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Research Project Ecology & Evolution 2

Biodiversity in artificial intertidal pools in the Wadden sea

Author: Jet Hiltermann – s3670473
Partner: Leah Merlijn – s3585891
Supervision: Lucía Irazabal Gonzalez and Britas Klemens Eriksson
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Abstract

Climate change, sea level rise and stormier seas ask for more coastal and marine infrastructure (CMI), such as dikes, to protect the habitants who live nearby the shore. However, dikes often serve as a poor habitat for flora and fauna, because they are made of concrete, are homogenic and they lack water-retaining features which all together suppresses the coastal biodiversity. The Netherlands is an example of an artificial shoreline with several dikes, which will be reinforcement in 2023. To enhance the biodiversity on the dikes, biological engineers try to mimic rocky shores, which have a similar ecology, by introducing artificial intertidal pools to the dikes. Since November 2021 there are 26 artificial intertidal pools at Lauwersmeerdijk in Groningen. This report compares the species diversity of artificial intertidal pools at Lauwersmeerdijk with its existing homogenous rocks. We did this by doing a biological survey and after this we analyzed the data in R. We expected that the pools would enhance the biodiversity on the dikes, however this was not the case.

Introduction

With almost 60 percent of the growing world population living within a distance of 100 km of shore, coastal ecosystems are being substituted by coastal and marine infrastructure (CMI) to fulfill anthropogenic transport, energy and urbanization needs (Perkol-Finkel and Sella 2015). Moreover, climate change, sea level rise and stormier seas, could result in flooding of the urban communities. The answer to reduce the damage of such hazards, is to build more dikes. These CMI's provide a habitat for multiple species, however they are still one of the main reasons for coastal marine biodiversity loss (Firth, Thompson et al. 2013, Perkol-Finkel and Sella 2015). As most marine life is located in the coastal area, the loss of biodiversity could have an impact on the whole marine ecosystem (Perkol-Finkel, Hadary et al. 2018). Dikes often serve as a poor habitat for flora and fauna, because they are made of concrete which has a high pH, they are homogenic, hard and they lack water-retaining features which all together suppresses the coastal biodiversity (Firth, Thompson et al. 2013, Perkol-Finkel, Hadary et al. 2018).

The Netherlands is an example of an artificial shoreline with several dikes in different parts of the country. Almost 30 percent of the land is below mean seawater level, which makes it very vulnerable for flooding, therefore a good water defense is more than needed to keep the urbanized areas dry and this has been going on for centuries (Hoeksema 2007). The natural coast consisted of brackish wetlands, mud flats and salt marshes (Nienhuis 1969, Wolff 2000) and had a flourishing biodiversity, however it is transformed over many centuries into a hard, homogenic artificial dike-shore which has a hugh negative effect on the coastal flora and fauna. The species that colonize these dikes are thought to be similar in ecology as the flora and fauna of natural rocky shores, however the communities on the dikes are suggested to be less diverse (Firth, Thompson et al. 2013). Natural rocky shores consist, just like artificial dikes, of hard rocks, however they are among other things heterogeneous and can contain water-retaining features which makes them able to host much more species then artificial dikes can. Due to weathering and erosion, intertidal pools can be formed on the natural rocky shores, which create isolated habitats that are very

heterogeneous in size, shape and composition and which promotes a stable ecological niche (Martins, Hawkins et al. 2007).

Mimicking nature could be a solution to the coastal biodiversity problem. Over the last decade ecological engineers manufactured artificial intertidal pools to increase the ecological value of CMI (Sella and Perkol-Finkel, 2015). These pools follow a *Nature-Inclusive Design* (NID), which refers to the including of biological and ecological principles, such as heterogeneous and bio-enhancing concrete, in the construction in such a way that it provides the presence of multiple habitat types. These structures could promote the coastal species richness and enhance the performance of marine ecosystems (Firth et al., 2014; Sella et al., 2021), however there has not been done much scientific research in the actual impact of the pools (Sella and Perkol-Finkel, 2015).

Since November 2021 there are artificial intertidal pools located at Lauwersmeerdijk, the Netherlands, and this dike will be reinforced by 2023. To provide more scientific insight on the impact of CMI on biodiversity and the importance of the artificial intertidal pools, this report presents the findings based on the research question: "Do artificial intertidal pools at Lauwersmeerdijk increase species diversity, compared to existing rock?". The artificial intertidal pools were created by the compagnies *ECOncrete* and *ReefSystems*, which both follow the NID approach (Perkol-Finkel, Hadary et al. 2018, "About Us | ReefSystems," n.d.). Our research was part of a bigger monitoring project, that's why also abiotic factors, such as the salinity and temperature, and percentage of coverage were measured. Our main question will be answered only by using the data that came out of a biological survey, which measures species richness, species evenness and species diversity. Species evenness shows how even species are distributed over a specific area. The higher the species evenness is, the more even the species are distributed. Species richness is the amount of species that are present in a certain area. Biodiversity is dependent on species richness and on the species evenness. The higher the richness and the species evenness, the higher the biodiversity (DeJong 1975, Stirling and Wilsey 2001, Nolan and Callahan 2006).We hypothesize that the artificial intertidal pools will enhance the species diversity, since it promotes multiple habitats and is less homogenic then the existing rock.

Materials and Methods

1. Study location

We conducted fieldwork at Lauwersmeerdijk; a 9 km dike located in between the Lauwersoog harbor (53.40964, 6.20664), and the Westpolder (53.37854, 6.29119) in Groningen, the Netherlands. In combination with the reinforcement plans several parties (Arcadis, ECOncrete, Heijmans, Heuvelman Ibis, ReefSystems, University of Groningen, Van Hall Larenstein University, Van Oord, Waterschap Noorderzijlvest) sought to improve the ecosystem on and surrounding the dike by adding artificial intertidal pools and reefs in November 2021. Our monitoring focused on 23 out of 26 intertidal pools (6 pools of ReefSystems and 17 of ECOncrete) installed in the intertidal zone of the dike with depths ranging from 0 to 3.4 m (Figure 1). Their location was determined with a GPS (Garmin eTrex 22x) and their elevation with a DGPS (Trimble R8 gps receiver and Trimble TSC3 controller).

Tidal ranges vary between 0.5-4 m. ("Taking Shape | Wadden Sea," n.d.), leaving the pools exposed during low tide.

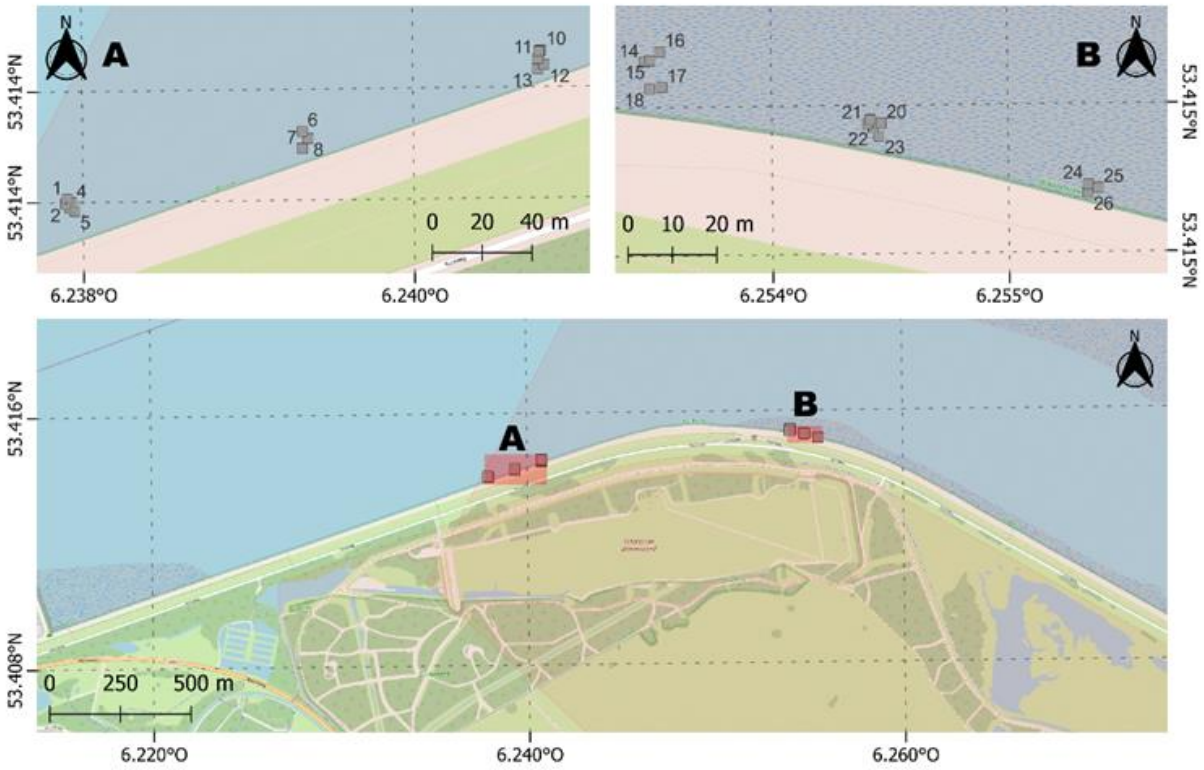

Figure 1. Map of study location at Lauwersmeerdijk
The grey boxes represent the placement of the artificial intertidal pools with their designated number. There are several groups consisting of 3 or 5 pools divided over two sites A and B.

2. Sampling method

The monitoring lasted ten days in May 2022 (2nd to 6th May and 16th to 20th May). We started sampling three hours before low evening tide and finished our sampling an hour after low tide. This research is part of a bigger monitoring project, therefore more abiotic and biotic factors were monitored than we eventually used for our results and discussion. For the abiotic factors, we measured the pools' elevation and we aimed to monitor the salinity and the temperature (Multiparameter Meter Multi 3320 2FA310 Xylem – WTW) at least twice per sampling day: once when arriving and once before finishing the fieldwork. The outside weather conditions were monitored during the two weeks by using weather forecasts, focusing on temperature, precipitation, and humidity. The biotic factors of the 23 pools were monitored in two different ways explained below.

2.1. Biological survey

For this type of monitoring, we reported all the species found within a 0.5x0.5 m quadrat - divided in 25 smaller quadrants - in the pools, on the new rock and on the old rock. We

counted the individuals of solitary species (snails, mussels, oyster, etc.) and we determined the percentage of coverage of covering species (algae, barnacles, etc.) for which the counting was not possible. We monitored all the sites as described below.

2.1.1. Pools

First, we took a 250 ml sample of water to take into the laboratory for further turbidity measures, and took the GPS coordinates for the pool. With an underwater camera (Denver AC-5000WMK2) we recorded a video of the inside of the pool to double-check in the laboratory for missing organisms. After this, we randomly placed the 0.5m x 0.5m quadrant onto the pool. Inside the quadrat, we: recorded all the species, counted all the solitary species, determined the overall percentage of coverage for the covering species and set a quantitative evaluation (0 scattered, 1 dense) for when the percentage of coverage was difficult to determine. We caught the species at the deeper parts of the pools with an aquarium net (SuperFish Aquarium Schepnetje 12 cm) and we identified them put in a white, hard plastic laboratory tray (Dynoplast Stjørdal AS art 151) with pool water. The unidentified species were taken into the laboratory in full 12 ml tubes for further assessment. To end we took a video of the, now more disturbed, pool for possible later assessment.

2.2.2. New rock and old rock

To monitored the new rocks we randomly placed the 0.5mx0.5m frame on the new rocks close to the measured pool. We recorded the exact location of the quadrant by using a GPS and marked it with nail polish for easier recognition. Inside the plot we recorded the same variables than in the pools on the on all parts of the rocks (rocks' surfaces, sides and underneath the rocks). We took samples from organisms we could not identify on the spot for further assessment.

2.2.3. Laboratory

For the species that we could not identify in the field, we used a stereomicroscope (Olympus SZ51 Stereo Microscope 0.8x - 4x) and an identification book (Hayward and Ryland 2017) to determine the lowest taxonomic level. The species identified under the microscope were counted and added to the species list. All were directly in the field stored in a cooling bag and after the field day put into a fridge of 4 °C.

2.2.4. Data analysis

For our sampling, we encountered several species that could not be counted, these species were recorded into percentage of coverage. Due to the differences in the type of data, we divided our data in "covering species" (*Semibalanus balanoides, Obelia sp., Fucus vesiculosus, Berkeleya rutilans, Gracilaria sp*., Green algae*, Porphyra sp., Pygospio elegans colony, Ulva sp.*) and "counted species"(*Aurelia aurita, Carcinus maenas, Corophium sp., Crangon crangon, Crassostrea gigas, Diadumene Cincta , Gammarus locusta, Haliplus confinis, Leptomysis gracilis, Littorina littorea, Melita palmata, Mesopodopsis slabberi, Mnemiopsis leidyi, Mnemiopsis leidyi* larvae*, Mytilus edulis, Nematod, Platichthys flesus,*

Praunus flexuosus, Praunus neglectus, Tergipes tergipes). The counted data are the counted individuals per species. After this, the data was grouped per location: inside the pool, new rock and old rock.

To formulate an answer to our research question, we should define the species evenness, species richness and biodiversity. For the richness we recorded all the species found at each location. The species are displayed in Table 1. We used the VEGAN R-package (Oksanen, Blanchet et al. 2013) to calculate species diversity using the following equation of the Shannon-Wiener Diversity Index (H):

$$H = -\Sigma pi * \ln(pi)$$

where pi stands for the proportion of the community made up of species i (DeJong 1975, Nolan and Callahan 2006).

The diversity was first calculated with the *Mnemiopsis leidyi* larvae. These were present in numbers of thousands, much more than the other species, but since they were very small we decided to take them out to see if they influenced the data.

The evenness was calculated with the following equation:

$$SEI = H / Ln((s-1) / Ln(n))$$

where SEI is the Shannon's Evenness Index, s is the number of species recorded, n is the total number of individuals in the sample, and H is the previously mentioned diversity index (DeJong 1975).

To visualize the data we made graphs of the richness per pool, and the diversity for both the count and coverage species (plotted the plots next to each other using library cowplot (Wilke, Wickham et al. 2019). The visual check (Q-Q plots) and the Shapiro-Wilk test showed that both the counted and covering data did not followed a normal distribution even after. Hence, we opted for the Kruskal-Wallis test since this is a non-parametric test that can be used for one variable (diversity) with 3 independent groups (we used our 3 locations). We obtained a significant result in the Kruskal-Wallis test, that is why we performed a Dunn's test (Ogle and Ogle 2017) with a Bonferroni correction, to establish the differences between sites.

Results

Species richness

The species richness can be seen in Table 1. A total of 28 different species were found over the three locations. 11 at the new rocks, 9 at the old rocks and 24 species at the pools. Then we looked at the richness per pool (Figure 2). The Kruskal-Wallis gave back a significant p-value of 0.02418. Performing a Dunn's test, this gives us a significant difference between the new rock and the old rock and a significant difference between the new rock and the pool.

Species evenness

The evenness was calculated with the Shannon's Evenness Index. This was not calculated separately, but within the R script for the diversity. The evenness was very low in the pools, and in both the rock locations the evenness was higher.

Biodiversity of covering species

Having the evenness, we can calculate the diversity for the data of the covering species and counted species. We used Shannon's Diversity Index to do so. In Figure 3 you can see the diversity of the covering species data. After performing a Kruskal-Wallis test on this (p= 0.31163) it turned out that these groups are not significantly different. So there is no increase of diversity in the covering species.

Table 1. All different species found, organized their phylum. Marked which species were present where.

Taxon	Species	New rock	Old rock	Inside
Cnidaria	*Aurelia aurita*			+
	Obelia sp.			+
	Diadumene cincta	+		
Ochrophyta	*Berkeleya rutilans*			+
	Fucus vesiculosus	+	+	+
Arthropoda	*Carcinus maenas*		+	+
	Leptomysis gracilis			+
	Corophium sp.			+
	Praunus flexuosus			+
	Melita palmata			+
	Semibalanus balanoides	+	+	+
	Gammarus locusta			+
	Praunus neglectus			+
	Mesopodopsis slabberi			+
	Haliplus confinis			+
	Crangon crangon			+
Mollusca	*Crassostrea gigas*	+	+	
	Tergipes tergipes			+
	Mytulis edulis	+	+	+
	Littorina littorea	+	+	+
Rhodophyta	*Gracilaria sp.*	+	+	
Chlorophyta	*Green algae*	+		+
	Ulva sp.	+	+	+
Ctenophora	*Mnemiopsis leidyi*	+	+	+
Nematoda	*Nematod*			+
Chordata	*Platichthys flesus*			+
Rhodophyta	*Porphyra sp*	+		
Annelida	*Pygiospio elegans*			+

Biodiversity of counted species

Figure 4 shows the diversity of the counted species data. The biodiversity was different for the different locations, this is already visible by eye, but also the Kruskal-Wallis test gives back a p value of 0.00589, which is smaller than 0.05 and thus significant. The Dunn's test gave a significant value between the old rocks and the pools (p= 0.0001), and between the new rocks and the pools (p= 0.006). This makes us state that the pools have the lowest biodiversity in the counted species of the three study locations.

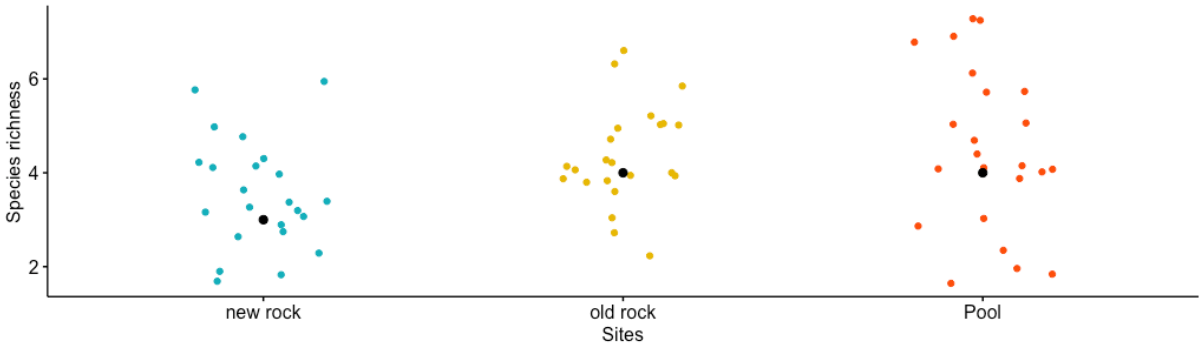

Figure 2. The richness per pool group at the different sites

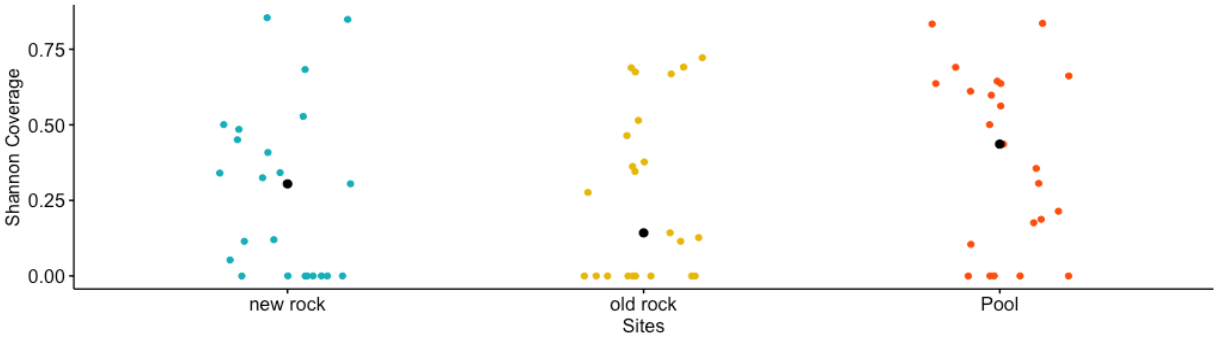

Figure 3. The diversity per pool group of the covering data

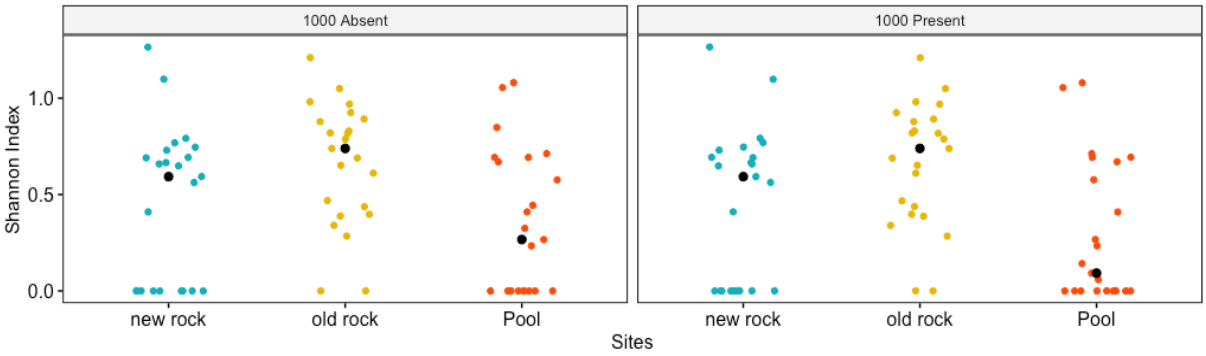

Figure 4. On the left: the diversity of the counted data with the Mnemiopsis leidyi larvae. On the right: the diversity for the counted data without the Mnemiopsis leidyi larvae present in the data.

Discussion

Species richness

The richness is the highest in the pools (species count is 24), this makes sense since the pools are water-retaining features in which floating/swimming species can stay during low tide. Both rock sites do not have these features, this makes the floating/swimming species, that might be surround it during high tide, float away during low tide, and this could be a possible explanation of the lower richness on the rocks. Beside the absence of water retaining features in rocks, the homogeneous concrete of which the rocks are made, could also be a reason why less different species were found on the rocks (Firth, Thompson et al. 2013).

Biodiversity of covering species

The biodiversity of the covering species followed our hypothesis that the diversity in the pools will be higher compared to the existing rock, however the Kruskal-Wallis test was not significant, which means that the different locations are not significantly different and we cannot use this result. An explanation for this high p-value is that the sample size was too small (Vargha and Delaney 1998).

Biodiversity of counted species

The Kruskal-Wallis test of the counted species showed a p-value lower than 0.05, which means that the biodiversity between the locations is different. Against our expectations in, the Dunn's test showed that this difference was because the pools were significantly less diverse than the existing rocks.

A low biodiversity is a result of a low richness and/or a low evenness (DeJong 1975). Therefore, an explanation of this could be the low evenness of the pools, which could be a result of species overruling in some pools. When looking to the data, this indeed seems to be the case. When *G. locusta* or *L. littorea* were present in a pool, they were present in a much higher amount than the rest of the species, which makes the distribution of species uneven and thus lowers the evenness.

An explanation for the uneven presence of these species in the pools could be their population dynamics. For example, *L. littorea* was most often present in a pool when also *S. balanoides* was present (however this is not an exclusive rule), and this makes sense as they both feed themselves on algae. A studie of Buschbaum shows that the presence of *L. littorea* directly reduces the growth rate of *S. balanoides*, however indirectly the grazing of *L. littorea* prevents *S. balanoides* to be overgrown by ephemeral algae (Buschbaum 2000). This process of population dynamics fluctuates constantly, which could have had an influence on the number of individuals (or percentage of coverage for *S. balanoides*) that we found.

M. leidyi larvae disrupted the evenness even more than *G. locusta* or *L. littorea* did. They were present only in a few pools and in counts of more than a thousand individuals. For this reason we decided to treat them as outliers and took them out of the data. However, after altering the our dataset, the species diversity for the counted species was still much lower in the pools compared to the existing old and new rock. This made us think that the evenness was much effected by outliers due to the small sample size (Vargha and Delaney 1998).

What probably would also help in elevating the evenness, is redoing the monitoring for the same pools, to decrease the amount of missed species. We only monitored the locations once, due to time pressure. There might have been more species present, however due to the water turbidity in some pools it was sometimes very hard to see at once. Furthermore, in the second week we were more aware of all the different species that were precent, which made us look more closely than we did in the first week. It might be the case that some species of the first week were actually other species that were very similar in morphology.

Another explanation of the low biodiversity in the pools is 'incubation time'. By which the time period is mend from the moment that the pools were placed until the moment of monitoring. Since the pools were placed half a year ago, which is quite recently, there is a chance that some coastal and marine flora and fauna needed more time to settle and colonize the pools (Lotze, Reise et al. 2005).

Furthermore the season could have an influence on the (amount of) species we found. We did fieldwork during the early spring, so repeating this research at the end of September might give a very different outcome (Winter, Haynert et al. 2018).

Future research

Unfortunately, the pools were not randomly distributed over our research field, therefore abiotic factors, such as salinity and temperature, could have played a role on their biodiversity. Consequently we were not able to compare the compagnies with each other. For future research it would be very interesting to be able to compare ECOncrete with ReefSystems to find out which pool is more promising. During the dike reinforcement of 2023, they could take into account that the different pools need to be randomly distributed. Another idea for future research is comparing artificial intertidal pools with natural intertidal pools, to find out if the artificial intertidal pools work the same as the nature ones.

Overfishing

Conclusion

To conclude, our hypothesis was that artificial intertidal pools at Lauwersmeerdijk would have a higher species diversity, compared to existing rock. Even though the results were not in line with the hypothesis, this results are promising because we did find a lot species, however we didn't have time to repeat the measurements for the same locations, therefore a follow-up study is recommended.

Acknowledgement

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