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Research Project Ecology & Evolution 2
Monitoring of intertidal pools

Biodiversity in artificial intertidal pools in the Waddensea

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Abstract

With the current climate change, sea level rise and the increasing of storms, hard coastal marine infrastructures are spreading. They are made of hard homogenic concrete rocks that are unsimilar to the natural structures, which led to a drop in coastal biodiversity. Engineers developed artificial intertidal pools, which are structures that try to mimic natural rock pools. These natural rock pools are less homogenous and provide a isolated habitat which is beneficial for biodiversity. We compared the biodiversity between these pools, old rocks and new rocks at the Lauwersmeerdijk in Groningen (NL) to see if it would be beneficial to implement more pools. The addition of the pools to the dike did not show an increase in biodiversity in this research

Introduction

The Netherlands has a heavily populated coastal line. The populations living around these coastal areas are protected with hard coastal and marine infrastructures (CMI) such as dikes. Nowadays, with climate change, sea level rise and the increasing of storms, these hard infrastructures are spreading. These dikes are made of hard concrete rocks, this differs very much from the original structure of the coast. The concrete is much more homogenic and less porous and therefore the arrival of dikes led to a drop in coastal biodiversity (Firth, Schofield et al. 2014), which is the variety of plant and animal life in a location. Biodiversity is made up by richness and evenness. 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 number of species that are present in a certain area. The higher the richness and the species evenness, the higher the biodiversity (Moore, 2013). A large part of the world has rocky shores. Rocky shores are coasts where rocks predominate. Intertidal rock pools, created by weathering and erosion in these rocky shores, could be the solution to this decrease of biodiversity in the homogenous CMI. These pools are heterogeneous and create an isolated area from the sea and both of these factors promote the coastal biodiversity (Martins, Hawkins et al. 2007). However, these pools do not exist on artificial structures, that is why engineers developed artificial intertidal pools, that try to mimic these natural rock pools. Artificial intertidal structures all follow a *Nature Inclusive Design* (NID) (Sella et al., 2021, Firth, L. B., et al., 2014), which means the texture and molds enable the creation of ecological niches which promotes the development of rich and diverse marine life. Often, they are made of bio-enhancing concrete, which is chemically balanced allowing more different marine species to develop on there ("Solution," n.d Econcrete). Another positive effect of NID coastal marine infrastructures is that they are a suitable habitat for calcium carbonate-shelled organisms, which leads to an increase in biocalcification; a process where these organisms bind CO₂ and use it to build their skeletons. This calcification leads to more calcitic crust which can protect CMI and reduce their maintenance costs (Borsje et al., 2011).

In November 2021 several parties (*ECOConcrete* and *ReefSystems*, (Perkol-Finkel et al., 2018; "About Us | ReefSystems," n.d.)) placed some artificial intertidal pools at the foot of Lauwersmeerdijk on the north of the Netherlands. This is part of a reinforcement project on the dike that will take place in 2023. Initially, 26 pools were placed, but 3 got dragged into the mud too deep to sample them. We will test if these pools increase species richness, and diversity compared to the existing rock. We hypothesize that the artificial intertidal pools

will increase the species diversity, since they promote a more heterogeneous, isolated and porous habitat than 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. 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.

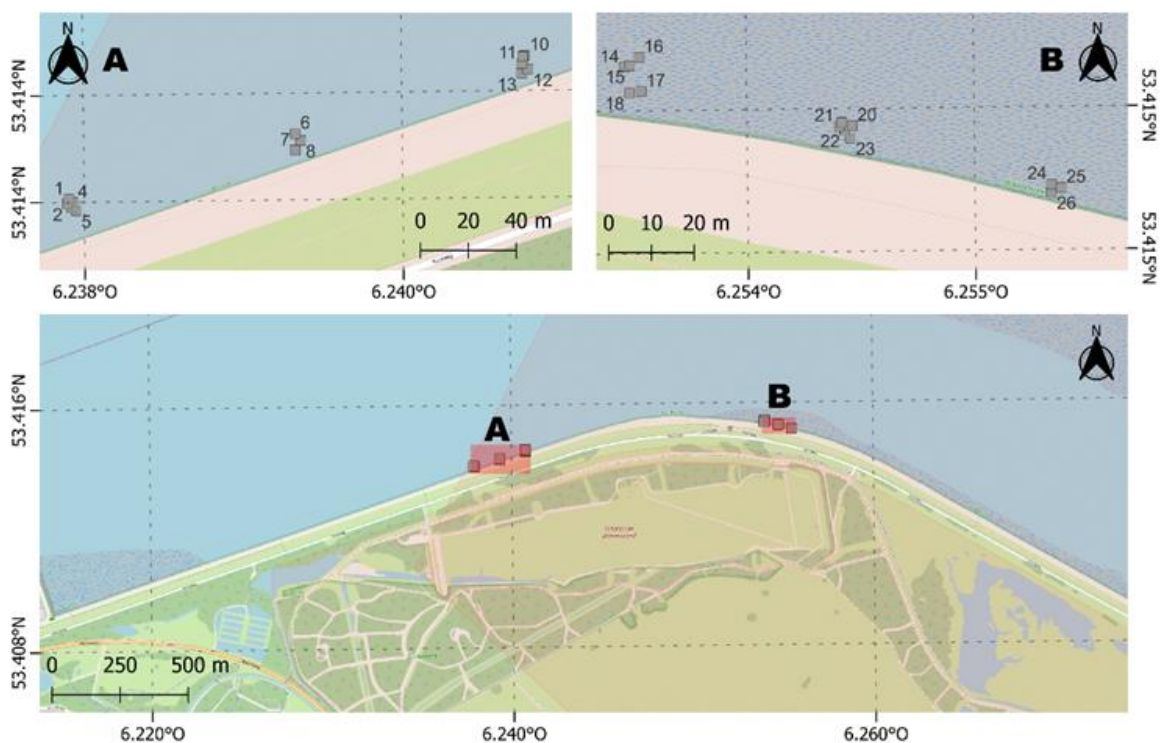


Figure 1. Map of study location at Lauwersmeerdijk. The grey boxes represent the placement of the artificial intertidal pools with their designated number. There are several groups consisting of 3 or 5 pools divided over two sites A and B.

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.

2.1.1 Map

Maps were produced using QGIS (version 3.24). The coordinate data from the dGPS was used for the maps. The coordinate reference system (CRS) used by the dGPS was EPSG: 28992 – Amersfoort/RD new.

2.1.2. Data analysis

The statistical analysis was done in RStudio (version 4.1.2). First the data was imported from Microsoft Excel using the readxl package (v.1.3.1, Wickham and Bryan, 2019). After this, the data was filtered based on the sample site using the dplyr package (v.1.0.7, Wickham et al, 2021). To calculate the percentage coverage of the species for each habitat type, the tabyl function of the janitor package (v.2.1.0, Firke, 2021) was used. The graphs were produced using the ggplot2 package (v.3.3.5, Wickham, 2016). The data was non-normal and could not be transformed using a logarithmic or square root transformation, so a Spearman's Rank Correlation Test was performed to find significant correlations between tidal elevation and the percentage coverage of species in each habitat type.

2.2. Biological survey

For this type of monitoring, we reported all the species found within a 0.5x0.5 m quadrant - divided in 25 smaller equal quadrants - in the pools, on the new rock and on the old rock. We counted the individuals of solitary species (*Littorina littorea*, *Mytilus edulis*, *Crassostrea gigas*, etc.) and we determined the percentage of coverage of covering species (*Berkeleya rutilans*, *Semibalanus balanoides*, etc.) for which the counting was not possible. We monitored all the sites as described below.

2.2.1. Pools

First, we took a 250 ml sample of water to take into the laboratory for further turbidity measures, and took the GPS (Garmin eTrex 22x) 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 quadrant, 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 to 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 monitor the new rocks we randomly placed the 0.5m x 0.5m frame on the new rocks close to the measured pool. We recorded the exact location of the quadrant by using a GPS (Garmin eTrex 22x) 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 at that moment for further assessment in the lab.

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 (-4).

2.2.4 Data analysis

To start working with the 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 "Count 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 count species are count data and the covering species are percentages and since these can't be mixed in one data analyses they were separated. 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 need the species evenness and the species richness.

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 for every new rock, old rock and pool using the following equation of the Shannon-Wiener Diversity Index (H):

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

here, pi means the proportion of the entire community made up of species i (Nolan, K. A., & Callahan, J. E. (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 check 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 Evenness index, *s* is the number of species recorded, *n* is the total number of individuals in the sample and *H* is the aforementioned diversity index.

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 (our 3 locations), to see if there was a significant difference between the groups..

To establish the differences between sites we performed a Dunn’s test (from the FSA library in R studio) with a Bonferroni correction.

Results

The 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 p-value of 0.02418. Performing a Dunn test gives us that there is a significant difference between the new rock and the old rock (*p* = 0.001435783) and a difference between the new rock and the pool (*p* = 0.003010717).

The evenness was calculated with 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, these locations mostly being overruled by one species. In both the rock locations the evenness was higher.

Having this we can calculate the diversity for both the count and coverage data. We used Shannon’s diversity index to do so. In Figure 3 you can see the diversity of the coverage 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 coverage data.

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

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

In Figure 4 you can see the diversity for the count data. The biodiversity was different for the different locations (Kruskal-Wallis $p = 0.00589$). Since the Kruskal-Wallis test gave back a value of $p < 0.05$ so there is a significant difference in between the different groups. In both cases this is the case between the old rock and the pool ($p = 0.0001$) and between new rock and old rock ($p = 0.006$) according to Dunn's test. Pools thus have the lowest diversity of the three locations.

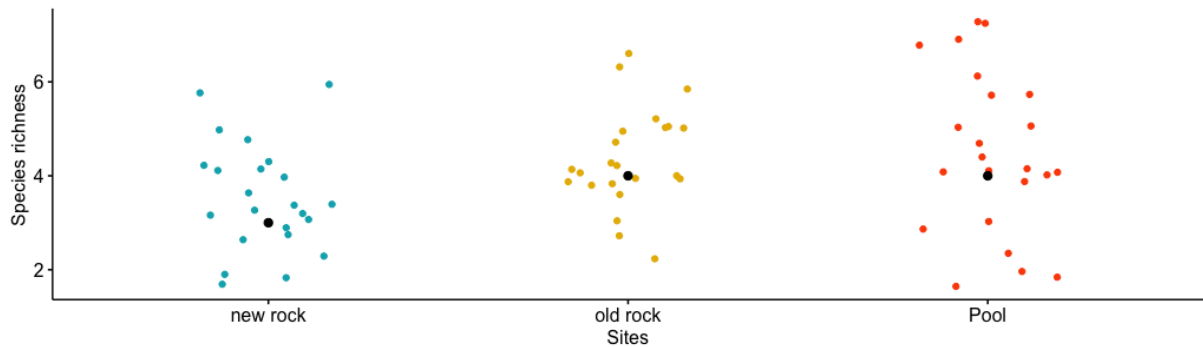


Figure 2. The richness per pool group at the different sites

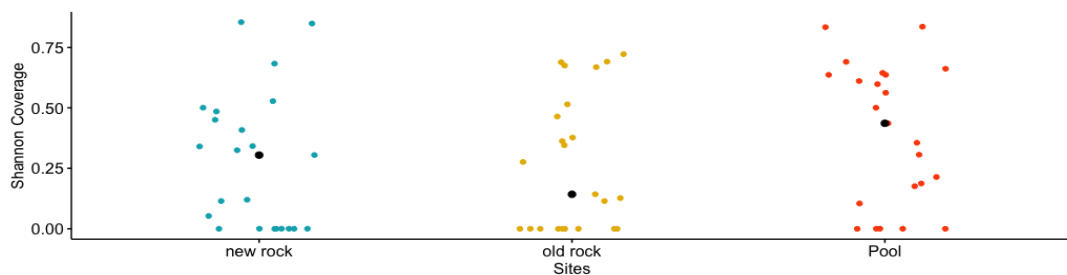


Figure 3. The diversity (H) per site of the coverage data

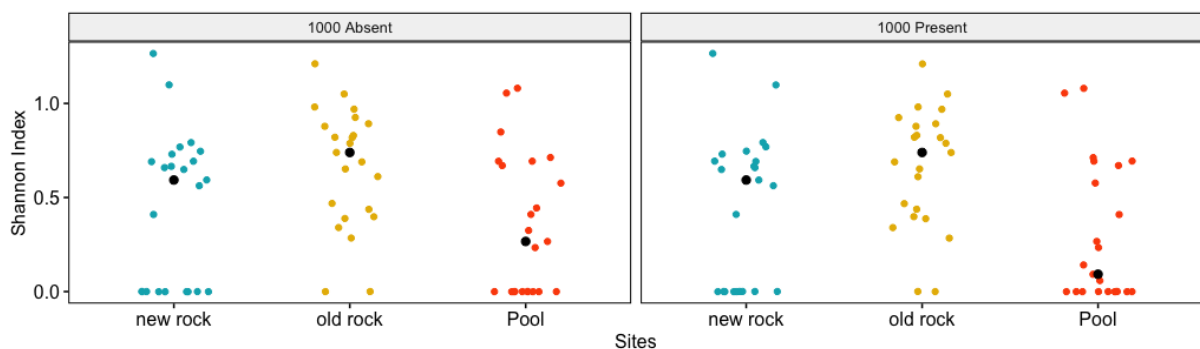


Figure 4. On the left: the diversity (H) of the count data per site with the *mnemiopsis leidyi* larvae included in the pools. On the right: the diversity (H) for the count data per site with the *mnemiopsis leidyi* larvae taken out of the pool data.

Discussion

For the covering species the biodiversity in the pools did not change from either of the rock types.

The species diversity of “Count species” in the pools is not higher than on the rocks. This is not what we expected. One explanation of this is that the evenness was much lower in some pools. Because the biodiversity is calculated using the richness and evenness, these could skew each other. Even though the richness per pool does not increase, the overall richness in all pools together is higher. But if the evenness is low, the biodiversity will also become lower (Stirling & Wilsey, 2001).

This low evenness may be due to the presence of *Gammarus locusta* or *Littorina littorea* in some pools. When they were present, they were immediately in a huge amount compared to the rest of the species that we found in that particular place. Another reason could be that the pools had more outliers, like the *Mnemiopsis leidy* larvae. Their presence in some pools were over a thousand individuals, which could make the evenness very unbalanced. However, when we took the data of the *Mnemiopsis leidy* larvae out of our dataset, the species diversity for counts was still much lower compared to the pools.

Another possibility of why we did not find an increase in biodiversity is that the pools have only been there for half a year. It is possible that some species need more time to settle and grow. In most studies the monitoring lasts over a year (Sella et al., 2021; Ido S. et al., 2015; Perkol-Finkel et al., 2018) Except, the new rocks do have a higher diversity than the pools, almost similar to the old rock, even though they have been there for the same amount of time as the pools. Ideally, we would have sampled for longer.

After the first week of fieldwork we spent a week analyzing in the lab, we discovered that some species which we thought were the same, were actually different. When going back the second week this made us more aware of different species than we did in the first week. Thus, the richness from week one can be lower than it should be.

Unfortunately, the pools were not randomly distributed over our research field. The engineers grouped the same pools together. Therefore abiotic factors, such as salinity and temperature, could have played a role on their biodiversity. Consequently we were not able to compare the companies 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.

Conclusion

With the current climate change, it is likely that there will be an on holding increase in CMI's and therefore dikes. With our results we had to reject our hypothesis of the pools increasing the local biodiversity compared to existing rock. We think this might be due to multiple reasons, under which time. However, there are studies that do show very promising results of increasing biodiversity with artificial pools (Firth, L. B., et al., 2014; Perkol-Finkel et al., 2018; Sella et al., 2021) and we believe that more research will show positive outcomes for the biodiversity.

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