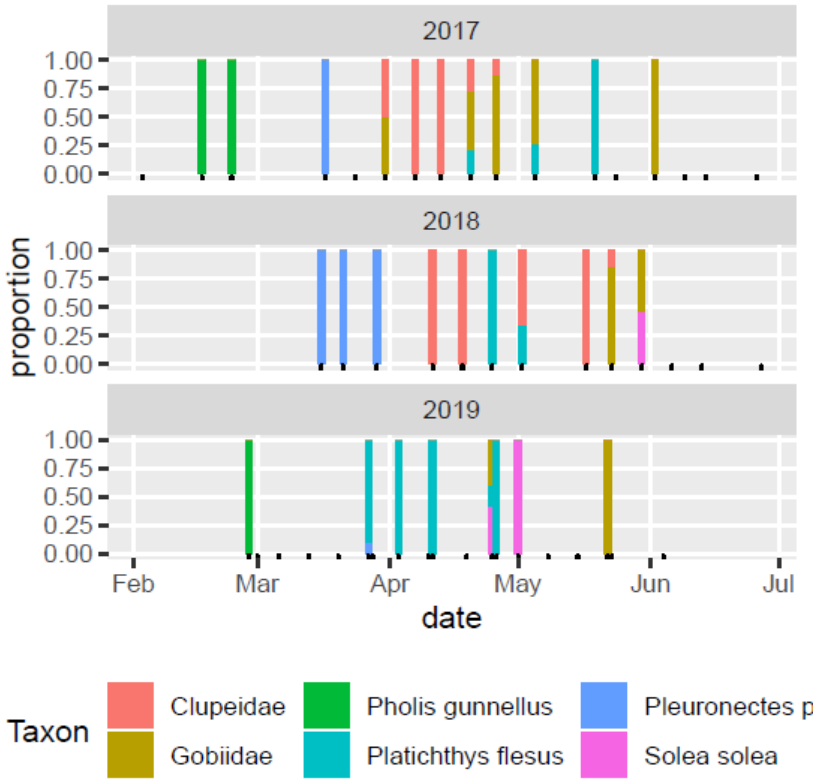


Figure 19. Species composition of fish larvae per sampling day in Cintropur filter samples, collected at the seawater intake point V722.

7.2.3 Specified results of fish larvae

The lengths of fish larvae in the Cintropur samples ranged between 4 and 30 mm, depending on the species (Figure 20). Herring larvae (Clupeidae) up to 30 mm length were impinged. Herring postlarvae (>40 mm) were not impinged. The Gobiidae had a length range between 8-20 mm. The modal length of flounder (*P. flesus*) larvae was 8 mm (range 7-12 mm). The smallest larvae (4 mm) were those of sole (*Solea solea*) and those of herring (*Clupeidae*) were the longest (32 mm).

The results are specified in the sections below.

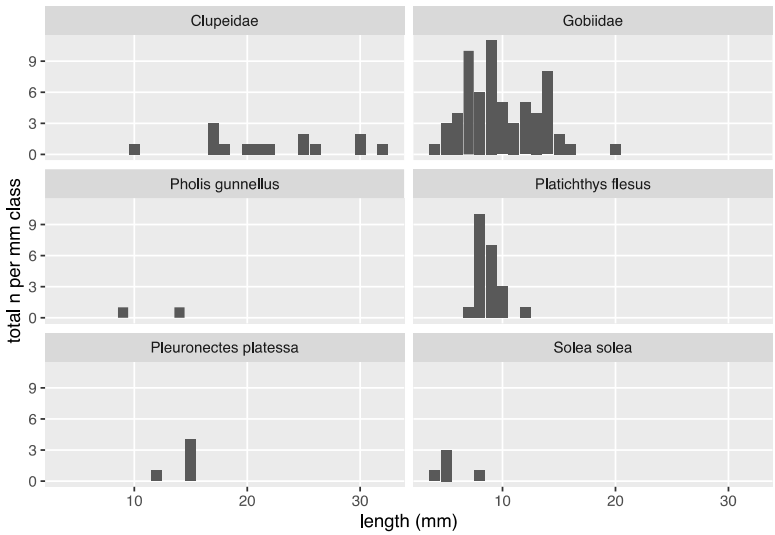


Figure 20. Overall length distribution of fish larvae found in Cintropur samples at the seawater intake point V722.

7.2.3.1 Gunnel (Pholis gunnellus)

Example of Pholis gunnellus yolk-sac larva.

The larvae of *Pholis gunnellus* appeared very early in spring (wk 8, even before March). In 2018, they were absent in the samples probably due to the rather late start of the sampling at the end of March. Among the impinged larvae of gunnel, there were yolksac larvae, which, considering their young age, most likely hatched in the vicinity of the salt water intake. The highest impinged concentration was 0.16 individuals per m^3 in 2017.

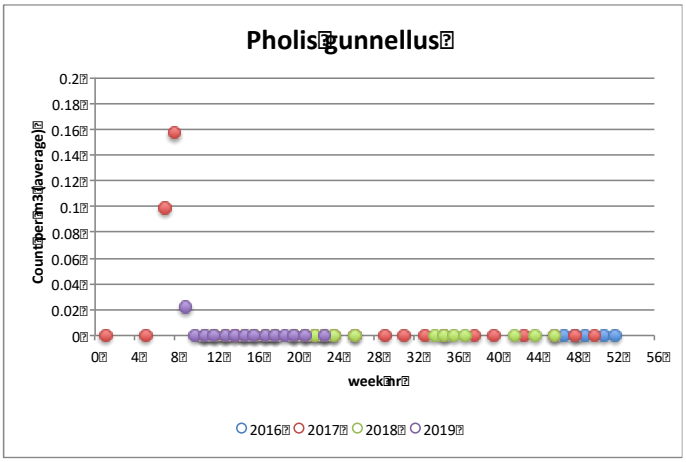


Figure 21. Concentrations of *Pholis gunnellus* (n per m^3), by week nr per year of sampling.

7.2.3.2 Flounder (Platichthys flesus)

Example of Platichthys flesus larvae.

The flounder *Platichthys flesus* is a flatfish species of which the larval stages enter the estuarine nursery areas in spring. At entrance in the Wadden Sea, the larvae are pelagic and they undergo a metamorphosis from symmetric and transparent larvae to fully pigmented larvae with both eyes on one side of the body, that settle on the sediment. Because of limited swimming ability, flatfish larvae use selective tidal stream transport.

Observations: Flounder larvae were found in the samples in concentrations up to 1.1 per m^3 (2017). Their seasonal occurrence was between mid-March and mid-May (wk 12-20). The larvae were in the process of metamorphosis and were still largely transparent with total lengths between 8-12 mm. The collected flounder larvae were often still alive.

Other flatfish species that were found in lower concentrations, and with a slightly different seasonal pattern, are plaice (*Pleuronectes platessa*) and sole (*Solea solea*). The seasonal occurrence of plaice is earlier, and that of sole later, compared to flounder (Figure 22, Figure 23, Figure 24).

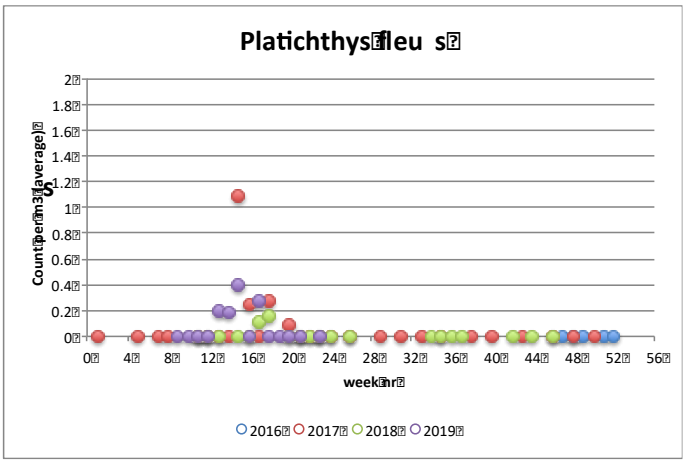


Figure 22 Concentrations of P. flesus (n per m^3), by week nr per year of sampling.

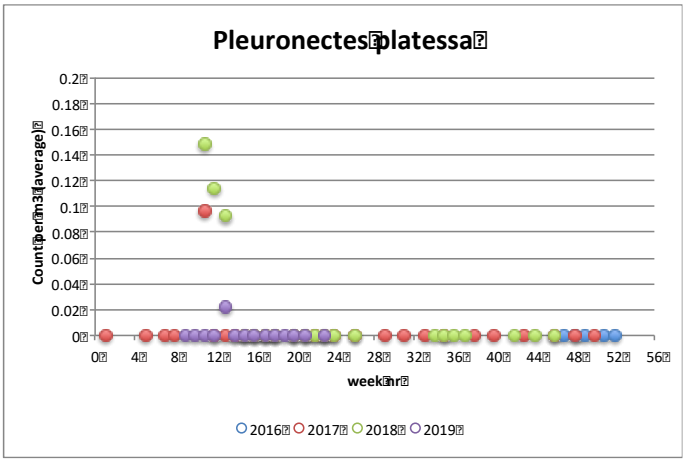


Figure 23 Concentrations of P. platessa (n per m^3), by week nr per year of sampling.

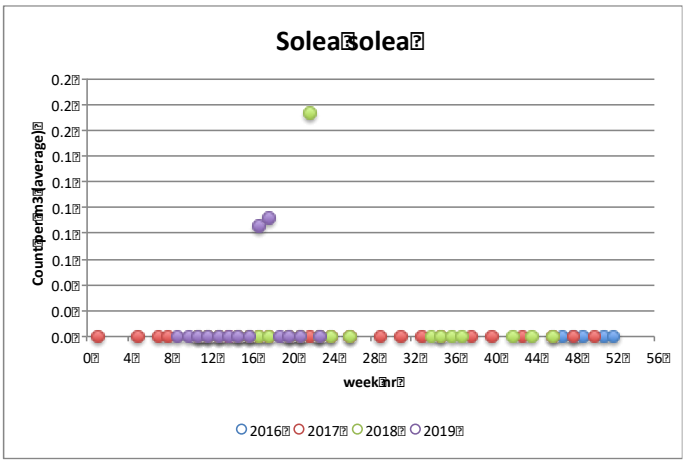


Figure 24 Concentrations of *Solea solea* (n per m^3), by week nr per year of sampling.

7.2.3.3 Clupeidae (Clupea harengus a.o.)

The clupeid larvae were not identified to the species level, but the specimens in the samples most likely belong to herring (*Clupea harengus*) and sprat (*Sprattus sprattus*).

Observations: larval stages of Clupeid fish were found in the samples in concentrations up to 0.6 individuals per m^3. The seasonal occurrence was between mid-March and the end of May (wk 12-22). Concentrations in the Cintropur samples were zero in 2019 (Figure 25).

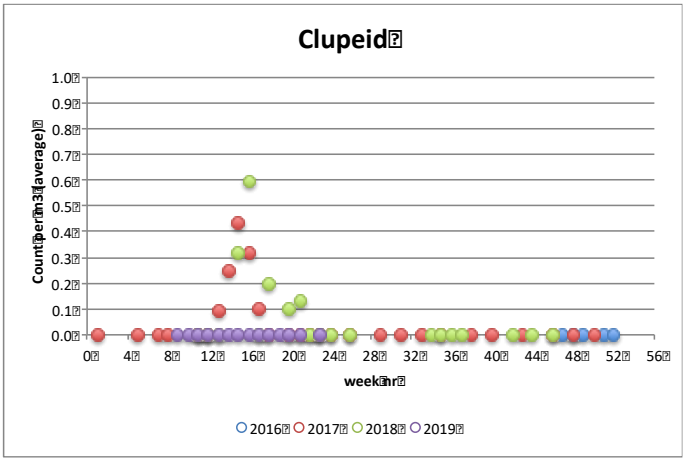
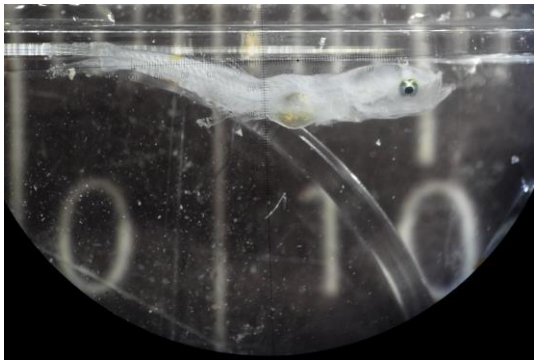


Figure 25. Concentrations of *Clupea sp.* (n per m^3), by week nr per year of sampling.

7.2.3.4 Gobies (Pomatoschistus sp.)



Example of Pomatoschistus sp. postlarva.

Pomatoschistus sp. is a very common fish species in the Wadden Sea. The eggs are attached to substratum.

Observations: Eggs containing embryos of gobies were sometimes found in the samples, but the majority of impinged gobies were (post)larval stages. The concentrations were highest in 2017, up to 1.6 individuals per m^3, and impingement occurred from mid-March and mid-June (wk 12-24).

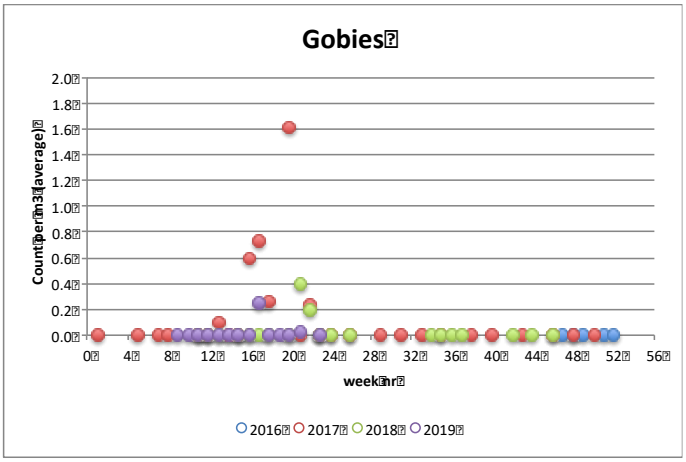


Figure 26. Concentrations of *Pomatoschistus sp.* (n per m³), by week nr per year.

7.3 Analyses of factors, influencing the intake of large organisms

In this section, different factors that may influence the impingement of larger organisms and fish larvae in the BE-installation are highlighted.

7.3.1 Performance of the salt water intake

Fouling of the protective screens on the intake pump may impact the performance of the water intake, potentially affecting (reducing) the intake of the larger marine organisms that cannot enter through the clogged or covered openings in the pump cover. As an indicator of the salt water intake performance, a parameter Delta was calculated.

$$Delta = \frac{h_{after} - h_{before}}{Q_{after} - Q_{before}}$$

where h is the water level inside the pump cover (m to Amsterdam Ordnance Datum = NAP) and Q is the water flow (m³/h) before and after a change in the setting of the flow rate (moment that pump settings are changed). In case of clogging, there is an increased friction for the water passing through the filter elements resulting in a head loss. Normally for every flow setting the head loss should be zero, though when the filter elements start to clog, a head loss starts to occur, which causes raised values of the calculated Delta value (m.h/m³). The moments of pump switches were identified in the logged data, and on these occasions the parameter Delta was calculated.

The performance parameter (Delta) of the salt water intake varied during the monitoring periods (Figure 6). The calculated Delta-values varied between 0 and 0.05. The two documented occasions, when the salt water intake screens were cleaned or replaced, are indicated by arrows in Figure 6. The application of wedge wire panels is indicated by a dark arrow. The star symbols indicate a temporary shutdown of the installation.

From the start of the monitoring November 2016 until June 2017, the Delta values were just about zero, indicating little clogging of the pump case. From mid-June 2017 onward, however, Delta started to increase until mid-July, at which point the PVC pump cover was cleaned by divers on 24 July 2017. The fouling was removed easily. The cleaning operation resulted in a drop in the Delta value which lasted until November 2017. Between November 2017 and January 2018, the Delta values indicated increasing clogging. Between January and March 2018, the Delta was slightly reduced without cleaning intervention, until another episode of increasing Delta at the end of April 2018. The next cleaning operation took place on 14 May 2018, with simultaneous replacement of the salt water pump.

The wedge wire panels, that were placed on October 4th, 2018, were cleaned on 30 January 2019; at that time only soft fouling was present, which was removed easily. The wedge wire screens showed similar patterns in Delta compared to the perforated screens, although the wedgewire screens had to be cleaned more often (Figure 27).

Clearly, the pressure drop, indicated by the Delta-parameter, is reduced immediately after cleaning. However, Figure 27 also shows that the resistance builds up again soon. During the summer months, this is most likely caused by fouling of filamentous algae that grow on the pump cover (despite the anti-fouling coating). Balanoid fouling could only settle on those surfaces where the antifouling coating had been damaged. At the shut-down periods of the installation, the Delta seems to drop due to natural processes (perhaps by episodes of low or elevated water temperatures).

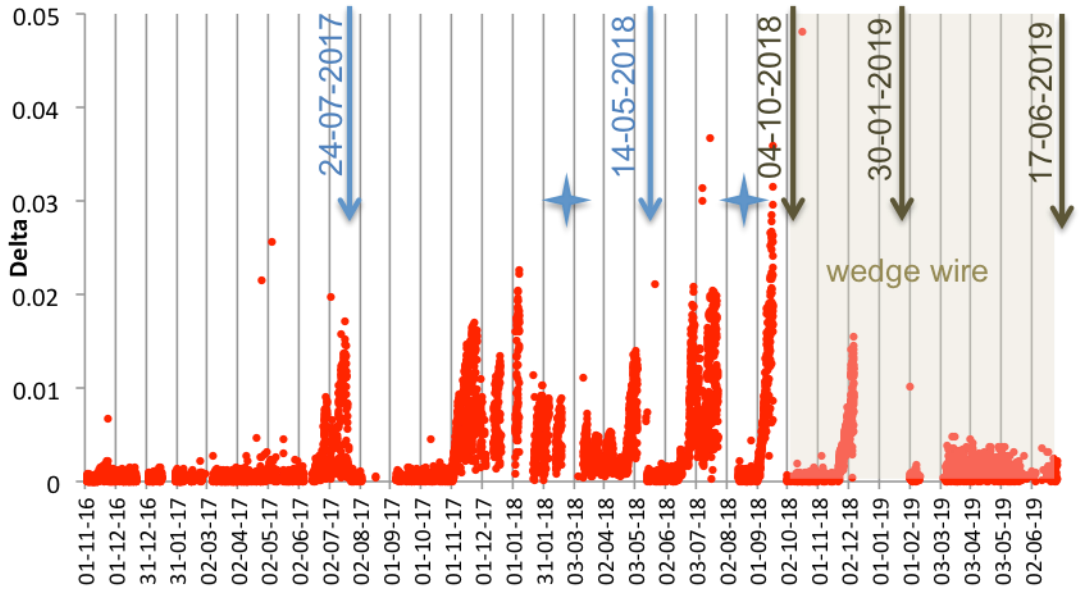


Figure 27. Plot of the Delta parameter against date, and two cleaning occasions indicated with blue arrows. The replacing of the pump cover is indicated with dark arrow. The stars indicate moments when the installation has been out of use for some time (shut-down).

7.3.2 Pump performance (Delta) and impinged concentrations

A plot of the concentration of selected Phyla against the fouling indicator (Delta) seems to indicate that high concentrations of impinged organisms occurred at low values of Delta (Figure 28; data of 2017 and 2018 with perforated pump screen). At a Delta value >0.005, only low concentrations (<5 n/m^3) of organisms were observed in the Cintropur samples.

However, when specified for Decapods and fish larvae per year, this only holds for 2017 and in 2018 and 2019 the patterns look very erratic (Figure 29). The concentrations of organisms were structurally low and the Delta values did not vary over a wide range. Therefore, no conclusions can be drawn - neither on the relation between the fouling parameter Delta and the intake of organisms, nor on differences between perforated and wedge wire screens.

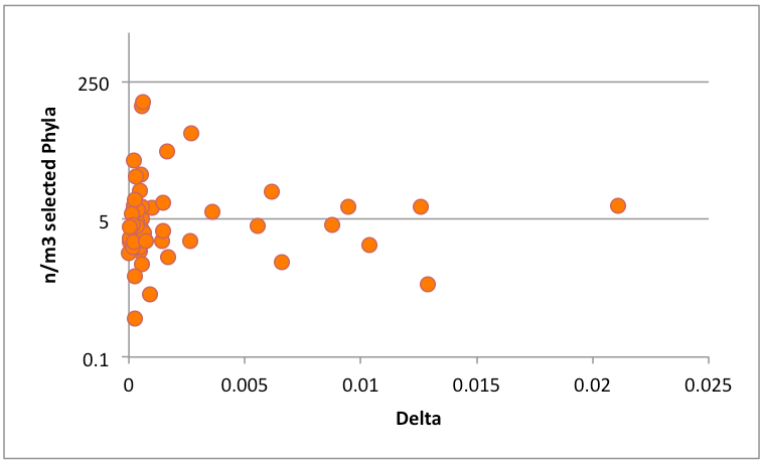


Figure 28. Plot of concentrations (n/m³; log-scale) of selected Phyla (Annelida, Arthropoda, Chaetognatha, Chordata, Mollusca, Platyhelminthes) against Delta fouling indicator.

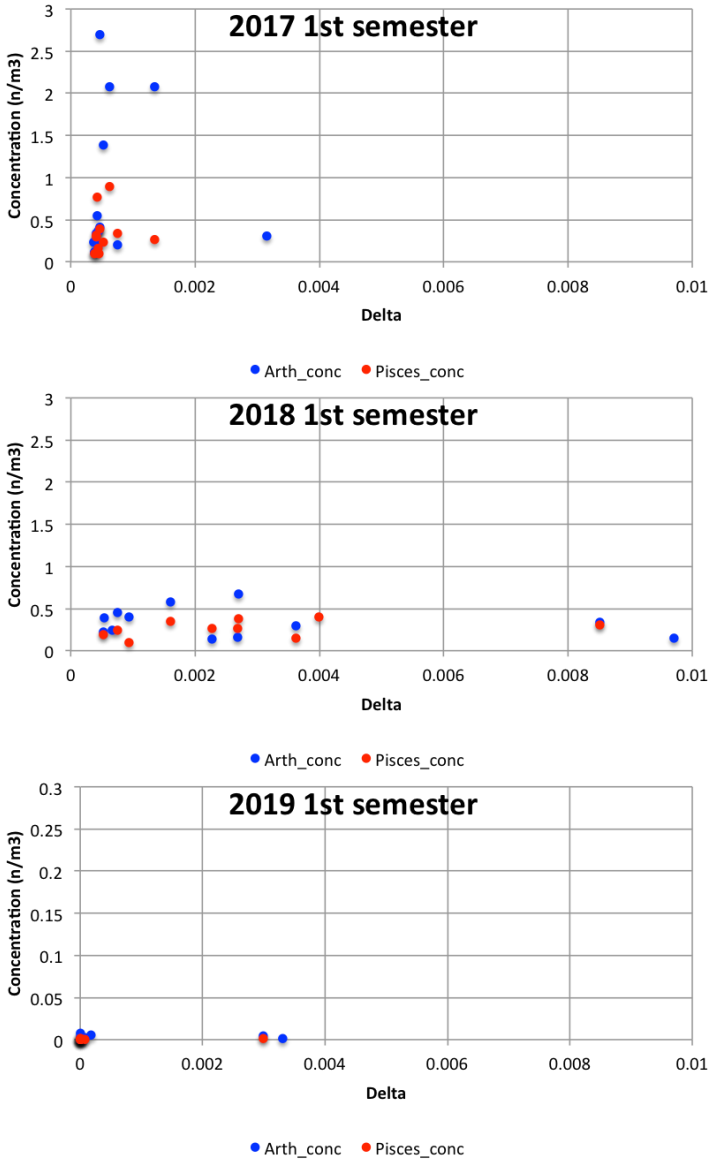


Figure 29. Plot of concentrations (n/m³) of selected Phyla: Arthropoda (shrimps, crabs and Mysids), Pisces (fish larvae) against the Delta fouling indicator (X-axis).

7.3.3 RV Stern samples vs. Cintropur samples

The comparison of the RV Stern samples with the Cintropur samples can be influenced by methodical differences. This was tested by applying combined statistical models.

A comparison of the AICc values of the different nested Generalized Linear Mixed Models (GLMM) shows that adding a zero-inflation component did not improve the models (Table 4). This means that the differences in detection power because of different volumes of water filtered by the two methods did not need to be accounted for in the model. The model with the lowest AICc was model M4 with filter type period as the only covariate (Table 5).

Table 4. AICc of the different models compared.

	df	AICc
M1	6.00	316.37
M1zi	8.00	321.34
M2	5.00	314.48
M2zi	7.00	319.31
M3	4.00	317.94
M3zi	6.00	322.62
M4	4.00	312.78
M4zi	6.00	317.46

Table 5. Results of model M4. Note the negative estimates due to log link and low values.

effect	component	group	term	estimate	std.error	statistic	p.value
fixed	cond		(Intercept)	-1.18	0.40	-2.97	0.00
fixed	cond		filterwedgewire	-1.58	0.54	-2.95	0.00
ran_pars	cond	yearweek	sd__(Intercept)	0.67			

Filter type period was the only significant covariate in any model at $p < 0.01$ in all models where it was included. The interaction between filter type period and sampling point in model M1 was not significant, and did not improve the model over a model with only filter type period as covariate.

Scaled residual plots show normally distributed residuals with non-significant deviation in a Kolmogorov-Smirnov test and Outlier test. Some residual patterns are still found in plots of standardized residuals versus predicted values.

Fish larval densities in samples taken near the Wadden Sea water intake point using a 1 mm mesh size plankton can be compared to fish larval densities in samples taken of the untreated saline water entering the RED pilot plant (point V722) using a 1 mm mesh size Cintropur filter.

We then investigated whether larval fish abundance in samples taken in the Wadden Sea near the seawater intake point were different from impinged larval fish abundance in samples taken within the REDstack installation, and whether the application of wedge wire screens changed the number of larval fish impinged in the RED pilot plant.

To do this we applied Generalized Linear Mixed Models (GLMM) using the R package glmmTMB (Brooks et al. 2017) where in the full model the average number of fish larvae in a sample i μ_i is a function of sampling point p (Wadden Sea or V722 inside the plant), intake screen type t (standard or wedge wire) and interaction between p and t.

Raw fish counts were corrected for sampling effort by adding volume of water filtered v as an offset. Models were adjusted for different background density of fish larvae by adding a random intercept for each of the 7 weeks w where both stations were sampled together. We also checked whether it was necessary to add a zero-inflation component to account for the difference in sampled volumes and thus detection power of plankton net and filter samples.

Models were fitted using a generalized Poisson distribution with log link.

$$M1\text{: } \log(\mu_i) = (1|w_i) + p_i + t_i + p_i * t_i + offset(\log(v)$$

Models were validated by checking for patterns in residuals using the R DHARMa package (Hartig 2019). Model selection was performed by comparing the Akaike's Information Criterion corrected for small sample sizes (AICc, Burnham and Anderson 2002) of the different models using the R MuMIn package (Barton 2019).

In comparing the results of the RV Stern versus Cintropur monitoring, one striking difference to be noted is, that some fish species (three-spined stickleback, glass eel, sand smelt and river lamprey) were caught with RV Stern but were never (during the 3-year sampling period) found in the Cintropur samples in the BE-installation.

The results of fish larvae caught both in RV Stern sampling and in Cintropur sampling in the different years show that, despite a limited number of simultaneous sampling events, the concentrations of the fish larvae are in the same order of magnitude both outside and in the installation with concentrations up to 1 fish larva per m^3 of sea water (Figure 30). The results of Figure 30 are summarised in a box-plot (Figure 31).

The box-plot shows that for most of the comparisons, there is no significant difference between the RV Stern and Cintropur sample concentrations for fish larvae. However, in May 2019 there may be slightly higer concentrations of fish larvae outside the installation (Figure 31) which can be attributed to differences in Gobiid larvae concentrations (Figure 30). For the other fish larvae there are no obvious differences.

This means that:

- fish larvae up to 30 mm total length were found in the installation
- fish(larvae) >30 mm total length were not impinged with the applied screens
- some fish monitored in the Wadden Sea were not found in the installation: glass eel, stickleback, sand smelt, river lamprey, Nilsson's pipefish, herring postlarvae
- fish larvae density impinged in the RED pilot plant (Cintropur samples) was similar to fish larvae density in the Wadden Sea near the intake point (RV Stern) for the species that were found in the Cintropur samples

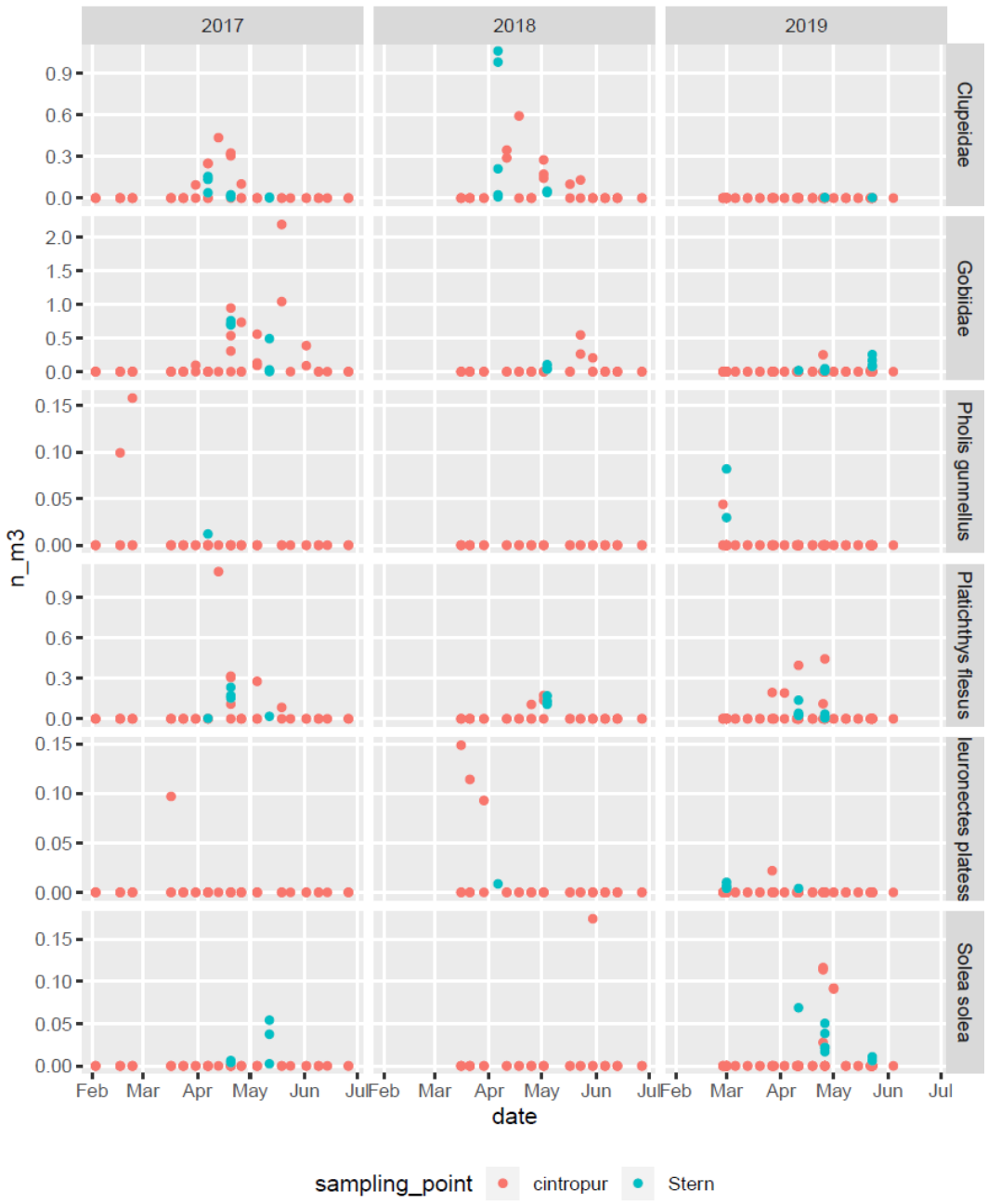


Figure 30. Density of fish larvae in RV Stern 1 mm CalCoFI net samples compared with density of fish larvae in Cintropur filter samples at the seawater intake point V722. In 2019, the wedge wire was applied. Note: different Y-axes scales.

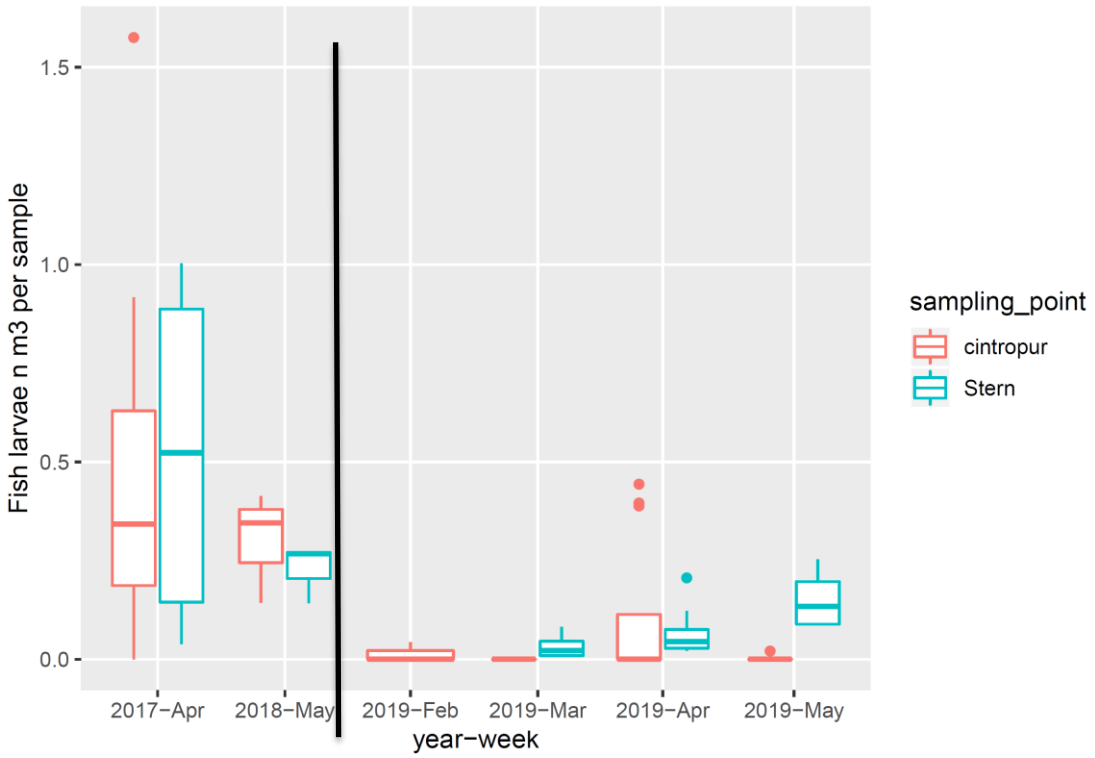


Figure 31. Density of fish larvae in RV Stern 1 mm CalCoFI net samples compared with density of fish larvae in Cintropur filter samples at the seawater intake for days on which both sampling points were sampled simultaneously. Black line indicates start wedge wire.

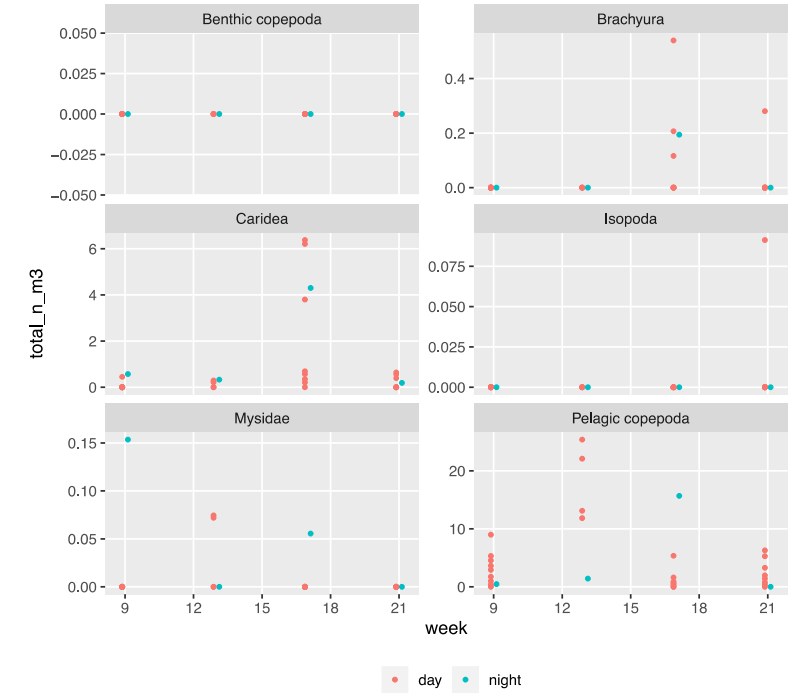
7.3.4 Cintropur samples: sandfilter backwash or no backwash, day and night

In 2019, a rapid sand filter (RSF) was tested upstream of the sample point V722. The RSF-backwash was partially intercepted at point V722 which may on occasion have interfered with the Cintropur sampling.

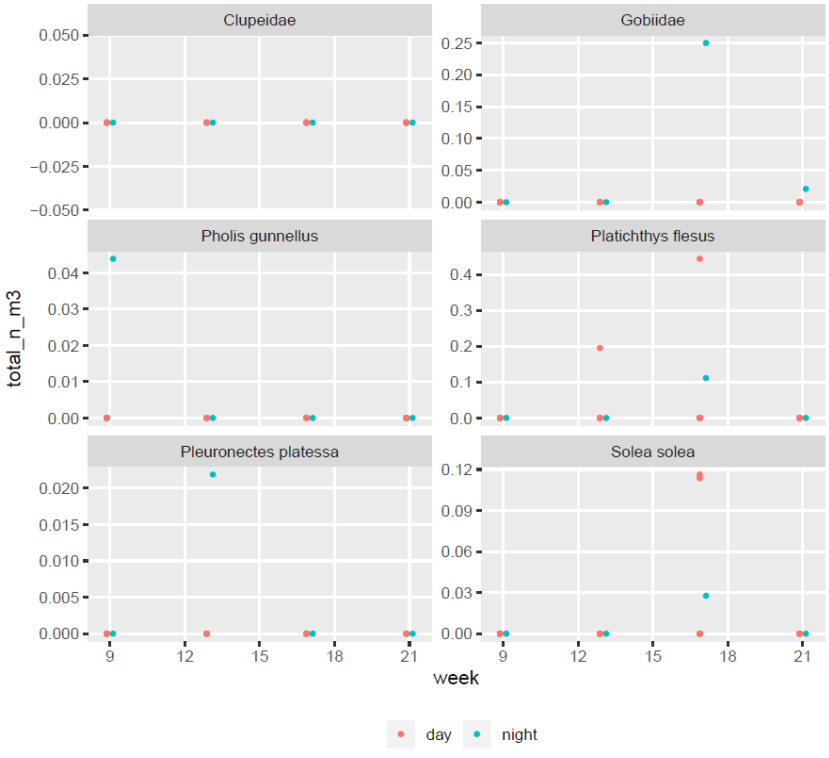
For both crustacean plankton and fish larvae, the concentrations were inherently very variable and apparently not different with or without sand filter backwash. However, the RSF-backwash may have had an effect on the measurements of concentrations in the installation, which can not be further evaluated with the available data.

In 2019, a limited amount of night samples was collected. A closer comparison revealed no striking difference in concentrations of either Crustacea or fish larvae in day or overnight samples, except perhaps for the gobies which were abundant in one night-sample (Figure 32).

In general, the concentrations of Crustacea and fish larvae were very low, which hampers the comparisons and does not allow drawing conclusions on these aspects.



a.



b.

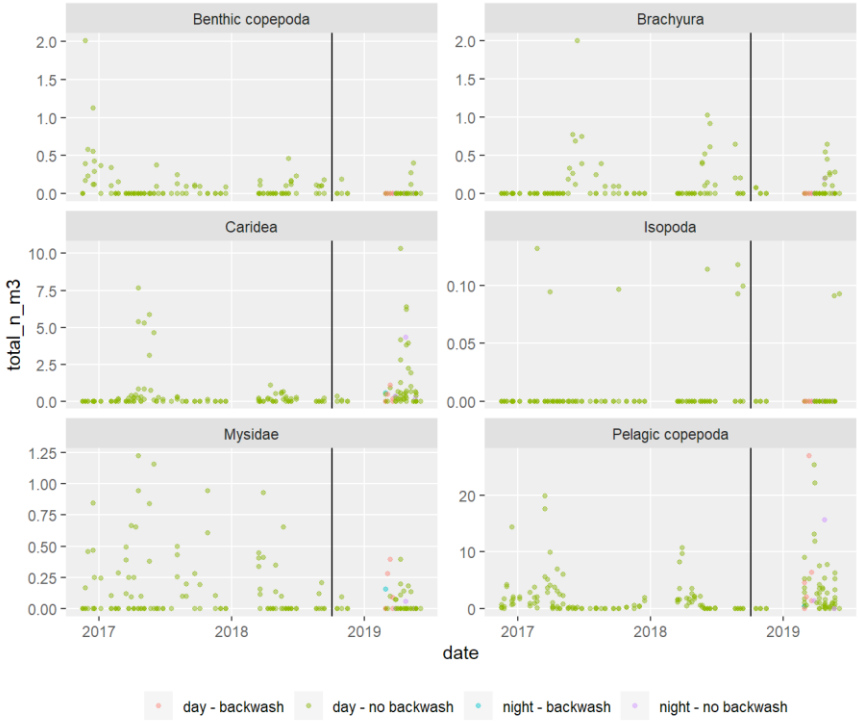
Figure 32. Density of a. Crustacea and b. fish larvae per Cintropur filter sample collected at the seawater intake point V722. Colours indicate whether the sample was taken during day or overnight. Selected weeks in 2019 where night samples were taken.

7.3.5 Cintropur samples: comparison of standard pump cover vs. wedge wire

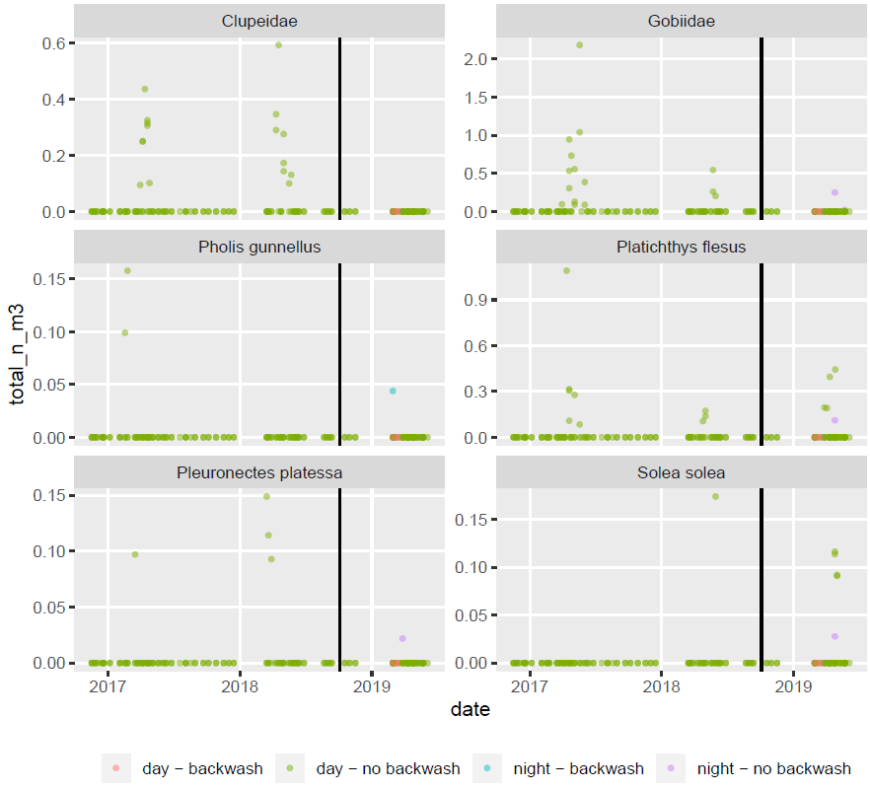
During the monitoring period, two different pump casings have been applied and this may have had an impact on the intake of large organisms. Samples between November 2016 and October 2018 were with perforated pump casing, samples between October 2018 and June 2019 were with wedge wire screens.

The concentrations of Crustacea and fish larvae of the different conditions (day and night, with or without backwash) are indicated in different colours in Figure 33 and the application of the wedge wire panels is indicated by a vertical black line.

In 2019, with wedge wire, there is no clear distinction in the crustacean sample concentrations versus the preceding period with perforated cover. Apparently, fish larval concentrations were lower after the introduction of the wedge wire compared to the preceding years.



a



b

Figure 33. Density of a. Crustacea and b. fish larvae per m³ Cintropur filter sample, collected at the seawater intake point V722. A vertical black line indicates when wedge wire intake screens were installed. Colours indicate whether the sample was taken during day or overnight, and whether during the sampling period a backwash cycle of an upstream sand filter occurred.

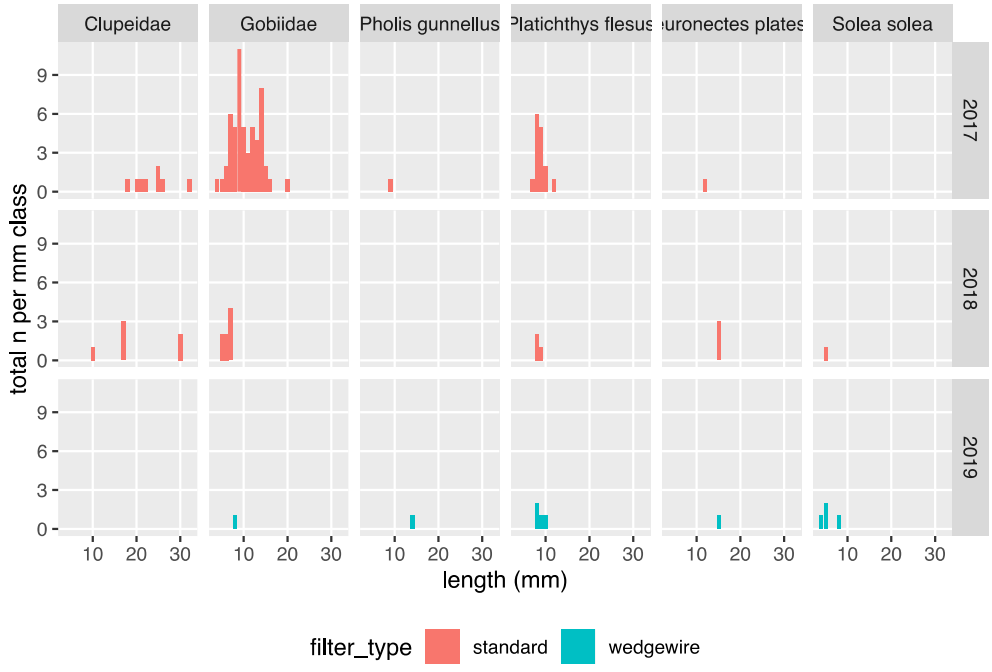


Figure 34. Per-year length distribution of fish larvae found in Cintropur filter samples at the intake point V722. Change to wedge wire intake screens in 2019 indicated by colours.

The length-frequency distributions of different fish species before (2017, 2018) and after (2019) application of the wedge wire did not differ to a great extent (Figure 34), and the concentrations of fish larvae were very low during the wedge wire season.

After the installation of wedge wire screens in October 2018, fish larvae densities in the 2019 samples taken at sampling point V722 were lower than in the previous situation, but this was also the case for the samples taken in the Wadden Sea (see section 6.2.2). Clupeid larvae were completely absent in the 2019 Cintropur samples, whereas in the RV Stern samples of the same year (near the sea water inlet) they were observed in very low densities of 0.004 and 0.003 larvae m^{-3}, equivalent to one fish larva per 333 and 250 m^3, respectively, on April 26 and May 23 (Figure 30).

Conclusions from this are:

- less fish larvae were impinged in 2019 than in previous years, but background fish larval density was also significantly lower during the period where the wedge wire screen filter was installed (2019) than in the preceding period
- therefore, with the available data, no conclusive effect of filter type (wedge wire vs standard) on the intake of fish larvae could be detected

7.4 Discussion on the impingement of large organisms

7.4.1 Methods

Large sample volumes are required for the sampling of fish larvae in particular, because they are present in the environment in rather low background concentrations (e.g. 0,003 individuals per m^3) which implicates that at an average sample volume of 10 m^3, less than one (on average 0,03 individual) would be caught.

The application of a Cintropur filter with a connected flow-meter made it possible to collect sample volumes up to 50 m^3 for the monitoring of larger organisms. After the application of a pump cover with wedge wire panels, the impression was that the samples became cleaner, containing less of organic debris than before. In this case, the sample volume can be increased without the risk of clogging the filter, except in case of occurring *Phaeocystis* algal bloom.

7.4.2 Comparison of impingement with background concentrations

After the application of the wedge wire pump protection it appeared that fish larval impingement had decreased, but RV Stern results indicated that background abundance of fish larvae in the environment had also decreased. Therefore, the decreased intake with wedge wire screens cannot be attributed to a better protection by this screen. The comparison with the RV Stern outside sampling has proven to be of high relevance by indicating that, compared to the previous perforated panels, no impact reduction of the impingement of fish larvae was provided by the application of wedge wire panels. Larger fish larvae (e.g. glass eel, herring post larvae) and small fish (stickleback, sand smelt, river lamprey), present in the RV Stern samples, were retained by the pump cover (both PVC and wedge wire) and were not impinged in the BE-installation. Apparently, perforated panels with 3 mm holes already provided considerable protection in keeping fish out of the water intake, compared to wedge wire panels. Both are capable of keeping (larval) fish >30 mm length out of the installation.

7.4.3 Seasonal variation

The impingement of large organisms showed clear seasonal patterns that recur each year. In fish larvae these patterns were the most pronounced: these are only impinged

in the first half of the year (February-June). Gunnel larvae may already be present earlier than in February, therefore it is advised to start the monitoring of large organisms in January, if environmental conditions do allow (the BE-installation is switched off during periods with frost risk and sea ice). Crustacean larvae were impinged in late spring and summer, whereas juvenile bivalves were impinged during summer.

The impinged abundances of large organism groups varied widely among samples and between years. It is important to at least cover the first half of the year (for fish larvae) or the whole year for other organism groups. The different fish species have a narrow timing of peak abundances, therefore high frequency sampling is needed to grab those.

7.4.4 Consequences for the impact assessment of impingement

Despite the limited number of comparisons with RV Stern, it appears that fish larvae up to a certain size (c. 30 mm length in the case of herring larvae) are impinged in the same order of magnitude of concentration that is present in the vicinity of the BE-installation. It means that the impingement of those larvae is not reduced by the applied pump protection device and that the quantity of impinged fish larvae (in the relevant season) will be quantitatively determined by the used volume of process water that is withdrawn from the Wadden Sea. This assumption is applied in the OOBE Synthesis.

The near-absence of herring larvae in 2019 is a striking observation. The majority of the herring larvae entering the Wadden Sea nursery belong to the autumn/winter spawning North Sea population, originating from Downs (Channel) and Banks spawning grounds (Dickey-Collas et al., 2010).

The North Sea herring collapsed in the mid-70^{ies} and again in the years 2000: recruitment (R (wr)0) has been relatively low since 2002, with very low recruitment in 2015 and 2017 (ICES, 2019). The recovery of the Downs population since the mid-70^{ies} collapse took 25 years, and presently the Downs stock even contributes c. 75% to the North Sea autumn spawning herring population. The review of Dickey-Collas et al. (2010) lists several causes of the collapse: variability in the transport of herring larvae from spawning grounds to coastal nurseries, overfishing of juvenile herring, changes in the zooplankton, variations in bottom temperature on the spawning grounds, predation by jellyfish, bottom-up processes, competition with other species or relations with the NAO-index (weather changes).

The recruitment of North Sea herring larvae has decreased since 2002, despite high spawning biomass of herring. This may be related to bottom up controls of herring recruitment strength (Alvarez-Fernandez et al., 2015) acting through increased mortality (cannibalism by older herring?) and slower growth (food limitation, small zooplankton) of pre-metamorphosing larvae. Feeding interactions between *Mnemiopsis leidyi* and juvenile herring (*Clupea harengus*), between June and September, were analysed by Kellnreitner et al. (2012). These two species showed a high overlap in their respective diets (copepods, meroplankton) during that study period. Due to the low predation impact of *M. leidyi* and the reduced temporal overlap, competition between the two species was estimated as low. Nevertheless, considering the high dietary overlap and the inter-annual variation in biomass and occurrence of both species and their zooplankton prey, competition in the Wadden Sea area cannot be excluded. However, *M. leidyi* is not very abundant in spring and is not likely to be either competitor or predator of herring larvae in this season (March-June) in the Wadden Sea.

Summarising the above, it can be understood that the near absence of herring larvae in the spring of 2019 near Breezanddijk can be caused by a recruitment failure originating in the North Sea, or in consideration of the modelling studies (Dickey-Collas et al., 2009), also by a late timing of larval transport and arrival in the Wadden Sea - in which case herring larvae may have been entirely missed during the OOBE-sampling (March-June, 2019). Fässler et al. (2011) suggest that the mortality of early-stage larvae does impact on the population dynamics in North Sea herring in the current productivity regime, implying a critical period in the determination of year-class strength. This would mean that the herring year-class strength is already largely determined in the phase before the larvae enter the Wadden Sea (larval length 10-30 mm) and that the added mortality by the water intake acts after this presumed critical phase.

The year-class strength of the flatfish populations (plaice, flounder, sole) is assumed to be determined to a large extent during the larval stage in the North Sea (Amorim et al., 2016). The mortality and selection of settlement in the nursery areas also have a considerable and density-dependent contribution but nevertheless only result in 'finetuning' of the yearclass strength of flatfish (Van der Veer et al., 2000). This leads to substantial annual fluctuations in the supply of flatfish larvae to the Wadden Sea. The plaice larvae, at entering the Wadden Sea, are already settling and have sizes that are largely kept out by the perforated or wedge wire panels. Flounder, however, settle in the Wadden Sea and have smaller sizes at metamorphosis, which make them more vulnerable to intake than plaice larvae. In the early nineties, mean concentrations of metamorphosing flounder of 5 up to 10 larvae per m^3 (depending on year, date and location) were observed in the Dollard (Ems estuary), eastern Dutch Wadden Sea, which is a nursery area where these larvae accumulate before settlement to a demersal life stage (Jager, 1999 – thesis). As such, the concentrations observed in- and outside the BE-installation were about 10% of that, much lower than the levels observed in the Dollard (which is an accumulation area). It is difficult to relate the intake to recruitment data, because these do not exist because of lesser commercial interest of flounder.

The gunnel lives in tidal areas and has its mating season in December-February (NNDF, www.verspreidingsatlas.nl). The eggs are guarded by the male until the moment of hatching. This implies that the yolksac larvae of the gunnel, that were found, are likely to be produced in the vicinity of Breezanddijk. The impingement draws from the local population and may therefore have a local impact. No population estimates are available since this species has no commercial interest and also has a rather sheltered habitat.

Gobies are very common in the entire Wadden Sea. (Not much information available).

For a complete impact assessment, the impact of a Blue Energy installation should be assessed in cumulation with other large-scale water intake acting on the populations. Furthermore, the indirect effects of the intake of herring, being a potential prey species for tern colonies (protected species under Natura 2000) in the Wadden Sea should be assessed (see Daenhardt, 2011).

7.5 Recommendations for future investigations BE-Breezanddijk

It would be an improvement for the monitoring of the BE-installation and for future investigations if at least two parallel water intake tubes would be constructed that can be sampled simultaneously: this would allow more and simultaneous comparisons of different 'treatments', concerning: wedge wire opening width, influence of rapid sand filter (RSF) backwash on the accumulation of organisms and their survival. This is of high relevance for the future upgrading of the BE-installation and the assessment of the impact of upgrading.

The effect of filtration with RSF could not be investigated in this study, because of the introduction during a late stage of the project, and due to the fully automated flushing cycle. In a future study this impact could be addressed. Regarding this, it is important that the backwash is not flushed into the pathway of the Cintropur monitoring.

The number of comparisons between day and night was limited in the present study, therefore no conclusions could be drawn, although for some species groups there may be day-night differences. In a future study this aspect deserves more attention and it implies that the sample volumes should be larger to allow overnight automated sampling without clogging the filter.

8 Small organisms

8.1 Introduction

In this chapter the possible impact of Reverse Electrodialysis power generation on zooplankton is investigated. Abundance, composition, seasonal patterns and survival of zooplankton interacting with the experimental Reverse Electrodialysis (RED) installation located at Breezanddijk, Netherlands were investigated in 2016 - 2019.

Additionally, the impact of organisms on the functioning of the RED installation at Breezanddijk was investigated.

8.1.1 Zooplankton monitoring

To investigate the possible impact of RED on zooplankton in the western Wadden Sea, data on the composition and seasonal patterns of zooplankton were needed. Zooplankton composition and seasonal patterns in the western Wadden Sea were largely unknown, with the last detailed study dating from 1973 - 1978 (Fransz and Arkel 1983). To address this data gap, a monitoring programme was carried out whereby zooplankton condition, density and composition at different sampling points in and around the RED installation at Breezanddijk were assessed at regular intervals, for both fresh water as well as seawater.

8.1.2 Zooplankton survival experiments

Zooplankton mortality in a RED installation can be caused by several factors, as reviewed in WP 1:

- Mechanical stress caused by:
  - shear stress near surfaces,
  - pumping,
  - filtration.
- Osmotic stress experienced when seawater and fresh water are mixed.
- Thermal stress experienced when seawater and fresh water are mixed.
- Predation:
  - by fouling organisms living on the installation surfaces,
  - by other zooplankton living in the process water,
  - at the brackish water outflow.

One of the possible mortality-causing factors for zooplankton in the RED process is the instantaneous mixing of seawater and freshwater. Mixing of seawater and fresh water can occur A) when filter residues of fresh water and seawater filters are combined to be discharged via the brackish water retour pipe, B) within the RED stacks and C) when filtered sea water and fresh water are combined in the system's bypass system which transports water that is not passed through the RED stacks. Here, we investigated mortality in two ways, by estimating the fraction of dead and alive organisms in samples taken at different points in the RED process, and by experimentally reducing salinity of unfiltered seawater samples by concentration and dilution with different amounts of filtered fresh water.

Salinity is a critical factor influencing diversity and distribution of aquatic organisms, including zooplankton (e.g. Kinne 1971; Telesh and Khlebovich 2010; Snoeijs-Leijonmalm, Schubert, and Radziejewska 2017). Beside salinity variation on a spatial

scale, salinity variation on a temporal scale (stability) is also an important factor (Attrill 2002; Whitfield et al. 2012).

Zooplankton can experience sudden changes in salinity when two water sources of different salinity are mixed (Kaartvedt and Nordby 1992; Kaartvedt and Aksnes 1992). Organisms can have several methods of regulating their ionic composition (Gilles 1975) but sudden change in salinity can cause mortality of zooplankton, as has been shown in copepods, with salinity reduction tolerance highly varying between species (Lance 1963; Calliari et al. 2008; Zajaczkowski and Legezynska 2001; Lee and Petersen 2003).

Mixing of freshwater and seawater occurs in natural systems, for example by glacial melting, river discharge, precipitation or mixing of stratified waters by storms, but can also be caused by anthropogenic factors such as sudden discharge of water from hydroelectric power plants and storm drains. In Blue Energy production seawater and fresh water are mixed, and this mixing might cause mortality of zooplankton entrained in the feedwater. Since the sensitivity to sudden salinity changes differs for different species, and little studies have been done on this salinity sensitivity in groups other than copepods, we performed an experiment where we exposed natural communities of Wadden Sea zooplankton to sudden salinity changes of varying magnitude.

8.2 Methods

In an initial evaluation of sampling points it was decided that samples taken at sampling points for fresh and saline filter residue (V715 and V725) could not be used for quantitative sampling because the irregular frequency of cleaning of the drum sieves would cause highly varying organism density depending on where in the cleaning cycle the samples were taken.

The focus of the monitoring in this project was on fauna in the seawater, but for comparison the fauna in the fresh water was also included in the monitoring. The following points were sampled:

1. V712, unfiltered fresh water,
2. V714, filtered freshwater from buffer tank,
3. V722, unfiltered seawater
4. Effluent from seawater drum sieve,
5. V724, filtered seawater from buffer tank,
6. V731, brackish water from buffer tank.

Samples were collected in square perspex buckets of 20 l volume. Sampled volume was 10 litres at each sampling point, except for a few occasions where organism density was very high or low. All samples were taken in duplicate.

Sample procedure was as follows for all points (except the points where a sample is taken directly from the drum sieve):

1. A flexible hose was connected to the sampling tab point,
2. The valve was opened and water is discharged for about five seconds to clear out any built-up residue in the sampling hose or valve,
3. The two sampling buckets were rinsed with sample water,
4. The two sampling buckets were filled to the desired volume using a low flow rate to minimise organism disturbance,
5. Samples were filtered over a plankton sieve with a diameter of 18 cm and a mesh size of 20 µm (when drum sieve mesh size was ~20 µm) or 50 µm (when drum sieve mesh size was increased to ~40 µm),

6. Filter residue was flushed from the filter into a 100 ml glass beaker using a spray bottle with filtered water.

For the samples taken directly from the drum sieve steps 1-4 were slightly different but the filtration steps were similar:

1. The drum sieve cover was opened,
2. The drum sieve surface was visually inspected for large holes or tears for one rotation of the drum sieve,
3. Sampling buckets were rinsed with water scooped from the container surrounding the drum sieve using 5l plastic beakers with handles,
4. Sampling buckets were filled to the desired volume using the plastic beakers,
5. Samples were filtered over a plankton sieve with a diameter of 18 cm and a mesh size of 20 µm (when drum sieve mesh size was ~20 µm) or 50 µm (when drum sieve mesh size was increased to ~40 µm),
6. Filter residue was flushed from the filter into a 100 ml glass beaker using a spray bottle with 50 µm filtered sample water.

Sampling hoses, buckets and filters were cleaned with demineralised water after all samples were taken.

Abundance of zooplankton organisms of different taxonomic groups was assessed by counting organisms in the sample in a modified Bogorov-Gollasch counting tray with 5 separate slits using a binocular microscope with 8x - 40x magnification. Condition of organisms was considered alive if movement was observed, and dead if organisms where immobile, with organisms poked with a cactus needle in case of uncertainty. Organisms were identified to

standardised taxonomic group levels, similar to taxonomic groups used in ballastwater treatment system certification tests. Calanoid and Cyclopoid copepods were combined in a category “Pelagic copepods” and Harpactacoid copepods in category “Benthic copepods” for nauplii as well as copepodites.

From 24-11-2016 onwards, dead and alive organisms were counted separately. Organisms that were not moving were considered dead. For microzooplankton groups ciliates and rotifers it was difficult to distinguish dead organisms in between the abundant detritus in the samples. For these groups, dead organisms were not counted. For copepod nauplii, dead organisms were counted, but it is possible that small dead nauplii were missed when counting with a stereomicroscope, so dead counts are likely an underestimation.

8.2.1 Additional sampling

Next to the regular monitoring programme, several other points were sampled occasionally to answer specific research questions:

- Niskin bottle samples were taken from NIOZ RV Stern near the seawater intake point in the Wadden Sea to test whether zooplankton densities at the Blue Energy pilot installation intake point were similar to in situ densities in the Wadden Sea.
- After installation of a fast sand filter (see report D2.2 for details) in 2019, sand filter effluent was sampled for sediment and zooplankton composition.
- To investigate possible accumulation of organisms inside the drum sieve drum, samples were taken directly inside the drum sieve drum.

- During the 2019 shellfish filtration pilot (described in report 3.2-3.3) effluent of the control tank and the tank containing mussels was sampled for sediment and zooplankton composition.

Any deviation from the standard sampling methods used in these tests will be mentioned in the results.

8.2.2 Analysts

Samples were analysed by NIOZ research assistants Dennis Mosk and Evaline van Weerlee and NIOZ postdoc Dr. Lodewijk van Walraven. From February - July 2018 MSc student Danila Uvarov assisted with the sampling.

8.2.3 Sampling frequency

The goal was to sample weekly in periods where high organism abundance is expected, which was roughly from March until November, and biweekly during winter when organism density was expected to be low, in November, December and February. Because the RED installation was not always operational, maintaining a weekly or biweekly sampling frequency was not always possible.

Because of time constraints, the fresh water side intake water and effluent was sampled at half the frequency of the seawater side. When fresh water was sampled one of the seawater sampling points (V724, buffer tank) was skipped.

The main sampling programme lasted from 2016 until end of 2018. In 2019 limited sampling was performed for experiments investigating survival of zooplankton after sudden salinity reductions. The undiluted control samples from these experiments are included in the monitoring results for sampling point V722.

In total, small organisms' samples were taken at V722 on 76 days. First samples were on Thursday, June 16, 2016, last samples were taken on Thursday, September 19, 2019.

8.2.4 Estimation of zooplankton biomass

To estimate the biomass contribution of each zooplankton group to total zooplankton biomass, we used literature-based biomass estimates for each group, where available (Table 6). This analysis was repeated twice to investigate how robust these estimates were; once for carbon content as done in Jaspers et al. (2018) for *Mnemiopsis leidy*i stomach contents and once for dry mass as done by Martens and Beusekom (2008) for the zooplankton community of the northern Wadden Sea.

Table 6. Individual biomass estimates (in µg /ind) and their sources used in the estimation of zooplankton biomass.

Species	ug_C_ind	reference	ug_ind	reference_dw
Cladocera	2.00	Jaspers et al. 2018		Pelagic copepod value used
Benthic copepod nauplii	0.17	Jaspers et al. 2018	0.80	Martens and van Beusekom 2008
Benthic copepoda	2.00	Jaspers et al. 2018	0.80	Martens and van Beusekom 2008
Pelagic copepod nauplii	0.17	Jaspers et al. 2018	0.11	Martens and van Beusekom 2008
Pelagic copepoda	2.10	Jaspers et al. 2018	6.40	Martens and van Beusekom 2008 (mean)
Balanoid nauplii	2.50	Jaspers et al. 2018	4.50	Martens and van Beusekom 2008
Balanoid cyprid	11.00	Jaspers et al. 2018	16.00	Martens and van Beusekom 2008
Bivalvia	2.20	Jaspers et al. 2018	0.08	Martens and van Beusekom 2008
Gastropoda veligers	2.20	Jaspers et al. 2018	2.00	Martens and van Beusekom 2008
Gastropoda adults	2.20	Jaspers et al. 2018	2.00	Martens and van Beusekom 2008
Mollusca other	2.20	Jaspers et al. 2018	2.00	Martens and van Beusekom 2008
Polychaete trochophores	4.30	Jaspers et al. 2018	0.15	Martens and van Beusekom 2008
Polychaete nectochaetes	4.30	Jaspers et al. 2018	10.60	Martens and van Beusekom 2008
Polychaete adults	4.30	Jaspers et al. 2018	10.60	nectochaete value used
Platyhelminthes	4.30	nectochaete value used	10.60	Martens and van Beusekom 2008
Rotifera	0.23	Jaspers et al. 2018	0.07	Martens and van Beusekom 2008
Ciliophora	0.23	Jaspers et al. 2018	0.07	using estimate for rotifera
Bryozoa cyphonauta	0.64	Wendt 2000 (average)	1.00	Martens and van Beusekom 2008
Rotifera+Ciliophora	0.23	Jaspers et al. 2018	0.07	using estimate for rotifera

8.2.5 Zooplankton survival: pilot experiments 2018

In 2018 experiments were carried out within the MSc internship of Danila Uvarov to test staining methods and get preliminary observations on Wadden Sea zooplankton survival at 1:1 seawater:fresh water dilution.

Western Wadden Sea zooplankton samples of 10 l were concentrated over a 50 μm filter in 100 ml glass beakers, after which either filtered fresh water or seawater was added for 1:1 seawater/fresh water dilution treatment or control, respectively. The beakers were kept for a minimum of 5 days in a climate room, during which mortality of plankton was followed. The experiments were repeated at 5.0, 10.0, 15.0 and 22.0 C. In these experiments, zooplankton mortality appeared to increase with increasing temperature. However, as mortality did not differ between control and experimental treatments, an effect of dilution could not be shown. Because of this, we decided to re-do these experiments with modified methods aimed at minimising background mortality by:

- minimising concentration of zooplankton samples,
- concentration of samples for dilution using reverse filtration,
- having a shorter experimental duration of 1 day.

We furthermore observed that effectiveness of Neutral Red staining commonly used in live-dead assays of plankton (Dressel, Heinle, and Grote 1972; Elliott and Tang 2009; Zetsche and Meysman 2012) varied highly between taxa, as also noted in previous studies. Rotifers for example stained well, even though clearly dead, and balanoid nauplii were poorly stained, even though clearly alive. Abundant detritus also complicated staining as the detritus absorbed some of the dye. Thus, in line with SOPs used for ballast water treatment certification at NIOZ we decided not to use stains in experiments, and assess viability by movement of organisms only.

8.2.6 Zooplankton survival: main experiment 2019

8.2.6.1 Sampling

All samples for this experiment were collected in 2019 at the REDstack experimental Blue Energy power plant located on the Afsluitdijk barrier in Breezanddijk, Netherlands. The experiment consisted of four different treatments: an undiluted control and three treatments were seawater was diluted with fresh water at a ratio of 1:1, 1:2 and 1:3, respectively. As seawater source untreated seawater from the Wadden Sea (sampling point V722) was used. As freshwater source 20 μm drum sieve filtered fresh water from Lake IJsselmeer (sampling point V714) was used. This water was filtered over a 50 μm filter to remove any remaining planktonic organisms larger than 50 μm.

Each treatment was replicated four times and treatments were randomly assigned to containers. 16 polypropylene 12 l Nalgene carboys were filled with 10 l of unfiltered seawater. Depending on the dilution factor, either 0 l, 5 l, 6.66 l or 7.5 l of seawater was removed from the carboy by reverse filtration using a funnel with 50 μm sieve attached to a PVC hose (Fig.). After this step the different dilution factors where achieved by topping up the carboys to 10 l again with filtered Lake IJsselmeer fresh water. All carboys where mixed by gently stirring using a metal rod, and conductivity was measured in each carboy using a handheld conductivity meter. Afterwards, carboys where transported to the Royal Netherlands Institute for Sea Research on Texel, Netherlands, where they where incubated in a temperature controlled room at temperatures matching the seawater temperature at collection date. The next day, the plankton in each bottle was concentrated first by reverse filtration, with the final ~1 l concentrated by pouring the sample over a 50 μm sieve and rinsing the sample in 100 ml beakers. All rinsing and dilution of samples was done using 50 μm filtered sample water of a salinity similar to the experimental treatment of the sample to prevent additional osmotic stress on organisms. This amounted to an experimental duration of 20 hours.

Abundance of zooplankton organisms of different taxonomic groups was assessed by counting organisms in the sample in a Bogorov-Gollasch counting tray using a binocular microscope with 8x - 40x magnification. Condition of organisms was considered alive if movement was observed, and dead if organisms where immobile, with organisms poked with a cactus needle in case of uncertainty. Organisms were identified to standardised taxonomic group levels used for all monitoring within the OOBE project, similar to taxonomic groups used in ballastwater treatment system certification tests. Calanoid and Cyclopoid copepods were combined in a category “Pelagic copepods” and Harpactacoid copepods in category “Benthic copepods” for nauplii as well as copepodites.

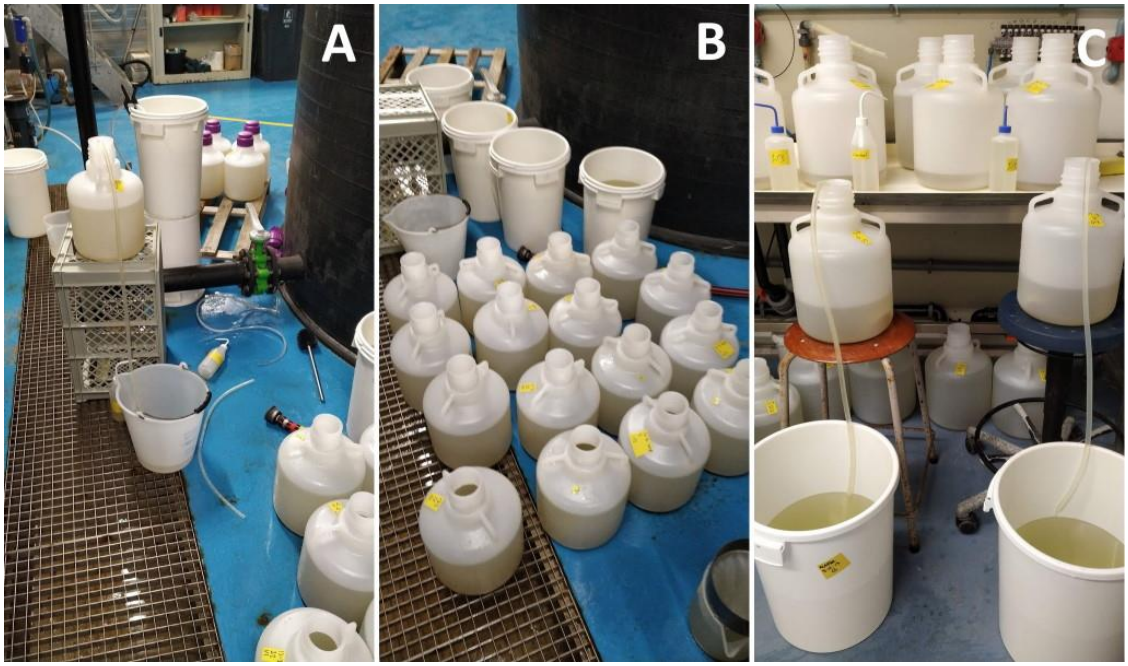


Figure 35. Photographic impression of sampling, A) filtering of samples at the RED power plant, B) All 16 carboys of a single experiment, C) filtering of samples in the NIOZ climate room.

It was difficult to distinguish dead organisms in between the abundant detritus and sediment in the samples (Figure 36), especially for rotifers and ciliates. Dead counts were only considered reliable for large conspicuous organisms such as copepods, cirripeds and polychaete nectochaetes. For analysis it was assumed that starting density and species composition of zooplankton in all containers at the start of the experiment was similar, except for naturally occurring variation. Differences in density of live organisms between control and treatments at the end of the experiment were assumed to be caused by mortality in the experiment.

Figure 36. Photograph of sample of the September replicate of the experiment showing abundant sediment and detritus in the sample.

In order to test if results were consistent across different water temperatures, seasons and to sample as wide range of taxonomic groups as possible, the experiment was repeated four times; monthly in March - May to cover the spring plankton bloom, and once in September in autumn to cover a possible autumn bloom.

8.2.6.2 Analysis

Data were analyzed for differences in live zooplankton counts between control and treatments, where any differences in live counts between control and treatments not explained by inherent variation were assumed to originate from mortality induced by the treatment. Treatment was included as dilution factor d where seawater is diluted with fresh water at seawater:fresh water ratios 1:d with d: 0 (control), 1, 2 or 3. We also investigated whether the relationship between zooplankton counts and dilution differed between the different replications of the experiments at different dates. For this we applied Generalized Linear Models (GLM) using the R package glmmTMB (Brooks et al. 2017) where in the full model the average amount of zooplankton organisms in a sample Y_i is a function of dilution factor d of sample i and seasonal replicate r (the four replicates in March, April, May and September) of sample i and an interaction between d and r. Dilution factor d was treated as continuous variable, but replicate r was treated as a factor because community composition was clearly different between different replicates.

$$
\begin{aligned}
M1: log(\mu_i) &= d_i + r_i + d_i * r_i \\
M2: log(\mu_i) &= d_i + r_i \\
M3: log(\mu_i) &= r_i \\
M4: log(\mu_i) &= d_i
\end{aligned}
$$

Models were fitted using a negative binomial distribution where variance increases quadratically with the mean, and a log link. Models were validated by checking for patterns in residuals using the R DHARMa package (Hartig 2019). Model selection was performed by comparing the nested models using likelihood ratio tests. The Akaike's Information Criterion corrected for small sample sizes AICc, (Burnham and Anderson 2002) of the different models was estimated using the R MuMIn package (Barton 2019).

Most rotifers and ciliates were small, close to the mesh size used for filtering of 50 μm, it was difficult to distinguish the smallest individuals of these groups using a low magnification stereomicroscope. Because of this, these groups were combined in the analysis and the analysis was repeated three times, once for all organism groups combined, once for all groups excluding rotifers and ciliates, and once for rotifers and ciliates combined.

8.2.7 Fouling experiments

To investigate whether fouling organisms still present in effluent in the RED installation settled on surfaces of pipes in the stack street area, a PE tube segment with spacers was designed to fit into one of the main distribution pipes of the right stack street. This tube segment was fitted on 08-04-2017. On 14-06-2017 the tube segment was removed (Figure 65), and a qualitative inventory was made of the organisms on:

- on the surfaces of the tube segment itself,
- in the surfaces of the stack street distribution pipe,
- in the sediment inside the stack street distribution pipe and adhering to the tube segment, extracting organisms and their remains by filtering the sediment over a 500 micron filter.

Afterwards the tube segment was cleaned and replaced. On 21-09-2017 a second and final check was performed using similar methods.

8.3 Results small organisms

8.3.1 Fresh water

An overall summary of seasonal patterns of the most common zooplankton groups found in lake IJsselmeer intake water is presented in Figure 37. Per-year daily averages of density of grouped zooplankton taxa found in lake IJsselmeer intake water are shown in Figure 38, and the proportion of the total zooplankton density in a sample of lake IJsselmeer intake water, per year and day, is shown in Figure 39. Most abundant zooplankton in lake IJsselmeer samples were rotifers, followed by ciliates, cladocera and copepods. In spring, rotifers were the first organisms to increase in abundance around february, followed by copepods in March and April. Bivalve larvae were only abundant at end of spring, early summer in May and June. Cladocera were present year-round, with highest abundances in the second half of the year. Seasonal pattern of ciliates was variable.

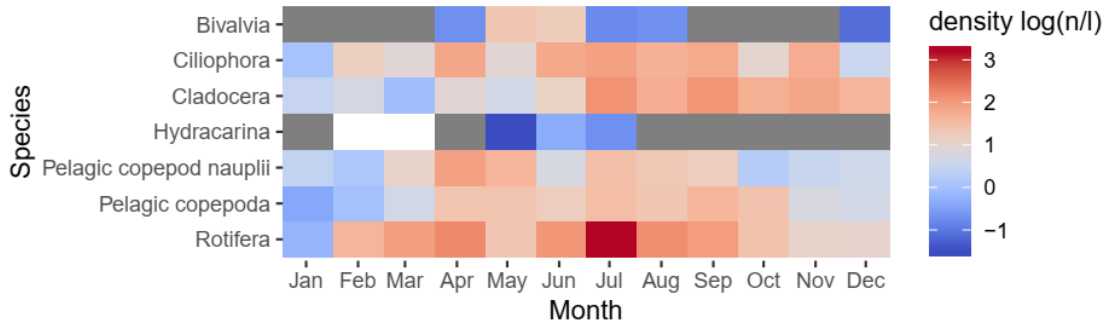


Figure 37. Summary of seasonal patterns of common plankton groups found at sampling point freshwater intake (V712), based on monthly averages of samples taken in 2016-2018. Grey: group was absent in this month.

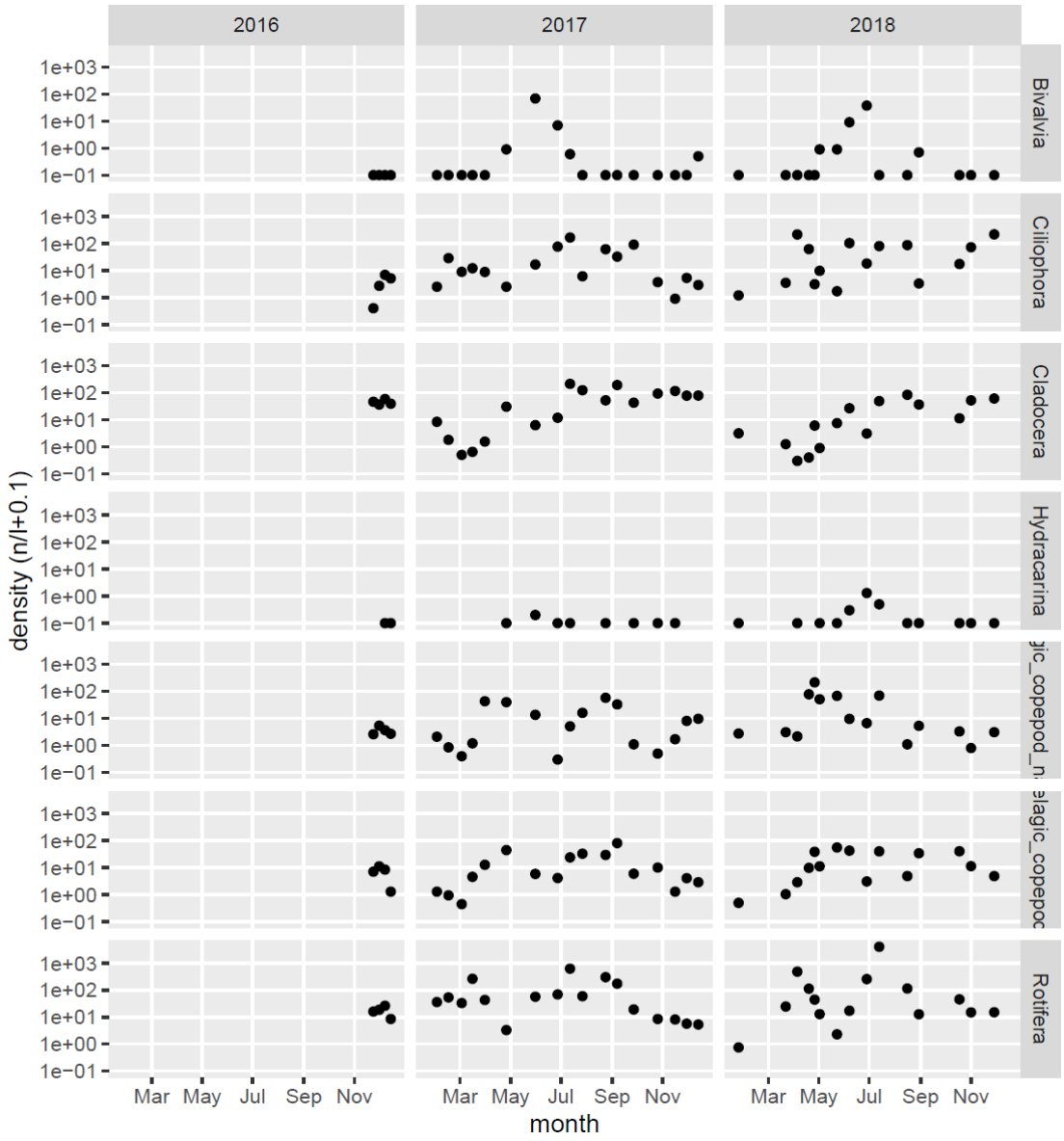


Figure 38. Mean density (n/l) per sampling day of major zooplankton groups in samples taken at sampling point V712, fresh water intake.

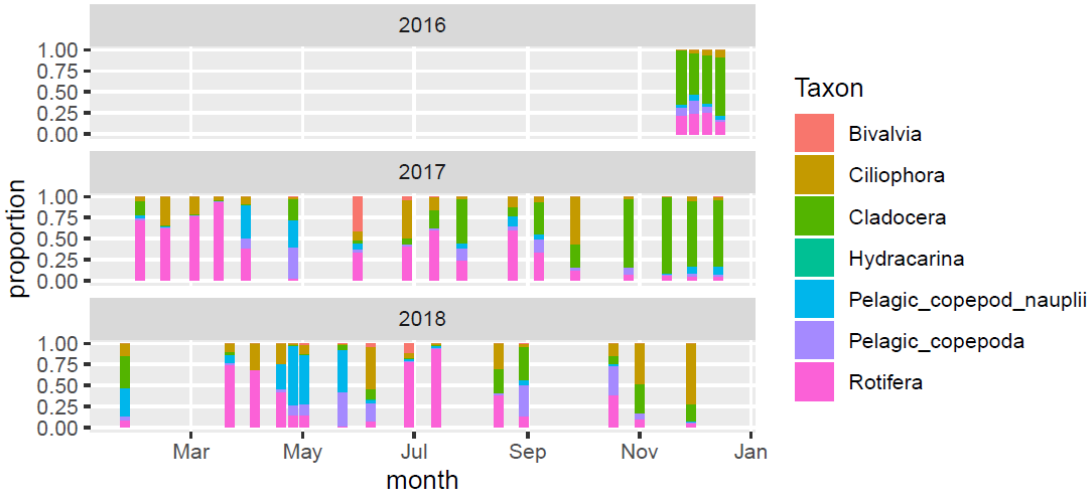


Figure 39. Proportion of total density per sampling day of major zooplankton groups in samples taken at sampling point V712, fresh water intake.

8.3.2 Seawater: Seasonal patterns

The abundance of most planktonic organisms varies seasonally, often by several orders of magnitude. In this section we present and describe the seasonal patterns observed in the different planktonic groups of small organisms monitored in the study at sampling point V722, saline intake.

8.3.3 Seawater: Seasonal patterns in density

In the following analyses summarising seasonal patterns samples taken before November 2016 were excluded as these were taken with a smaller mesh size and rotifers and ciliates were not counted separately.

An overall summary of seasonal patterns of the most common zooplankton groups found in Wadden Sea intake waters is presented in Figure 40. Per-year, daily averaged densites of grouped zooplankton taxa are shown in Figure 41, and the proportion of the total zooplankton density in a sample, per year and day, is shown in Figure 42.The figures show a zooplankton community that is highly varying in composition and abundance, but with common patterns that are repeated every year, or at least in the intensively sampled years 2017 and 2018.

Copepods were present on almost every sampling day, but their total contribution to zooplankton density was often lower than 50% or even 25%, and copepods reach peak densities in April.

In early spring (February and March) the zooplankton community is dominated by meroplanktonic larvae of polychaetes, which decrease in density until May when they are almost absent.

Larvae of barnacles can also be abundant, and are present from February to October. Especially in May and June, mollusc larvae of both gastropods and bivalves can be very abundant, and often contribute up to 50% of the total community by density.

Rotifer density was variable, with high densities observed in April–June but also in August–November. The same holds for ciliates, which are most abundant in April–May and July–November. Overall, density of zooplankton is highest in spring (March–June).

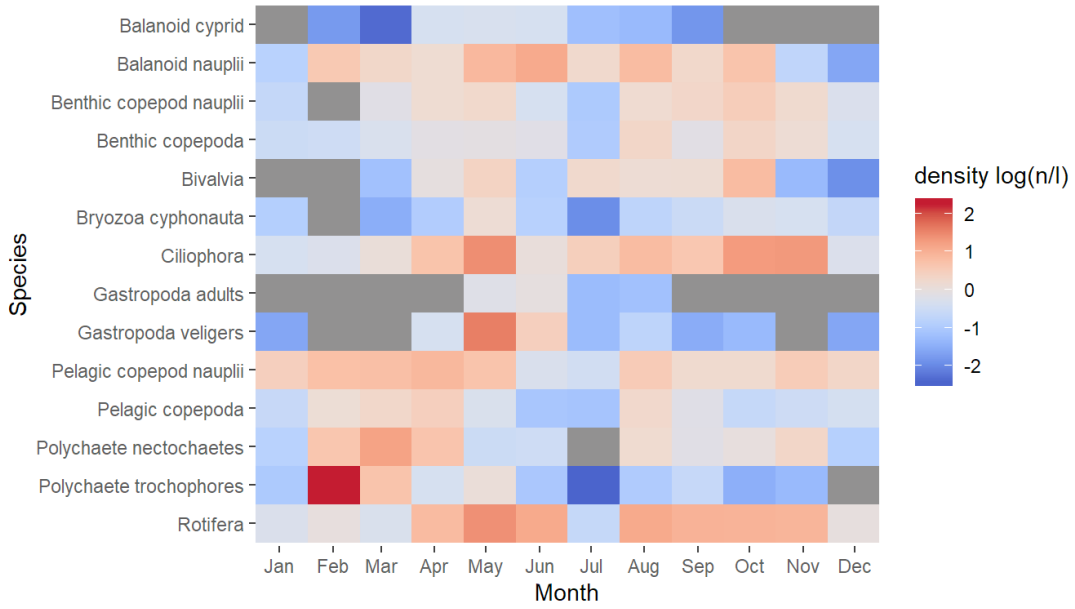


Figure 40. Summary of seasonal patterns of common plankton groups sampled at sampling point seawater intake (V722), based on monthly averages of samples taken in 2016-2019. Grey: group was absent in this month.

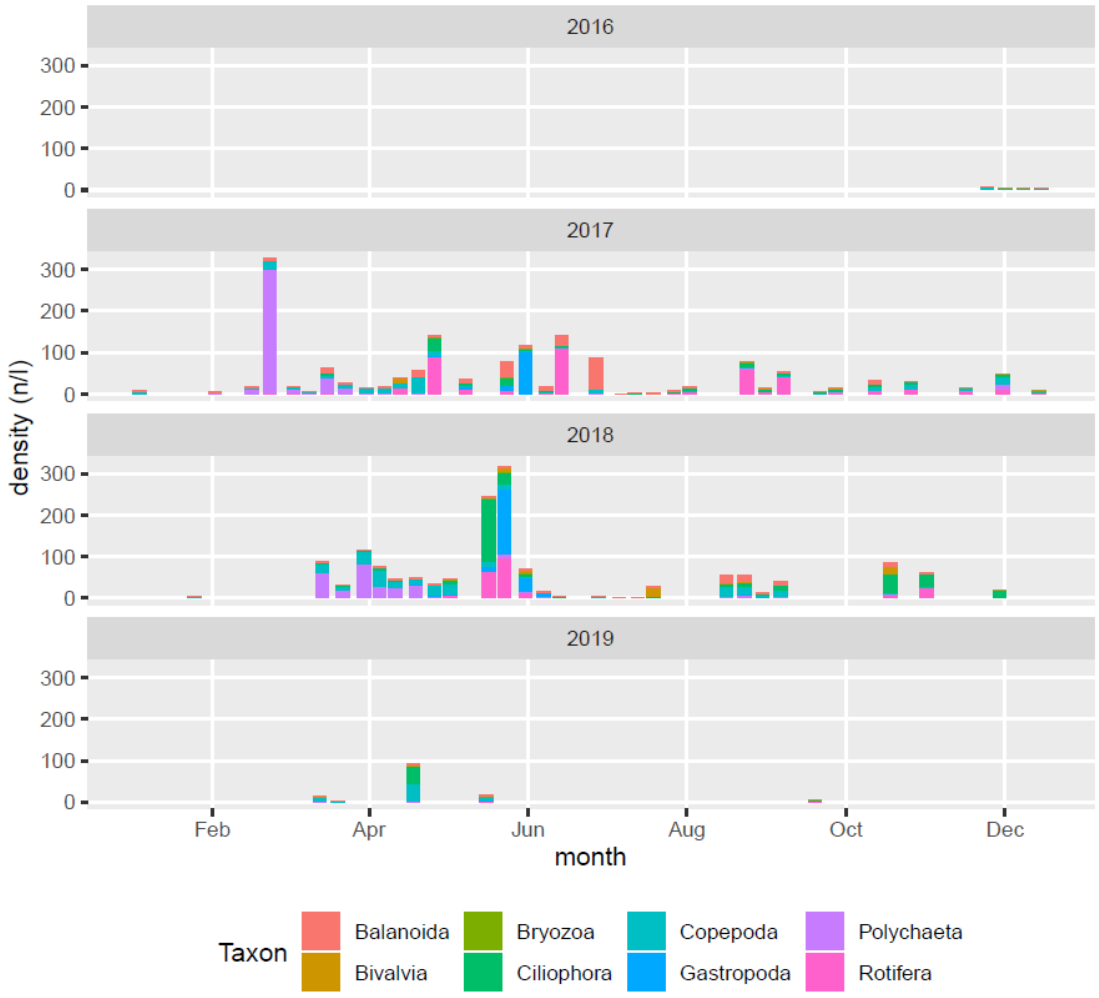


Figure 41. Mean density (n/l) per sampling day of major zooplankton groups in samples taken at sampling point V722, seawater intake. *A high polychaete density of 569 n/l on 2017-02-23 was set at 300 n/l to limit y axis range.

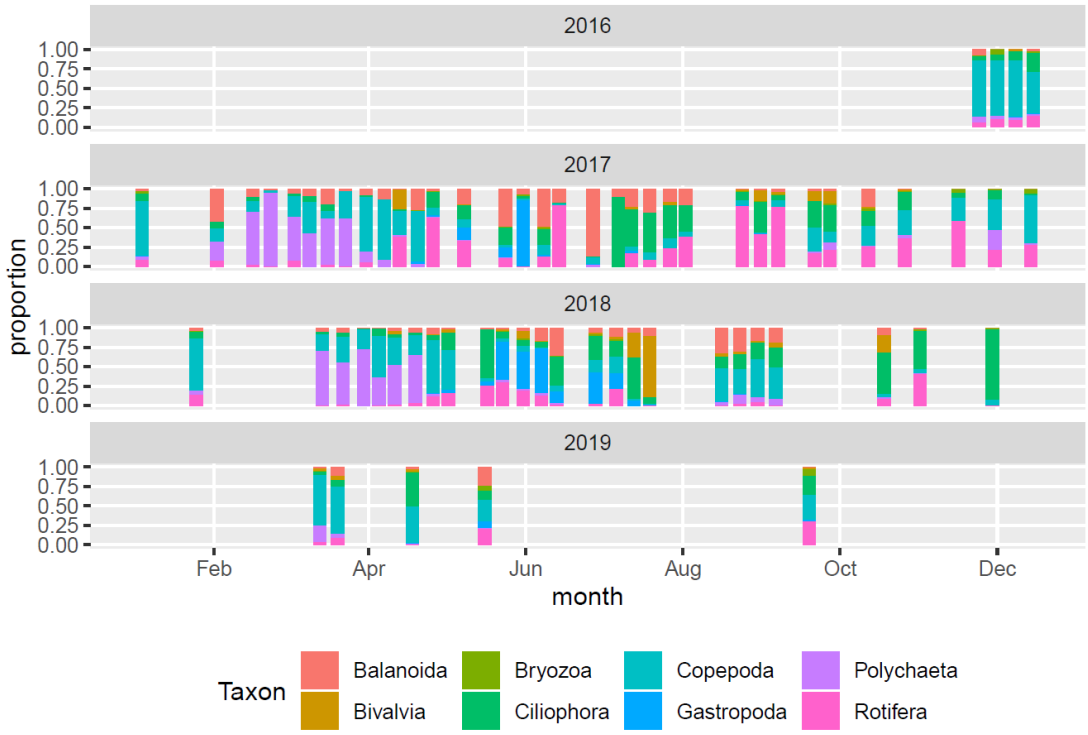


Figure 42. Proportion of total density per sampling day of major zooplankton groups in samples taken at sampling point V722, seawater intake.

8.3.4 Seawater: Seasonal patterns in biomass

Biomass was estimated using two sets of individual carbon content estimates, carbon content cf Jaspers et al. (2018) and dry mass cf Martens and van Beusekom (2008). These estimates produced similar results overall, but the carbon content estimates produced extremely high biomass values for bivalve and gastropod larvae, as well as polychaete trochophores and nectochaetes, as these groups or life stages did not have separate estimates for carbon content.

Because of this, we chose to use the dry mass estimates of Martens and van Beusekom (2008) for estimating biomass, presented below.

The average dry weight of zooplankton of the taxa considered was 95.7 µg/l (s.e.: 18.0 µg/l). Maximum zooplankton biomass was 913.3 µg/l on 30-03-2018, minimum zooplankton biomass was 0.8 µg/l on 07-07-2017. Patterns in estimated biomass were similar to patterns in density, with high biomass during spring, and in 2017 also in early summer, and low biomass in the rest of the year (Figure 43).

Copepods estimated biomass was on average 11.5 µg/l (s.e.: 2.46 µg/l), highest on 20-04-2017 at 130.1 µg/l and lowest on 7-7-2017 at 0 µg/l. Highest copepod biomass occurred in spring (end of March, April and May), with in 2018 a peak at the end of summer in late August/early September as well (Figure 44).

The contribution of each group to the total biomass was highly variable, with patterns similar to patterns in density (Figure 45). In early spring, zooplankton biomass consisted mainly of polychaete larvae, which gradually decrease and are replaced by copepods and balanoid larvae. In summer biomass is low, and consists mainly of balanoid and gastropod larvae. End of summer and autumn, biomass consists of a mix of mainly copepods, polychaete larvae and ciliates. In the end of autumn and winter, the biomass consists mainly of polychaete larvae and copepods, although sampling in this period was limited.

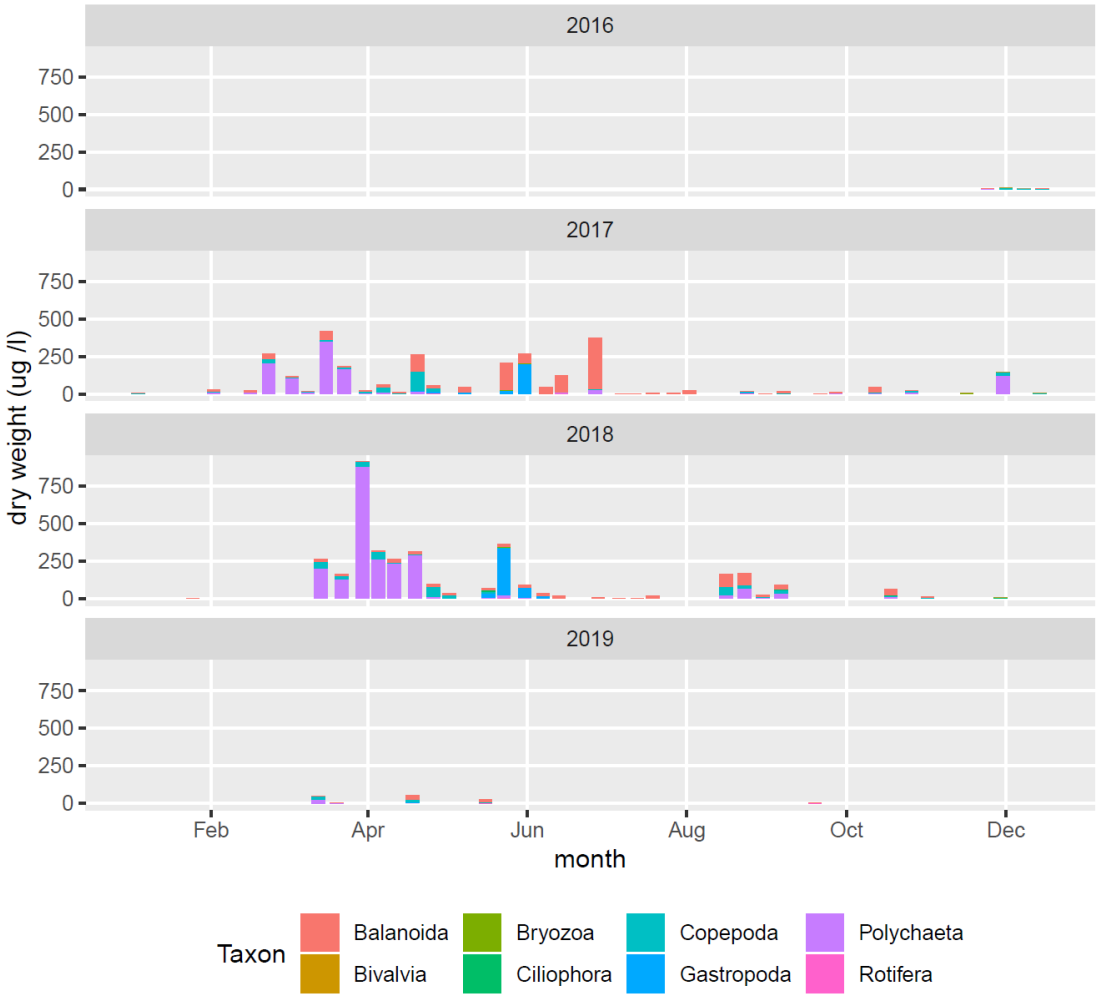


Figure 43. Estimated dry weight (µg/l) per sampling day of major zooplankton groups in samples taken at sampling point V722, seawater intake.

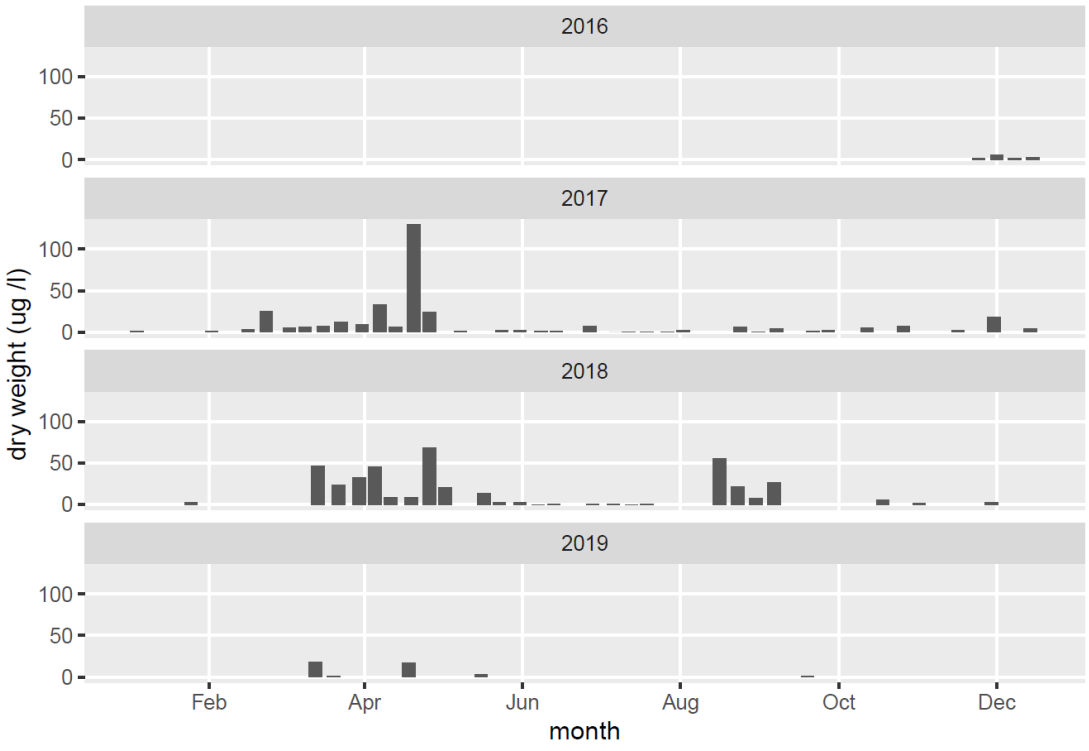


Figure 44. Estimated dry weight (µg/l) per sampling day of copepods (all stages) in samples taken at sampling point V722, seawater intake.

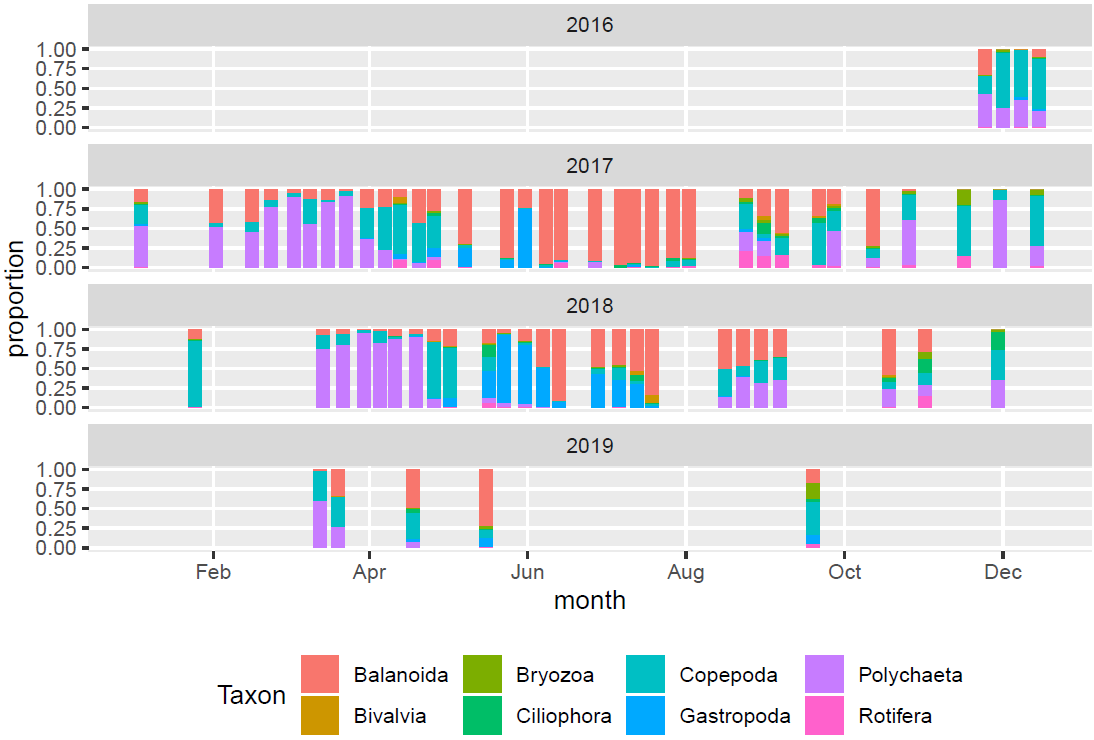


Figure 45. Estimated proportion of total biomass (dry weight) per sampling day of major zooplankton groups in samples taken at sampling point V722, seawater intake.

By assigning the taxa included in the above biomass estimates to either holoplankton or meroplankton, depending on their life cycle, we could estimate the relative contribution of meroplankton versus holoplankton to zooplankton biomass in the Wadden Sea near Breezanddijk.

Figure 46 (dry weight per day) shows that most peaks in biomass are composed of organisms belonging to the meroplankton. Expressed in proportion of the total biomass, meroplankton contributes the majority of zooplankton biomass on most days sampled (Figure 47). On average 72.6 % (se: 3.0 %) of zooplankton biomass is estimated to consist of meroplankton, and only on 9 out of 68 days the proportion of meroplankton is less than 50 % of the total, all these days being in April, May, November and December. The highest proportion of meroplankton biomass is found in early spring and summer, the lowest proportions of meroplankton are found in late spring and winter.

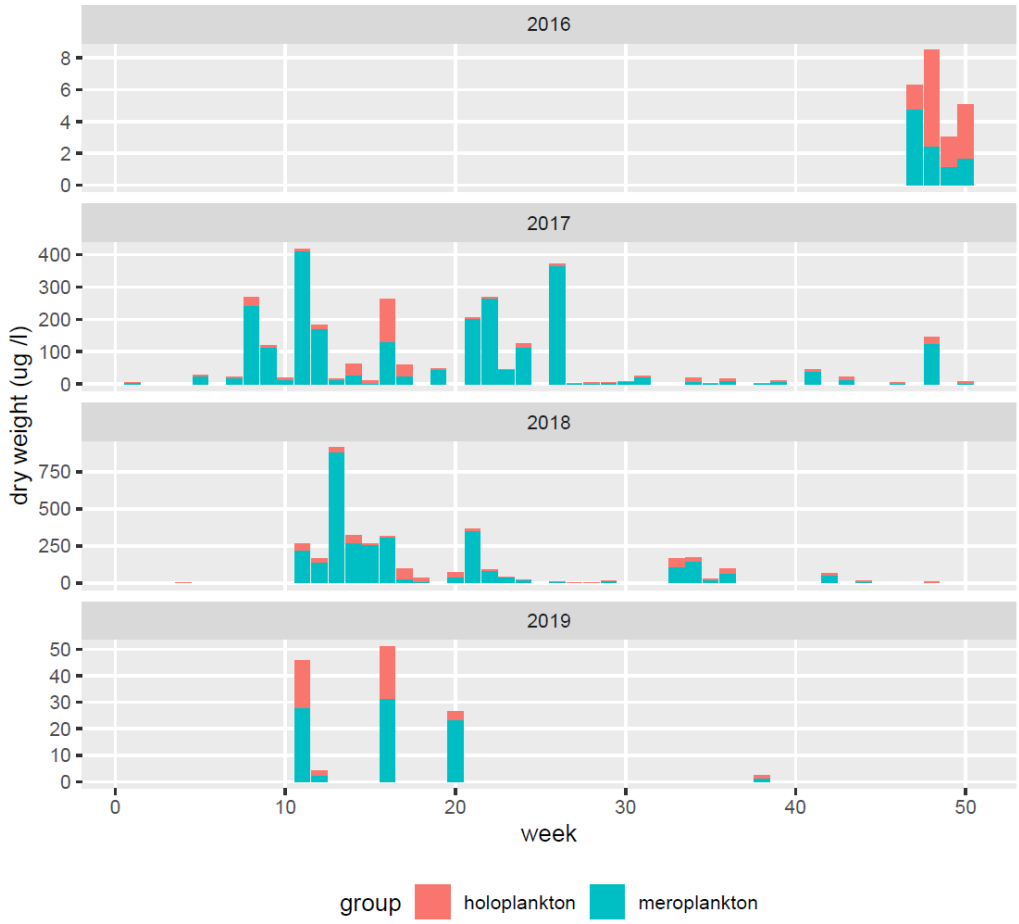


Figure 46. Estimated dry weight (µg/l) per sampling day of meroplankton and holoplankton in samples taken at sampling point V722, seawater intake.

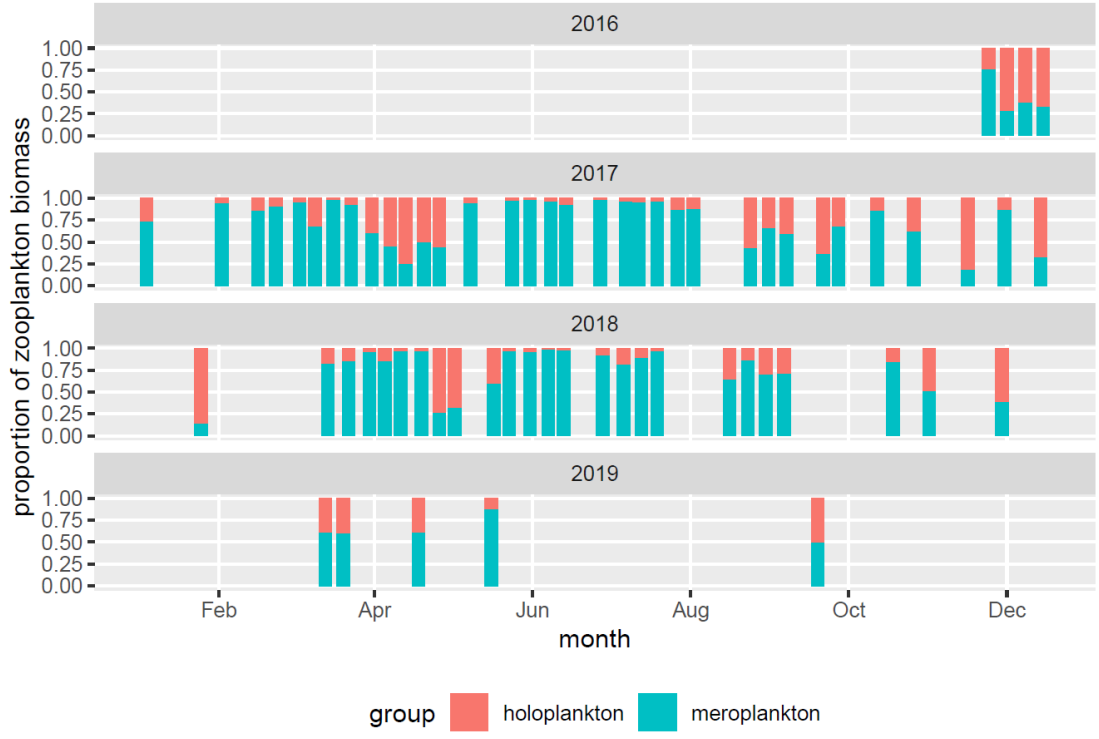


Figure 47. Estimated proportion of total biomass (dry weight) per sampling day of meroplankton and holoplankton in samples taken at sampling point V722, seawater intake.

8.3.5 Efficiency of filtration of small organisms in the installation

8.3.5.1 Initial intake system 2016 - 2018

The efficiency of the drum sieve in retaining fouling and non fouling zooplankton was investigated by comparing organism densities in the water before and after the filtration step. After filtration two points were sampled for the seawater side: samples were taken directly after filtration within the drum sieve itself, and from the buffer tank where the water accumulates before distribution to the stacks.

As a first step, the ratio r between the concentration before (C_{before}) and after (C_{after}) filtration was estimated as $r = C_{after}/C_{before}$. If this ratio is smaller than one, it means that organism density after filtration was lower than before. If the ratio is higher than one, organism density was higher after filtration than before. In the figure below, some examples of the ratio r for different organism groups are shown. Contrary to expectations organism densities after filtration were sometimes higher than before filtration for some groups and in some periods, mainly the first half of 2017 (Figure 48). Explanations for this could be:

1. Temporal variation in organism concentrations in the intake water (the "background concentration"). We tried to minimise the effect of this by taking all samples consecutively with as little time in between as possible, but the patchy nature of zooplankton populations means that inherent variation can be high.

2. Temporal variation in organism concentration in the effluent caused by irregular frequency of drum sieve cleaning.

3. Variation induced by subsampling and different analysts. We tried to minimise this by having each replicate analysed by a different analyst whenever possible.

4. Reproduction of organisms within the installation. This was deemed unlikely because of the continuous water flow within the installation.

5. Accumulation of organisms within the drum sieve. This was investigated by comparing organism density within the drum sieve drum with organism density in the effluent.

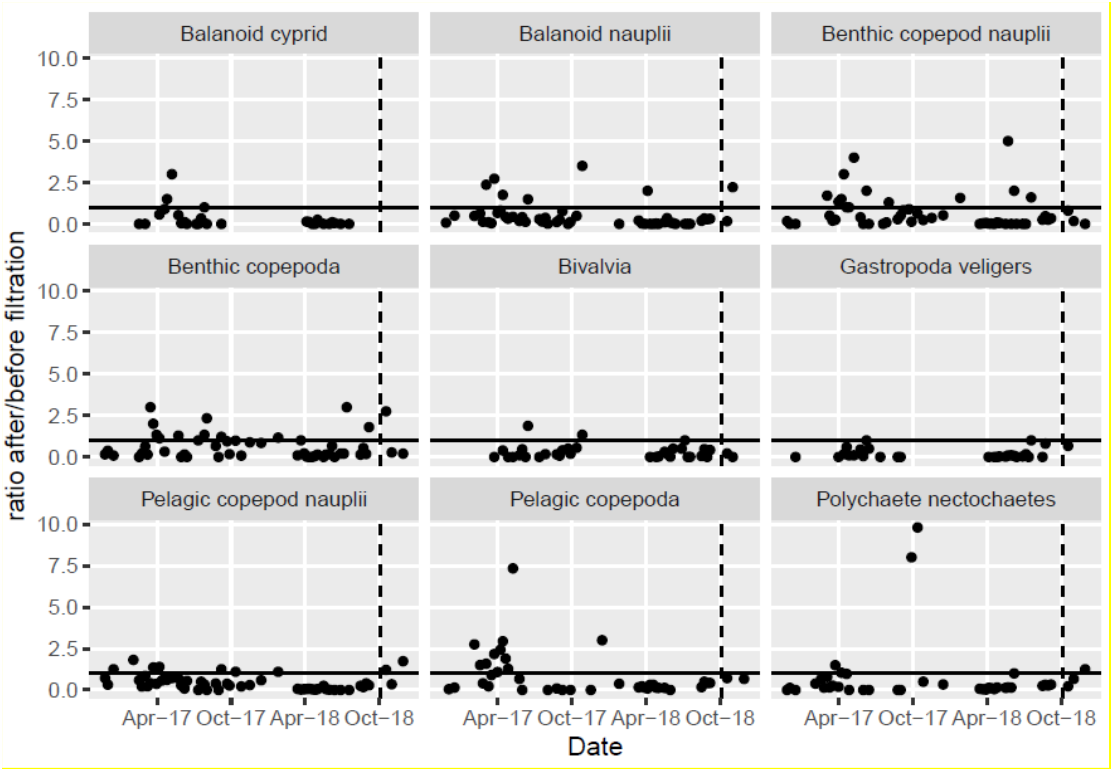


Figure 48. Ratio between organism concentration after filtration and organism concentration before filtration in seawater at REDstack Breezanddijk 2016-2018 for six selected groups of organisms. A horizontal straight line highlights the ratio 1:1. A vertical dashed line shows the switch to wedgewire screens on the intake system.

8.3.5.2 Accumulation in drum sieve

To investigate whether organisms accumulate inside of the drum sieve drum, on several days additional samples were taken inside the drum. This shows that organisms indeed accumulate inside the drum sieve. Densities inside the sieve were higher than densities at all other sampling points for abundant groups (Figure 49).

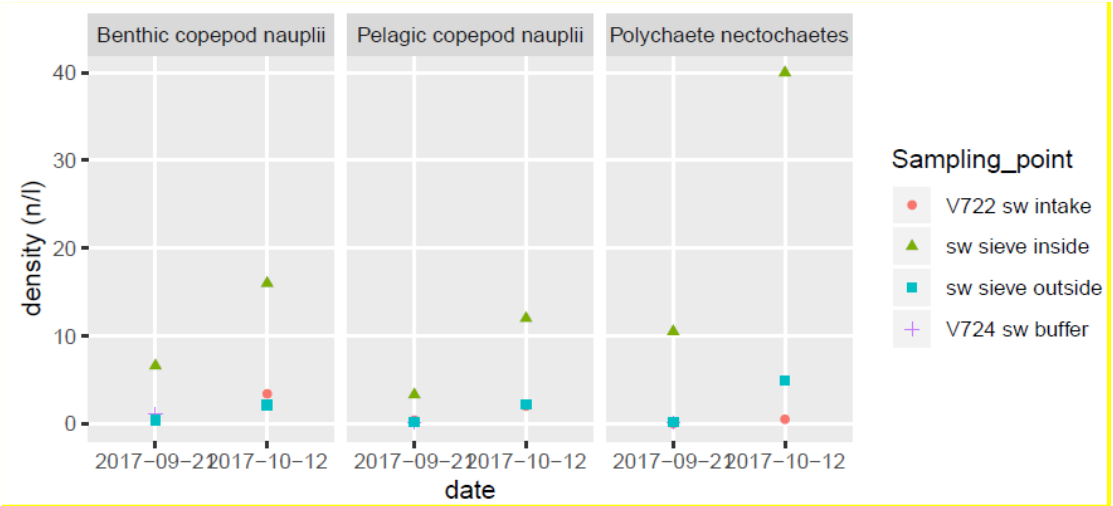


Figure 49. Comparison of organism concentrations in seawater at four different sampling points sampled on 21-09-2017 and 12-10-2017.

8.3.5.3 Renewed intake system 2019

In october 2018 the intake screens on the seawater intake were changed to 0.5 and 1 mm wedgewire screens, and drum sieve mesh size was changed back to 20 μm. Though limited sampling took place in 2019, this change did not appear to influence filtration efficiency of the drum sieve which was still variable, with individuals of most zooplankton groups still present after filtration (Figure 48).

8.3.5.4 Comparison of filtration systems 2019

In 2019 two additional sets of duplicate samples were taken at five different locations; unfiltered Wadden Sea water, drum-sieve filtered water from the buffer tank, rapid sand filter effluent and shellfish filtration experiment effluent from mussel-containing tank as well as empty control tank (Figure 50). Highest zooplankton densities were found in shellfish filtration effluent, seawater intake, but interestingly also in sand filter effluent. Lowest densities were found in drum sieve effluent taken from the seawater buffer tank. In the sand filter effluent larger organisms such as balanoid nauplii and adult copepods were almost absent. This might be because large organisms are physically retained by the sand filter, but small organisms such as copepod nauplii can move between the fiter medium particles, and actively avoid being absorbed in the porous filter medium.

The filtration efficiency of the different filtration systems was also investigated for all particles including sediments, using a portable laser-diffraction based particle size analyzer, the LISST-200X. Methods and results of these analyses are presented in a companion report.

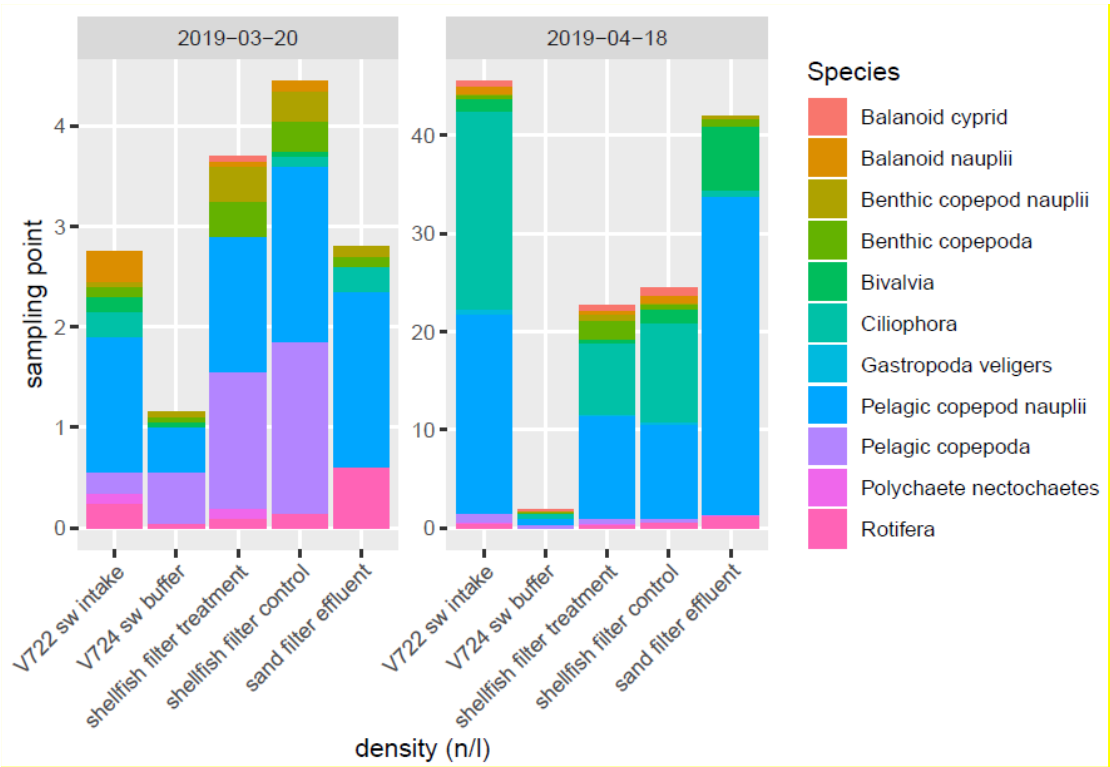


Figure 50. Zooplankton density (n/l) sampled at five different sampling points within the RED installation on March 3, 2019 and April 18, 2019. Average of duplicate samples.

8.3.6 Zooplankton survival: monitoring

From the counts of live and dead organisms in the samples the percentage of dead organisms can be estimated, summarized in Table 7. Below figures show average

percentage of dead organisms for each sampling day for the most common groups of organisms present. The highest percentage of dead organisms were observed in the brackish retour pipe (Figure 56), where fresh water and seawater are combined. Freshwater cladocera had the highest percentage of dead organisms at 90 %, followed by pelagic and benthic copepods (65 % and 58 %), balanoid cyprids (49 %), bivalvia (27 %), pelagic copepod nauplii (27 %) and gastropod veligers (15 %). For other groups less than 5 % was dead. For groups that are exclusively marine such as balanoids and polychaete larvae, the fraction of dead organisms in brackish water can be used as an indicator for survival. However, as the plankton in the brackish retour pipe is of both fresh water as well as seawater origin, taxa that occur in both fresh water as well as seawater could come from either source. Because of this, mortality after mixing of seawater and fresh water was investigated separately in an experiment where seawater intake water was diluted with filtered fresh water to exclude organisms of fresh water origin.

For filtered and unfiltered fresh water the percentage of dead organisms was 5 % or lower, except for benthic copepods (33 % in unfiltered water, 23 % in filtered water) and pelagic copepods in filtered water (7 %). In fresh water, less than 5 % of cladocera was dead (Figure 51 and Figure 52).

For filtered and unfiltered seawater, the fraction of dead organisms was high for fresh water cladocera (95 %), but also for balanoid cyprids (24 % in unfiltered water, 54 % in filtered water). In benthic copepods the percentage of dead organisms was between 6 and 9 %. For gastropod veligers the percentage of dead organisms was 8 % in filtered water. For all other groups, less than 5 % was dead (Figure 53 and Figure 55).

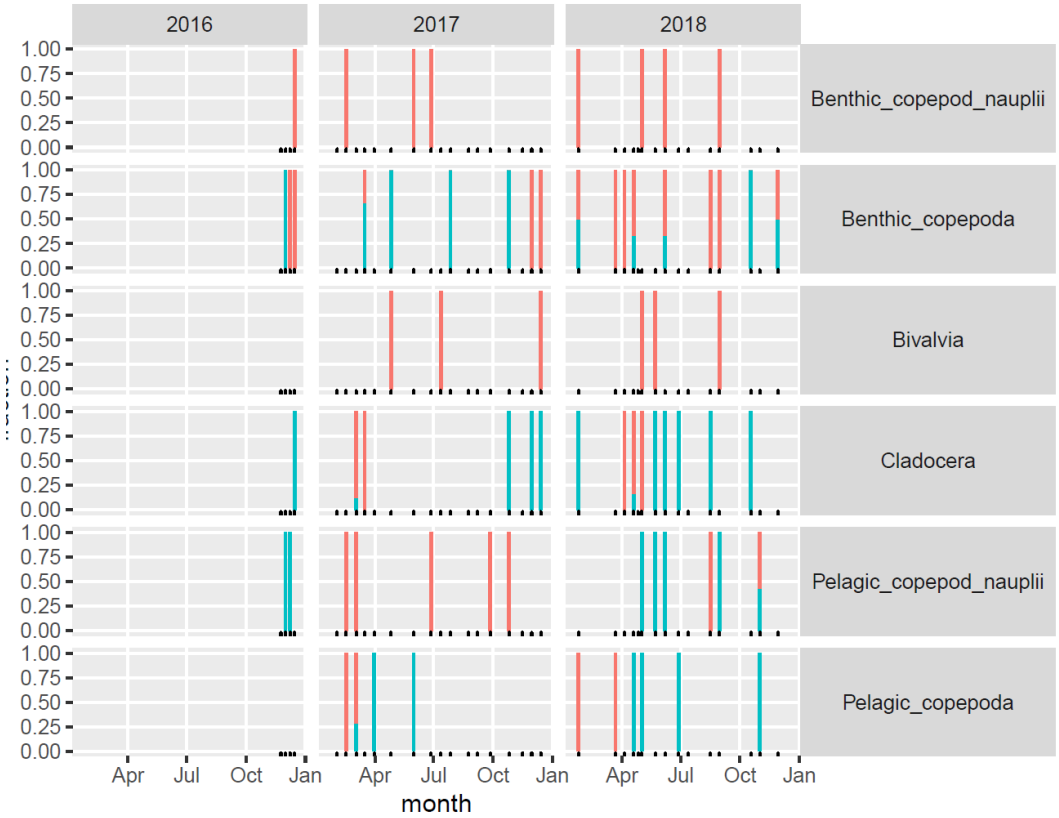


Figure 51. Fraction dead and alive organisms at the freshwater intake of the RED power plant at Breezanddijk, per week in 2016 - 2018. Red = alive, blue = dead.

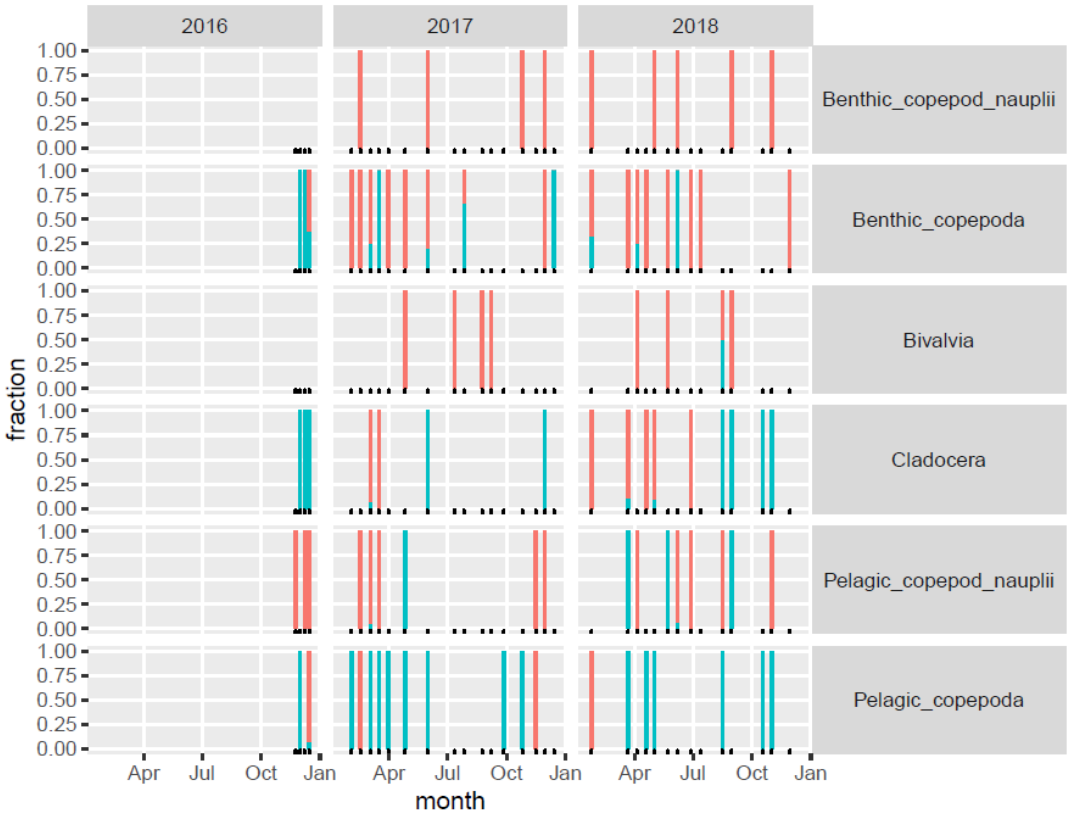


Figure 52. Fraction dead and alive organisms in the fresh water drum sieve after filtration in the RED power plant at Breezanddijk, per week in 2016 - 2018. Red = alive, blue = dead.

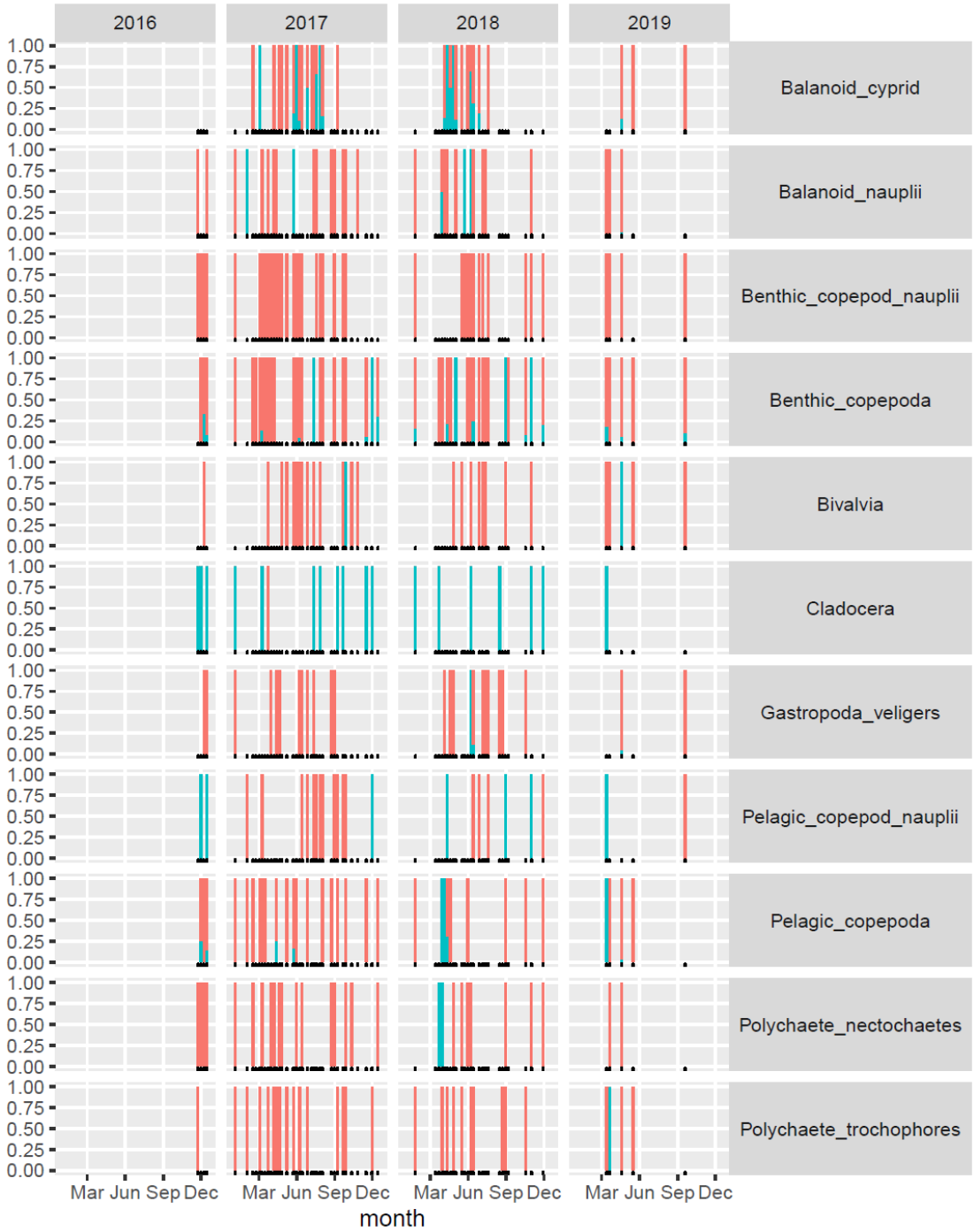


Figure 53. Fraction dead and alive small organisms at the seawater intake of the RED power plant at Breezanddijk, per week in 2016 - 2019. Red = alive, blue = dead.

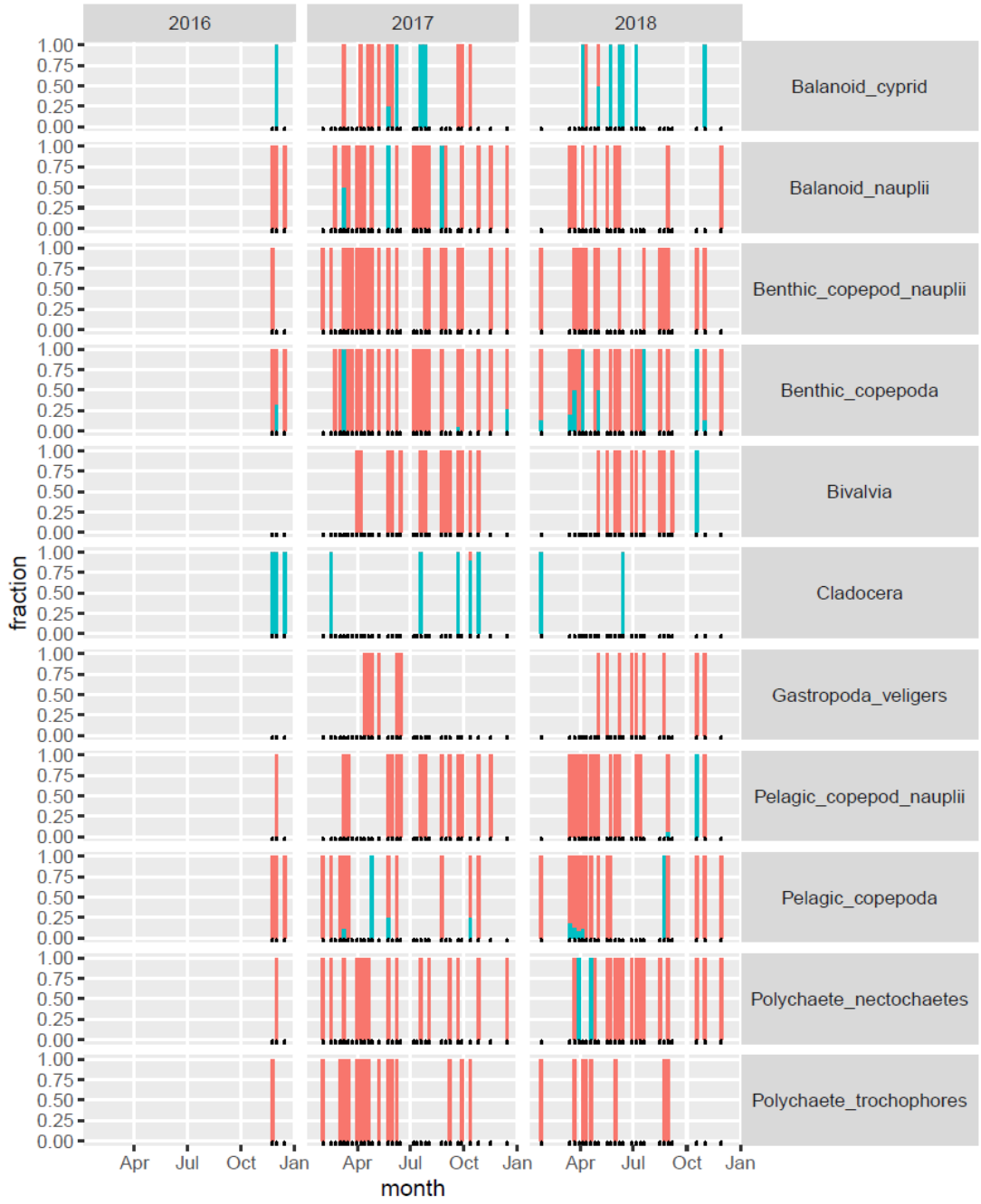


Figure 54. fraction dead and alive organisms in the seawater drum sieve after filtration in the RED power plant at Breezanddijk, per week in 2016 - 2018. Red = alive, blue = dead.

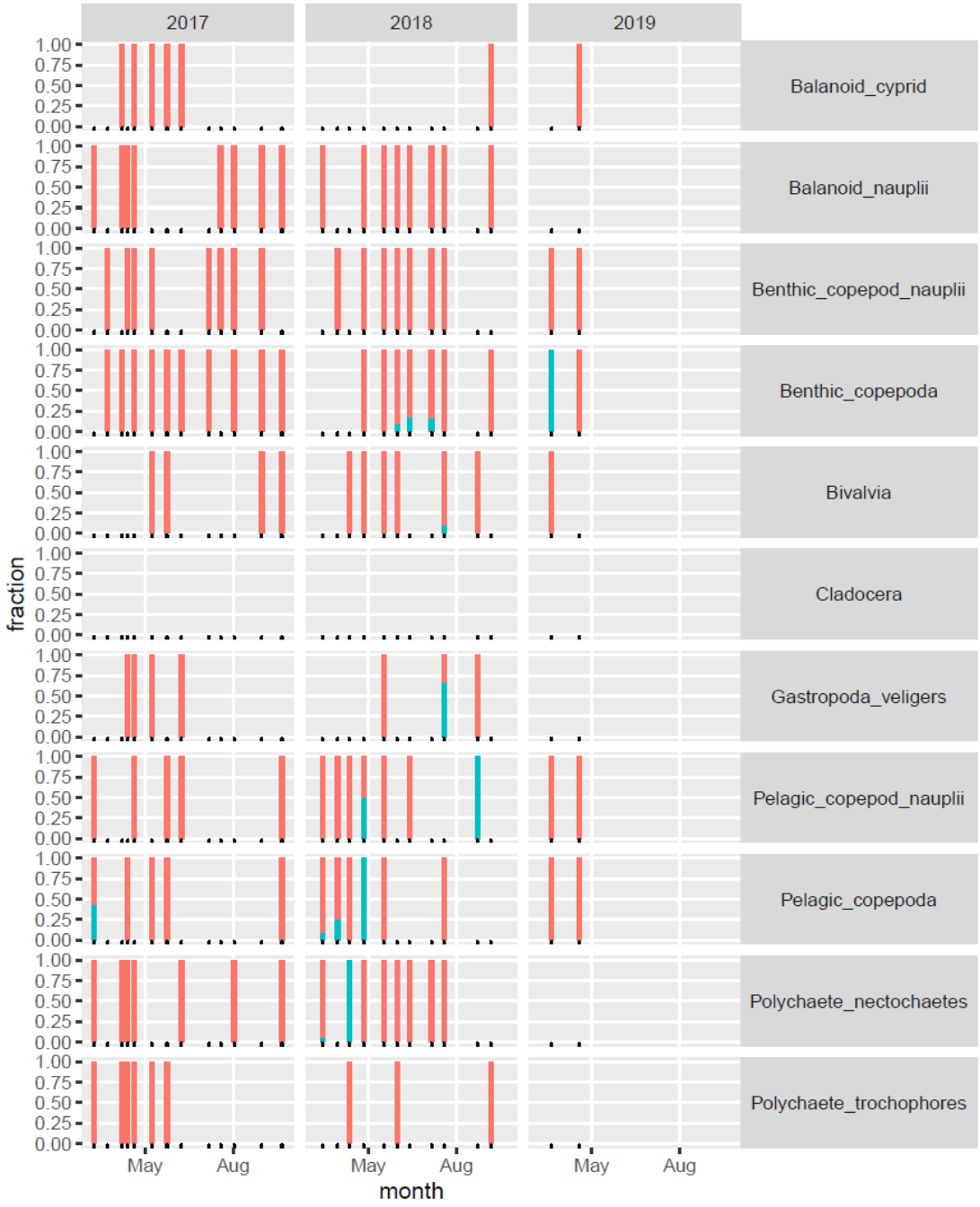


Figure 55. fraction dead and alive organisms in the seawater buffer tank of the RED power plant at Breezanddijk, per week in 2016 - 2018. Red = alive, blue = dead.

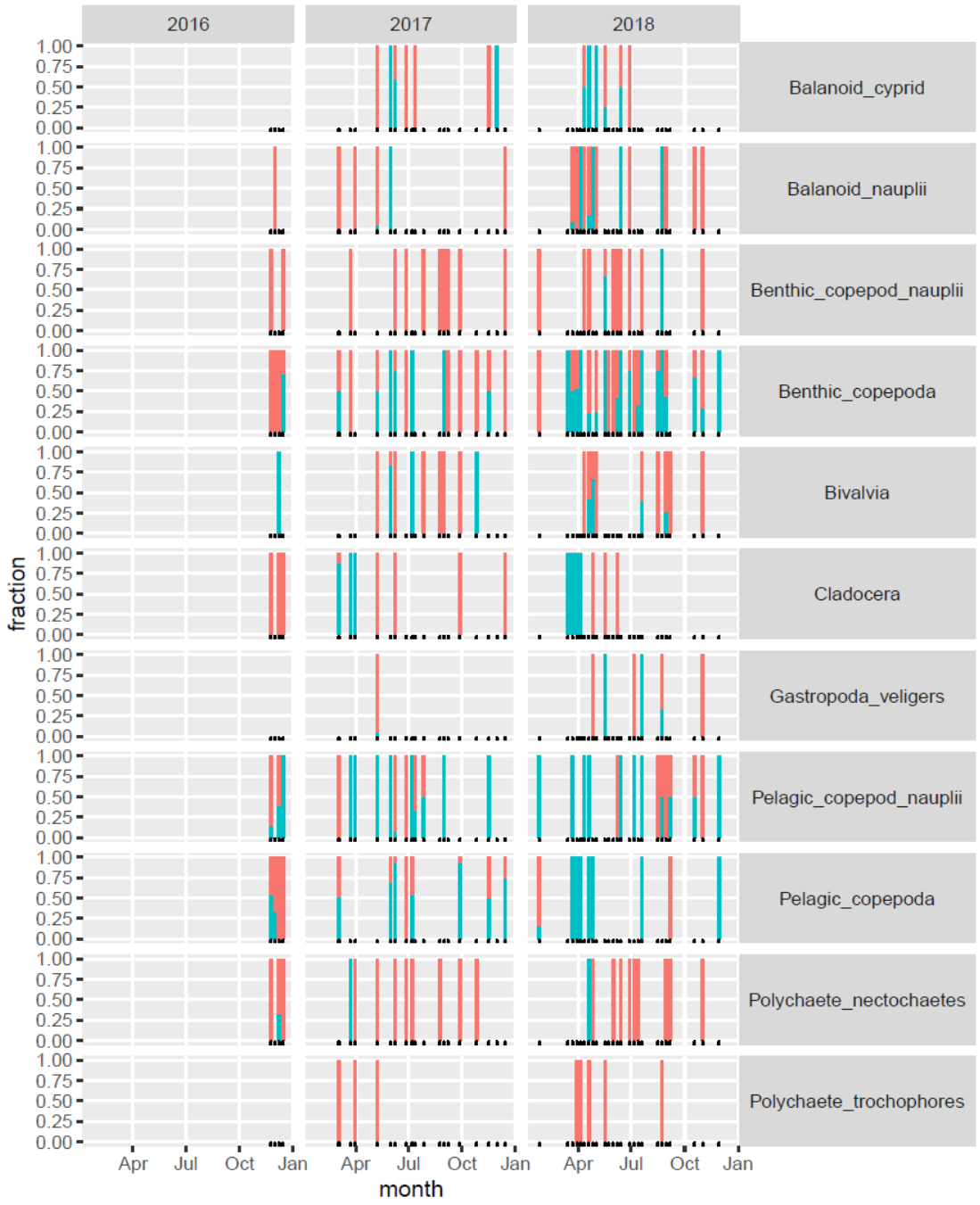


Figure 56. fraction dead and alive organisms at the brackish retour discharge of the RED power plant at Breezanddijk, per week in 2016 - 2018. Red = alive, blue = dead.

Table 7. Means and standard errors of the fraction of dead small organisms in samples taken at various sampling points in the RED power plant at Breezanddijk, 2016 - 2019. V712: fresh water intake, V722: seawater intake, V724: seawater buffer tank and V731: brackish retour pipe.

group	Sieve_fresh	Sieve_saline	V712	V722	V724	V731
Balanoid cyprid		53.75 (6.36)		23.94 (4.02)		48.75 (6.33)
Balanoid nauplii		3.34 (2.13)	50 (11.79)	1.22 (0.82)		4.05 (2.38)
Benthic copepod nauplii						2.63 (1.75)
Benthic copepoda	22.76 (5.81)	9.25 (2.97)	32.92 (6.69)	6 (1.8)	8.05 (4.65)	57.5 (5.33)
Bivalvia	4.55 (2.59)	0.15 (0.1)	0.72 (0.38)	0.28 (0.16)	0.77 (0.54)	30.7 (6.22)
Cladocera	5.2 (1.16)	99.17 (0.36)	2.59 (0.85)	95 (2.71)		98.38 (0.99)
Gastropoda veligers				0.57 (0.26)	8.33 (4.62)	15.35 (4.28)
Pelagic copepod nauplii	1.05 (0.38)	0.18 (0.12)	2.05 (1.25)	0.28 (0.11)	3.14 (2.37)	26.86 (4.71)
Pelagic copepoda	6.71 (1.77)	3.22 (0.91)	4.73 (1.35)	2.88 (0.89)	5.35 (2.35)	64.59 (4.55)
Polychaete nectochaetes		0.31 (0.22)		0.03 (0.02)	0.54 (0.31)	1.05 (0.85)
Polychaete trochophores				2.56 (1.94)		

8.3.7 Zooplankton survival: experiments

The experiment was repeated four times in March, April, May and September 2019, at four different water temperatures; 6.0, 10.5, 13.0 and 16.0 C (Table). Salinity of the Wadden Sea water used for dilutions and control ranged between 26 and 24.

The amount of organisms counted in a sample varied considerably, also within treatments (Fig.). Highest densities were observed in April, with on average 57.17 (12.2) ind l^{-1} and lowest densities were observed in September with on average 6.0 (1.5) ind l^{-1} (Table).

Table 8. Sampling dates, treatment, conductivity (mS/cm), salinity and water temperature (gr. C) per treatment.

Date	Treatment	Temperature	Conductivity	Salinity
2019-03-13	Control	6.00	26.12	25.86
2019-03-13	1_1	6.00	14.00	13.09
2019-03-13	1_2	6.00	9.63	8.75
2019-03-13	1_3	6.00	7.23	6.43
2019-04-18	Control	10.50	29.07	25.59
2019-04-18	1_1	10.50	16.30	13.59
2019-04-18	1_2	10.50	12.12	9.86
2019-04-18	1_3	10.50	9.26	7.38
2019-05-16	Control	13.00	31.18	25.85
2019-05-16	1_1	13.00	17.41	13.66
2019-05-16	1_2	13.00	12.57	9.60
2019-05-16	1_3	13.00	9.34	6.96
2019-09-19	Control	16.00	31.32	24.06
2019-09-19	1_1	16.00	17.67	12.85
2019-09-19	1_2	16.00	12.16	8.57
2019-09-19	1_3	16.00	9.40	6.50

8.3.7.1 Community composition

The zooplankton community composition and abundance differed between the four replications of the experiment in different months (Figure 57). In March the community was dominated by copepods and polychaete larvae, with ciliates, rotifers and bivalves present in low numbers. In April copepods were abundant but the highest density was observed in ciliates, with bivalve veligers being the third most abundant group. In May rotifers were the most abundant group, followed by balanoid larvae, ciliates, copepods, nematodes and bryozoa. In September the community consisted mainly of ciliates and rotifers, with copepods, bryozoa and other groups present in low numbers.

Table 9. Mean and standard error of zooplankton density in control treatments (n per l sample) for the most common taxonomic groups.

Group	Date	n_l	se	dilution_factor
Copepoda	2019-03-13	8.28	0.63	0
Balanoida	2019-03-13	0.10	0.07	0
Bivalvia	2019-03-13	0.55	0.16	0
Gastropoda	2019-03-13	0.00	0.00	0
Polychaeta	2019-03-13	2.77	0.66	0
Nematoda	2019-03-13	0.22	0.13	0
Rotifera	2019-03-13	0.55	0.16	0
Ciliophora	2019-03-13	0.60	0.11	0
Bryozoa	2019-03-13	0.05	0.03	0
Copepoda	2019-04-18	31.43	5.26	0
Balanoida	2019-04-18	1.62	0.25	0
Bivalvia	2019-04-18	1.67	0.81	0
Gastropoda	2019-04-18	0.38	0.09	0
Polychaeta	2019-04-18	0.15	0.06	0
Nematoda	2019-04-18	0.10	0.07	0
Rotifera	2019-04-18	0.20	0.11	0
Ciliophora	2019-04-18	21.29	5.45	0
Bryozoa	2019-04-18	0.32	0.13	0
Copepoda	2019-05-16	4.85	0.83	0
Balanoida	2019-05-16	4.17	0.68	0
Bivalvia	2019-05-16	0.02	0.02	0
Gastropoda	2019-05-16	1.62	0.15	0
Polychaeta	2019-05-16	0.08	0.02	0
Nematoda	2019-05-16	1.18	0.27	0
Rotifera	2019-05-16	3.77	1.42	0
Ciliophora	2019-05-16	2.12	0.80	0
Bryozoa	2019-05-16	1.03	0.43	0
Copepoda	2019-09-19	1.82	0.21	0
Balanoida	2019-09-19	0.02	0.02	0
Bivalvia	2019-09-19	0.12	0.05	0
Gastropoda	2019-09-19	0.15	0.06	0
Polychaeta	2019-09-19	0.00	0.00	0
Nematoda	2019-09-19	0.10	0.04	0
Rotifera	2019-09-19	1.80	0.36	0
Ciliophora	2019-09-19	1.42	0.64	0
Bryozoa	2019-09-19	0.50	0.16	0

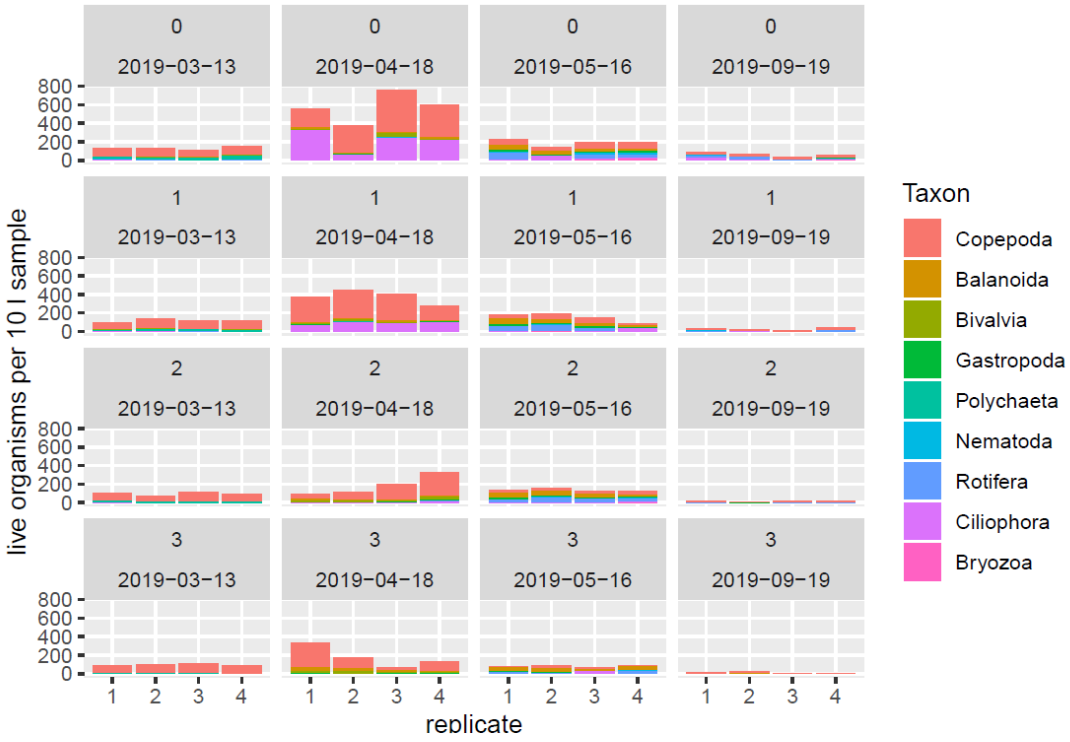


Figure 57, Amount of live organisms per sample for each treatment at each temperature level, for the main taxa observed in the samples.

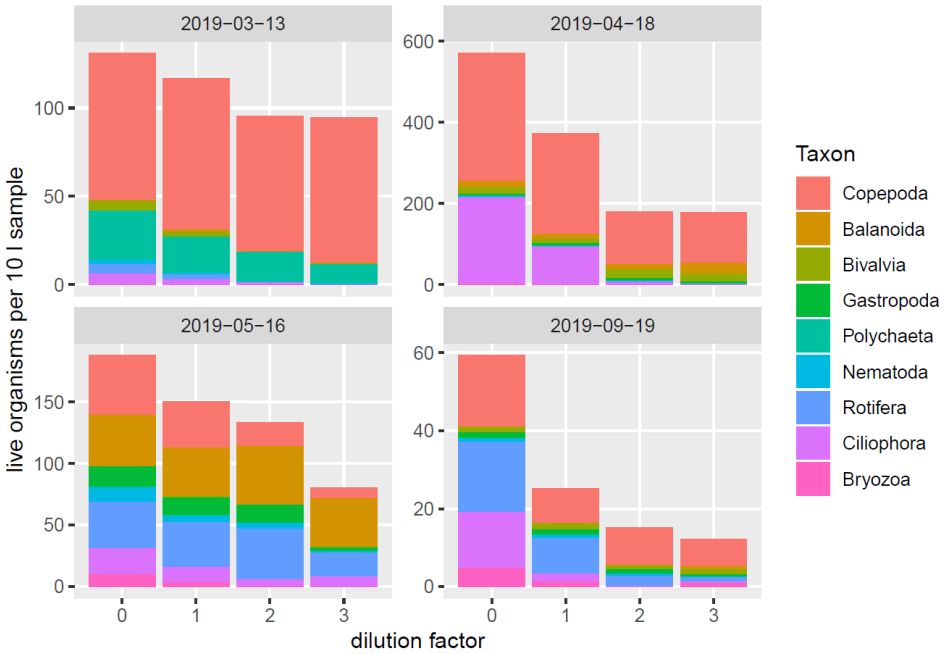


Figure 58, Count of live organisms per sample averaged for each treatment at each date, for the main taxa observed in the samples.

Table 10. Mean and standard error of zooplankton density (n per l sample) per treatment, all zooplankton groups combined.

dilution_factor	Date	n_l	se
0	2019-03-13	13.12	1.95
1	2019-03-13	11.70	1.51
2	2019-03-13	9.55	0.99
3	2019-03-13	9.45	0.59
0	2019-04-18	57.17	12.22
1	2019-04-18	37.34	5.06
2	2019-04-18	18.06	6.20
3	2019-04-18	17.75	6.29
0	2019-05-16	18.85	4.63
1	2019-05-16	15.08	4.37
2	2019-05-16	13.38	2.12
3	2019-05-16	8.07	2.52
0	2019-09-19	5.95	1.54
1	2019-09-19	2.52	0.95
2	2019-09-19	1.52	0.45
3	2019-09-19	1.23	0.50

8.3.7.2 Analysis

An analysis of differences in zooplankton counts for either the total community, rotifers and ciliates separate, or the total community excluding rotifers and ciliates, revealed differences between these groups. For the total community, the full model M1 with an interaction between dilution factor and date was chosen as the best model as it had the lowest AICc and likelihood ratio tests showed that it was a significant improvement over a simpler model ($\chi^2(3) = 17.03, p = 7 \times 10^{-4}$). Figure 59 shows observed versus fitted values for the model. Overall the fit appears to be good, but in May at dilution factor 2 the model appears to underestimate counts.

For the total community, but excluding rotifers and ciliates model M2 had the lowest AICc Likelihood ratio tests showed that model M1 was not an improvement over the simpler model M2 ($\chi^2(3) = 5.68, p = 0.13$) but M2 was a significant improvement over simpler model M3 ($\chi^2(1) = 30.3, p = 3.7 \times 10^{-8}$). Thus model M2 was chosen as the best model. Figure 60 shows observed versus fitted values for the model. Overall the fit appears to be good, but in March the model appears to overestimate counts in the control and underestimate counts in dilution factor 3.

For rotifers and ciliates model M1 had the lowest AICc and likelihood ratio tests showed that model M1 was an improvement over the simpler model M2 ($\chi^2(3) = 43.9, p = 1.6 \times 10^{-9}$). Model M1 was chosen as the best model. Figure 61 shows observed versus fitted values for the model. Again overall the fit appears to be good, but in April at dilution factor 1 the model appears to underestimate counts.

All models had residuals distributions that were not significantly different from normal (checked with QQ plots) although plots of residuals versus fitted values still showed slight patterns in residuals. A summary of the results of each model is shown in Table 11.

Table 11. Results of best models for each set of organism groups considered.

organisms	model	term	estimate	std.error	statistic	p.value
all	M1	(Intercept)	4.87	0.13	37.82	0.00
all	M1	Date2019-04-18	1.44	0.18	8.08	0.00
all	M1	Date2019-05-16	0.42	0.18	2.29	0.02
all	M1	Date2019-09-19	-0.90	0.19	-4.82	0.00
all	M1	Treatment	-0.12	0.07	-1.72	0.09
all	M1	Date2019-04-18:Treatment	-0.30	0.10	-3.17	0.00
all	M1	Date2019-05-16:Treatment	-0.14	0.10	-1.45	0.15
all	M1	Date2019-09-19:Treatment	-0.43	0.11	-4.03	0.00
all excl. rotifers/ciliates	M2	(Intercept)	5.00	0.10	49.98	0.00
all excl. rotifers/ciliates	M2	Date2019-04-18	0.81	0.11	7.24	0.00
all excl. rotifers/ciliates	M2	Date2019-05-16	-0.17	0.11	-1.46	0.14
all excl. rotifers/ciliates	M2	Date2019-09-19	-1.92	0.13	-15.07	0.00
all excl. rotifers/ciliates	M2	Treatment	-0.23	0.04	-6.16	0.00
rotifers/ciliates	M1	(Intercept)	2.59	0.23	11.29	0.00
rotifers/ciliates	M1	Date2019-04-18	3.07	0.30	10.22	0.00
rotifers/ciliates	M1	Date2019-05-16	1.54	0.30	5.20	0.00
rotifers/ciliates	M1	Date2019-09-19	0.87	0.31	2.83	0.00
rotifers/ciliates	M1	Treatment	-0.91	0.17	-5.22	0.00
rotifers/ciliates	M1	Date2019-04-18:Treatment	-0.63	0.22	-2.91	0.00
rotifers/ciliates	M1	Date2019-05-16:Treatment	0.68	0.20	3.39	0.00
rotifers/ciliates	M1	Date2019-09-19:Treatment	-0.18	0.23	-0.77	0.44

GLM model parameters were used to estimate the percentage of zooplankton surviving one day after dilution of seawater samples with different fractions of fresh water (Figure 62). Overall survival of zooplankton decreased with increasing dilution factor, with the slope of the decrease differing across dates. Overall survival was highest in March and lowest in September. The model for all organisms excluding rotifers and ciliates (model M2) does not include an interaction term between treatment and date but only separate intercepts at each date level, thus the slope of the relationship between dilution factor and survival is similar for all replicates at the different dates. For these organisms, survival decreased with increasing fresh water dilution to a lowest survival of 50 % at dilution factor 3. The model for rotifers and ciliates only showed the highest decrease in survival with increasing dilution factor. At all dates except June survival was lower than 50 % at dilution factor 1 already, with below 10 % survival at dilution factor 3. In April rotifer and ciliate survival was similar to survival of the other groups. This shows that differences in survival of the whole community between dates of are driven by differences in survival in rotifers and ciliates.

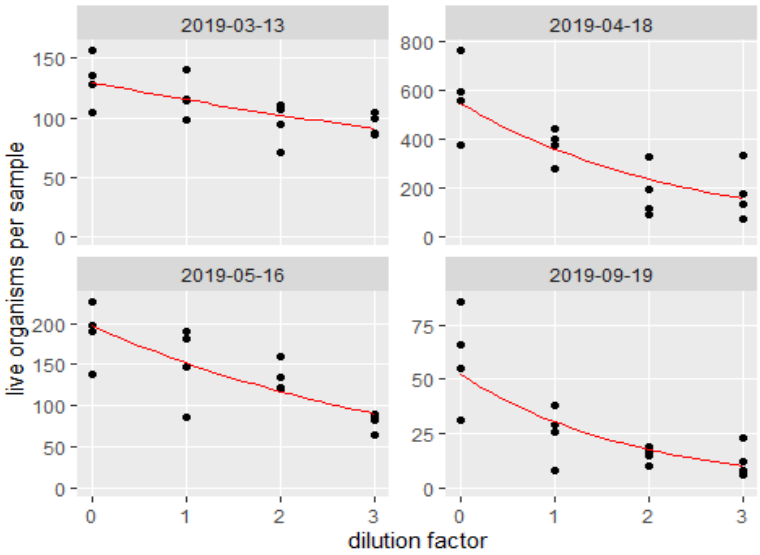


Figure 59. Observed counts of total live organisms per sample versus fitted estimates of model M1.

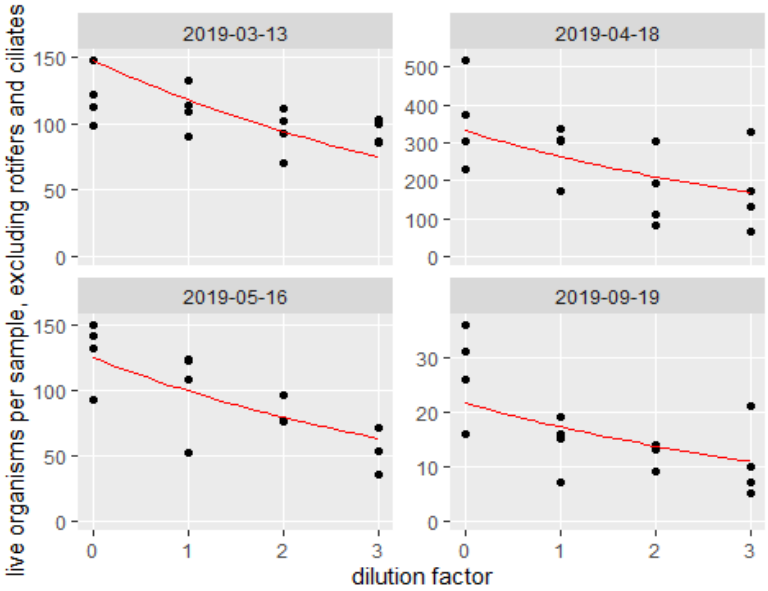


Figure 60. Observed counts of total live organisms excluding rotifers and ciliates per sample versus fitted estimates of model M2.

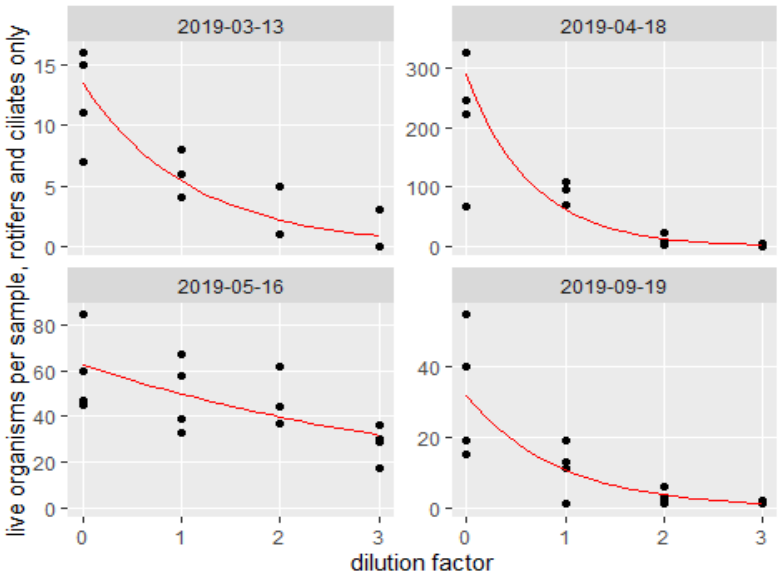


Figure 61. Observed counts of rotifers and ciliates per sample versus fitted estimates of model M1.

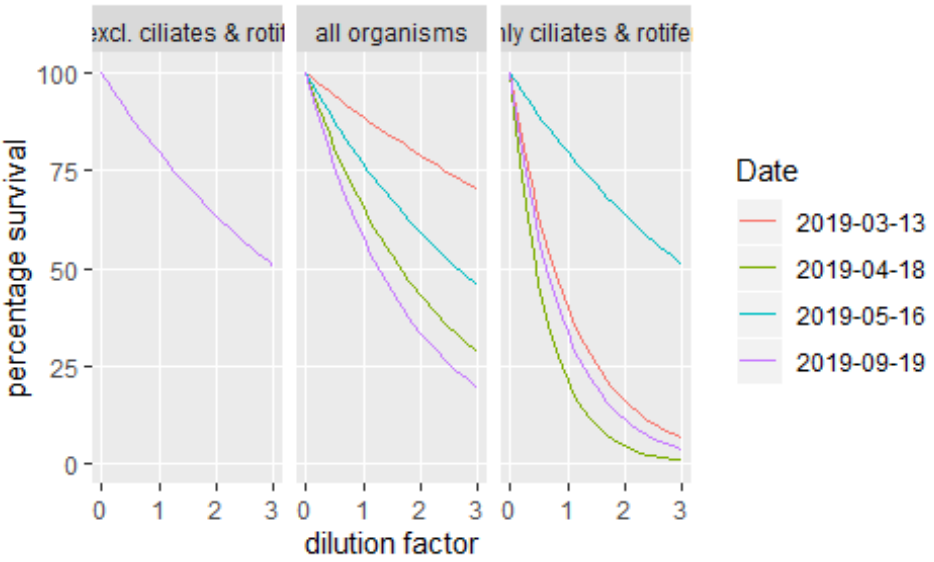


Figure 62. Estimated survival of Wadden Sea zooplankton one day after dilution of seawater samples with fresh water in a seawater:freshwater ratio 1:x, where x was the dilution factor. Estimates are based on separate GLM models for different organism groups.

At the currently (as of September 2019) used dilution level in the RED facility in Breezanddijk of 1:1, survival is estimated as ranging between 58 % (in September) and 89 % (in March) for the total community, 80 % for all organisms excluding rotifers and ciliates (all months) and between 21 % (April) and 80 % (May) for rotifers and ciliates. On average, survival at 1:1 dilution is estimated as 72.4 % for the total community, 80 % for all organisms excluding rotifers and ciliates and 43.8 % for rotifers and ciliates.

8.3.7.3 Response of different zooplankton taxa to instantaneous salinity reductions

A summary of counts for the most common zooplankton taxa is given in Figure 63 for holoplankton groups and Figure 64 for meroplankton groups. Occurrence of most planktonic taxa varied by orders of magnitude between the different replications of the

experiment in different months. Also between samples within treatments, variation was often high. For several groups on several dates, organisms were absent or present in low numbers, such as adult copepods in May and September, Balanoid nauplii in March and September, Balanoid cyprids, Gastropod veligers in March and Polychaetes in May and September. This limits quantitative analysis of mortality for specific taxa. Nevertheless, differences in live organism counts at different dilution factors were not the same for each taxonomic group, and a qualitative description of observed patterns is included below.

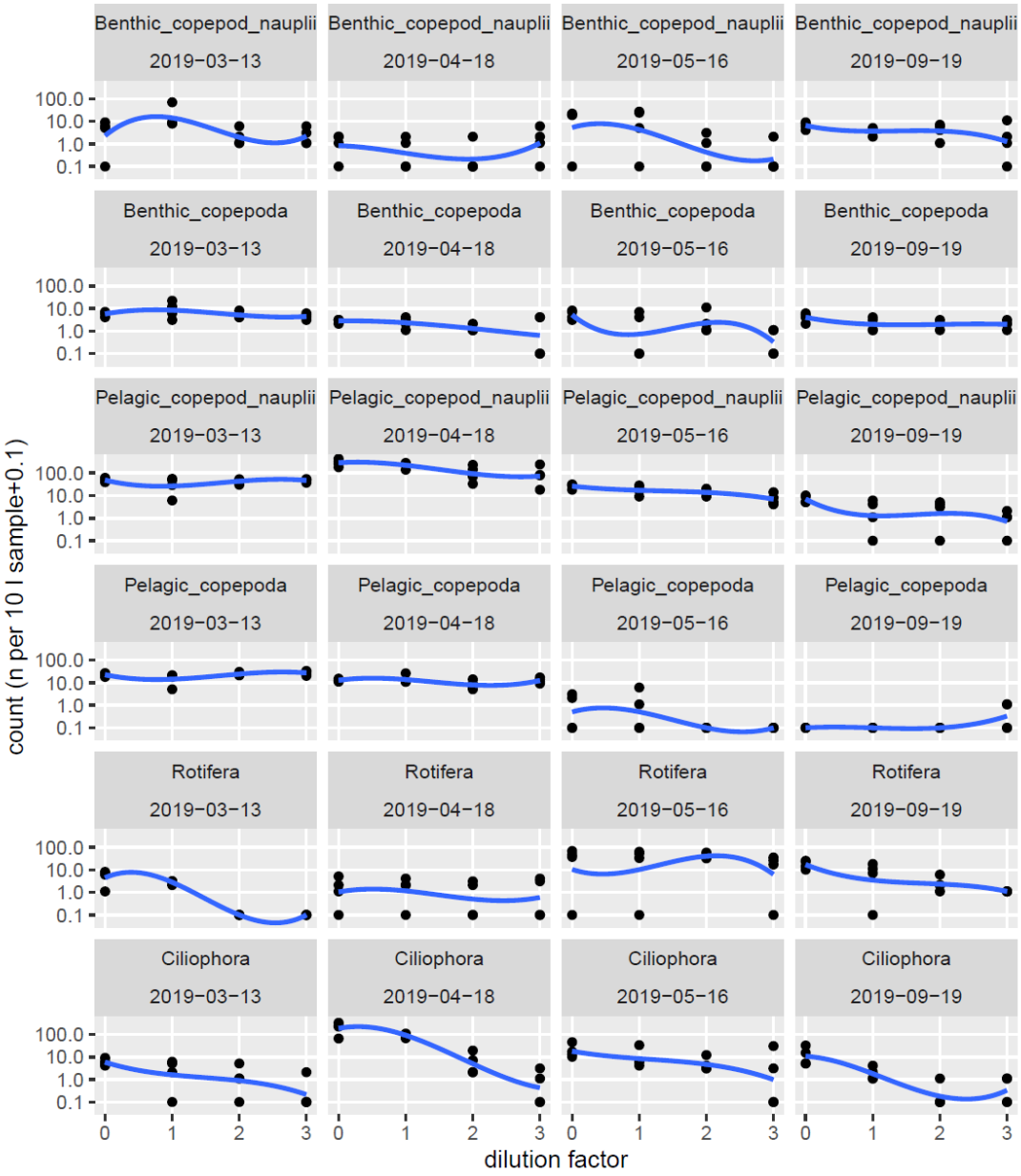


Figure 63. Counts of live holoplankton of various taxonomic groups, grouped per treatment and experimental replicate. Note the vertical log scale. 0.1 was added to all counts to facilitate plotting on a log scale. Smoothing splines (k=3) added.

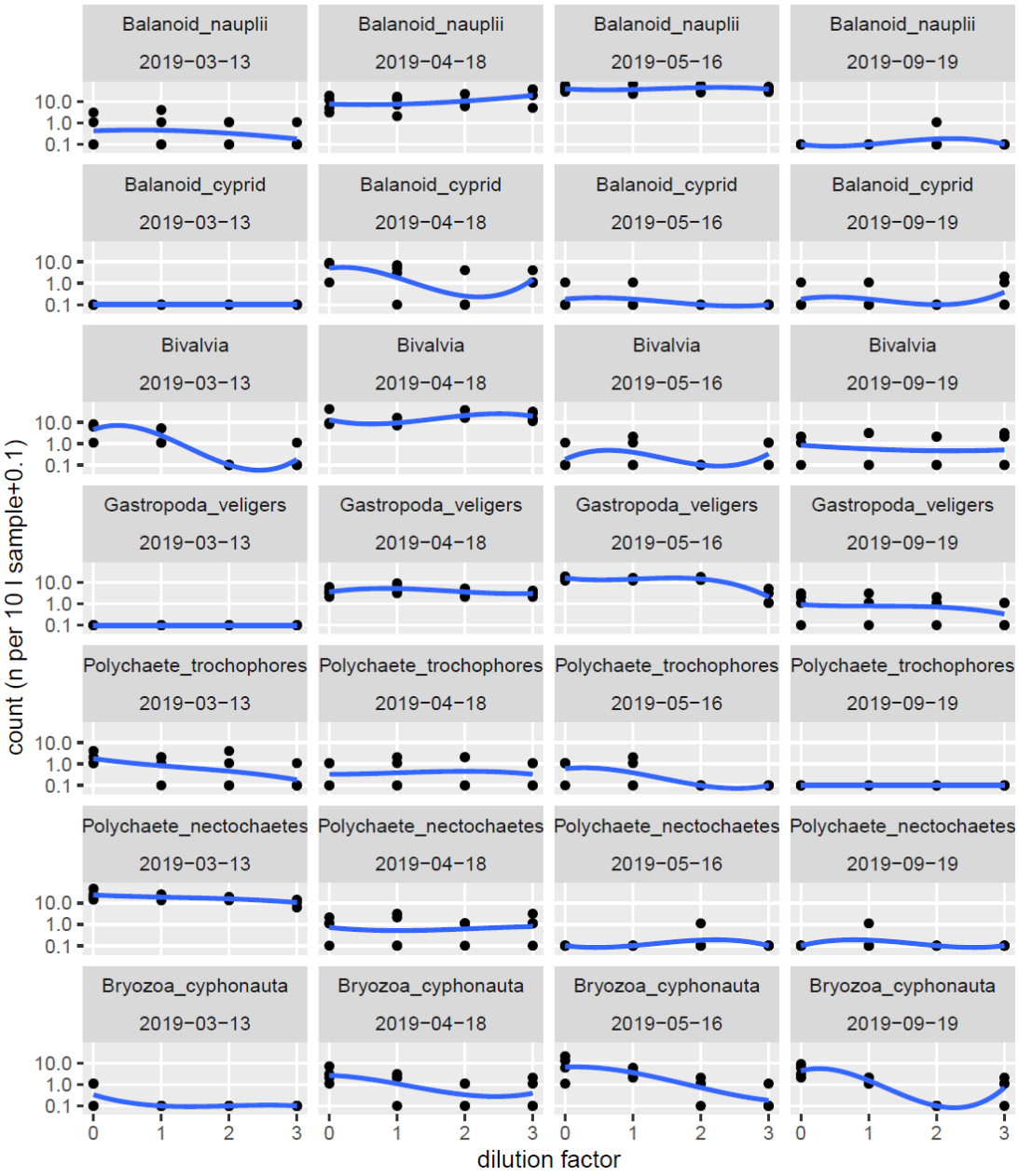


Figure 64. Counts of live meroplankton of various taxonomic groups, grouped per treatment and experimental replicate. Note the vertical log scale. 0.1 was added to all counts to facilitate plotting on a log scale. Smoothing splines (k=3) added.

Copepods

In April, copepod densities where highest at 31.4 (5.3) ind l^{-1}. In September, density of copepods was lowest at 1.8 (0.2) ind l^{-1}. Benthic copepods (Harpactacoida) and their nauplii were present in low densities of generally less than one ind l^{-1}. Density of pelagic copepods (Cyclopoida and Calanoida) was generally higher than one ind l^{-1} in March, April and May, although in May this was mainly nauplii. In May and September copepodites were absent in samples with dilution factor 2 and 3 in May and dilution factor 0, 1 and 2 in September.

Ciliates

The ciliates showed the highest reduction in counts with increasing dilution, with ciliats often not found at the highest dilution levels of 1:2 and 1:3. Ciliate densities were highest in April and lowest in March.

Rotifers

Rotifer densities were highest in May and lowest in March. In March, rotifers where absent in 1:2 and 1:3 SW:FW dilutions. In later months there was no clear trend in rotifer counts with increasing dilution, except for a small decrease in September counts.

Balanoids

Balanoid naupllii density was highest in May and lowest in September, while cyprid density was highest in April. Both nauplii as well as cyprid larvae of barnacles showed no clear trend with increasing dilution levels.

Bivalves

Bivalve density was highest in April and lowest in May. In March there was a clear reduction in bivalve counts at 1:2 and 1:3 SW:FW dilution, but this was not observed in later months.

Gastropods

Gastropod veligers were absent in March, with highest densities in May. They were generally only present in low numbers <1 ind l^{-1}, without a clear trend in counts between treatments, except that in May there appeared to be a reduction in counts between dilution factor 2 and 3.

Polychaetes

Polychaetes, both trochophores as well as nectochaetes, did not show a clear response to different dilution levels.

Bryozoa

Bryozoa cyphonautes larvae density was highest in May and lowest in March. In April, May and September there was a decrease in bryozoa cyphonauta larvae counts with increasing dilution levels.

8.3.8 Fouling experiments

First check 14-06-2017

During the first check of the PE pipe segment a large volume of sediment was found at the bottom of the stack street distribution pipe, with a thickness of around 5 cm in the centre (Figure 65B), Sediment from inside the pipe and stuck to the various surfaces was rinsed off, collected and filtered over a 500 micron sieve, as well as sediment . The pipe segment itself was still quite clean (Figure 65A), but several fouling organisms had attached themselves to the PE surface. Organisms found living on the pipe segment surface were:

- Tubes made of sediment, made by either polychaetes or amphipods such as *Jassa* sp.
- Hydroid polyp colonies (*Clytia* sp.)
- Barnacles, ca. 10 ind.
- Peritrichous ciliates (e.g. *Zoothamnium*)

In the sediment collected from inside the pipe and rinsed off the wide range of organisms was found as well:

- 18 Foraminifera
- 5 Gastropod veliger larvae
- Cockle *Cerastoderma edule* shells, 4 doublettes of 1 mm diameter
- Razor clam *Ensis leei* shells, one live ind. Of 7 mm length, 22 empty doublettes of 2 -3 mm length
- 2 empty doublette of *Scrobicularia plana*
- One empty shell of *Peringia ulvae*
- One *Alitta* sp. Polychaete of 25 mm length

Barnacles (about 50 in total) were also observed growing on other surfaces inside the stack street distribution pipe. Barnacle size ranged from 4 to 6 mm, species was mainly *Balanus crenatus*.

Second check 21-09-2017

At the second check of the pipe segment, the same samples were taken as in the first check. This time the following organisms were observed on the pipe segment surface:

- *Molgula manhattensis* tunicates 56 ind.
- *Corophium* sp. amphipods 2 ind.
- *Polydora* sp. Polychaete 1 ind.

In sediment collected from the inside of the pipe and rinsed off the various surfaces of pipe terminating cap and components the following organisms were found:

- *Molgula manhattensis* 21 ind.
- *Polydora* sp. 6 ind.
- *Streblospio* sp. 4 ind.
- Barnacle fragments of ca. 20 ind.
- Ca. 320 *Peringia ulvae* shells of which about 10 % was still alive
- *Cerastoderma edule* 2 mm, 1 ind.

- *Ensis leei* 2 ind of 3 and 4 mm.

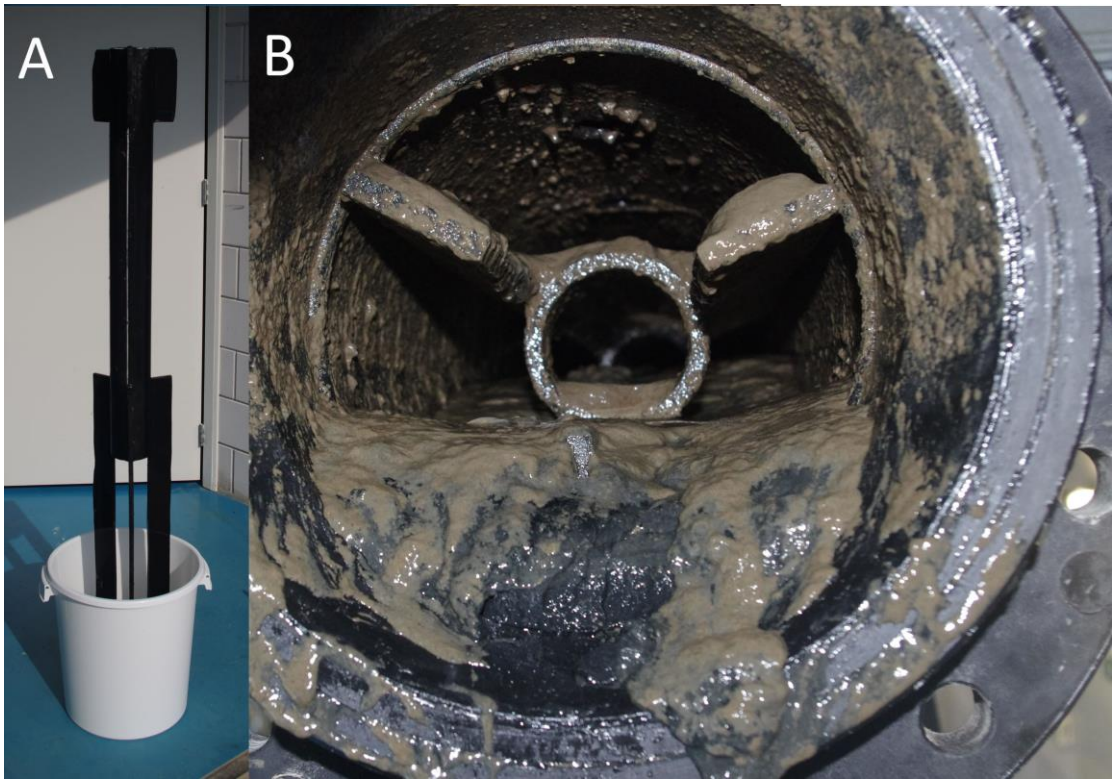


Figure 65. Pipe segment installed at REDstack Breezanddijk left stack street to investigate fouling. B. interior of right stack street main distribution pipe showing the tube segment on the first check date of 14-06-2017.

8.4 Discussion small organisms

8.4.1 Methods

In a preliminary study, documented in the WP 2.5 report, the estimates of zooplankton density and species composition in this study were found to be representative for density and composition occurring *in situ* in the Wadden Sea, near the intake point. Basically, the system can be comparable to a very large and a very long plankton pump, and will likely suffer the same advantages and disadvantages of this commonly applied method of zooplankton sampling.

Differences in efficiency between plankton nets and pumps have been studied extensively (e.g. Harris et al. 1986, Powlik et al. 1991, Masson et al. 2004) with varying results. Avoidance will be higher at lower pumping speeds. According to Harris et al. (1986), a pump with a filtration rate of 2.8 m^3/hr is as effective as a 200 micron plankton net for fish larvae.

One important aspect that has to be taken into account is that organisms encountered at the intake could not only originate from the plankton, but also from the community living in and on surfaces of the pipes transporting the water from the sea into the installation. Organisms like harpactacoid copepods and nematodes were found living in and on the silty, detritus –rich layer that formed on the inside of the pipes. Predation by sessile organisms such as barnacles, mussels and hydroids could also lead to an

underestimation of density, but because of the relatively high filtration rate we think this impact is low.

8.4.2 Seasonal patterns

There are very little studies on zooplankton species composition and seasonal patterns in the western Wadden Sea. Van Walraven et al. (2017) studied gelatinous zooplankton, but for non-gelatinous zooplankton the most recent comprehensive study dates from 1983 (Fransz & van Arkel 1983), documenting seasonal patterns in zooplankton from 1973 – 1978. The seasonal patterns and succession of the different meroplanktonic groups is still similar, with a peak in polychaete larvae and fish larvae in spring, followed by balanoids and mollusc larvae in spring, although the densities of polychaete larvae observed by Fransz and van Arkel on the station closest to Breezanddijk are lower than found in the present study (Figure 66).

Fransz and van Arkel hypothesize that after a strong winter mortality in the benthos can lead to a decline in meroplankton. This is not confirmed by the present study, as high densities of meroplankton, especially polychaetes, were found after the cold spring of 2018. Densities of copepods in spring appear to be of a similar order of magnitude in 1973–1978 compared to 2017 and 2018. Summer and autumn densities of copepods however, appear to be much lower in the present study and in general, although in 2018 an autumn peak of copepods was still observed.

One explanation of reduced zooplankton biomass in summer and autumn, might be the predation by the invasive ctenophore *Mnemiopsis leidyi*, which occurs in high densities in the area in this period, and can exert a significant predation pressure on the zooplankton (van Walraven et al. 2017).

In the same period as Fransz and van Arkel, Baretta & Malschaert (1988) studied the distribution and abundance of zooplankton in the Ems estuary, from 1974–1977. They found similar seasonal patterns, but again with higher copepod abundance in summer and autumn than in the present study.

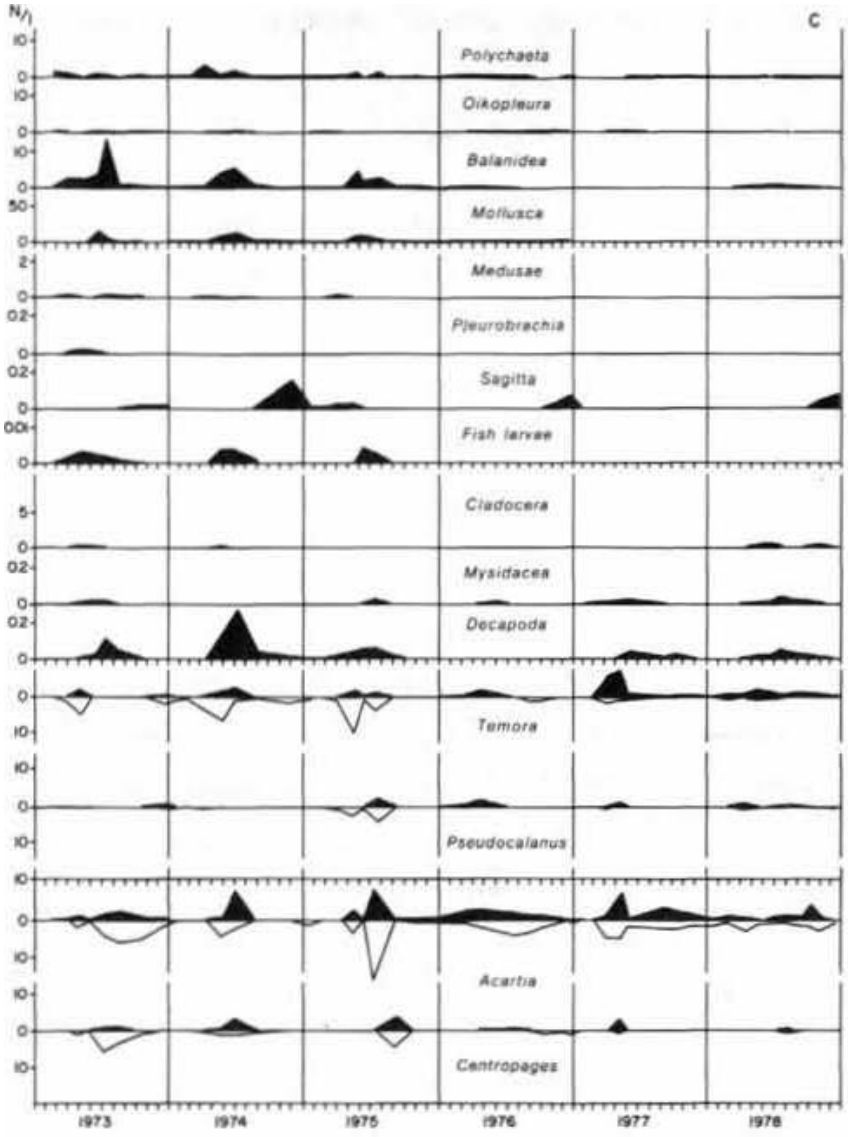


Figure 66. Seasonal patterns in zooplankton density (n/l) in the western Wadden Sea from Fransz & van Arkel 1983 with nauplii below, and adults above the x axis.

8.4.3 Importance of meroplankton

This study shows that for the inner western Wadden Sea the majority of the zooplankton biomass is contributed by meroplanktonic larvae of benthic organisms, with copepods only contributing to a minor part of the zooplankton biomass for most of the year except late spring. Meroplanktonic larvae typically occur in short bursts of weeks to months; firstly, the polychaetes, then the balanoids and then the gastropods and bivalves. Fluctuations in the abundance of benthic organisms might thus also influence biomass of zooplankton.

8.4.4 Impact of entrainment in Blue Energy power plants on zooplankton

8.4.4.1 Water temperature change

In conventional power plant cooling systems, organism survival decreases with increasing water temperatures (Mayhew et al. 2000), but temperature change Δt in these systems is generally in the order of 10 °C (Bamber and Seaby 2004). Temperature changes would occur naturally when fresh water is discharged into the Wadden Sea and both water masses have different temperature. However, mixing of water masses is not

instant and so temperature changes would be more gradual than the sudden temperature changed experienced in the RED process. Maximum Δt estimated for REDstack Breezanddijk was 1 °C at 1:1 seawater:fresh water ratio, for the whole period except two occasions during a summer heat wave. We assume that the impact of this minor temperature change is negligible compared to the impact of salinity changes.

8.4.4.2 Impact of salinity shock

The Wadden Sea zooplankton community mainly consists of euryhaline species, capable of living in a wide range of salinities (Fransz and Arkel 1983) and this experiment shows that the community can also partly survive sudden reductions in salinity, with mortality increasing with increasing dilution factor.

Analysis of highly variable zooplankton sampling data can be challenging, where counts can fluctuate by orders of magnitude on small temporal and spatial scales. In the salinity shock experiment, we tried to minimize between-sample variation in starting organism concentration and community composition by randomizing container assignment, sequence of filling of containers with source water and counting the samples by different analysts. In order to mimic in situ conditions as much as possible, samples were not concentrated and taxonomic composition of samples was not altered, to limit crowding, predation and oxygen shortage. A downside of this approach was that densities of several organism groups were often too low to be able to disentangle experimental mortality from naturally occurring variation.

A separate analysis of the response to changing salinity levels of ciliates and rotifers versus other members of the zooplankton community revealed that mortality is highest in ciliates and rotifers, with ciliates likely being the most sensitive to changing salinity. In most crustacean, mollusc, polychaete and bryozoan groups there appeared to be little mortality with increasing dilution factor, but these groups combined did show significant decrease in counts with increasing dilution factor which, converted in an estimate for survival, ranged from 75 % at dilution factor 1 to 50 % at dilution factor 3.

Possible relationships between dilution factor and survival suggested in Figure 63 for holoplankton groups and Figure 64 for meroplankton groups will have to be verified using additional experiments with either laboratory cultures, higher organism concentrations or higher sample volumes. Nevertheless, focusing on the response of the whole -or part of the- community we found differences in community response to changing salinity levels at the four different dates at water temperatures 6, 10.5, 13 and 16 °C. Water temperature in the area generally varies from 0 °C to 20 °C (H. M. Van Aken 2008), so it would be interesting to expand the experiment to test community response for the full range of water temperatures observed in the area. Survival of different species of *Acartia* copepods 20 hours after salinity shock was found to differ between different temperature levels, but with no overall trend, with differences mainly attributed to differences between experimental temperature level and temperature at time of capture (Lance 1963). In the same study, a decrease of survival with increased dilution factor was observed with survival strongly decreasing for dilution factor higher than 2 (less than 30 % seawater). Similar results were obtained by Calliari et al. (2008). Both these studies found that sensitivity to salinity shock differed between species of *Acartia*.

While Lance (1963) mainly focused on survival after 20 hours, copepods were kept at the reduced salinities for 5 to 9 days. For most treatments mortality continued after the first 20 hours, especially at 15 % - 25 % seawater dilutions (dilution factor 1:5 - 1:3), which is expected as acclimation to a new salinity can take several days (Khlebovich 1974). Thus, estimates of survival after less than one day as presented here and in

Calliari et al. (2008) could overestimate actual survival. On the other hand, keeping organisms in lowered salinities for longer periods would not be an accurate representation of the RED process, as once discharged the diluted water would mix with seawater again and organisms would return to normal salinity levels. Lance (1963) shows that a percentage *Acartia bifilosa* copepods immersed in 15 % or 20 % seawater survived when returned to 100 % seawater, with survival greatly varying with immersion duration, from almost 100 % survival at 1-minute immersion to 0 % survival at 16 and 17 minutes of immersion. This could apply to organisms passing through a RED installation as well, where plankton survival might be increased by minimising the residence time of organisms in the installation, and/or designing the brackish outflow in such a way that the discharged brackish water is quickly mixed with seawater.

The estimated survival percentages in this experiment include only the effect of salinity reduction and mortality caused by pouring, mixing, filtering and transport of the samples. Mortality caused by other downstream and upstream factors such as shear stress, mechanical disturbance caused by filtering and pumping, and predation could lead to lower survival than estimated here. Also, besides direct mortality, salinity shock can also have non-lethal consequences for zooplankton, such as decreased feeding activity and movement (Calliari et al. 2008) which could affect feeding, reproduction, growth and susceptibility to predation.

8.4.4.3 Impact of intake pump, transport and filtration.

The fraction of dead organisms in intake waters was generally low, at or below 5 %, for both fresh water as well as seawater, with the exception of balanoid cyprids in seawater, benthic copepods in fresh water and cladocera in seawater, where the fraction of dead organisms was higher. The fraction of dead organisms in filtered water samples was generally higher or equal than that of the intake water. Compared to mortality induced by salinity change, the mortality induced by pumping, transport and filtration seems to be minor.

Mortality of zooplankton was only assessed for the drum sieve filtration method. The impact of sand filtration on mortality of zooplankton could be different.

8.4.4.4 Cladocera in seawater: indicator for frequency of secondary entrainment of organisms?

Freshwater cladocera in seawater intake likely originated from the brackish water discharged by the RED installation at Breezanddijk, or fresh water discharge near Den Oever and Kornwerd. If brackish water discharged by the installation in the harbour of Breezanddijk is partly taken up again at the intake point just outside of this harbour, one would expect elevated densities of freshwater organisms -such as cladocera- in the intake water. Presence of cladocera in the intake water could thus perhaps be used as a tracer of the amount of discharged brackish water re-entering the installation a second time. Mean density of cladocera was 42.54 ind l^{-1} in fresh water intake water, and 0.05 ind l^{-1} in seawater intake water, which means average density of cladocera in seawater was 0.12 % of average cladocera density in fresh water. This would suggest that the amount of brackish water re-entering the system was negligible.

8.4.4.5 Conclusions survival small organisms

A sudden decrease in salinity, such as caused by the RED process, will lead to mortality of zooplankton, depending on dilution level, community composition and/or possibly seawater temperature. The extent of this mortality increases with increasing dilution level, and varied for different dates with different water temperatures and community composition.

Ciliates and to a lesser extent rotifers appeared to be the most sensitive to salinity reduction, with crustaceans, gastropods polychaetes being the least sensitive.

A dilution of 1:1 SW:FW will thus impact only part of the zooplankton community, while higher dilution levels of 1:2 and 1:3 will likely impact most taxa. At higher temperatures the mortality of zooplankton after 1:1 SW:FW dilution might be higher, but this would need further experiments to be confirmed. Avoiding most of the mortality caused by salinity reduction would be possible by separate discharge of fresh and marine filter residue to its respective water sources, instead of mixing these residues together for discharge in the marine water source, as recommended in our review (Jager and Van Walraven 2017).

At the current dilution level of 1:1 average Wadden Sea zooplankton survival following dilution of 1:1 SW:FW is estimated at around 72.4 %, excluding other mortality factors. Mortality by other factors is estimated to be minor compared to mortality by osmotic shock. For the estimation of basin-wide effects of Blue Energy, we recommend running calculations with different levels of mortality to assess the sensitivity of large-scale effects to different levels of mortality.

The impact of a Blue Energy power plant on tidal basin scale is further explored in the synthesis D 4.3. A 100 MW Blue Energy plant using 100 m3/s of seawater takes in 8.64 * 10^6 m^3 of seawater each day. Compared to an average volume of the Mardiep tidal basin of 2890 *10^6 m^3 (Duran-Matute et al. 2014) this means every day 0.3 % of the water in the basin is filtered by the BE power plant. The impact of the power plant on organisms is then based on mortality of the organisms and the exposure time of the organisms to mortality by Blue Energy, which is based on the time the organisms are present in the water column and susceptible to being entrained in the Blue Energy plant.

Generation time of holozoöplankton ranges from several days for rotifers and ciliates to several weeks or months for copepods, depending on water temperature, with higher generation times at lower temperatures (Gilloly, 2000). Residence time of meroplankton in the water column is also highly variable, but generally in the order of 3-4 weeks, as also found in the monitoring performed for this project. If we take an average generation time of one month, the probability of being entrained in the Blue Energy plant would be 0.3*30=10% for each generation. Combined with a mortality probability of 50-100 % this would lead to an additional mortality of 5-10 % of zooplankton. This is a significant amount. However, as mortality is highest for groups with the shortest generation times; rotifers and ciliates, the overall impact of mortality on the population level is expected to be limited.

8.4.5 Impact of different intake systems on filtration efficiency.

The switch to a different intake system using wedgewire screens occurred in october 2019, when the main monitoring programme had ended. This limited collection of additional samples, but the limited data available suggest that the switch did not impact drum sieve filtration efficiency for zooplankton (Figure 48). This is as expected, since the width of the wedge wire slits (0.5 and 1 mm) still far exceeds the size of most zooplankton organisms except for groups like large copepods and fish larvae.

8.4.6 Fouling

Fouling is caused by settling of organisms from the intake water on installation surfaces, including RED membranes. Fouling can influence the operation and proper functioning of the installation. Fouling organisms mostly belong to the meroplankton, living

pelagically before settling on substrates or in sediments. Suitable settlement substrates can be found in the installation, in which case fouling is a fact. Fouling is dependent on the suitability of the substrate for settlement, the development stage of the organisms and suitable settlement conditions.

As efficiency of the drum sieve filtration was not 100 % fouling organisms were still present in the effluent distributed to the RED stacks. Our tests using a PE pipe segment installed inside one of the stack street distribution pipes allowed us to present an overview of main fouling organisms present (Figure 67). Fouling organisms not only settled on the surfaces of the pipes, but also colonised the sediment layer that formed at the bottom of the pipe. That fouling organisms could settle and grow inside the pipes is shown by their size, with the largest organisms being several millimeters in size.

Fouling can be prevented by keeping daylight out of the installation, which inhibit the growth conditions needed by algae and most cyanobacteria. Fouling can be reduced by applying prefiltration. Most of the fouling organisms are larger than 50 microns in size. Filtration of the Blue Energy process water by drum screens with mesh size of 50 micron (or less) should in theory be able to protect the stacks from fouling. Other options include influencing the conditions and the suitability of the substrate for settlement using anti-fouling coatings or ultrasonic vibrations. Switching fresh- and marine feedwaters may also inhibit fouling, although this is also used in the current system where fouling organisms were found. Furthermore, mechanical or chemical cleaning can be applied. Design and cleaning interval of pipes could be optimised to minimise sediment buildup, as high densities of organisms were observed settling on and in the sediment.

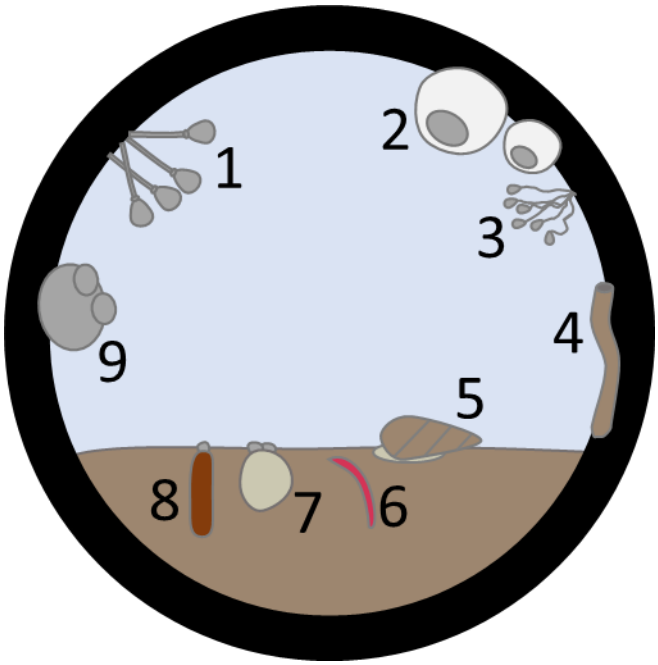


Figure 67 . Schematic overview of fouling organisms encountered in the Blue Energy plant stack street pipes. 1) Colonial hydroids *Clytia* sp. 2) Barnacles 3) Colonial ciliates e.g. *Zoothamnium* 4) Tubes made of sediment by polychaetes or amphipods 5) *Peringia ulvae* snails 6) Polychaeses 7) Cockles *Cerastoderma edule* 8) Razor clams *Ensis leei* 9) Tunicates *Molgula manhattensis*.

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