Effects of the installation of two electricity cables on the condition index of the Blue Mussel (Mytilus edulis) from an intertidal mussel bed

Author(s): J.R. Graal

Wageningen University & Research report 23.002

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Author(s): J.R. Graal

Van Hall Larenstein University of Applied Sciences

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Name	Jan-Willem Ronald Graal
Students registration number	000018505
Programme	Coastal and Marine Management
Department	Van Hall Larenstein University of Applied Sciences
Supervisors	S.T. Glorius
Place	Den Helder
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Contents

Summary

Two energy cables have been installed between Ameland and Holwerd. These cables cross an intertidal mussel bed (*Mytilus edulis*) The effect of the installation of energy cables on an intertidal mussel bed are unknown. In this study the effect of the cable construction on the condition index of mussels is investigated using a 'before-after', 'control-impact' experimental design. Mussel samples were taken from the two cable transects and a reference transect prior the installation, several week after the installation and about a year after the first samples were taken. From each sample several mussels (within different length classes) were taken for determination of the individual shell length and ash free dry weight (AFDW). With these parameters the condition index is calculated. A GLM was fitted to determine the relation between AFDW/length ~ length, and the condition index at a shell lengths of 20, 30 and 45 mm were calculated using this model. The effect of the cable constructions on the mussel condition was investigated by fitting several GLM's with terms for transect, time and their interaction.

Results show that the construction of the cables did not affect the mussel condition on the short term (several weeks after construction) but long term effects (appr. one year after construction) cannot be ruled out. Long term effects were only found for one of the cable transects (size classes 20 and 30 mm) and a bordering transect (size class 45 mm). The condition of these mussels stayed behind, compared to their counterparts in the reference site. It is not clear why no effects were found on the other cable transect but it is possible that it's position, more towards the gully inlet, tampered the effect of the construction works due to a higher water refreshment rate. It is also possible that particles that are brought into suspension during the construction, were transported further into the gully, leading to lower sedimentation loads in the upstream cable and higher in the downstream cable trajectory. Possible effects are most likely to occur very locally to the installation works, but it is not limited to proximity. Effects further away have been observed.

The results found in this study apply only to this mussel bed in combination with this installation method. Results found here do not automatically apply to other beds. Effects of cable construction on mussel condition might depend on the position of the bed and construction within the gully system (determining the water refreshment rates), but also on size class (and therefor age) of the mussels as is shown here.

1 Introduction

The Wadden island of Ameland is building towards the Netherlands' sustainability goals of the energy supply being almost completely sustainable and CO_2 neutral (Ministerie van Algemene Zaken, 2022). Ameland is a small municipality of 268,5 km^2 (BZK, 2022) counting 3757 inhabitants (CBS, 2022) in the north of the Netherlands. The renewable energy produced by solar farms must be transported to a substation in Holwerd (Liander, 2019) on the mainland, some 10 kilometres south of Ameland. Two electricity cables embedded in the Wadden soil carry the electricity to the substation. However, a solar farm built on the island in 2016 (Eneco, n.d.) caused the cables would reach its capacity (Liander, 2019).

Two new cables would solve this. Liander manages the electricity network in Friesland (Liander, 2022), the province in which Ameland and Holwerd are located. The new cable route crosses an intertidal blue mussel bed (*Mytilus edulis*), as shown in figure 1. Mussel beds have important features in the Wadden Sea and are legally protected as important components in the ecosystem (Glorius et al., 2019). The problem is, that the effects of the installation of an electricity cable through an intertidal mussel bed have not yet been researched. The permit for the installation of the cable was granted to Liander under the conditions, that the effects to the mussel bed are monitored and any lasting effects must be restored.

This study is part of a larger study by Glorius et al. (2022). In the research of Glorius et al. (2022) the effects of the installation of the electricity cable on a intertidal mussel bed has been researched, regarding mussel density and fresh weight, bed area and coverage with mussel patches (= location were mussels aggregate). This complementary study focusses on the effect on the condition index of the mussels on this specific mussel bank, which is based on the shell length and ash free dry weight (AFDW) of the mussel flesh.

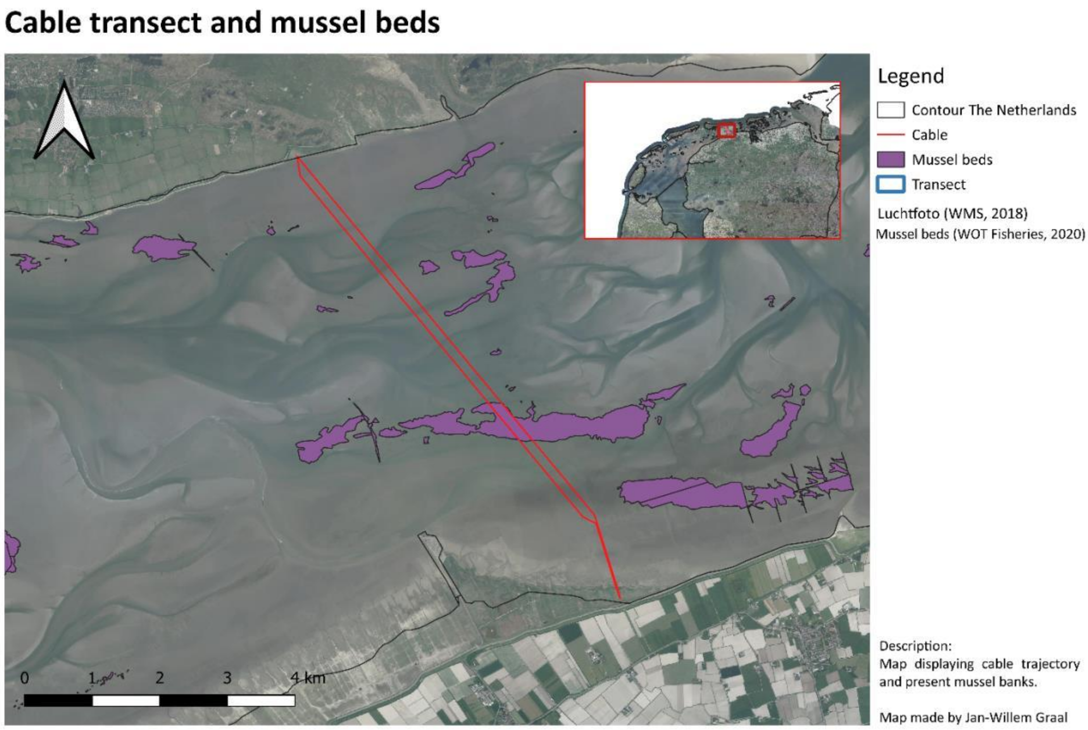

Figure 1. *The cable routes in red crossing a tidal mussel bed. The contours in dark purple display the position of the mussel beds as measured by WMR early in 2020 with regards to the WOT Fisheries program.*

1.1 Construction of the cables

The two power cables are constructed parallel to one another with a distance of 150 meter in between them. For the construction of each cable, a slit was made in the soil using a plow. The plow was mounted on sled, which was dragged along the transect. Once the plow lifted the soil, the cable was laid into the ground simultaneously. When the cable was in the slit, the lifted soil fell back into place. The following high tide closed the slit (Alsema, 2021).

1.2 Ecology of mussel beds

Beds of blue mussels (*Mytilus edulis*) have an important function in the Wadden Sea ecosystem, as they provide a good structure and functioning of habitat type H1140 'Mudflats and sandflats not covered by seawater at low tide' within the Dutch Nature Protection Act (Glorius & Meijboom, 2021). At low tides they are available as an important food source for non-diving birds, such as oystercatchers and herring gulls. While submerged the mussels are eaten by several crabs and many fish. The pseudo-faeces, selected particles that are filtered (Kooijman, 2006), consist for a large amount of organic material and has a high food value for benthic animals (Dankers & Fey-Hofstede, 2015). Within and around the mussel bed, richer areas are created as a result of pseudo-faeces and many organisms can profit (Dankers & Fey- Hofstede, 2015).

Around 40% of new formed mussel beds dies the first winter (van der Meer et al., 2018). This can be due to (a combination of) a storm causing too much wave action, an excess of predators, currents or orientation relative to the wind and wave direction (Dankers & Fey-Hofstede, 2015). During the second year of a new bank about half of the mussels succumb. After 5 years, the reduction of surface area and number of mussels in beds is slow and when the bed is older, it is more likely it will exist longer (Dankers & Fey-Hofstede, 2015; van der Meer et al., 2018).

1.3 Properties of mussel beds

Subareas 1 and 3 of the mussel bed, which positions can be found in figure 2, have been formed in the summer of 2019 and consist of young mussels (Glorius et al., 2022). In these areas no Japanese oysters (*Crassostrea gigas*) are found. In subarea 2 mussels have been found frequently since 1995,the year the WOT-fishery measurements started and since the year 2000 every single year. The mussel population in this subareas is therefore more diverse, consisting of mussels of different age classes. In this part of the bed, Japanese oysters are also found . For a more in depth historical overview of the mussel bed, see paragraph 4.3 and 'Bijlage 1' in Glorius et al. (2022).

1.4 Possible effects

Possible direct effects of construction works on mussel(bed) according to Glorius et al. (2022) might be harm to the seabed structure, or the mussel bed, burial or destruction of mussels. Indirect effects are also possible. Sediment in- and outside the cable trajectory can be brought into suspensions and subsequently fall upon the mussels. Suspended solid levels could increase because of the installation works, which could result in lower mussel food intake, possibly causing a decline in condition and increase in winter mortality. Besides this, there is a possibility that a gully will form over the cable trajectory and split the mussel bed into two due to erosion of mussels.

1.5 Condition index

Condition indices are widely used by ecologists (Labocha et al., 2013). They can be used to measure animal's body condition as an estimate of fitness. In morphometrics literature three indices of body condition have been heavily used. The ratio index (body mass/body size), the slope-adjusted ratio index (based on regression slopes generated from a reference population), and the residual index (the residuals of a regression of body mass on body size) (Jakob et al., 1996). This study uses the ratio index and is described in chapter 2.5.

2 Assignment

It is unknown what the effects are of the construction of energy cables on the condition of intertidal blue mussel (*Mytulis edulis*) from a mussel bed in the Wadden Sea. Furthermore it is unknown on what time- and spatial scale effects take place and whether effects are different for different mussel cohort (= year class determined by shell length).

The main question is:
'What are the potential effects of the construction of energy cables on the condition of mussels from an intertidal mussel bed between the island of Ameland and Holwerd (mainland) in the Dutch Wadden Sea?'

The sub questions are:
1. On what time frame do effects take place?
2. Are effects determined by mussel cohort/length?
3. On what spatial scales do effects take place?

3 Materials and Methods

This study follows a Before-After-Control-Impact (BACI) experimental design. In this chapter this design will be explained. Also an overview is given of the sample locations and -times as well as the methods used for collecting and sorting the samples and for determining the condition index. The statistic used to determine construction effect are also described in this chapter.

3.1 BACI

This study uses a Before-after-control-impact (BACI) design in order to distinguish the effects of the cable construction on the mussel condition from naturally induced effects on mussels conditions caused by e.g. storm events and presence of predators. BACI designs are an effective method to evaluate natural and human-induced perturbations on ecological variables (Conner et al., 2016). In a BACI design one or multiple characteristics are collected of an area with disturbance and another without, both before and after the disturbance has occurred. If the area with disturbance develops differently than the area without, an effect has taken place (see figure 2) (Stewart-Oaten & Bence, 2001).

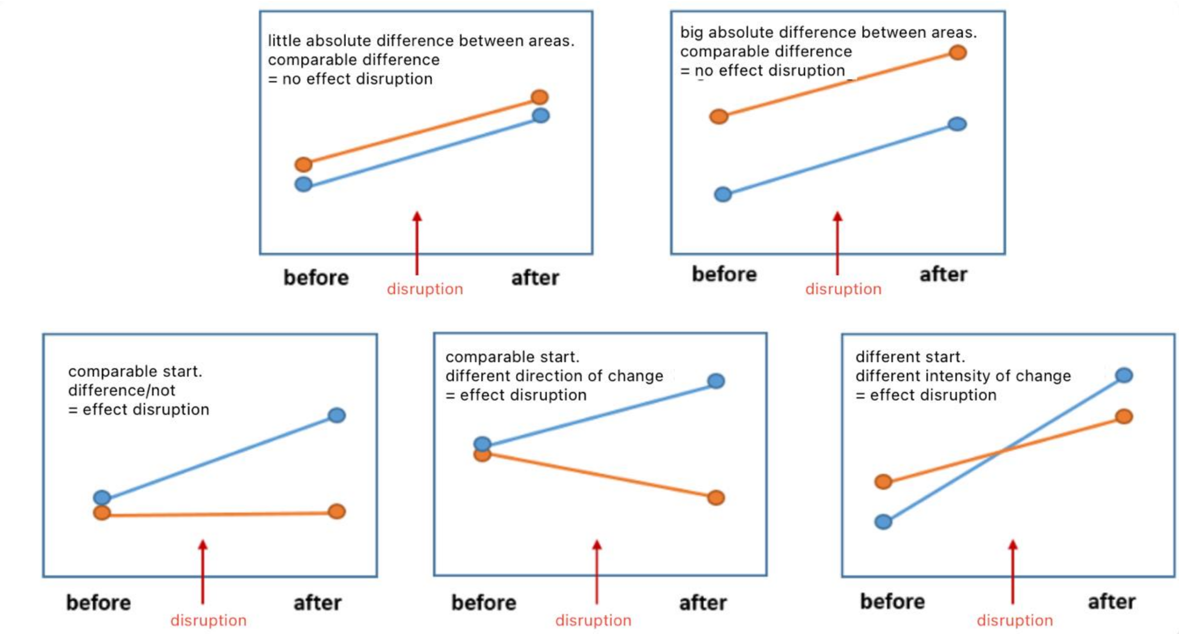

Figure 2. *Possible theoretical outcomes of a study which follows a BACI-design. The two upper panels display scenarios without effect of the disturbance, where start values are equal of different, but the changes are identical. The lower three panels shows an effect of disturbance where the direction or intensity of the changes differ.*

The effect of the disturbance can be isolated from the autonomous developments. Examples of autonomous developments that can occur and influence the present mussels are the occurrence or absence of spawning, predation of birds and influences of weather.

3.2 Sample locations and times

Mussel samples have been taken at three different dates. T0 is May 25, 2021, T1 is November 4, 2021 and T2 is May 31, 2022. T0 is prior to the construction of the cables, T1 is a few weeks post construction and T2 is around the same time of year, one year after T0. The eastern cable was installed between the 25th and 29th of August and the wester cable was installed on the 14th of September (Glorius et al., 2022). The method taken in the field follows the methodology used in inventories made for WOT-nature on tidal mussel beds, as described in Glorius & Meijboom (2020).

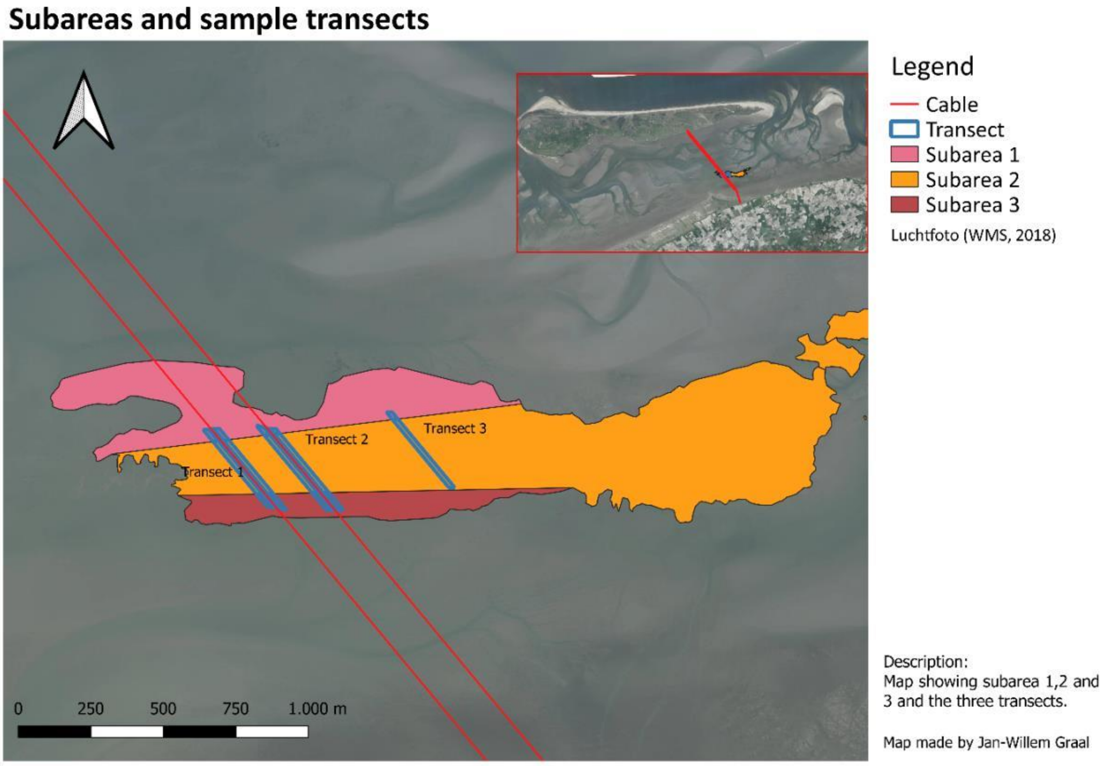

Figure 3. *Mussel bed divided in three subareas, with the two electricity cables in red, and three sample transects in blue.*

Within subarea 2 there are three transects visible. Transect 1 and 2 are the trajectory of the energy cables and transect 3 is the reference transect and serves as a control group. Transects 1 and 2 are both 15 meters wide. This is the width Liander was allowed to work in for installing the cables. On the left (east) and right (west) of transect 1 and 2, an extra transect was of 15 meters wide was created. These are the edges. This means that a total of seven transects are present. The eastern transect edge of cable 1 is 'edge 1.1', cable 1 is 'transect 1.2' and the western transect edge of cable 1 is 'transect 1.3'. The same logic follows for cable 2 (transect 2.1, 2.2 and 2,3). The reference transect does not have extra transects and consists only of transect 3. The edge transect are used to investigate if possible effects extend to a larger area.

The cable transects are 15 meters wide, because this was the width Liander was allowed to work in according to the permit. In reality, the contractor hired by Liander only used 7,5 meters of this workspace.

3.3 Mussels

The mussels used in this study originates from samples taken by Glorius et al. (2022). In this report a detailed explanation of the followed procedures to collect the samples can be found.

In short, samples were taken by pushing an aluminium frame with a dimension (cm) of 16 (w) x 32 (l) x 10 (h) into the soil of a mussel patch until the top was level with the mussel surface (figure 3). A label with a sample number was pinned next to the frame and a picture was taken from right above and the surroundings. With a handheld GPS a waypoint was made for capturing the sampling location.

Figure 4. *Photos of the sampling. Left: Positioning of the sampling frame on a mussel patch (before pushed into the ground). Right: A photo of the direct environment of the sample location and on the right the 1 mm sieve.*

After this, the top layer of 10 cm was scraped from the frame and the material was sieved through a mesh size of 1 mm to remove sand, sludge, and organisms <1 mm. The sample was put into a plastic bag, labelled and sent to the laboratory for analysis.
Next, the lengths (in mm) were measured of all living mussels, other living shellfish and empty shells. The living mussels have been subdivided into three groups of different length groups (≤20 mm, >20 ≤40 mm, >40 mm) and stored in the freezer at -20°C.

All mussel samples were collected from randomly selected mussel patches. The footprint of the sled used for the cable construction was smaller than the width of the transect. For the T1 and T2 sampling campaign, mussel patches located inside the trajectory of the sled (visible in the field) were sampled when possible.

A power analysis described in the monitoring plan of Glorius et al. (2021), resulted in the decision to collect 8 samples at each location. At each sampling time, 8 samples were collected in transects 1, 2 and 3. At both sides of transect 1 and 2, also eight samples were taken (four at each side). This resulted in 40 samples per sampling time and 120 samples in total.

Note considering the T1 and T2 sampling campaign. Glorius et al. (2022) found in their study that mussel patches damaged by the sled first break down into multiple smaller patches that hardly survive afterwards. As a result of this, during the T2 measurement (about one year after the installation) most mussel patches in the trajectory of the sled disappeared and only samples outside this trajectory (but still inside the transect) could be sampled.

3.4 Ash free dry weight

The mussels of each sample were initially divided into 11 length classes of 5 mm. >5-≤10, >10-≤15, etcetera, until >55-≤60 (mussels larger than 60 mm have not been found). Then, per length class, 2 mussels were randomly picked from each sample. This means that 11 length classes times 2 mussels times (40 samples T0 + 40 samples T1 + 40 samples T2), 2.640 mussels in total, were planned to be processed. In reality not all samples carry 2 mussels of each size class. Especially the smallest and largest size classes lacked mussels in many samples. In total 1943 mussels have been processed.

The process of the mussels went as followed. Once the mussels were measured for length and put into size classes of 5 mm, they are numbered and put in crucibles. Next, the shell was removed from the flesh. The shell is thrown out and the tissue was placed again in the crucible. The crucibles were dried in a drying oven at 60°C for 48 hours. This is the standard method at Wageningen Marine Research. The mussels cooled down in a desiccator at room temperature (±20 °C). This temperature and length of time was also used by Nalepa et al. (1993) for drying *Dreissena polymorpha,* the zebra mussel and by Langdon & Önal (1999) for *Mytilus galloprovincialis*, the marine mussel.

Once the mussels had cooled down the weight would be measured (±0.1 mg) using a Mettler XPE204. Following this, the mussels were reduced to ash in a muffle furnace at 560 °C for two hours. This is also the standard method for burning mussels to ash at Wageningen Marine Research. Also Kamermans (1993) used this temperature and length of time for his research on cockles (*Cerastoderma edule*), which are quite similar in size and weight. Once again cooled down in a desiccator at room temperature, the mussels were measured for weight again (±0.1 mg). AFDW is determined by subtracting the ash weight from the dry weight (Weil et al., 2019).

3.5 Condition index

The condition of the mussels is defined as the ratio between AFDW of the mussel flesh and the shell length. This is achieved by dividing the AFDW of the flesh by the shell length of the mussel.

As mentioned earlier, the smaller and larger length classes lacked mussels, as they did not appear all of the time in the samples. Therefore, a general linear model (GLM) was made from the collected samples, for length against condition. There was a relation between AFDW/length ~ length. For each sample a GLM was fitted to determine this relation.

Multiple transformations were tested on the GLM's for AFDW and the condition index. The tested transformations for condition are a linear condition GLM, an exponential condition GLM, a linear root condition GLM, an exponential root condition GLM, a linear root length & condition GLM and an exponential root length & condition GLM. The transformations tested for AFDW are a linear AFDW GLM, an exponential AFDW GLM, a linear root AFDW GLM, an exponential root AFDW GLM, a linear root length & AFDW GLM and an exponential root length & AFDW GLM. The exponential root length & condition GLM showed the best spread residuals, and was therefore used.

The best model describing the relation best was used to calculate the condition index at fixed shell lengths of 20, 30 and 45 mm. These analysis were performed with the program 'R' (R Core Team, 2022). Figure 4 shows an example of the model for a sample.

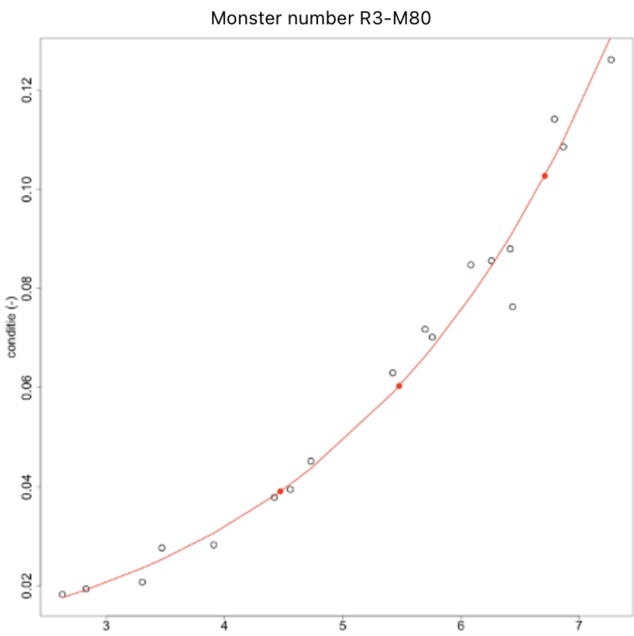

Figure 5. *Example of model used to calculate condition index at shell lengths of 20, 30 and 45 mm. Black dots are data and red dots are model results.*

3.6 Statistical analysis

Statistical analysis is performed in the program ‘R’. The (univariate) parameters ‘time’, ‘location’, ‘length’, ‘condition’ and ‘AFDW’ will be studied in order to find out whether the installation of the cables have effect on the mussels. Below this is the regression (smallest quadrants) model that fits best.

$P = \beta_0 + \beta_1 \times time + \beta_2 \times location + \beta_3 \times time \times location + \varepsilon$

P = dependable variables (parameter)
β0=intercept and β_{1-3} are the regression coefficients.
time = T0 - T1, T0 - T2
location = cable (1.2, 2.2), edge (1.1, 1.3, 2.1, 2.3) or reference (3)
ε = not explained variation

Multiple models were constructed to investigate the effects between different sites and time frames. Models containing T0 and T1 were used to investigate short term effects, and models containing T0 and T2 to investigate long term effects. Each model contained one of the sites (cable 1 or 2, and side 1 or 2) as well as the reference site.

4 Results

In this chapter the condition indices of the mussels at different site and sampling times are presented using boxplots. A boxplot tell the following information. The median is the thick stripe in the middle of the boxplot. This marks the mid-point of the data and divides the box into two. The box is 50% of the data. The lower end of the box is the lower quartile Q1 and the upper end of the box is the upper quartile Q3. The minimum score is the lowest condition (excluding outliers) and the maximum score is the highest condition (excluding outliers). The circles are outliers.

Model outcomes for the 'location x time' interaction terms are shown in this chapter as they indicate an effect of the construction works. Results for the terms reflecting autonomous processes (e.g. sampling time and location) are presented in the annexes.

The results of the data are reported with a decimal point (.) instead of a comma (,) (in derogation of the Dutch SI).

The results stated in this report only apply to the samples as they have been received.

4.1 Mussel condition 20 mm-shell length

In figure 4 the condition index of the 20 mm shelled mussels is shown. The green boxplots are condition index scores of T0, orange is T1 and green is T2. The condition is displayed for cable 1, cable 2, edge 1, edge 2 and the reference transect.

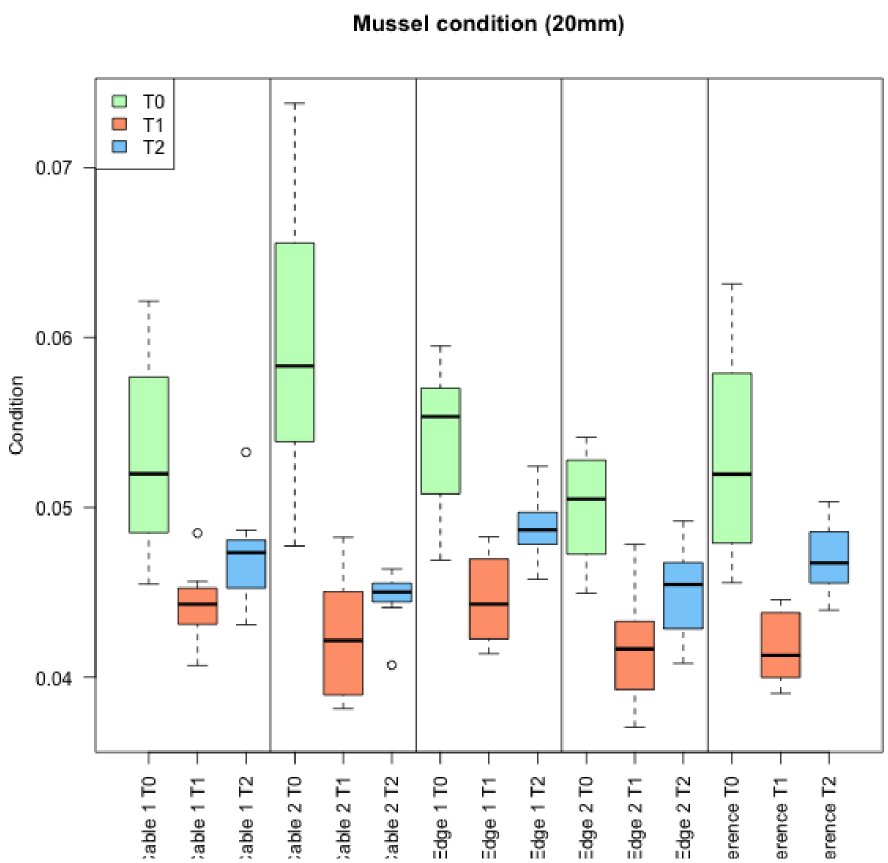

Figure 6. *The condition of the mussels with 20 mm long shells from cable 1, cable 2, edge 1, cable 2 and the reference transect. T0 is green, T1 is orange and T2 is blue.*

Figure 4 shows that, for all sites, the condition index for the 20 mm-shell mussels is the highest at the T0 sampling event, prior the installation of the cables. A big decline in condition is visible from T0 to T1. The parameter 'time' is significant from T0 to T1 for the reference transect against cable 1, cable 2, edge 1 and edge 2 (see Annex 1). The condition is also visibly a lot lower during T2 than T0. 'Time' is also significant for condition from T0 to T2 for the reference transect against all cables and edges. Although not statistically tested, the condition seems to increase between T1 (winter 2021) and T2 (spring 2022), see Figure 4. The cables and edges display a similar condition index progression over time as the reference transect.

20 mm	**T0-T1**		**T0-T2**	
	Estimate	P-value	Estimate	P-value
Cable 1	-0.0026	0.4393	-0.0002	0.9402
Cable 2	0.0059	0.1517	0.0089	**0.0286**
Edge 1	-0.0018	0.5426	-0.0007	0.7907
Edge 2	-0.0029	0.3069	-0.0010	0.7096

Table 1. The estimates and P-values of the statistical interaction terms 'location x time' and condition for the 20 mm shell mussels for models that compare cable 1, cable 2, edge 1 or edge 2 to the reference transect for T0-T1 and T0-T2 separately. Significant P-values are printed in bold.

Table 1 shows the estimates and P-values for the interaction terms of the different models for mussels with a shell length of 20 mm. The estimate tells the following. For cable 1, the model first looks at the condition at T0 of cable 1 and the reference transect. Then, it creates a line from the T0 to, for example, T1. The estimate tells the difference between T0-T1 for cable one and T0-T1 for the reference transect. A negative estimate describes that the cable has a better conditional score than the reference transect. A positive estimate tells that the reference has a better conditional score. The larger the estimate is, the bigger the difference. If a difference in increase or decrease is severe enough, it is significant. Cable 1 has from T0 to T1 a better condition than the reference transect, because the estimate is -0.0026. This difference in development is small, and not large enough to be significant. This also can be seen in figure 4, where the orange boxplot of cable 1 is slightly higher than the one of the reference transect.

There is a significant difference (P=0.02) in condition at cable 2 between T0 and T2 with an estimate of 0.0089. This is a positive estimate and therefore means that the mussels from the reference transect have better a condition. This shows that, although the condition decreases at all sites between T0 and T2 (see text above) this decrease was more severe at cable 2 compared to that of the reference site. All other investigated situations were not significant (P>0.05) which implies a similar development compared to the reference site.

4.2 Mussel condition 30 mm-shell length

In figure 5 the condition index of the 30 mm shelled mussels at the different sites and sampling times are shown. The green boxplots are condition index scores of T0, orange is T1 and green is T2. The condition is displayed for cable 1, cable 2, edge 1, edge 2 and the reference transect.

'Time' was not significant, except from T0 to T2 for cable 2 and edge 2 (see Annex 2).

When looking at the condition index score for the reference transect with the other cables and edges in figure 5, it can be seen that the development from T0 to T1 and T2 remains fairly equal. The same is true for cable 1 and edge 1. In cable 2 a decline is visible from T0 to T1 and an even larger decline from T0 to T2. Edge 2 seems to follow the same development, but not as strongly.

When looking at the boxplots of edge 2 and sampling times T0 and T1, it can be seen that the medians lay on a similar point on the condition index. However Q1 of T1 is lower than the minimum score of T0. There are no outliers on both boxplots. This means that more than 25% of the T1 mussels have a lower score than the lowest score of T0, meaning that edge 2 has a similar decline over time as cable 2, but not as strong.

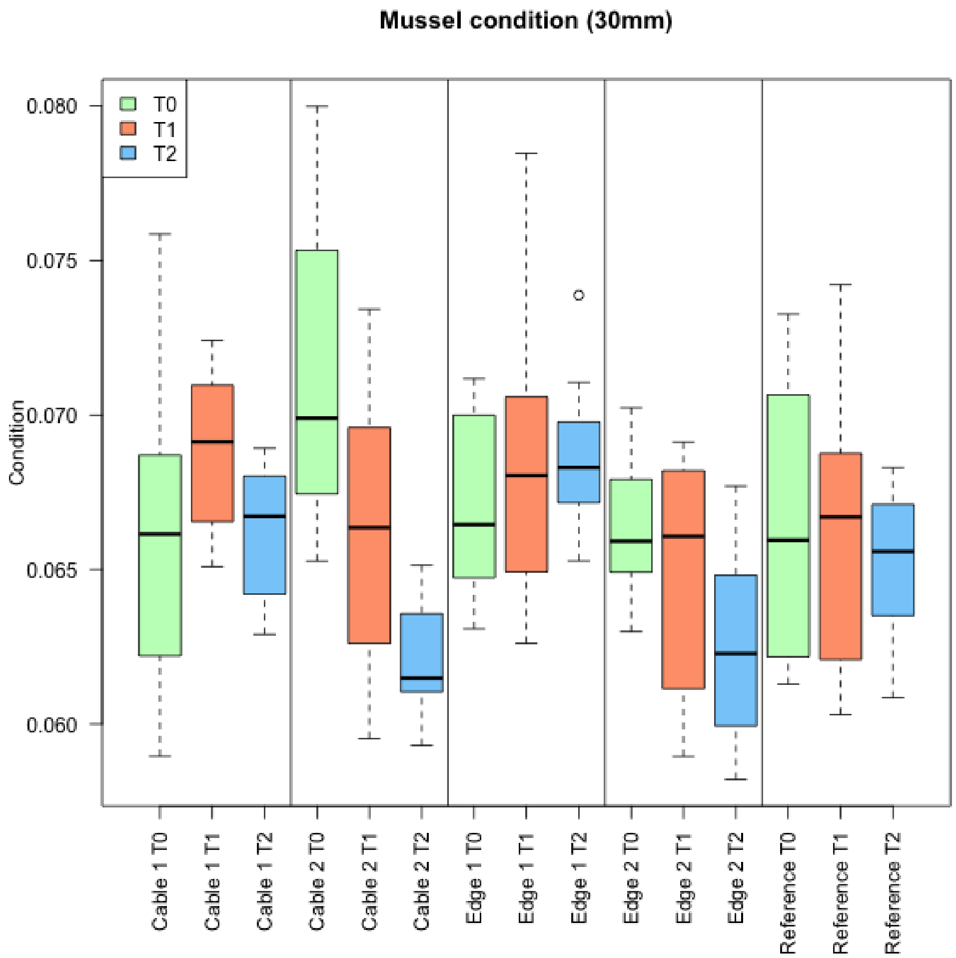

Figure 7. *The condition of the mussels with 30 mm long shells from cable 1, cable 2, edge 1, cable 2 and the reference transect. T0 is green, T1 is orange and T2 is blue.*

30 mm	**T0-T1**		**T0-T2**	
	Estimate	P-value	Estimate	P-value
Cable 1	-0.0030	0.3619	-0.0014	0.6123
Cable 2	0.0047	0.1874	0.0079	**0.0092**
Edge 1	-0.0017	0.5802	-0.0029	0.2273
Edge 2	0.0011	0.6862	0.0025	0.2939

Table 2. The estimates and P-values of the statistical interaction terms 'location x time' and condition for the 30 mm shell mussels for models that compare cable 1, cable 2, edge 1 or edge 2 to the reference transect for T0-T1 and T0-T2 separately. Significant P-values are printed in bold.

Table 2 shows the estimates and P-values for the interaction terms of the different models for mussels with a shell length of 30 mm. None of the interactions are significant but one. 'Location x time' is significant for the reference transect against cable 2 from T0 to T2 (P=0.009). The estimate is positive and therefore means that increase in condition was significantly better at the reference transect than at cable 2.

4.3 Mussel condition 45 mm-shell length

In figure 6 the condition index of the 45 mm shelled mussels is displayed via boxplots. The green boxplots are condition index scores of T0, orange is T1 and green is T2. The condition is displayed for cable 1, cable 2, edge 1, edge 2 and the reference transect.

Figure 6 shows that cable 1, cable 2, edge 1, edge 2 and the reference transect all develop similarly. During T0 all conditions are relatively low (but most of the time higher compared to 25 and 30 mm) and at T1 they have increased. Condition is at T1 the highest at all transects. After T1, all conditions drop at T2 at intermediate values.

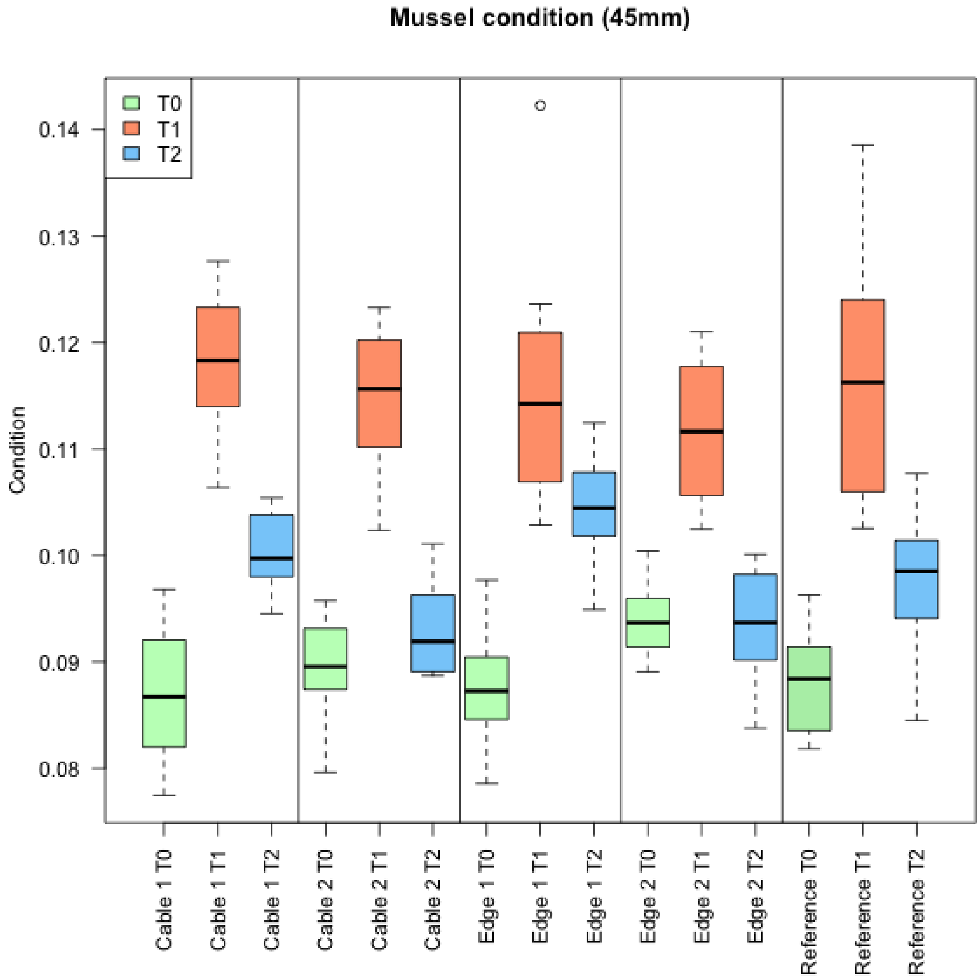

Figure 8. *The condition of the mussels with 45 mm long shells from cable 1, cable 2, edge 1, cable 2 and the reference transect. T0 is green, T1 is orange and T2 is blue.*

The term 'time' is significant from T0 to T1 for all situations tested here (see Annex 3). So at all sites the condition increased between spring (before construction) and winter (after construction) of 2021. The same is true for cable 1 and edge 1 for T0 to T2. 'Time' is not significant for the reference transect against cable 2 and edge 2 for T0 to T2.

Table 3 shows the estimates and P-values for the interaction terms of the different models for mussels with a shell length of 30 mm. Like the 20 mm and 30 mm mussels, there is one significant interaction. Not cable 2 but edge 2 is significant (P=0.02) between T0 and T2. The estimate is positive and means that increase from T0 to T2 was severely larger at the reference than at edge 2. between T0 and T1 no significant effects were found the condition in the cable- and edge sites developed in a similar way as in the reference site.

45 mm	T0-T1		T0-T2	
	Estimate	P-value	Estimate	P-value
Cable 1	-0.0025	0.6768	-0.0039	0.3440
Cable 2	0.0033	0.5633	0.0058	0.1493
Edge 1	0.0000	0.9942	-0.0074	0.0830
Edge 2	0.0108	0.0590	0.0098	**0.0167**

Table 3. The estimates and P-values of the statistical interaction terms 'location x time' and condition for the 45 mm shell mussels for models that compare cable 1, cable 2, edge 1 or edge 2 to the reference transect for T0-T1 and T0-T2 separately. Significant P-values are printed in bold.

4.4 Results overview

There is a big difference in development of the condition between the 20 mm- and 45 mm-shell length mussels. The 20 mm-shell mussel mussels have a relatively high condition at T0 (even though it is lower than the larger classes at T0). The condition then dropped during winter (T1) and increased again slightly at T2.

The 45 mm-shell mussel saw the opposite development. The large mussels scored low on the condition index T0, then increased at T1, and after the winter it had dropped again at T2.

The middle class is less coherent. The general development of the condition seems to stay at the same level. Cable 2 sees a clear decrease in condition from T0 to T2. This is also slightly visible at edge, where the decrease from T0 to T1 is small, but the decrease of T0 to T2 is well visible. This shows that only one of the two cable transects show a negative development for the condition of the mussels.

The interaction term 'location x time' accounts for possible effects caused by the installation of the cables. No short term (T0-T1) cable effects have been found. However long term effects were found. For the small (20 mm-shell) and medium (30 mm-shell) sized mussels the interaction term was significant for cable 2 in comparison to the reference transect. For the large (45 mm-shell) mussels, edge 2 tested significant for the long term.

5 Discussion

Currently only one mussel bed is used for this report. This means that the results are only true for this specific mussel bed. All mussel beds have different properties and this has different consequences. Young mussel beds have a smaller chance of survival than older beds (Dankers & Fey-Hofstede, 2015). The presence or absence of oysters influence the chance of survival and the resilience. The coverage and density of mussel beds influence how much nutrients are available for other patches nearby (Dankers & Fey-Hofstede, 2015).

The installation of this cable was conducted in a very specific manner. If the installation was performed any other way, the result might deviate from the ones found in this study. For these reasons can the results from this research not be automatically be extended to other situations.

Sample M104 till M115 have dried 21 hours and 30 minutes extra within the drying oven at room temperature (±20 °C). This is due to the malfunction of the heating element in the oven. This malfunction has occurred in the night of the 21st and 22nd of November. When the animal tissue has reached a constant weight dry weight is achieved (Bloomer et al., 1971). This happens when all water is removed by drying (Houghton, 2008). It is possible that the mussels have taken in moist out of the air at room temperature, but the data did not deviate from others, and was therefore used in the analysis. The mussels had the normal 48 hours of drying at 60 °C immediately after the malfunction was noticed.

No short term effects (T0 to T1) have been observed. This is most likely, because the time between the installation of the cables and T1 only differ 2 weeks. This time period is probably too short for mussels to gain enough in flesh weight to observe a change in the mass of the flesh. For future research, it is advised, to have a longer period of time between the installation of the cable and collecting the samples after installation.

Long term effects have been found for the 20 mm-, 30 mm- and 45 mm-shell mussels. Small and medium sized mussel effect was found at cable 2 and the large sized mussel effect was found at edge 2. The fact that these effects all take place on and beside cable 2 and not on cable 1 is noticeable. Something in that area has caused a decline in condition on the long term for the mussels in the direct environment.

Bayne and Worral (1980) saw a similar development with dry flesh weight (DFW) of 60-mm shell length *Mytilus edulis* at the locations Lynher and Cattewater in England, as the 45 mm-shell mussels in this research. They saw that due to seasonal variability the DFW changed accordingly. During summer months the DFW increased and during winter the DFW decreased. During September, October and November the DFW was the highest, because the mussels had all summer to grow.

Figure 6 can be explained, that during T0 and T2 (both in may) the condition was low, due to the prior winter time. The mussels sampled at that moment were probably starting to strengthen and grow, but still had a lower DFW. T1 (November) has a high condition, probably because of the prior summer time.

This explanation seems contradictory to the development of the 20 mm-shell mussels, because the condition changes in the opposite manner. This difference might be the results of the life phase of the mussel. Young (small sized) mussels might invest its energy into the growth as seen here with the 20 mm group while full grown mussels invest their energy in fat an reproduction organs. The 45 mm-shell mussels, like the 60 mm-shell mussels of Bayne and Worrall (1980), have to invest less energy into shell growth, and can invest more in the soft tissue. The ratio of length and AFDW, which is used to calculate the index, grows. A study by Hilbish (1986) indicates that the rates of growth in shell and soft tissue do not occur simultaneously, and that shell growth precedes the growth of soft tissue. The small 20 mm-shell mussels invest more in the shell growth than the 45 mm-shell mussels, which probably will give the result, that the mussels has grown in length and in flesh weight, and therefore decreased in condition. This does not mean that the flesh weight or DFW has decreased, but merely indicates that the shell has grown relatively quicker.

Salt water reaches the mussel bed through a tidal inlet west of Ameland and through a tidal inlet east. This is visible in figure 1. This probably means that parts of the mussel bed which are closer to the inlets, receive fresher water, with more nutrients. Cables 1 and 2 seems to lay on gullies from the west inlet. This means that the fresh sea water is received from west to east of the cables. If this is true, the mussels near cable 1 (located further west) experience the new tide with before cable two. This also means that the mussels form cable 1 get to filter the nutrients in the water before the mussels from cable 2. The higher refresh rate for cable 1 mussels might explain the results. The reference transect looks to be located more on the west tidal inlet, and therefore has fresher water than cable 2.

In water with low currents and flowrate, sediment accumulates. The wantij is the area where to tidal currents meet in opposite direction. At the wantij, the flowrate is very slow. A lot of sediment is brought in suspension (Rijkswaterstaat, n.d.). Cable 2 seems to be in the middle of the wantij and therefore the mussels filter water with a relative higher concentration of suspended particles and a lower concentration of nutrients.

Adding to this, the sediment brought into suspense by the installation works might have been transported further to the end of the gully of the western inlet, where cable 2 is located. This would cause an increase in sediment near cable 2, which does not occur at cable 1, causing different food availability.

To test whether this is true, in future research for finding the effects caused by the installation of cables, it is advised multiple reference transects must be used. If the set-up of a future project looked like the one in this research, it is advised, that on either side of the cables transects multiple reference transects are placed. This is more expensive and requires more time, but this would clear up if the decline in cable two has to do with a higher water refresh rate, near the tidal inlets.

6 Conclusion

Short term effects, about two weeks after installation of cable, have not been found. Long term effects, about a year after installation, have been found. It is unclear if the effects which have been found derive from the installation, but this cannot be excluded.

Mussel length does not seem affect the effects of installation of the cables. For all three length classes significant differences in development of condition have been found.

When effect were found it all happened at or near the cable 2 transect. Two out of three significant effects happened on the cable 2 transect itself and one happened on the edge of cable 2. These results suggest a possible cable effect is most likely to occur very locally to the installation works, but is not necessarily limited to this area. Edge 2 demonstrates that effects can also occur in the near proximity of the installation area.

Potential effects of the construction of the energy cables on the condition of mussels from an intertidal mussel bed between Ameland and Holwerd are that the condition of mussels of different sizes can decrease. If effects appear, it will most likely happen over a longer period of time, for example a year post installation.

7 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. The organisation has been certified since 27 February 2001. The certification was issued by DNV.

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Justification

Report 23.002

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

Approved: S.T. Glorius
Colleague scientist

Signature: 

Date: 27 juni 2023

Annex 1 Model results shell length 20mm

Statistics (‘Time’, ‘Location x Time’) for 20 mm shell mussels for reference against cable 1, cable 2, edge 1 and edge 2, and T0 against T1 and T0 against T2

* = P ≤ 0,05; ** = P ≤ 0,01; *** = P ≤ 0,001

Shell size:	Location	Time	Parameter	Estimate	Standard err.	T-value	P-value	
20 mm	Reference - Cable 1	T0-T1	Time	-0.0086	0.0024	-3.5947	0.0012	**
20 mm	Reference - Cable 2	T0-T1	Time	-0.0173	0.0029	-6.0262	0.0000	***
20 mm	Reference - Edge 1	T0-T1	Time	-0.0095	0.0021	-4.5808	0.0001	***
20 mm	Reference - Edge 2	T0-T1	Time	-0.0083	0.0020	-4.1430	0.0003	***
20 mm	Reference - Cable 1	T0-T2	Time	-0.0058	0.0023	-2.4646	0.0201	*
20 mm	Reference - Cable 2	T0-T2	Time	-0.0150	0.0027	-5.4601	0.0000	***
20 mm	Reference - Edge 1	T0-T2	Time	-0.0053	0.0020	-2.5945	0.0149	*
20 mm	Reference - Edge 2	T0-T2	Time	-0.0050	0.0020	-2.5603	0.0161	*
20 mm	Reference - Cable 1	T0-T1	Location	0.0000	0.0023	0.0153	0.9879	
20 mm	Reference - Cable 2	T0-T1	Location	-0.0066	0.0029	-2.3001	0.0291	*
20 mm	Reference - Edge 1	T0-T1	Location	-0.0011	0.0021	-0.5119	0.6127	
20 mm	Reference - Edge 2	T0-T1	Location	0.0030	0.0020	1.4976	0.1454	
20 mm	Reference - Cable 1	T0-T2	Location	0.0000	0.0023	0.0152	0.9880	
20 mm	Reference - Cable 2	T0-T2	Location	-0.0066	0.0027	-2.4033	0.0231	*
20 mm	Reference - Edge 1	T0-T2	Location	-0.0011	0.0020	-0.5232	0.6050	
20 mm	Reference - Edge 2	T0-T2	Location	0.0030	0.0020	1.5456	0.1334	
20 mm	Reference - Cable 1	T0-T1	Location x Time	-0.0026	0.0034	-0.7850	0.4393	
20 mm	Reference - Cable 2	T0-T1	Location x Time	0.0060	0.0041	1.4734	0.1518	
20 mm	Reference - Edge 1	T0-T1	Location x Time	-0.0018	0.0029	-0.6163	0.5427	
20 mm	Reference - Edge 2	T0-T1	Location x Time	-0.0030	0.0028	-1.0406	0.3069	
20 mm	Reference - Cable 1	T0-T2	Location x Time	-0.0003	0.0033	-0.0756	0.9403	
20 mm	Reference - Cable 2	T0-T2	Location x Time	0.0090	0.0039	2.3066	0.0287	*
20 mm	Reference - Edge 1	T0-T2	Location x Time	-0.0008	0.0029	-0.2679	0.7907	
20 mm	Reference - Edge 2	T0-T2	Location x Time	-0.0010	0.0028	-0.3762	0.7096	

Annex 2 Model results shell length 30mm

Statistics ('Time', 'Location x Time') for 30 mm shell mussels for reference against cable 1, cable 2, edge 1 and edge 2, and T0 against T1 and T0 against T2

* = P ≤ 0,05; ** = P ≤ 0,01; *** = P ≤ 0,001

Shell size:	Location	Time	Parameter	Estimate	Standard err.	T-value	P-value	
30 mm	Reference - Cable 1	T0-T1	Time	0.0027	0.0023	1.1553	0.2581	
30 mm	Reference - Cable 2	T0-T1	Time	-0.0051	0.0025	-2.0366	0.0512	
30 mm	Reference - Edge 1	T0-T1	Time	0.0014	0.0022	0.6505	0.5207	
30 mm	Reference - Edge 2	T0-T1	Time	-0.0015	0.0020	-0.7324	0.4700	
30 mm	Reference - Cable 1	T0-T2	Time	0.0001	0.0020	0.0510	0.9597	
30 mm	Reference - Cable 2	T0-T2	Time	-0.0092	0.0020	-4.6192	0.0001	***
30 mm	Reference - Edge 1	T0-T2	Time	0.0016	0.0017	0.9629	0.3439	
30 mm	Reference - Edge 2	T0-T2	Time	-0.0038	0.0017	-2.3066	0.0287	*
30 mm	Reference - Cable 1	T0-T1	Location	0.0004	00023	0.1766	0.8612	
30 mm	Reference - Cable 2	T0-T1	Location	-0.0048	0.0025	-1.9345	0.0632	
30 mm	Reference - Edge 1	T0-T1	Location	-0.0006	0.0022	-0.2542	0.8012	
30 mm	Reference - Edge 2	T0-T1	Location	0.0002	0.0020	0.0867	0.9315	
30 mm	Reference - Cable 1	T0-T2	Location	0.0004	0.0020	0.2026	0.8409	
30 mm	Reference - Cable 2	T0-T2	Location	-0.0048	0.0020	-2.4041	0.0231	*
30 mm	Reference - Edge 1	T0-T2	Location	-0.0006	0.0017	-0.3341	0.7408	
30 mm	Reference - Edge 2	T0-T2	Location	0.0002	0.0017	0.1049	0.9172	
30 mm	Reference - Cable 1	T0-T1	Location x Time	-0.0030	0.0032	-0.9273	0.3620	
30 mm	Reference - Cable 2	T0-T1	Location x Time	0.0048	0.0035	1.3513	0.1874	
30 mm	Reference - Edge 1	T0-T1	Location x Time	-0.0018	0.0031	-0.5595	0.5803	
30 mm	Reference - Edge 2	T0-T1	Location x Time	0.0012	0.0028	0.4082	0.6863	
30 mm	Reference - Cable 1	T0-T2	Location x Time	-0.0014	0.0028	-0.5125	0.6123	
30 mm	Reference - Cable 2	T0-T2	Location x Time	0.0079	0.0028	2.7992	0.0092	**
30 mm	Reference - Edge 1	T0-T2	Location x Time	-0.0029	0.0024	-1.2343	0.2273	
30 mm	Reference - Edge 2	T0-T2	Location x Time	0.0025	0.0024	1.0695	0.2940	

Annex 3 Model results shell length 45mm

Statistics (‘Time’, ‘Location x Time’) for 45 mm shell mussels for reference against cable 1, cable 2, edge 1 and edge 2, and T0 against T1 and T0 against T2

* = P ≤ 0,05; ** = P ≤ 0,01; *** = P ≤ 0,001

Shell size:	Location	Time	Parameter	Estimate	Standard err.	T-value	P-value	
45 mm	Reference - Cable 1	T0-T1	Time	0.0311	0.0043	7.1806	0.0000	***
45 mm	Reference - Cable 2	T0-T1	Time	0.0253	0.0040	6.2855	0.0000	***
45 mm	Reference - Edge 1	T0-T1	Time	0.0286	0.0048	5.9067	0.0000	***
45 mm	Reference - Edge 2	T0-T1	Time	0.0178	0.0039	4.5570	0.0001	***
45 mm	Reference - Cable 1	T0-T2	Time	0.0134	0.0029	4.6068	0.0001	***
45 mm	Reference - Cable 2	T0-T2	Time	0.0036	0.0028	1.2941	0.2062	
45 mm	Reference - Edge 1	T0-T2	Time	0.0168	0.0029	5.7826	0.0000	***
45 mm	Reference - Edge 2	T0-T2	Time	-0.0004	0.0027	-0.1559	0.8772	
45 mm	Reference - Cable 1	T0-T1	Location	0.0011	0,0042	0.2663	0.792	
45 mm	Reference - Cable 2	T0-T1	Location	-0.0013	0.004	-0.3351	0.7401	
45 mm	Reference - Edge 1	T0-T1	Location	0.0005	0.0048	0.1019	0.9196	
45 mm	Reference - Edge 2	T0-T1	Location	-0.0058	0.0039	-1.4962	0.1458	
45 mm	Reference - Cable 1	T0-T2	Location	0.0011	0.0029	0.3839	0.7039	
45 mm	Reference - Cable 2	T0-T2	Location	-0.0013	0.0028	-0.4843	0.632	
45 mm	Reference - Edge 1	T0-T2	Location	0.0005	0.0029	0.1691	0.8669	
45 mm	Reference - Edge 2	T0-T2	Location	-0.0058	0.0027	-2.1264	0.0424	
45 mm	Reference - Cable 1	T0-T1	Location x Time	-0.0025	0.0060	-0.4214	0.6768	
45 mm	Reference - Cable 2	T0-T1	Location x Time	0.0033	0.0057	0.5848	0.5634	
45 mm	Reference - Edge 1	T0-T1	Location x Time	0.0000	0.0068	0.0073	0.9943	
45 mm	Reference - Edge 2	T0-T1	Location x Time	0.0108	0.0055	1.9677	0.0591	
45 mm	Reference - Cable 1	T0-T2	Location x Time	-0.0040	0.0041	-0.9624	0.3441	
45 mm	Reference - Cable 2	T0-T2	Location x Time	0.0058	0.0039	1.4826	0.1493	
45 mm	Reference - Edge 1	T0-T2	Location x Time	-0.0074	0.0041	-1.7976	0.0830	
45 mm	Reference - Edge 2	T0-T2	Location x Time	0.0099	0.0039	2.5431	0.0168	*

Wageningen Marine Research
T +31 (0)317 48 7000
E: marine-research@wur.nl
www.wur.eu/marine-research

Visitors’ address

- Ankerpark 27 1781 AG Den Helder
- Korringaweg 7, 4401 NT Yerseke
- Haringkade 1, 1976 CP IJmuiden

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