Flat oyster (Ostrea edulis) settlement on scour protection material

Innovation Eco Scour Protection in offshore windfarm Borssele V

Author(s): Linda Tonk, Sterre Witte, Pauline Kamermans

Wageningen University & Research report C063/20

Flat oyster (Ostrea edulis) settlement on scour protection material

Innovation Eco Scour Protection in offshore windfarm Borssele V

Author(s): Linda Tonk, Sterre Witte, Pauline Kamermans

Wageningen Marine Research
Yerseke, January 2020

CONFIDENTIAL no

Wageningen Marine Research report C063/20

This document is produced for the project **Offshore Wind Farm Borssele Site V** - innovation concept *Eco-friendly scour protection*.

The project **Offshore Wind Farm Borssele Site V** is contracted by **Two Towers B.V.**, a consortium of Van Oord Offshore Wind B.V., Investri Offshore, and Green Giraffe.

The innovation concept *Eco-friendly scour protection* is subcontracted by Royal Netherlands Institute for Sea Research, Wageningen Marine Research, Bureau Waardenburg B.V., HZ University of Applied Sciences, and Roem van Yerseke.

Keywords: flat oyster, settlement, offshore windfarm, scour protection.

Client: Van Oord
Attn.: Remment ter Hofstede
Schaardijk 211
3063 NH, Rotterdam

This report can be downloaded for free from https://doi.org/10.18174/526156
Wageningen Marine Research provides no printed copies of reports

Wageningen Marine Research is ISO 9001:2015 certified.

Contents

Summary

European flat oysters (*Ostrea edulis*), important ecosystem engineers with several important ecosystem services, have near to disappeared from the Dutch North Sea. In order to reintroduce the flat oyster population in the North Sea, the availability of hard substrate for initial settlement is critical. Such substrate is offered in offshore wind farms where further anthropogenic disturbances of the seabed are restricted, making them a promising site for restoration efforts. Nature inclusive designs of scour protection around the base of the monopiles in wind farms could greatly improve the ecosystem contribution of offshore wind farms. However, the best type of substrate to use as scour protection to aid oyster settlement is unknown. In the project EcoScour(Protection), settlement success of oysters on different types of substrates is compared in order to find the best substrate for application in an eco-friendly scour protection in offshore wind farm Borssele V. The substrate types tested are: fresh mussel shell, weathered mussel shell, granite, sandstone, silex, marble, concrete, ECOncrete, roof tile, steel and BESE-elements. Settlement success is compared using three different measures: 1) total numbers of spat settled - in order to compare total spat collected per type of substrate provided in identical baskets, 2) settlement per kg of substrate - to find optimal substrates with regards to transport, and 3) settlement per cm^2 of substrate - to compare success between substrates independent of the available space to settle on.

The substrates were deployed at three different locations; the saltwater lake Grevelingen in the Netherlands, an oyster spatting pond in New Quay, Ireland and a natural bay in Tralee, Ireland. In the Grevelingen settlement of oyster spat per basket was most successful on weathered mussel shells, while granite was most successful at both locations in Ireland. Per kg fresh mussel shells were the most successful substrate at all locations. In terms of spat collection per cm^2, granite (New Quay and Tralee) and marble (lake Grevelingen) were also successful and more so than mussel shells.

The final choice of substrate for application in wind farms can depend on several factors. (1) When considering number of spat per mass of substrate, mussel shells are the substrate of choice. (2) When taking into account the cost-effectiveness for application in eco-friendly scour protection, working with construction materials such as granite or (E)concrete or by-products such as silex or marble (both from quarrying) are good options. (3) In view of longevity of the ecosystem and persistence even after decommissioning of the wind farm, substrates that are originally found in the North Sea could be considered. For that purpose, mussel shell or (red) granite gravel would be the best choice of oyster spat collection substrates to apply in eco-friendly scour protection.

1 Introduction

The experiment described in this report is part of the project plan Borssele V- EcoScour (Protection). The designation of Borssele V as an innovation site situated in the Borssele Wind Park, 20 km located off the coast of the Netherlands provides an opportunity for oyster reef development (Kamermans et al., 2018) and to gain knowledge on best reef initiation practices. Within this project different outplacement methods for long-term establishment of live European flat oysters on two scour protections around monopiles in Borssele V are tested. The work described in this report is part of work package 1: outplacement method which consists of five tasks (1.1 literature review, 1.2 oyster spat settlement, 1.3: oyster spat sensitivity to movement and cleaning, 1.4: suitability of using adults for outplacement and 1.5: outplacement). As part of the objective to test outplacements methods, different substrates for oyster spat settlement were tested (task 1.2), to provide insight in which substrate is most likely to be successful in collecting European flat oyster spat. Transport and biosecurity treatment of the substrates with spat are tested in task 1.3. In this report we focus on task 1.2 on the question which substrate is most likely to be successful in collecting oyster spat, based on experimental studies. Part of these substrates containing spat will be used for outplacement in task 1.5.

1.1 Background

The demand for production of clean energy, combined with intensified space use of the North Sea has resulted in the demand for ‘nature inclusive’ design of wind farms. Therefore the contribution of wind farms to recovery of biodiversity is investigated (North Sea 2050 Spatial Agenda, 2014). This demand offers perspective for reintroduction of European flat oysters (*Ostrea edulis*), a keystone native oyster species in the North sea that has near to disappeared from the North Sea due to human disturbances such as overfishing, introduction of diseases and habitat destruction (Beck et al., 2011, Berghahn and Ruth, 2005, Kamermans et al., 2018, Korringa, 1946, Lotze et al., 2006, Smaal et al., 2015, Thurstan et al., 2013). The flat oyster is included in the OSPAR List of Threatened and/or Declining Species and Habitats for the North-East Atlantic (OSPAR, 2008).

Flat oysters form reef-like beds that provide various ecosystem services that contribute to a diverse system (Bouma et al., 2009, Smyth et al., 2016). Flat oysters are filter feeders and increase the water quality by means of filtration of the water (Beck et al., 2011, Grabowski and Peterson, 2007). The reef-like structure of flat oyster beds increases the richness in micro habitats on the otherwise sandy bottom of the North Sea and thereby offering a higher habitat complexity (Beck et al., 2011, Grabowski and Peterson, 2007, Smyth and Roberts, 2010). In turn, an increase in habitat complexity offers a range of unique niches and provides shelter, thereby increasing the biodiversity and the stability of the community (Buhl-Mortensen et al., 2010, Smyth and Roberts, 2010). Oyster beds offer substrate for other benthic organisms to settle on as was already described by Möbius (1877) and recently elaborated by Smyth and Roberts (2010). Oyster beds provide a nursery and habitat for commercial fish species contributing an estimated average yearly economic value of US$5500–US$99,000 per hectare of oyster bed in the US (Jonathan et al., 2012). It should be noted that the economic value is related to the density of oysters that differs per region. In addition, oyster beds attenuate waves, and can therefore function as natural coastal protection in shallow areas (Borsje et al., 2011, Steven et al., 2011). These ecosystem services potentially make oyster reefs valuable for the North Sea ecosystem. Restoring oyster beds and introducing oysters in areas where they occurred historically, therefore offers a promising perspective for increasing the natural value of the intensively used North Sea ecosystem (Smaal et al., 2015).

Projects aimed at restoration of natural flat oyster beds and introduction of flat oyster populations in new areas such as wind farms are initiated around northwest Europe, for example in The Netherlands, Germany, Denmark, Sweden, Ireland and the United Kingdom. For an overview of the projects in Europe see Pogoda et al. (2019). Restoration projects have shown that larval recruitment, environmental conditions, hydrographics and especially presence of suitable settlement substrate are essential for successful establishment of oysters (Kennedy and Roberts, 1999, Smyth et al., 2016). The availability of hard substrate (e.g. stone or shell material) is crucial for establishment of a flat oyster population and is therefore a major limiting factor for recovery of oyster beds in the North Sea, where the bottom largely consists of soft sediment (Korringa, 1946, Möbius, 1877, Smyth et al., 2016). Offshore wind farms are considered as a potential location for restoration of flat oyster beds. The scour protection of the monopiles may offer hard substrate necessary for flat oysters to settle on (Kamermans et al., 2018, Smaal et al., 2017, Smaal et al., 2015). Additionally, there are no seabed-disturbing activities (such as bottom trawling fisheries) allowed in offshore wind farms which has been argued to be a prerequisite for the restoration of submersed oyster beds (Gercken and Schmidt, 2014, Smaal et al., 2015). The Dutch government has set goals to increase the share of renewable energy sources to 16% of the total energy source, which requires the construction of new offshore wind farms in the North Sea (Kamermans et al., 2018). Figure 1 gives an overview of the existing and designated wind farms in the Dutch Exclusive Economic Zone. Intensified space use (for example by fisheries, exploitation of fossil fuel, shipping and construction of offshore wind farms) of the North Sea has resulted in the demand for a 'nature inclusive' design of off-shore wind farms where the contribution of wind farms to restoration of ecosystems and increased biodiversity is investigated (IenM, 2014, Wilson and Elliott, 2009).

Monopile wind turbines are protected from scour (erosion of seabed around the foundation pile) by scour protection; usually in the form of rock dump around the pile (Whitehouse et al., 2011). A fine filter layer immobilises the sediment and an armour layer with larger debris that can sustain current and wave action stabilises this filter layer. One of the proposed ways to increase nature-inclusivity of wind farms, is through introducing flat oysters to an eco-friendly scour protection.

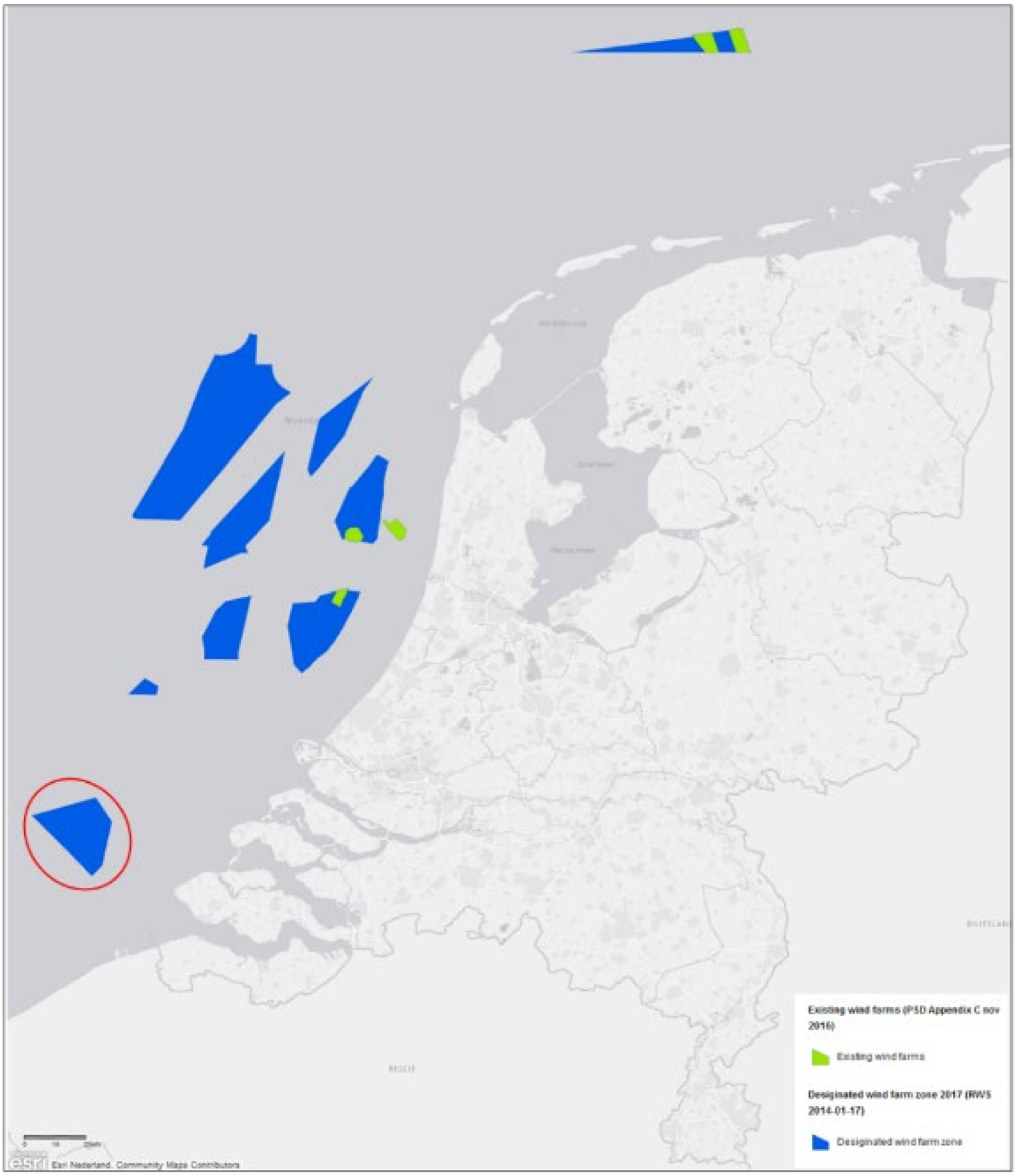


Figure 1: Map showing the existing and future wind farm locations in the Dutch exclusive Economic Zone in the North Sea. The red circle indicates the Borssele wind farm location, where the suggested substrate will be applied on a eco-friendly scour protection. Source data map: Heijden, 2019.

The scour protection around windfarms offers potential settlement substrate and habitat for oysters, however the most effective way of introducing flat oysters in wind farms is still unknown. Part of the offshore wind farm Borssele V, situated in the south of the Dutch Exclusive Economic Zone (Figure 1), has been designed for experiments with introduction of flat oysters in order to increase the (local) biodiversity (Kamp, 2016). Different scenarios for the introduction of flat oysters exist: 1) growing an oyster population from larvae that are naturally present in the water column, by providing the right substrate at the right time, 2) relocating spat on substrate to the wind farm, or 3) placing a combination of suitable hard substrate for settlement of flat oyster larvae and adult flat oysters as brood stock, after this settlement takes place in the OWF. Oysters are most vulnerable during the larval phase and the spat phase, with losses occurring due to predation or changes in water temperature, salinity and hydrography (Mackenzie, 1970, Shumway, 2011). A population of oyster spat is likely less robust than a population of adults and it will take more time before a self-sustaining population is formed (Oord, 2018). An advantage of scenario two, letting flat oysters settle naturally in a relatively controlled environment on hard substrate that can be translocated to the offshore wind parks, is that it avoids the need for sourcing the oysters from natural beds and reduces the mass of oysters that needs to be outplaced (Sas et al., 2019). In addition, going through larval and spat phases in a relatively controlled environment before being outplaced to an OWF, might increase the success of a restoration project.In this project a combination of spat and adults will be used for outplacement. However the focus in this report is on task 1.2, testing the most suitable substrate for collecting oyster spat.

Flat oysters start their life as a male and reach sexual maturity from the age of 2-3 years, then they may change sex within one year depending on water temperature (Korringa, 1952) (Figure 2). Eggs are held in the gills and mantle cavity and then fertilised by sperm that is extracted from the water column by the same mechanism used for feeding and respiration (Sawusdee et al., 2015). After 7-10 days, the fertilised eggs reach the veliger stage and they are released into the water column (Figure 2). The larvae then go through a planktonic development stage (10-30 days), during which they grow up to 290-360 µm depending on food availability and water temperature (Robert et al., 2017). When a suitable location is detected, the larvae glue their left valve to the substrate using a crystalline cement material (MacDonald et al., 2010)(Figure 2). The settlement phase is the only moment in the life cycle of the flat oyster when they can cement to the substrate, they do not relocate afterwards (Cranfield, 1973).

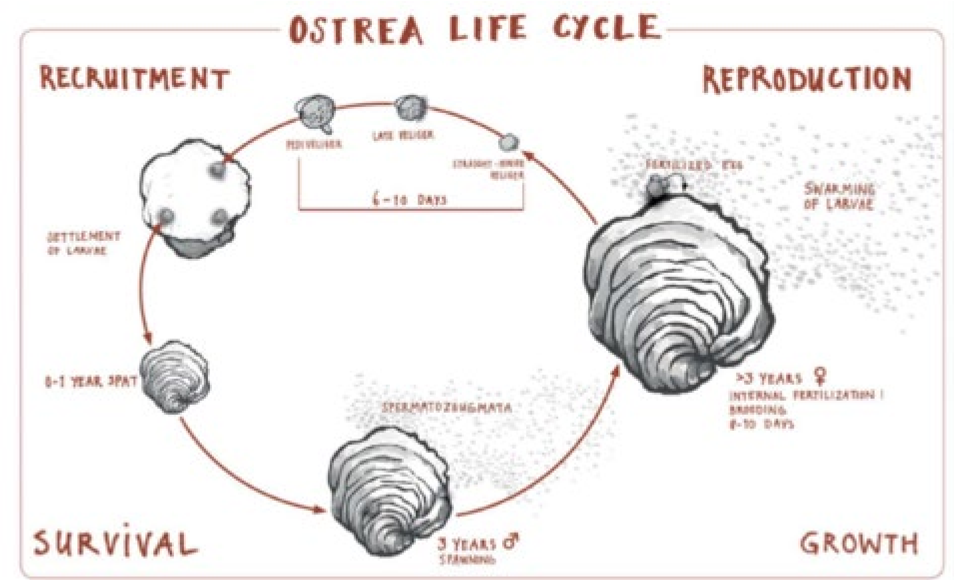


Figure 2: illustration of the life cycle of Flat oyster showing the critical stages in reproduction, recruitment, survival and growth. Source image: Sas et al., 2019.

In order to assess the potential of establishment of flat oysters in wind farms, the process of settling of oyster spat needs to be better understood. Metamorphosis and settlement of oyster larvae depends on a range of environmental cues and it has been shown that larvae can delay metamorphosis when suitable cues are absent (Cole and Jones, 1939, Coon et al., 1990). Important factors for settlement of sessile organisms include temperature, light, proximity of other organisms (e.g. conspecifics, prey, predators, biofilm), sound, topography and chemical composition of the substrate (Knights and Walters, 2010, Koehl, 2007, Lillis et al., 2013, Mesias-Gansbiller et al., 2013, Rodriguez-Perez et al., 2019). It has been shown that the availability of hard substrate is crucial for settlement of flat oyster larvae, and that different substrates have different success rates, though between different shell substrates no differences were found (Christianen et al., 2018, Rodriguez-Perez et al., 2019, Smyth et al., 2016, van den Brink, 2012).

Part of the offshore wind farm Borssele V, situated in the south of the Dutch Exclusive Economic Zone (Figure 1), has been designed for experiments with introduction of flat oysters in order to increase the (local) biodiversity (Kamp, 2016). Knowledge that enables selecting the optimal moment and procedure for oyster spat treatments, prior to outplacement to offshore field locations, including optimizing survival of oysters and reducing the risk of outplacing non-native species is needed. In this report the focus lies on selecting the right substrate, which is important for the success of a restoration project. Different types of substrate could be applied to enrich scour protection with settlement of flat oysters, of which some potentially more successful than others. The research described here aims to find the best substrate for this purpose. A total of eleven substrate types were chosen based on their applicability in scour protection, aquaculture and restoration projects. In a second phase, also indicated as task 1.5 outplacement which is not within the scope of this report, the spat on substrate as a result of the experiments in task 1.2 settlement will be outplaced to the North Sea. In order to find out whether a preference exists for settlement on these different substrates field experiments were conducted at three easily accessible near-shore locations where flat oysters occur. A location in the western part of Lake Grevelingen, near the Brouwersdam, was chosen since Lake Grevelingen is one of few locations in the Dutch waters where flat oysters still occur naturally (Smaal et al., 2015). However, *Bonamia ostrea* occurs in the lake (Engelsma et al., 2010). The eukaryotic parasite *B. ostrea* causes (lethal) infections in the gill area of infected oysters and leads to increased mortality (Culloty and Mulcahy, 2007). To avoid introduction of the parasite in the North Sea the introduction of oysters from infected areas is not advised. Therefore, a *Bonamia* free location Tralee Bay in Ireland was selected as a second location to perform the settlement experiment. In Tralee Bay a large population of native flat oysters occurs which is used as a natural resource for oyster fisheries. In addition, due to easy accessibility and high larvae numbers the experiment was performed in a New Quay spatting pond in Ireland. The latter is again a *Bonamia* infected area. The amount of oyster settlement depends the available substrate and the number of larvae in the water column. The latter depends on the size of the adult oyster population or brood stock. Differences in settlement of flat oysters on different types of substrate are expected to be found if there is a preference for a certain substrate. Besides substrate type, timing of the deployment of the substrate is crucial and needs to be timed according to the presence of oyster larvae in the water column. The settlement peak of flat oyster larvae is thought to be two weeks after a peak in larvae numbers is observed (Maathuis et al., 2020). If substrates are deployed too early before settlement is expected, biofouling could potentially hamper settlement of oyster larvae. If the substrates are deployed too long after the larvae peak, settlement is more likely to be missed (van den Brink et al. submitted)..

1.2 Research objectives

In order to optimise settlement and outplacement of flat oysters on scour protection in offshore wind farms, it is critical to use the most suitable substrate for settlement. This substrate can then be used to enrich the scour protection around wind farms to encourage successful establishment of flat oyster beds in offshore wind farms in the North Sea. In task 1.2 oyster spat settlement, we aim to provide insight in which substrate is most likely to be successful in collecting spat, based on experimental studies. The research question is formulated as follows:

> Do differences in spat settlement on eleven different types of substrate occur and which substrate types are most successful in collecting oyster spat per collector, per kg substrate and per surface area (cm^2)?
>
> Hypothesis: no differences in settlement of *O. edulis* between the different substrate types.

1.3 Approach

Differences in settlement of flat oysters on different types of substrate are expected to be found if there is a preference for a certain substrate. In order to assess this, field experiments were conducted at three different locations; the saltwater lake Grevelingen in the Netherlands, an oyster settlement pond in New Quay, Ireland and a natural bay in Tralee, Ireland (Figure **,** methods section). At each location, ten types of substrate (eleven in the Grevelingen) were deployed in the water column and the amount of flat oyster spat that settled on the different substrates was compared. In the Netherlands, the Pacific oyster (*Crassostrea gigas*) is present in higher numbers than the flat oyster, therefore the settlement of the Pacific oyster was also monitored. The settlement was compared in absolute number of spat, number of spat per kg substrate and number of spat per cm^2 on each type of substrate at the three locations. Total or absolute number of spat indicates the settlement success per basket. Settlement per kg substrate is evaluated because it might be advantageous to transport lighter substrates when outplacing substrates in the offshore wind farm. Settlement per cm^2 corrects for the available space to settle on. If a preference for a type of substrate exists, a significant difference in oyster spat settlement is expected when analyzing settlement per cm^2. It is then evaluated which substrate was most successful and which properties of the different substrate types potentially influenced the settlement success.

2 Materials and Methods

2.1 Set up of field experiment

2.1.1 Substrate material

In order to test the settlement success of oysters on different substrates, field experiments were conducted where settlement of flat oyster spat on eleven different substrates was compared. The substrate types used were fresh mussel shells, weathered mussel shells, granite, sandstone, silex (a.k.a. flint), concrete, ECOncrete, fragments of roof tile, BESE-elements, steel and marble (Grevelingen only). Figure 3 gives an overview of the different types of substrates used and the basket containing the substrates for deployment in the water column. Table 1 provides an overview of some of the properties of the substrate materials. The different substrates were placed in identical baskets (diameter 15 cm, height 40 cm) made of oyster mesh, a polyethylene mesh structure used in oysters cultivation, with a mesh size of 2x2 cm (Figure 3). The baskets with substrate were deployed in the top layer of the water column at approx. 20 to 30cm. Weight and volume of each basket with contents was determined before deployment of the baskets.

Figure 3: A) basket used to collect the substrates, in this example filled with silex. B) overview of the different substrates used in the field experiments. From top left to bottom right: mussel shells, granite, sandstone, silex, marble, concrete, ECOncrete, roof tiles, BESE-elements, steel.

Table 1: overview of the properties of the substrate materials used in the field experiments

Substrate type	Composition	Chemical name	Unit weight (kg/m3)	Rugosity	3D complexity
Granite (clean)	quartz and other minerals	SiO_2	2700	medium	medium
Cooked mussel shells (fresh)	calcium carbonate	$CaCO_3$	2930	smooth	high
Cooked mussel shells (weathered)	calcium carbonate	$CaCO_3$	2930	smooth	high
Concrete	calcium, aluminium, iron and silicon	Ca, Al, Fe, SiO_2	2400	medium	medium
ECOncrete	calcium, aluminium, iron, silicon and proprietary admix	Ca, Al, Fe, SiO_2	2400	high	medium
Sandstone	quartz, feldspar, calcite, silica, clay	SiO_2, $KAlSi_3O_8$ – $NaAlSi_3O_8$ – $CaAl_2Si_2O_8$, $CaCO_3$	1900	medium	medium
Reinforcing steel	alloy of iron and carbon	Fe, C	7800	smooth	high
Treated BESE	starch, cellulose	$C_{27}H_{48}O_{20}$, $C_6H_{10}O_5$	1500	smooth	high
Rooftiles	clay (alumnium silicates)	AlSi	1600	smooth	medium
Silex	quartz	SiO_2	1390	medium	medium
Norwegian marble	carbonate minerals	$CaCO_3$	2700	medium	medium

Shell material has been suggested to be one of the most successful materials for collecting oyster spat and is widely used in aquaculture (Kamermans et al., 2018, Korringa, 1952). The thin organic coating (periostracum) that forms the outermost layer of the fresh mussel shell (in this report fresh is defined as no more than 3 months old after the flesh has been removed) is known to repel biofouling as long as this layer remains intact. Older, weathered shells (over a year old after the flesh has been removed) with a non-intact periostracum are thought not to have this anti-biofouling mechanism. Granite is regularly used for scour protection around monopiles. Sandstone was included as a substrate in the experiment because it is one of the natural hard substrates that occur in the North Sea (Veenstra, 1969). Additionally, sandstone could potentially be used as a matrix for 3D printed reef structures. Silex or flint is another material that occurs naturally in the North Sea (Veenstra, 1969) and is available at relatively low cost as it is obtained as by-product during the extraction of marl in the south of the Netherlands. Norwegian marble is obtained as a by-product during extraction of granite (pers. comm. Tim Raaijmakers) and was included as a substrate because it is rich in calcium carbonate, potentially beneficial for settlement of oyster spat. Concrete can be obtained as a by-product from construction or can be cast in many different shapes, which could make for a convenient substrate for application in offshore wind farms. ECOncrete is concrete that is enriched with a "proprietary admix integrating by-products and recycled materials" and textured to increase settlement of organisms and enhance the ecological value of the areas where it is used (ECOncrete, 2019). Roof tiles were historically used to collect oyster spat (Korringa, 1976) and were typically covered in lime to facilitate removal of the oysters when applied in oyster culture. The roof tiles used in this research were untreated. BESE-elements are biodegradable modular 3D structures partly made of starch from potato waste that are intended to be used for restoration of ecosystems (BESE-products, 2019). Steel is chosen as a substrate because it could be used to construct structures, e.g. that are elevated above the seafloor, reducing the chance of burial underneath the sediment.

2.1.2 Location

The substrates were deployed at three different locations; the saltwater lake Grevelingen in the Netherlands, an oyster spatting pond in New Quay, Ireland and a natural bay in Tralee, Ireland (Figure 4). The spatting pond is owned by the Redbank Shellfish company, an outdoor nursery area specially equipped for production of oyster spat, in New Quay (Ireland, 53°09'25.9"N 9°04'00.2"W). The spatting pond is the most controlled of the three trials, it is set up to optimise the number of oyster spat so the highest number of flat oyster spat is expected to be found in this trial. When the water temperatures exceeded 15 °C, brood stock of adult flat oysters was placed in the pond. Once the first oyster larvae were detected in the water, water in the settlement pond was no longer refreshed and the temperature was kept relatively constant.

Figure 4: Map of the three locations of the field experiment. Pinpoints indicate the different locations. NQ: New Quay, TR: Tralee Bay, GR: Lake Grevelingen.

Even though the expectation is to find the highest settlement rates in this pond, this location is not suitable for breeding oysters for outplacement at Borssele V from a legislative perspective, as it is located in an area that is infected with *Bonamia ostrea* (Sas et al., in prep.).
Substrates were also deployed in Tralee Bay (Ireland, 52°16'18.8"N 9°51'43.3"W) on longlines of the Tralee Oyster Fisheries Society parallel to the surface of the water that are tightened between anchored buoys. Tralee Bay is a natural bay in Ireland where flat oyster farming and oyster fishing takes place. The substrates were placed on longlines in an area of the bay where oyster farmers collect their spat yearly. The natural setting is more exposed to waves and ocean swell, as well as settlement of other organisms on the substrates. However, *B. ostrea* has not been found in the bay (Sas et al., in prep.), making it a potential location to source flat oyster spat for outplacement in wind farms. In addition, the field experiment was carried out in Lake Grevelingen in an off-bottom mussel farm near the Brouwersdam (The Netherlands, 51°44'45.2"N 3°49'46.1"E) (Figure 4). Besides flat oysters, the Pacific oyster is also present in large numbers in these waters and, based on earlier experiments by Kamermans et al. (2004) and van den Brink et al. (2012), Pacific oysters are expected

to be found on the substrates in the experiments in much higher frequencies than flat oysters. In the Grevelingen the substrates were randomly distributed along longlines in a mussel culture plot. Visual inspection of the substrates was carried out with a GoPro to follow the level of biofouling. The expectation was that at this location much more biofouling occurs than at the other locations, therefore the baskets at this location were cleaned by picking off the fouling by hand every two weeks. At all three locations the baskets with different types of substrate were randomly distributed in space, approx. 30 cm apart. In Lake Grevelingen the baskets were randomly distributed along a double longline of 30 m. In Tralee Bay the baskets were randomly distributed along one longline of approx. 60 m. In the settlement pond of New Quay the baskets were randomly distributed along the four sides of the pond (Figure 4). Table gives an overview of the number of baskets that were deployed at the three different locations. Note that a total of 60 extra baskets were deployed containing sandstone (n=20), mussel shells (n=20) and silex (n=20). These baskets were destined for outplacement in the North Sea (task 1.5) and are not part of the experiment described in this report.

Table 2: overview of the number of replicates per substrate left at the end of the experiment at each location.

Location	**Mussel fresh**	**Mussel weathered**	**Granite**	**Sandstone**	**Silex**	**Marble**	**Concrete**	**ECOncrete**	**Roof Tile**	**BESE**	**Steel**
New Quay	5	5	5	5	5	0	5	5	5	5	5
Tralee	2	0	4	10	5	0	4	4	3	5	3
Grevelingen	12	4	5	6	6	5	5	5	5	5	5

2.1.3 Larvae monitoring & timing of deployment

To determine the settlement peak the larvae numbers were monitored at the experimental locations in New Quay (daily from the 9th June until the 23rd of August 2019) and Lake Grevelingen (weekly in week 24, 25, 27 and 29 of 2019). To determine the number of oyster larvae, 100 litres of water was filtered through a 55 µm plankton net. The collected organic material was kept chilled and immediately transported to the lab, where it was fixated with 4% formaldehyde. The larvae number was determined by microscope. The substrates were introduced in the water column a week before a peak in settlement of flat oyster larvae was expected. Figure A gives an overview of the larvae measured in the water column in New Quay and Lake Grevelingen, unfortunately no larvae collection was available in Tralee Bay. The figure also shows spat settlement on settlement plates in New Quay. Settlement occurs shortly after the larvae peak, and the number of larvae in the water column was higher in New Quay than in Lake Grevelingen. The substrates were introduced in the water column in late June or early July and retrieved at the end of September or early October 2019 and the number of oyster spat was counted directly after retrieving the substrates (see Table 3). Figure 5B shows the temperature measured in New Quay and Lake Grevelingen. No temperature measurements were done in Tralee Bay.

Table 3: Dates of deployment and retrieval of the baskets with substrates

Location	**Date deployment**	**Date retrieval**
New Quay	25 June 2019	23 September 2019
Tralee	1 July 2019	25 September 2019
Grevelingen	1 July 2019	7 October 2019

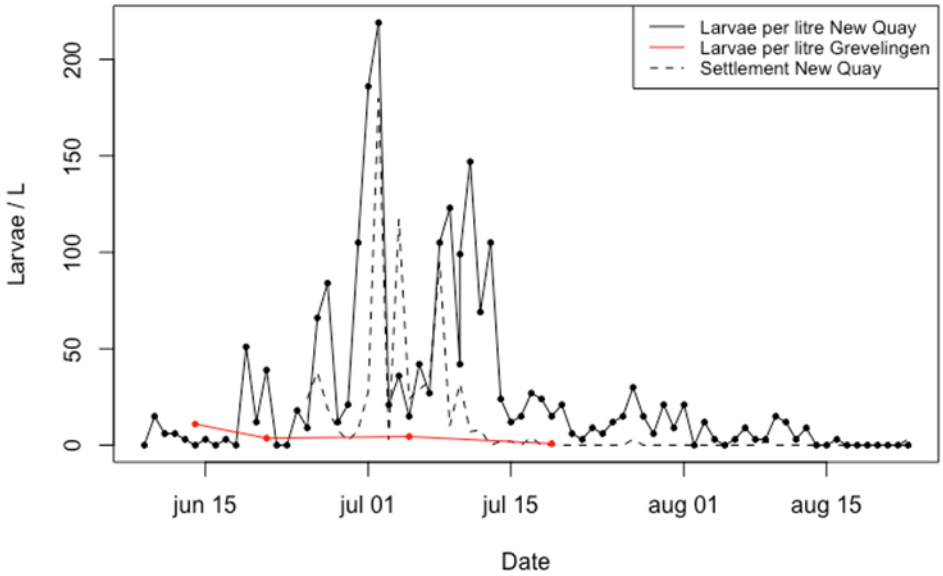


Figure 5A: overview of the number of O. edulis larvae counted per litre of water over time (solid lines). Black indicates New Quay, red indicates Grevelingen. The dashed line indicates settlement of O. edulis larvae in New Quay.

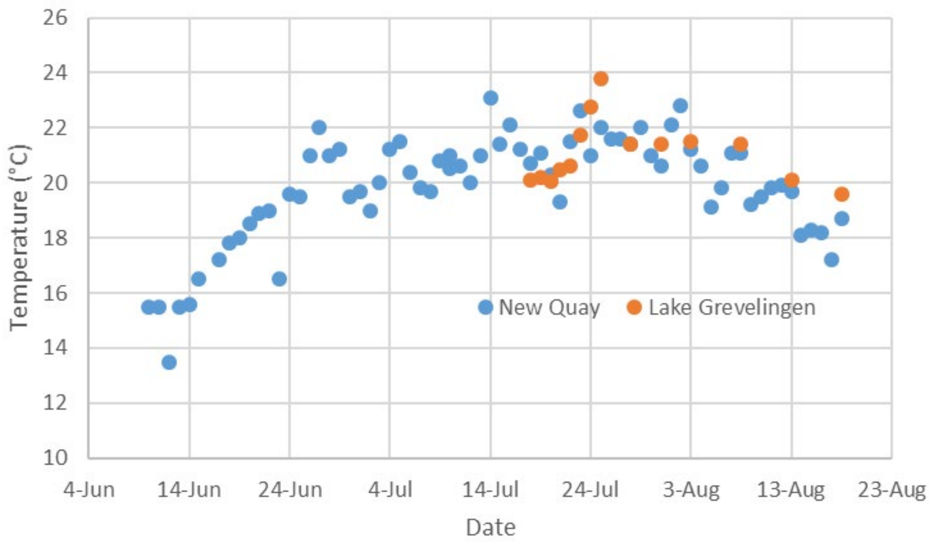


Figure 5B: Temperature measured in New Quay (blue) and Grevelingen (orange).

2.2 Counting oyster spat

Before counting the spat, biofouling was removed and if necessary, the substrate was cleaned using filtered seawater. Cleaning the substrates that were deployed in the Grevelingen was the only way that the oyster spat, which was sometimes only a few millimetres in size, could be counted visually. After recollection, the substrates were weighed again. When the number of spat was estimated to be over 250 individuals per basket, a subsample was taken. In order to take a subsample, the substrate was spread out evenly and split into equal parts by eye. Depending on the amount of spat on the substrate, a quarter or half a basket was counted first and more substrate was counted when the subsample was still too small. A minimum of a 100 spat were counted when taking a subsample. When sufficient oyster spat was counted, the substrate subsample (spat on substrate) was weighed (wet weight) in gram. The total number of spat in a basket (N) was then calculated using:

$$N = \left(\frac{W_t}{W_s}\right) * n_s$$

Where W_t is the total (wet) weight of the substrate (g), W_s is the (wet) weight of the substrate subset that was counted (g) and n_s is the number of spat counted in the subset.

When counting the spat, a distinction was made between living and dead individuals where possible. Both living and dead spat were counted as dead spat has once settled on the substrate and therefore could indicate a difference in settlement preferences between substrates.

2.2.1 Fraction living spat

In addition to the number of total spat on the different substrates, the fraction live spat of the total spat that was present on the substrates were calculated. The fraction living spat of the total spat per basket was compared between the substrates, in order to determine whether differences in survival existed between different substrates.

2.2.2 Other observations

Besides counting the oyster spat notes were taken on the degree of biofouling, whether a clear preference was seen between rough or smooth sides of the substrate (for instance ECOncrete showed a clear rough and smooth side). Furthermore observations such as preference for a shaded side (top and bottom) of the basket were written down.

2.3 Measuring three-dimensional substrate surface

In order to better compare the settlement success of the different substrates, the three-dimensional surface area of the different substrates was estimated. The 3D substrate surfaces that were used in this research, were an approximation of the surface and not an actual measured 3D surface for each basket. This approximation had to be made since it was not possible to estimate the surface area of the substrate in each individual basket due to logistical issues and time constraints. In selecting the methodology for determining the 3D surface, three different methods were tested; photogrammetry, 3D scanning (using a handheld 3D scanner and a laser scanner) and paraffin wax dipping (Holmes, 2008, Veal et al., 2010). Considering time constraints, wax dipping was the most convenient and precise method of determining the 3D surface area techniques as compared to foil wrapping, dye or latex dipping, photogrammetry, several 3D scanning methods and X-ray CT scanning (Naumann et al., 2009, Veal et al., 2010). There has been discussion on the use of a single layer of wax versus double wax dipping; single wax dipping is argued to be more precise for complex surface structures, whereas double wax dipping is suitable to compare between substrates with different surface structures and surface roughness (Veal et al., 2010).

For photogrammetry, many photos are taken of an object from different angles and a computer program is used to construct a 3D model from these photos. The advantage of photogrammetry is that it is relatively cheap as any type of photo camera can be used. The programs for creating the model are available from free to paid professional licenced programmes (like Agisoft PhotoScan Professional (Version 1.2.6), the photogrammetry software we tested for this research). Some photogrammetry programs offer the functionality to calculate the surface area of an object based on measured distances between markers that are placed on or next to the object. However, aligning the photographs is a time-consuming process and if too few photos are taken, the program will not be able to align the photos and construct a 3D model.

Laser 3D scanning uses laser beams combined with a rangefinder to determine the distance between an object and the scanner from different angles to create a 3D image of the object. The laser scanner that was tested for this research was the Robin PSI PlantScreen™ at Wageningen Plant Research of Wageningen University and Research. Unfortunately, the triangulation scanning unit was not suitable for the purpose as it is designed for scanning plants and therefore did not generate an appropriate model of the substrates used in the field experiments. Therefore, Artec's handheld Eva structured light 3D scanner was also tested at NIOZ, Texel. It projects a grid of light on the substrate and then uses three lenses to capture the object. Using triangulation, the accompanying software can construct the 3D model relatively easily and it can automatically calculate the 3D surface of the area. This method proved to be a quick way to accurately generate a digital 3D model of the substrate and calculate the surface area, although capturing small pieces of substrate proved to be difficult.

While digital 3D reconstruction of substrates is an elegant way to estimate surface area, an alternative relatively quick and cost-efficient way to estimate the surface area is wax dipping. This technique is often used to estimate the surface area of corals (Holmes, 2008, Veal et al., 2010). To estimate the surface area, the substrates are dipped in melted paraffin wax. The increase in weight between either the substrate and substrate with wax, or between the weight of the substrate after a first dip and the weight after a second time of wax dipping is taken as indication for the surface area of the substrate. This is based on the premise that the wax layer always has the same thickness and a certain amount of wax therefore always covers an equal surface area of the substrate. A larger surface area of the substrates needs more wax to cover the complete area and therefore leads to a larger difference in weight. In the context of this research wax dipping is the most suitable technique to measure the surface area, considering the large number of substrates of which the surfaces needed to be determined. However, the wax alters the substrate and could therefore not be used before deployment of the substrates. The surface of a random size mix of pieces of substrate representative of the substrates in the baskets was therefore used for wax dipping instead.

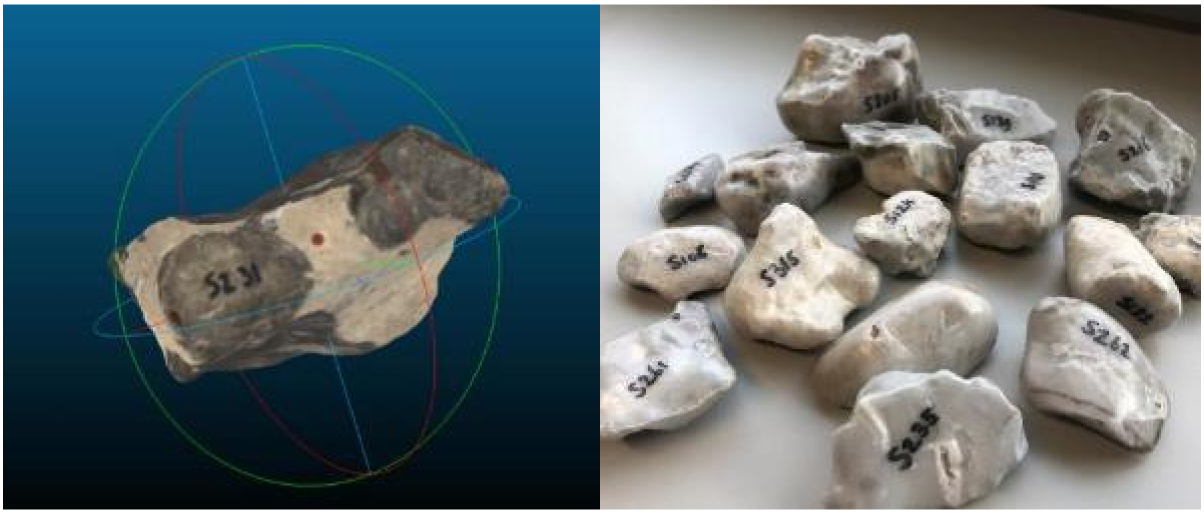


Figure 6: A. example of a three-dimensional model of a piece of silex. B. some pieces of silex dipped in paraffin wax.

For the purpose of this research, a combination of two of the methods as described above was eventually used to determine the 3D surface area of the different substrates. The average surface of the substrates was determined using double wax dipping and a calibration curve was calculated using a handheld 3D scanner (Figure gives an example of what the two methods look like). Wax dipping was conducted based on the wax dipping method by Stimson and Kinzie (1991) as described by Veal and colleagues (2010) and Holmes (2008). For the wax dipping, a representative subset of the substrate in the baskets was measured. The subset of pieces of substrate was taken to be sufficiently high so that the R-squared for the substrate weight:wax weight curve was at least 0.75 for each different substrate (arbitrarily chosen, Annex 1- wax dipping). Paraffin wax was melted "au bain marie" in a double boiler with water that is maintained at 65 °C. The temperature was continuously measured using HANNA HI 93510N with a liquid probe. The substrates were weighed to the nearest 0.1g, then the substrates were dipped into a wax bath with melted paraffin wax for two seconds (Paraplast Tissue Embedding Medium). After dipping, the substrate was shaken to rid it of any excess wax. After this first wax dipping, major irregularities in the substrate are sealed which reduces the chance of breaking the assumption that the wax layer has the same thickness over the whole surface area. Substrates with different textures potentially have different wax adhesion, as adhesion to a smooth surface might be different from adhesion to a rough surface. The first round of wax dipping allows for comparing between different substrates as the second wax layer attaches to the first layer rather than substrates with a different roughness. After the first dip, the wax is allowed to cool to room temperature before the substrate is reweighed. After weighing the substrate with the first wax layer, the substrate is dipped, cooled and weighed again. The weight difference between the first and second wax layer is taken as measure for the surface area of the substrate.

A factor to convert the weight difference between the first and second wax dip to three-dimensional surface area was calculated based on a calibration curve constructed through 3D scanning of a subset of the substrates. Five different sized pieces of every type of substrate that were used for wax dipping were also scanned using Artec's handheld Eva scanner. 3D models of the substrates were created with Artec Studio (version 14) and used to calculate the surface area. Unfortunately, it was not possible to construct 3D models of all substrates. The mussel shells were too thin to have enough overlapping points to align the outside and inside of the shell. Smaller pieces of granite, silex, marble and sandstone also had too little overlap between scanned sides to create an accurate model. The surface area of the steel wire frames, which was always cut to the same dimensions (cylinders with a diameter of 2.3 mm and a length of 14 cm crossed in a hash shape), was calculated and added to the calibration curve. A calibration curve could be constructed using the digitally calculated three-dimensional surface area and the weight difference between the first and second wax dip. The calibration curve was forced to intercept the y-axis at 3.41 mm^2, which is the minimum possible surface where there is no substrate but just a drop of wax. This surface was calculated as the calculated surface of a sphere with a radius that is the average thickness of two wax layers:

$$s = 4 * \pi r^2$$

Where s is the surface (cm^2) and r is the average thickness (cm) of the second wax layer on a standardised surface. This average thickness was measured using plastic tags with a thickness of 3 mm (n=30). The tags were dipped in wax two times, and after each dip the increase in thickness was measured using Mitituyo digital callipers. The average thickness of the second wax layer was 0.521 mm.

Figure 7 shows the weight difference between the first and second wax layer compared to the actual measured 3D surface of the substrates. The relation between the 3D surface and the weight of the second wax layer is defined with the following conversion factor:

$$surface = 3.41 + \ 27.48 \ * weight\ difference$$

Based on the relation in the calibration relation (Figure 7), the surface area (cm^2) per weight (kg) was determined for the different substrates. Using the measured wax weight difference, the average surface area per kg was calculated for each substrate. The mass of the substrate is then multiplied with this average surface area per kg to obtain a measure for the three-dimensional surface area of the substrate in each basket.

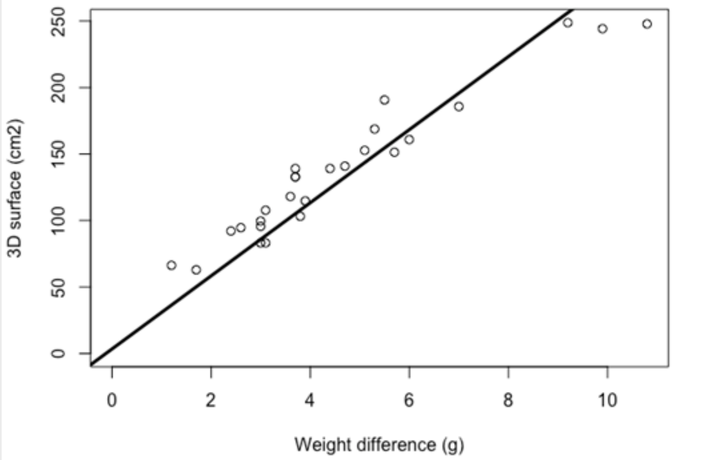


Figure 7: Relation between the difference in weight between the first and second wax dip and the three-dimensional surface as measured in cm^2 using 3D scanning. This relation was used as calibration curve to calculate the total three-dimensional surface area that corresponds with the weight of the substrates in each basket.

2.4 Data analyses

Data analyses were executed in R (version 3.5.0). Packages used were plyr, emmeans, multcomp and viridis (R package version 0.5.1.). Total numbers of settled oyster spat (live plus dead) at the different locations were compared. If the locations differ significantly, the settlement of oysters on different substrates were analysed separately for each location.

2.4.1 GLM

Settlement on the different substrates is compared using number of oyster spat per basket, number of spat per kg of substrate and number of spat per cm^2 using generalised linear models (GLM, a generalization of linear regression that allows for response variables that have a non-normal distribution of residuals and for the magnitude of the variance of each measurement to be a function of its predicted value). This method was chosen, as the variables were not normally distributed as they were count data (quasipoisson distribution).

The model used was:
glm(count ~ substrate type + location, family=poisson) (Generalised Linear Model)

The fraction living of the total spat found was also compared between substrates for the different locations using GLM with a quasibinomial data family for proportional data. The fraction of living spat for the different substrates were compensated for differences in total numbers of spat observed at the different locations and substrates by weighing it by the number of observations.

The model used was:
glmer(count ~ (1 | location) + substrate type, family=poisson) (Mixed model)

2.4.2 Tukey post-hoc test

Differences in weight to surface area ratio between the different substrates were tested using an ANOVA with a Tukey post-hoc test. A Tukey post-hoc test was used to determine which substrates differed significantly ($p<0.05$) from other substrates. Substrates where no oyster spat was found at all were excluded from post-hoc testing.

2.4.3 Overall settlement success

A summary table was made using the Tukey post-hoc groups. Substrates in the highest group scored +1 for each measure, substrates in the intermediate group scored 0 and substrates in the lowest significant group scored -1. These scores were added for each measure of settlement success (total spat per basket, spat per kg of substrate, spat per cm^2 of substrate) to arrive at a measure for overall success (final score) of all the different types of substrates used in the field experiment.

3 Results

3.1 Biofouling of substrates

When counting the number of spat that settled on the substrates in the baskets at the three different locations of the experiment, a few things stood out. The degree of biofouling noticeably differed per location (Figure 8). In the spatting pond of New Quay, there was virtually no biofouling visible on the substrates when the baskets were retrieved at the end of the experiment after being submerged for three months. In Tralee Bay some substrates were covered in *Ectopleura larynx*, or ringed tubularia, a hydroid in the *Tubulariidae* family. *Spirobranchus triqueter,* a brushworm from the *Serpulidae* family was also regularly observed on the different substrates. Different species of anemones, sponges, bryozoa, saddle oysters (*Anomiidae*), scallops (*Pectinidae*) and other molluscs were found regularly. On the substrates in Lake Grevelingen, almost no flat oyster spat was found and most of the oyster spat found were Pacific oysters*.* In addition, the substrates in Lake Grevelingen were inspected visually every two weeks, and a difference in the rate of progression of biofouling on the different types of substrate was observed. The first four weeks, fresh mussel shells showed less biofouling than the other substrates in this location. After the first month, this difference in degree of fouling was no longer visible. At the end of the experiment *Caprellidae* (a family of amphipods commonly known as skeleton shrimps) and mussels (*Mytilus edulis*) were present in high numbers. Even though the substrates were located in the top of the water column, a muddy substance covered most of the substrates that were deployed in the Grevelingen.

Figure 8: Illustration of the different degrees of biofouling on the baskets with substrates in different locations as they were collected to be counted. A) New Quay; almost no biofouling. B) Tralee; high degree of biofouling. C) Grevelingen; high degree of biofouling by mussels (*Mytilus edulis*) and skeleton shrimps (Caprellidae) is visible.

3.2 Spat on substrate

An overview of the field experiment results is given in this section. The result tables with the means, standard errors and p-values for all the different substrates, can be found Annex II.

Large differences in total number of oyster spat on the substrates were found between the three locations (Figure 9). A Tukey post hoc test shows a significant difference between the total number of spat (per basket) found in New Quay and Tralee. In Lake Grevelingen almost no flat oyster spat (n=20, the total amount of flat oyster spat found on all substrate) was found on the different types of substrate, however more spat of the Pacific oyster (n=755) was observed. Therefore, we chose to analyse the differences in settlement between substrates for Pacific oysters rather than flat oysters for Lake Grevelingen. The locations were analysed separately because of the differences in spat settlement between the locations. Table gives an overview of the average settlement and standard deviation for the different measures (total spat per basket, spat per kg substrate and spat per cm^2) per substrate at each location.

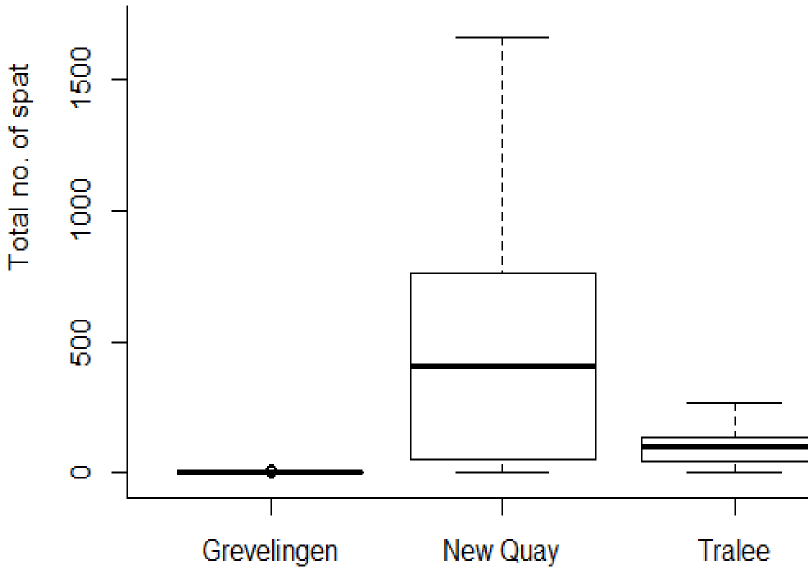


Figure 9: Boxplot showing the total number of flat oyster spat collected on all the baskets for the three different locations. Boxes represent the median, quartiles and interquartile outliers in total numbers of spat per location.

At all locations, most spat was found on the more shaded bottom of the basket, rather than at the top (Figure 10). In the settlement pond in New Quay the shaded north-facing side of the pond was preferred by flat oyster spat. A total of 11081 spat settled on the north side, versus 3733 on the east side, 4545 on the south side and 5735 on the west side. On the mussel shells, most of the spat had settled on the concave inside of the shells and also the hollow parts of the roof tile seemed to be preferred over the convex side (Figure 11). On concrete, roof tile and steel, most of the spat was found on the rough edges of the substrate. Settlement on BESE-elements occurred at the centre of the structure, almost no spat was found at the edges. The smoother surface of the substrate seemed to be less preferable to settle on. Although the settlement on ECOncrete was mainly on the smooth sides, rather than on the more complexly structured side. On the natural rock substrates, no such clear preferences for areas on the substrate could be distinguished. Differences in spat size from several mm to 1.5cm were detected (Figure 11).

Figure 10: Left, topside of the basket where less spat was detected. Right, underside of the basket where most spat was detected.

Figure 11: Top left, spat in the concave part of a mussel shell. Top right, size differences in spat. Bottom, spat size up to 1.5 cm.

Table 4: Summary of the mean total number of spat (per basket), mean number of spat per kg substrate, mean number of spat per cm^2 for the different types of substrate in the different locations of the experiment. The substrate that is performing best is indicated in bold, the standard deviation is indicated in brackets. Missing substrate replicates are indicated with a grey empty box; marble was only deployed in Lake Grevelingen and weathered mussels were lost in Tralee.

	Mussel	**Mussel (w)**	**Granite**	**Sandstone**	**Silex**	**Marble**	**Concrete**	**ECOncrete**	**Roof tile**	**BESE-elements**	**Steel**
Spat per basket											
New Quay	976.1 (357.8)	796.7 (210.2)	**1120.8 (796.7)**	307.6 (179.3)	458.9 (252.2)		594.7 (272.1)	687.4 (555.5)	58.4 (30.3)	2.8 (2.4)	15.6 (4.9)
Tralee	150.5 (160.5)		**206.8 (32.1)**	86.2 (43.3)	108.4 (11.9)		161.3 (43.8)	89.3 (21.7)	106.7 (30.3)	0	1.4 (1.2)
Grevelingen *C. gigas*	29.3 (14.6)	**30.25 (7.5)**	12.6 (7.4)	9 (3.8)	9 (4.0)	9.2 (5.4)	3.8 (2.5)	4.8 (3.5)	3.8 (0.8)	0.8 (0.8)	0
Spat per kg											
New Quay	**1320.5 (571.1)**	884.5 (398.5)	265.9 (196.6)	88.5 (51.3)	119.4 (75.0)		113.4 (63.8)	218.1 (153.9)	17.6 (8.6)	18.2 (15.3)	10.3 (3.2)
Tralee	**145.9 (153.0)**		47.5 (7.2)	24 (11.3)	24.3 (11.3)		34.6 8.9)	27.1 (5.7)	30.5 (8.4)	0	0.85 (1.0)
Grevelingen *C. gigas*	**28.6 (15.0)**	21.7 (4.1)	2.6 (1.5)	2.3 (1.0)	2.3 (1.0)	3.2 (2.0)	0.8 (0.6)	1.4 (0.9)	1.1 (0.3)	1.8 (1.7)	0
Spat per cm^2											
New Quay	0.1 (0.06)	0.1 (0.04)	**0.3 (0.3)**	0.05 (0.03)	0.2 (0.1)		0.2 (0.1)	**0.3 (0.2)**	0.02 (0.001)	0.002 (0.0001)	0.009 (0.0003)
Tralee	0.02 (0.01)		**0.06 (0.009)**	0.02 (0.007)	0.03 (0.005)		0.05 (0.01)	0.04 (0.008)	0.03 (0.008)	0	0.0007 (0.0008)
Grevelingen *C. gigas*	0.003 (0.002)	0.002 (0.0005)	0.003 (0.002)	0.001 (0.007)	0.004 (0.002)	**0.004 (0.003)**	0.001 (0.0009)	0.002 (0.001)	0.001 (0.0003)	0.0002 (0.0002)	0

3.3 Total spat per basket

In New Quay, a total number of 25094.8 flat oyster spat were counted. Some of the baskets were counted using subsets, which is why some numbers counted are not an integer. On average 501.9 spat were counted per basket, the maximum number of spat was counted on granite. The maximum observed number of spat in one basket of granite was 2336.1, on average 1120.8 spat were found per basket. The lowest number of spat was counted on BESE-elements, where on average 2 spat were found per basket. Figure 12A shows boxplots of the total number of spat found on the different substrates in each basket. The types of substrate are divided into statistically similar clusters. Mostly no significant difference in flat oyster spat settlement was found between the different types of substrate. However, spat settlement on roof tile is significantly lower than settlement on mussel, ECOncrete and granite. Spat settlement on granite is significantly higher than settlement on sandstone. Note that marble was not deployed at the Irish sites.

In Tralee, due to weather circumstances some of the replicates were lost. However, for sandstone, additional baskets were used that were deployed for a different experiment. We opted to include these samples in the analysis to obtain a larger sample size. The resulting sample size is shown in Table , methods section. Although the baskets with mussel shells were retrieved, most of the shells presumably broke due to wave action and subsequently had fallen through the mesh of the baskets. As a result, for spat on mussel shells only two (partially filled) baskets could be counted. A total number of 3858 flat oyster spat were counted. On average, 96.5 spat were counted per basket. The maximum number of spat (n=264) was counted on mussel shells, while granite showed the highest average number of spat per basket (n=206). No spat was collected on BESE-elements, and on steel an average of 1.33 only spat per basket was observed. Figure 12B shows boxplots of the total number of spat found on the different substrates in each basket. Granite collected a significantly higher number of flat oyster spat than sandstone and ECOncrete. Settlement of flat oyster spat on the remainder of the substrates did not differ significantly.

In Lake Grevelingen, additional baskets for mussels (n=5+5), sandstone (n=6+1) and silex (n=5+1) were hung in the water column and spat in these extra baskets was also counted (Table , methods section). At this location, only 20 flat oyster spat were found in total, of which one was dead. One spat was found in a basket with roof tiles and one in a basket with granite. The rest of the flat oyster spat was found on mussel shells and weathered mussel shells. In contrast, 755 Pacific oyster spat were found, of which 97% was living. Due to lack of flat oyster spat, the analysis for settlement was carried out with the number of Pacific oyster spat. The highest number of Pacific oyster spat was found on the fresh mussel shells (n=351), whereas on average the most spat per basket was observed on weathered mussel shells (n=30.25). No spat was observed on steel, and the lowest average number of spat per basket was collected on BESE-elements (n=0.8). Figure 12C shows boxplots of the total number of Pacific oyster spat found on the different substrates in each basket. The types of substrate are divided into three statistical clusters based on a Tukey post-hoc test. On mussel shells and weathered mussel shells, significantly more spat was observed than on most other substrates. There was no significant difference in spat collection on weathered mussel shells and granite, and granite also did not collect significantly more spat than the remainder of the substrates. The remainder of the substrate types did not show a significant difference in spat collection.

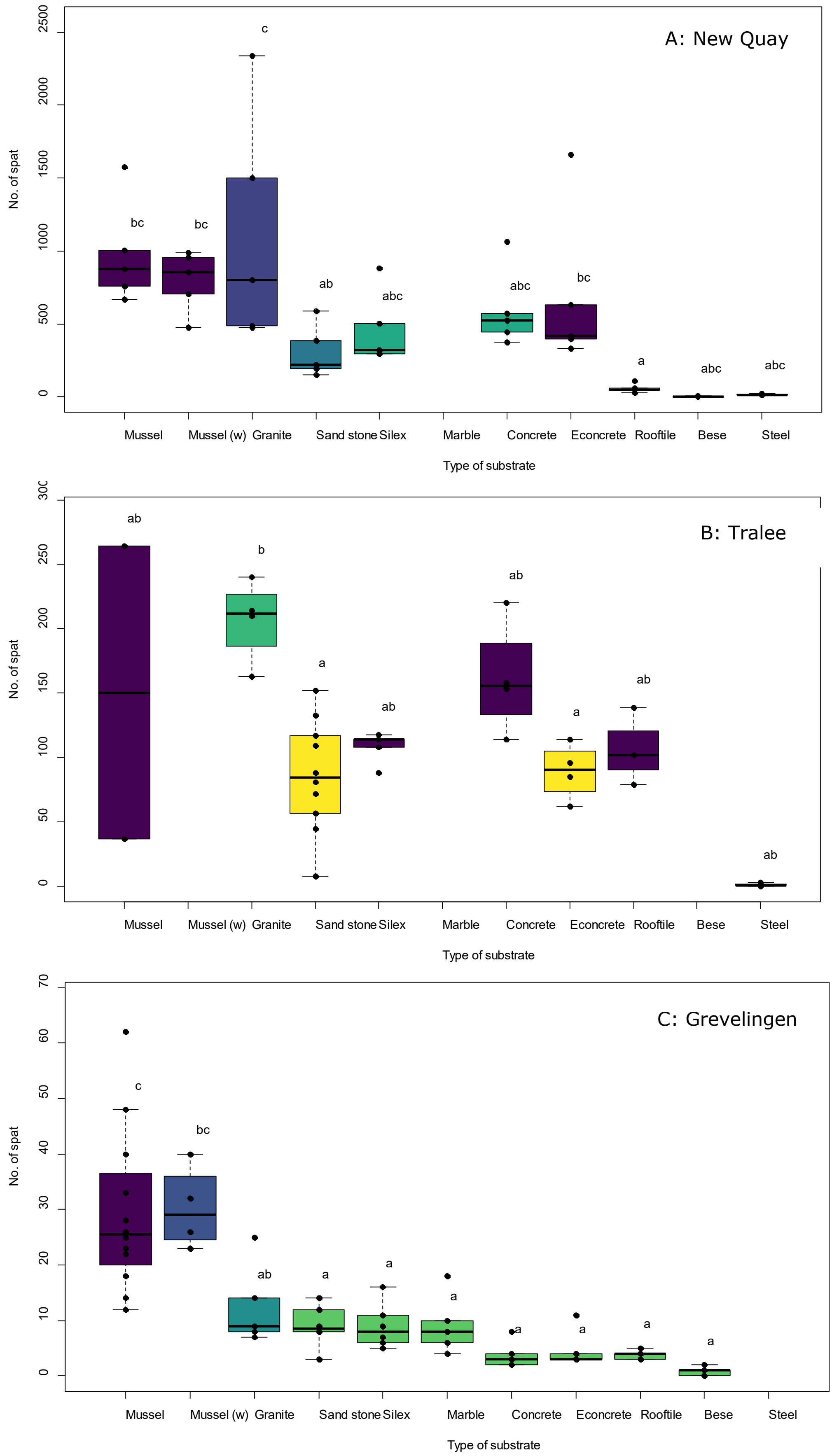


Figure 12: Boxplots showing the total number of spat per basket on the different types of substrates for the three different locations. Boxes represent the median, quartiles and interquartile outliers in total numbers of spat per location. Statistically insignificant substrates share a group, indicated by the letter(s) above the box and by colour. Substrates that share at least one letter do not differ significantly.

3.4 Spat per kg substrate

In New Quay, the highest number of flat oyster spat per kg substrate was found on fresh mussel shell. On average 1320 spat per kg was found per basket and a maximum of 2286 spat per kg was found in one basket. The minimum non-zero spat per kg (or lowest amount of spat found, not counting baskets with no spat) was found on steel (n=7.3). Figure 13A shows boxplots of the number of spat found per kg on the different substrates in each basket. Statistically insignificant substrates share a group, indicated by the letter(s) above the box and by colour. Substrates that share at least one letter do not differ significantly. There are two statistical groups of numbers of spat per kg for the different substrates in New Quay. Fresh and weathered mussels form one group, on these substrates significantly more spat per kg was observed than on all other substrates. The rest of the substrates form another group as there are no significant differences.

In Tralee the highest number of spat per kg was found on fresh mussel shell. On average 145.9 spat per kg was found per basket on this substrate, the maximum amount of spat per kg found in one basket was 254.1 on fresh mussel shell. Note that the standard deviation is high (SD=153.0) because only two baskets could be retrieved and counted. The minimum non-zero number of spat per kg was found on sandstone, where 2.9 spat were counted per kg in one basket. Figure 13B shows a boxplot of the number of spat found on the different substrates in each basket. The settlement of flat oyster on mussels differs significantly from all substrates except for steel. No significant difference was found between the other substrates.

In Lake Grevelingen, the highest number of Pacific oyster spat per kg was found on fresh mussel shell. On this substrate an average of 28.6 spat per kg and a maximum of 343.6 spat per kg was found per basket. No significant in spat per kg differences were found between fresh and weathered mussel shells. The lowest non-zero number of spat settlement was found on concrete (0.38 spat per kg in one basket). Figure 13C shows a boxplot of the number of spat found on the different substrates in each basket. Settlement of Pacific oyster spat on the two types of mussels is significantly higher than all other substrates except for steel. No spat was collected on steel.

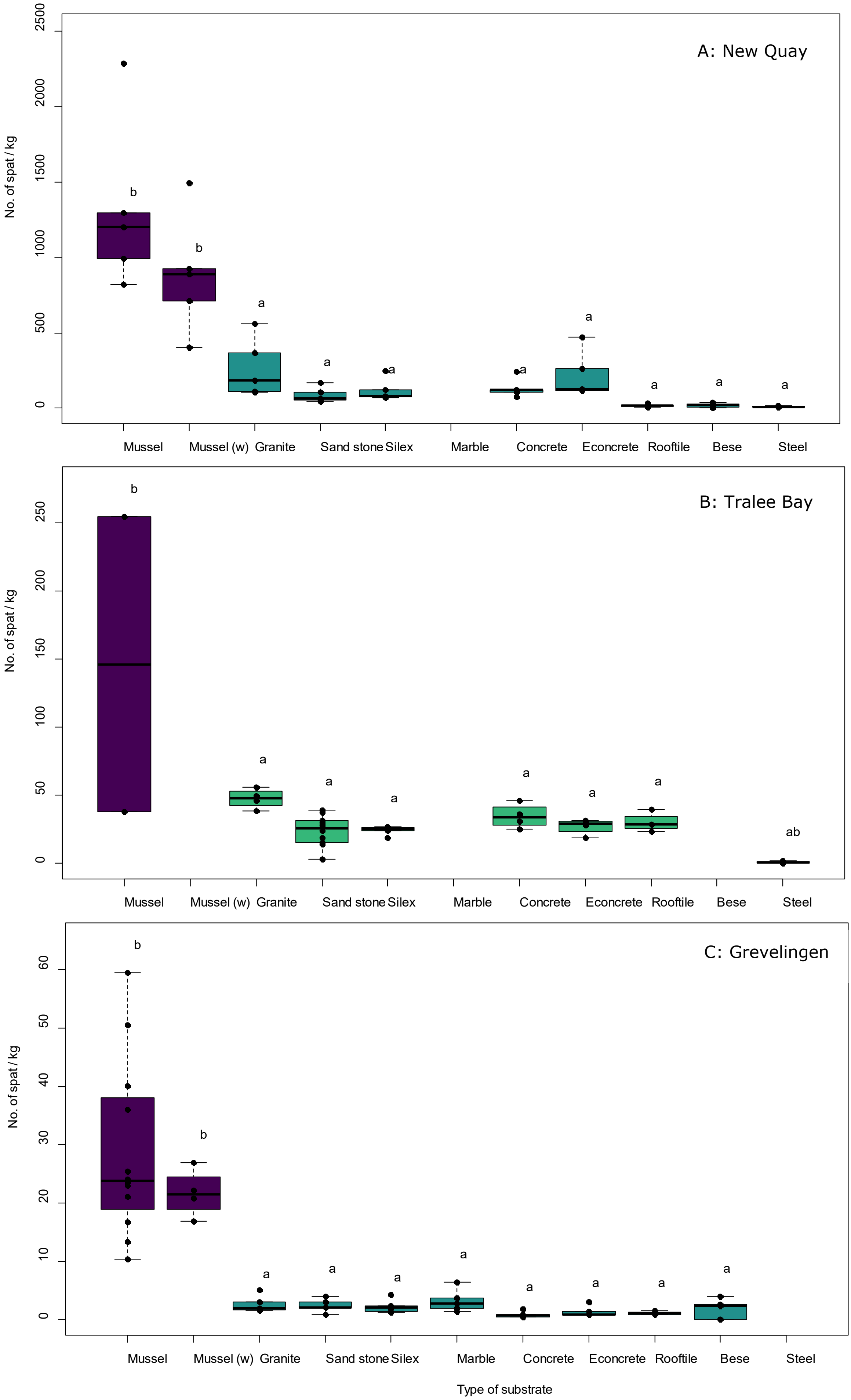


Figure 13: Boxplots showing the total number of spat per kg on the different types of substrates for the three different locations. Boxes represent the median, quartiles and interquartile outliers in total numbers of spat per location. Statistically insignificant substrates share a group, indicated by the letter(s) above the box and by colour. Substrates that share at least one letter do not differ significantly.

3.5 Spat per surface area

The flat oyster spat per cm^2 in New Quay shows that granite is the most successful substrate. On average 0.34 spat per cm^2 per basket was found on this substrate and highest density in one basket was 0.73 spat per cm^2 on granite. The minimum non-zero spat per kg (or lowest amount of spat found, not counting baskets with no spat) was found on weathered mussel shells; 0.004 spat per cm^2 found in one basket. Figure 14A shows a boxplot of the number of spat found on the different substrates in each basket. Settlement per cm^2 on granite was significantly higher than on weathered mussel, sandstone, steel and roof tile. Settlement on ECOncrete was significantly higher than settlement on roof tile, sandstone and steel. There were no significant differences in number of spat per cm^2 found between the other substrates.

In Tralee, the best substrate for settlement of flat oyster per cm^2 was granite. On average 0.06 spat per cm^2 were found per basket and in one basket a maximum settlement of 0.07 spat per cm^2 was found. The worst performing non-zero substrate was sandstone (0.001 spat per cm^2). Figure 14B shows a boxplot of the number of spat found on the different substrates in each basket. Granite shows a significantly different number of flat oyster spat per cm^2 than roof tile, mussel, sandstone and steel. Concrete shows a significantly higher settlement than mussels, sandstone and steel. Steel and sandstone collected significantly less spat than roof tile, ECOncrete, silex as well. No spat was found on BESE-elements.

In Lake Grevelingen settlement of Pacific oysters per cm^2 was highest on marble with an average settlement of 0.004 spat per cm^2 and maximum in one basket of 0.008 spat per cm^2. Spat settlement was lowest on BESE-elements (average 0.0002 spat per cm^2). Figure 14C shows a boxplot of the number of spat found on the different substrates in each basket. Settlement on BESE-elements is significantly lower than settlement on marble, silex, granite and fresh mussel. Settlement on marble was significantly higher than on concrete, roof tile and BESE-elements. No significant difference in settlement per cm^2 was found between the other substrates. No spat was found on steel.

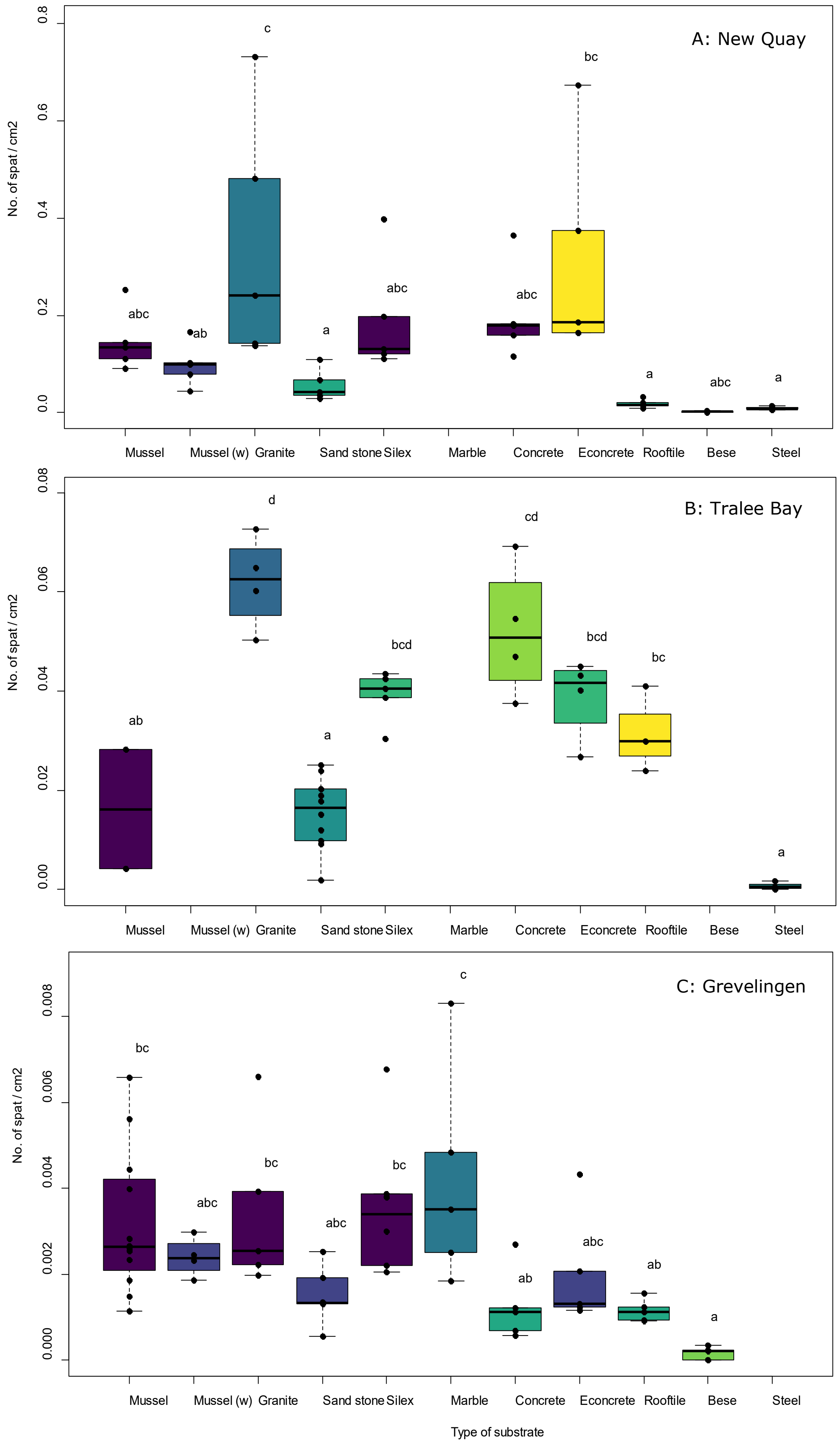


Figure 14: Boxplots showing the total number of spat per cm² on the different types of substrate for the three different locations. A) New Quay. B) Tralee bay. C) Lake Grevelingen (C. gigas spat). Boxes represent the median, quartiles and interquartile outliers in total numbers of spat per location. Statistically insignificant substrates share a group, indicated by the letter(s) above the box and by colour. Substrates that share at least

3.6 Fraction living spat

On average 86% of the flat oyster spat and 94% of the Pacific oyster spat found on the substrates was alive. However the texture and colour of some substrates made it difficult to distinguish living from dead spat (for example on ECOncrete). This may have resulted in a bias between substrates due to an underestimation of dead spat since it was more difficult to spot dead spat on substrates such ECOncrete and marble.

In New Quay, the fraction live spat differed per substrate. Only one spat was found on BESE-elements resulting in a 100% survival for this substrate. The sample size therefore needs to be taken into account when interpreting these results. The second best was found on weathered mussel shell, where average survival was (94%). The lowest average survival (68%) was found on steel. Figure 15A shows boxplots of the fractions living spat of total spat found on the different substrates in each basket. The substrates can be divided into different statistical groups. The fraction living spat on weathered mussel was significantly higher compared to the other substrates except for fresh mussel shell. The fraction living spat found on concrete and ECOncrete was significantly lower compared to both types of mussel shells and granite. Fraction living spat on rooftile, BESE-elements and steel did not differ significantly from any of the other groups, which is likely due to the relatively small number of flat oyster spat found on these substrates.

In Tralee, the highest average survival was found on mussel shell (99.4%; slightly higher than roof tile with 99%), and the lowest survival (95%) was found on sandstone. Figure 15B shows that there was no significant difference in fraction living flat oyster spat found on the different types of substrates. This is possibly the result of the loss of replicates and a relatively small number of observations of dead spat.

In Lake Grevelingen, the highest average survival of Pacific oyster spat was found on granite and roof tile (100%), and the lowest survival (86%) was found on silex. Figure 15C shows boxplots of the fractions living spat of total spat found on the different substrates in each basket. The fraction of living spat on fresh mussel shells differed significantly from the fraction on sandstone and silex. No significant difference in fraction of living spat was found between the other substrates. No dead spat was found on rooftile and granite, resulting in a very high standard error for these two substrates.

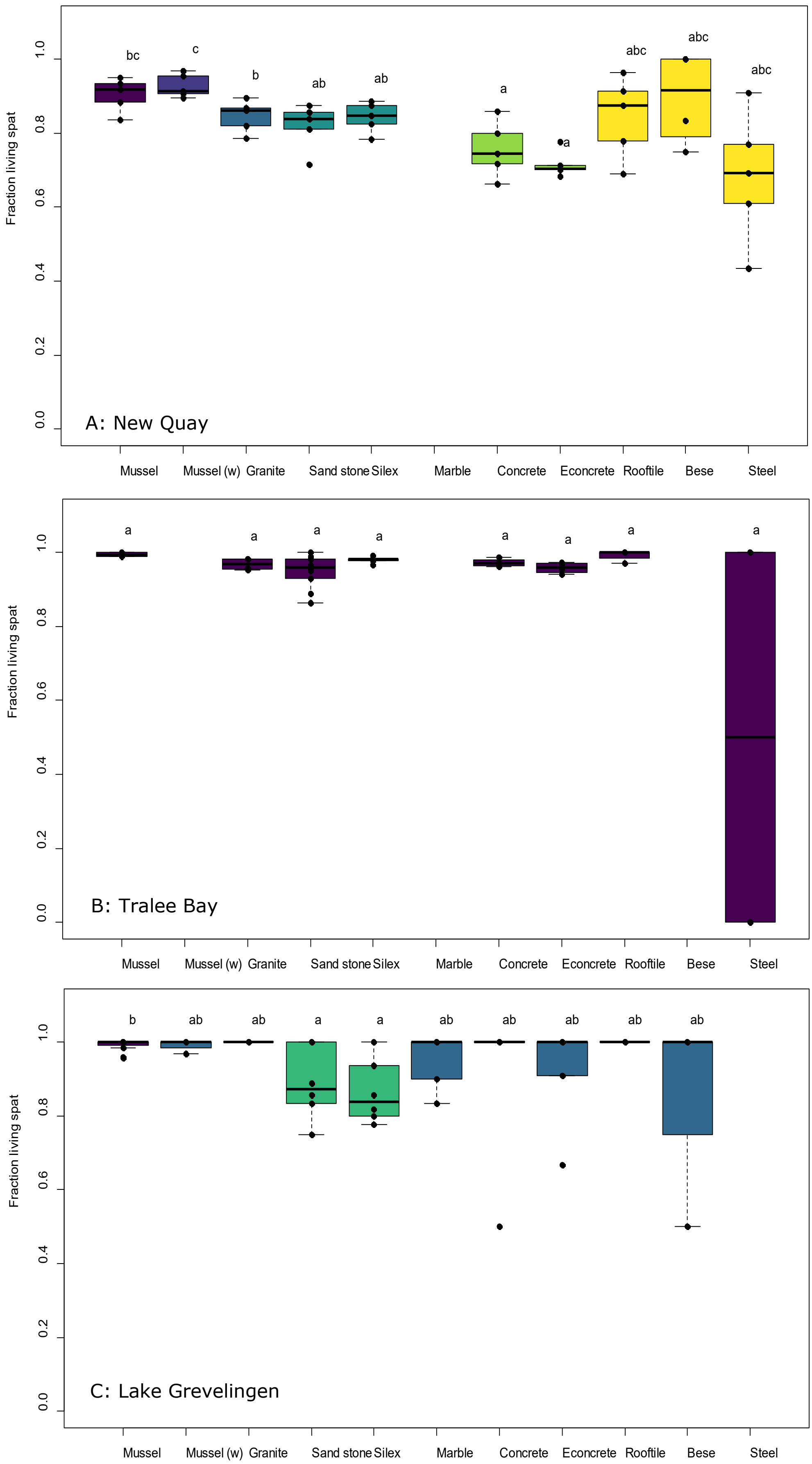


Figure 15: Boxplots showing the fraction of living spat out of the total spat on the different substrates for locations: A) New Quay, B) Tralee bay and C) Lake Grevelingen (C. gigas). Boxes represent the median, quartiles and interquartile outliers in total numbers of spat per location. Statistically insignificant substrates share a group, indicated by the letter(s) above the box and colour. Substrates that share at least one letter do not differ significantly.

3.7 Overall settlement success

Table gives an overview of the overall settlement success for the combination of the different measures of success (total spat per basket, spat per kg, spat per cm^2) of the different substrates for the different locations as described above. In New Quay mussels and weathered mussels performed best over the combined measures of settlement success with a score of 3 and 2 respectively. Roof tile and sandstone performed worst, both with a score of -3. In Tralee mussel was the best performing substrate with a score of 2, followed by granite, silex, concrete and steel with a score of 1. Sandstone was the worst performing substrate with a score of -3. In Lake Grevelingen, mussel and weathered mussel performed best. BESE-elements have the lowest overall settlement success with a score of -3, followed by roof tile and concrete which both have a score of -1.

Table 5: Overview of the overall settlement success for the combination of the three measures of settlement success for all types of substrate at the three different locations of the field experiment.

Substrate	New Quay	Tralee	Grevelingen
Mussel	3	2	3
Mussel (w)	2		3
Granite	1	1	1
Sandstone	-3	-3	-1
Silex	0	1	-1
Marble			-1
Concrete	0	1	-2
ECOncrete	1	-1	-1
Roof tile	-3	0	-2
BESE-elements	0		-3
Steel	-2	1	

4 Discussion & conclusions

4.1 Discussion

4.1.1 Main findings

In this research, field experiments were conducted to find the most suitable substrate for settlement of native European flat oysters with the aim to introduce flat oysters into the offshore wind farm Borssele V. Settlement success of oysters on various substrates were compared based on three different metrics (total settlement per basket, settlement per weight of substrate and settlement per surface area of substrate). Different results were found for each of these measures. Settlement per basket was highest for mussel shells and granite. Mussel shells proved to be the best substrate for oyster spat settlement per kg. In terms of spat collection per substrate area, granite, ECOncrete and marble were most successful. Overall settlement success which combines the different measures at all three locations was highest for fresh mussel shells. The less successful substrates vary more in terms of success, depending on the location. Many of the substrates showed no significant difference in settlement.

4.1.2 Oyster settlement

In terms of total spat settled on the different substrates per basket, mussel shell performed relatively well at all three locations. This is not unexpected, several studies have found oysters to settle more readily on oyster shell or alternatively other bivalve shells (Christianen et al., 2018, Korringa, 1952, van den Brink, 2012). In New Quay and Tralee granite also collected a lot of spat per basket. BESE-elements and steel collected the least total number of spat, though not significantly less than most other substrates. When analysing spat settlement per kg of substrate, mussel shell performed significantly better than the rest of the substrates at all three locations. The settlement success of oyster spat observed in the field experiment differed considerably per location, which is likely due to environmental conditions at the locations. In the settlement ponds, the circumstances are optimised for settlement of oysters with a controlled water circulation. At the other two locations, there were more uncontrolled factors such as wave action and biofouling. In Lake Grevelingen, almost no flat oysters were found even though larvae of flat oysters were detected in the water column (Figure **,** method section). According to several oyster farmers flat oyster spat fall was indeed very low in the western part of the Grevelingen (pers. comm. Tony van der Hiele). In Lake Grevelingen Pacific oyster spat was found in higher numbers. This exotic oyster has spread along the North Sea coast quickly over the last decade (Troost, 2010), as it is not susceptible to the Bonamia parasite that affects flat oysters, is more opportunistic during the larval stage and does not have a similar brood care as flat oysters (Ó Foighil and Taylor, 2000, Smaal et al., 2015).

Data of the number of oyster larvae in the water column was only available for New Quay and Lake Grevelingen. Therefore settlement success could not be compared between all three field experiment locations. Differences in spat settlement between locations are something that should be kept in mind when outplacing spat on substrate or aiming for collection of spat in situ. For collection of spat for outplacement, a controlled situation similar to the settlement ponds would be most effective. This hypothesis was confirmed by Spencer and Gough (1978), who found a lower survival in spat settlement experiments at sea, than in a nursery setting. However, the settlement ponds in New Quay cannot be used for this purpose as they are situated in an area where the Bonamia parasite is present (Culloty and Mulcahy, 2007)**.** Introduction of oysters from a Bonamia infected area is against regulation.

In previous experiments in the Dutch Voordelta (in the South of the Netherlands), less spat was collected when substrate was deployed too early (Sas et al., 2018). The assumption was, that this was due to the amount of biofouling on the substrate that hampers settlement of oyster larvae. Therefore, in our experiment substrates were deployed just before the main settlement peak of flat oysters was expected, allowing little time for a biofilm to develop. An interesting observation was that mussel shells seemed to attract less biofouling in the first few weeks of the field experiment than the other substrates. Mussel shells are known to repel biofouling through several mechanisms, a distinct microtopography (Bers et al., 2006) and chemical repellents (Bers et al., 2006) as long as their periostracum (a thin organic coating which is the outermost layer of the shell) is intact. In our field experiment there was no significant difference in settlement success between fresh and weathered oysters.

4.1.3 Settlement per surface area

The three-dimensional surface of the substrate is a measure for the total available space on which oyster spat can settle. Although total spat collection and spat collection per kg of substrate are useful for cost-efficiency calculations, analysis of the settlement success per cm^2 of substrate allows for comparing substrates based on available settlement space. Because of this correction, mussels performed worse when considering settlement per cm^2 of substrate than for the other measures of settlement success. In the field experiment, granite performed well in New Quay and Tralee (significantly better than mussel) in terms of settlement per area (cm^2) of substrate. In Lake Grevelingen marble showed the highest settlement per cm^2 but showed no significant differences with other substrates except concrete, roof tile and BESE-elements. Kamermans et al., (2004) found settlement between 0.0013 and 0.0067 flat oysters per cm^2 and between 0.65 and 6.31 Pacific oysters per cm^2 in Lake Grevelingen in 2003. Their substrates collected significantly more oyster spat than the substrates in our field experiments, which in the Grevelingen only collected a maximum of 0.0001 flat oysters per cm^2 and 0.004 Pacific oysters per cm^2. Kamermans and colleagues (2004) also found that mussel shells collected less oyster spat than the other collector types in their research (Chinese hats and tubes). Freeman and Denny (2003) found that settlement per cm^2 of substrate ranged between 0.4 and 3.4 for eastern oysters (*Crassostrea virginica)*. In experiments by Theuerkauf et al., (2015) a maximum of 0.03 spat per cm^2 was found. Nalesso and colleagues (2008) found that settlement of mangrove oysters (*Crassostrea* spp.) was highest on shell material, with an average settlement of 0.2 spat per cm^2. In our experiments the highest settlement per cm^2 was found on granite and ECOncrete in the spatting ponds of New Quay (both 0.3 spat per cm^2). Even though oyster settlement per cm^2 is the most suitable measure to compare between studies, settlement also depends strongly on environmental conditions and species. This could explain differences in settlement between these studies and our field experiments and needs to be taken into account.

Oysters only settle and metamorphose when there is suitable hard substrate available to settle on (Brown et al., 2010, Korringa, 1952). It has been suggested that the availability of hard substrate was more important for settlement of flat oysters than the nature of the substrate (Smyth et al., 2016). This would mean that the substrate with the highest relative surface area (mussel or BESE-elements) would collect most oyster spat per basket, but when correcting for available space to settle the preference for substrate type becomes visible. As mussel shells have more surface area for a similar weight than the other substrates except for BESE-elements, this substrate has a relatively high surface complexity. The research by Smyth and colleagues however compared settlement on two types of shell material, whereas this research compares more dissimilar types of settlement substrates. Nestelrode et al. (2007) argue that interstitial space and settlement space are important factors in settlement success of the Eastern oyster (*Crassostrea virginica*). This principle might also be applicable to flat oyster, leading to increased settling with increased substrate complexity. Although BESE-elements are structurally complex, the surface is smooth and settling organisms are more exposed, which could explain the lack of settlement of oysters on this substrate. On ECOncrete on the other hand, more settlement was observed on the smoother side as opposed to the more structured and rougher side.

4.1.4 Fraction living spat

Fractions of living spat found on the substrates were generally high, with average fractions living spat ranging between 68% and 100%. High survival (ranging between 99% and 97%) of Eastern oyster spat in an experimental setting was also found by Theuerkauf et al. (2015). There was not one substrate that showed a significantly higher or lower fraction of living spat in all three locations. No significant differences in the fraction living of total spat counted were found between substrates in Tralee, whereas for New Quay weathered mussel was the most successful substrate. In Lake Grevelingen silex and sandstone performed worst, but the fraction of living spat on these substrates was only significantly lower than the fraction of living spat on fresh mussels. However, the texture and colour of some substrates made it difficult to distinguish living from dead spat (for example on ECOncrete), resulting in a bias or potential underestimation of dead spat between substrates. Possibly dead spat that has fallen off further biased the results towards a higher spat survival. Factors that might contribute to differences in survival between locations are food availability and temperature. Food availability is a factor that can seriously limit growth, survival and mortality which is highest in the first 5-10 weeks (Utting, 1988). Temperature is also an important factor for survival of oyster larvae and spat. Experiments with extreme temperatures (10 °C and 32.5 °C) show significantly lower spat survival (Davis and Calabrese, 1969). Water temperatures in our experiments from settlement in July where between19 °C and didn't exceed 25 °C in summer (Figure 5B). Temperatures in North-Western European coastal areas generally fluctuate between 5 °C in winter and 23 °C in summer.Parameters measured in the water column such as pH, particulate matter and salinity were found not to affect oyster spat survival (Nell and Holliday, 1988, Utting, 1988). Extreme values for either of these environmental factors are likely to affect oyster spat survival but are not likely to have occurred in our field experiments.

4.1.5 Settlement cues

There are multiple factors other than substrate type that are of importance when considering the settlement of benthic organisms. It is unknown how large the role of the different substrates is compared to other environmental cues.

Spat settlement appeared to be higher on the more shaded underside of the substrates in the baskets. In addition, spat settlement was higher on the shaded North side of the settlement pond in New Quay. Shaded areas are not a prerequisite for settlement, but oysters do show preference for shade when choosing a location. Eastern oyster larvae settle more often on shaded surfaces than on illuminated areas (Baker & Mann, 1998; Shaw et al., 1970) and negative phototaxis (movement away from light) is also observed in the flat oyster (Cole & Jones, 1939; Walne, 1979). A less exposed part of the substrate might also offer some refuge from predation. Important predators flat oysters are crabs (Mascaró and Seed, 2001a, Mascaró and Seed, 2001b) and of young Eastern oysters are crabs, starfish, flatworms and oyster drill (Galtsoff, 1964). Brown shrimp predate on young Pacific oyster in the Wadden Sea (Weerman et al., 2014). Substrate that offers protection from predators might be favourable in this aspect. This could be a reason why oysters prefer to settle on the inside of mussel shells.

Occurrence of individuals of the same species was shown to be a vital cue for settlement of many sessile species (Jensen and Morse, 1990). Aggregation of conspecifics has an important ecological function, as it reduces the risk of predation and sedimentation as well as increase the chance of fertilisation (Gercken and Schmidt, 2014, Whitman and Reidenbach, 2012). Rodrigues-Perez et al. (2019) found a significant increase in settlement of flat oyster larvae when cues from conspecifics were present. The settlement near conspecifics is likely due to hydrophilic signalling molecules in the water column. For larvae of *Crassostrea sp.* NH_3 and L-DOPA, excreted by conspecifics, induces the typical searching behaviour exhibited by settling larvae (Pawlik and Hadfield, 1990, Whitman and Reidenbach, 2012). In order to optimise the survival of spat at outplacement, it is vital to know if density dependence plays a role in survival. If the spat mortality increases with spat densities, it is essential for survival of spat that the densities are not too high. Little information is available on density dependent mortality and growth in oyster spat. Juvenile Sydney rock oysters (*Saccostrea* *commercialis*) did not show a response in survival rate to increased stocking density (Holiday et al., 1991), but stocking density did result in reduced growth rate of Pacific oysters (Marshall and Dunham, 2013). However, the latter depends on environmental conditions such as food availability. If food is not limiting stocking densities can be high without affecting the growth rate. Therefore, under optimal conditions it can be assumed that there is a net positive influence of conspecifics on settlement of flat oysters.

Presence or absence of specific habitat-related microbial films plays an important role in the settlement of sessile organisms as they are a good indication of habitat type (Bao et al., 2007, Cameron and Hinegardner, 1974, Campbell et al., 2011, de Brito-Simith et al., 2017). Biofilm can provide organisms with information about the presence of food and indicates that a surface is not temporary (if a certain substrate is stationary it will develop a biofilm with time which is a potential indication for permanency) or toxic (Unabia and Hadfield, 1999). Permanence of the surface is indicated by the maturity of the biofilm, and the recruitment of sessile organisms is positively correlated with biofilm age (Bao et al., 2007, Campbell et al., 2011). Although for effective settlement of oyster larvae the biofilm should not be too old (van den Brink et al., submitted). Larvae of flat oysters have been shown to settle more readily on substrate with a biofilm that is associated with an environment where adult oysters grow (Rodriguez-Perez et al., 2019). Certain bacteria (e.g. *Alteromona colwelliana* and *Shewanella colwelliana*) are known to induce settlement of flat oysters, which is likely due to the production of L-DOPA and GABA by the bacterial film (Mesias-Gansbiller et al., 2013).

Bed topography influences the shear stress as well as the flux of oxygen and food particles to the bed and is therefore an important factor to take into account with regards to spat settlement. Higher (microscopic) rugosity (roughness) of the substrate results in increased settlement of larvae of flat oysters compared to smooth surfaces (Cole and Jones, 1939, Korringa, 1946). It has been suggested that reduced shear stress as a result of 3D bed topography helps increase settlement of oyster larvae (Whitman and Reidenbach, 2012). The topography is of direct influence on the hydrodynamics above the bed. The amount of hydrodynamic stress influences the possibility to settle because too much stress sweeps the spat away from the substrate (Crimaldi et al., 2002). Oyster larvae likely sense deformations and accelerations of the water using cilia or statocysts, thereby sensing the topography (Budelmann, 1988, Mackie et al., 1976). When assessing why one substrate is more attractive to settle on than the next, this rugosity might play a role.

Historically oysters are typically found attached to natural substrates rich in calcium carbonate, such as shells or coralline algae (Fitt et al., 1990). A study by the Danish Shellfish Centre (unpublished, but recorded by van den Brink, 2012) also reported that stacked discs (a.k.a. Chinese hat collectors) coated in lime (calcium carbonate), collected more than double the amount of oyster spat than uncoated Chinese hats. Calcium-rich substrates might be good settlement substrates, as oysters need calcium to build their shell as they grow. Just as our results show, oysters farmers suggest that mussel shells collect the highest total numbers of spat, making them the most cost-effective spat collector compared to Chinese hats and the plastic tubes that were used as spat collectors in the study by (van den Brink, 2012). Marble is also rich in calcium and therefore expected to be attractive for settlement of oysters if the calcium concentration is indeed a reason why shell material is one of the preferred substrates for oysters. Our settlement results from Lake Grevelingen indicate that this might indeed be the case, as settlement per cm^2 substrate was highest for marble. Marble could be a good substitute for the light shell material that is more likely to wash away due to hydrodynamics. Alternatively, a lime coating of substrates could be considered. However, a coating is often brittle and could therefore pose the risk of losing oyster spat. Other substrate types that do not contain calcium, such as granite also performed well in our field experiment. This could indicate that calcium, although it might play a role, it is not the primary factor in determining settlement for spat of flat oysters.

Coolen (2017) found that the species composition of the benthic community on natural hard substrate was different from that on artificial substrates in the North Sea. Therefore the type of substrate (natural versus artificial) may also influence the settlement of flat oysters. Our mussel shell material performed best in terms of settlement success per kg and in the Grevelingen also per basket. In lake Grevelingen, the artificial substrates performed worse in terms of settlement per cm^2 than the natural substrates. However, this was not the case in New Quay (where concrete and ECOncrete performed relatively well) and Tralee (where concrete, ECOncrete and rooftile performed well). Natural substrates in the form of biogenic reefs such as peat, polychaete and shellfish banks were naturally present in North Sea but have largely disappeared due to bottom disturbing activities (Beck et al., 2011, Berghahn and Ruth, 2005, Kamermans et al., 2018, Korringa, 1946, Lotze et al., 2006, Möbius, 1877, Smaal et al., 2015, Thurstan et al., 2013). A good choice for alternative natural substrate would then be shell material, however shells might be more prone to influences of current and wave action. In some areas of the North Sea, natural inorganic hard substrates occur (such as the Klaverbank, the Borkumse stenen, Helgoland and Sylt) (Michaelis et al., 2019, van Moorsel, 1993). Sizes of the geogenic hard substrate depend on the glacial history and the mobility of the sandy seafloor (Michaelis et al., 2019). The main gravel types in the Southern Bight of the North Sea consist of flint or silex, limestone, sandstone quartz, quartzite and some igneous rocks (Veenstra, 1969). Therefore, silex, sandstone and (red) granite can also be considered natural substrates, although naturally occurring mainly in gravel form. An additional reason to use natural substrate is that it might form a more natural system, thereby potentially allowing it to remain in the North Sea even after decommissioning of the wind farm. This would of course be desirable, as the complete mature ecosystem associated with an oyster reef takes time to develop.

4.2 Conclusions & recommendations

In this research, the settlement success of oyster larvae on different types of substrates was analysed. Our field experiment at three different locations showed that per basket mussels and granite were the most successful substrates for oyster settlement out of ten different substrate types. Based on settlement success per kg substrate mussel shells were most successful. In order to compare settlement success between different substrates and between studies, settlement per cm^2 is the most suitable measure. This way the effect of the space available for settlement is accounted for. It is therefore advisable to express settlement success in terms of settlement per surface area. Granite, ECOncrete and marble (Grevelingen) were most successful per surface area. However, the final choice of substrate not only depends on how successful it is in oyster spat collection but also on several other aspects such as cost-efficiency, applicability, and longevity or durability of the material. When considering number of spat per mass of substrate, mussel shells are indeed the substrate of choice. When taking into account the cost-effectiveness for application in eco-friendly scour protection, working with construction materials such as granite or (E)concrete or by-products such as silex or marble (both from quarrying) are good options.

4.2.1 Recommendations for outplacement-substrate

- When choosing a substrate for oyster spat collection aspects such as cost-efficiency, applicability, and longevity or durability of the material should be taken into account.
- When taking into account the cost-effectiveness for application in eco-friendly scour protection, working with construction materials such as granite (widely used in scour protection) or (E)concrete or by-products such as silex or marble (both from quarrying) are good options.
- Mussel shells appeared too fragile for exposed conditions in OWFs, when using shells as collectors for outplacement in OWF’s more robust shells such as flat oyster shells should be considered. Although more robust than mussel shells, oyster shells are also a lightweight substrate and therefore prone to hydrodynamic action. In order to apply shells as collector material and to avoid them from washing away they need to be deployed in a cage or container. Another option could be to mix the shell material with gravel, which could be a viable option to avoid washout as indicated by pilot studies in a flume tank (Rigter et al., 2019).
- In view of longevity of the ecosystem and persistence even after decommissioning of the wind farm, substrates that are originally found in the North Sea could be considered like shell material or (red) granite gravel. The drawback of these materials is stability.

- Although BESE-elements and steel were the least successful substrates in terms of settlement of oysters, they could potentially be used in combination with shell material to contain shells after outplacement.
- Outplacement of already settled spat in an OWF should be considered versus the more natural process of spat settlement in situ by introducing adult oysters as a brood stock. The advantage of settlement of spat in a controlled environment such as a spatting pond is a higher number of spat per substrate can be realised. It is however hard to find such spatting ponds in areas that are *Bonamia* free. In addition, survival rates of settled spat during transportation from the spatting pond to the OWF currently represent a factor of uncertainty but may outweigh risk factors such as sufficient larvae in the OWF or predation of freshly settled spat, versus more robust older spat.
- Of the initially planned substrates for outplacement (silex, sandstone and mussel shells) that were deployed in Tralee Bay, the mussel shells were lost. In addition, other substrates such as granite and concrete performed better then silex and sandstone. Considering the costs involved with shipping time and transport involved with outplacement an opportunistic approach is advisable that, besides silex and sandstone, granite and concrete (provided enough material is available) will also be used for the outplacement trial in Borssele V. In addition 70 L oyster shell will be deployed in Tralee Bay in June 2020 to collect oyster spat and to be used in the same outplacement trial in Borssele V.

4.2.2 Recommendations for future research

- Based on the research described here future research is recommended using a similar experimental approach on the most successful substrate materials granite, (E)concrete and marble at an outplacement location offshore.
- When a similar type of experiment is considered in Lake Grevelingen, the focus should be on location (smaller experiments determining best spat fall locations in Lake Grevelingen). Once a location is determined where chances of *O. edulis* settlement are better, marble or other calcium rich substrates could be tested for *O. edulis* settlement as well as more robust oyster shells instead of mussel shells.
- Future research is recommended to examine whether differences in substrate rugosity result in differences in settlement. Hydrodynamics around the substrates or even settlement on substrates itself could for example be tested in a flume. This wave tank allows to precisely control the current velocities and measure the flow velocities around an object.
- Development of a specific biofilm might be an interesting way to make a specific substrate more attractive for settlement of flat oysters. Further research is needed to asses if this can increase oyster settlement.

5 Acknowledgements

We would like to thank Denis O’Shea, Iarfhlaith Çonnellan and their employees for placing and maintaining the oyster substrate as well as their valuable input for the experiments. We would also like to thank Pim van Dalen for preparation of the baskets and fieldwork assistance. In addition we thank Emiel Brummelhuis and Jack Perdon for field assistance. Niels Wagenaar, Tony van der Hiele and various students of the Hogeschool Zeeland for helping out with counting the spat. Tjeerd Bouma and Jim van Belzen for input for the experiments. Sander Holthuijsen (NIOZ Texel) for assisting with the 3D scanning methods, Lydia Meesters for her help with the 3D scanners in Wageningen and Marinka Puyenbroek for advising in the process of photogrammetry. Finally we would like to thank Roy Osinga from ECOncrete and Truls Sverdvik from Norock (marble) for providing substrate material.

6 Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. This certificate is valid until 15 December 2021. The organisation has been certified since 27 February 2001. The certification was issued by DNV GL.

References

BAO, W.-Y., SATUITO, C. G., YANG, J.-L. & KITAMURA, H. 2007. Larval settlement and metamorphosis of the mussel *Mytilus galloprovincialis* in response to biofilms. *Marine Biology : International Journal on Life in Oceans and Coastal Waters,* 150**,** 565-574.

BECK, M. W., BRUMBAUGH, R. D., AIROLDI, L., CARRANZA, A., COEN, L. D., CRAWFORD, C., DEFEO, O., EDGAR, G. J., HANCOCK, B., KAY, M. C., LENIHAN, H. S., LUCKENBACH, M. W., TOROPOVA, C. L., ZHANG, G. & GUO, X. 2011. Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. *BioScience,* 61**,** 107-116.

BERGHAHN, R. & RUTH, M. 2005. The Disappearance of the Oysters from the Wadden Sea: A Cautionary Tale For No-Take Zones. *Aquatic conservation : marine and freshwater ecosystems.,* 15**,** 91-104.

BERS, A. V., PRENDERGAST, G. S., ZÜRN, C. M., HANSSON, L., HEAD, R. M. & THOMASON, J. C. 2006. A comparative study of the anti-settlement properties of mytilid shells. *Biology Letters,* 2**,** 88-91.

BESE-PRODUCTS. 2019. *BESE-products* [Online]. Available: https://www.bese-products.com/ [Accessed January 14 2020].

BORSJE, B. W., VAN WESENBEECK, B. K., DEKKER, F., PAALVAST, P., BOUMA, J., VAN KATWIJK, M. M. & DE VRIES, M. 2011. How ecological engineering can serve in coastal protection. *Ecological engineering,* 37**,** 113-122.

BOUMA, T. J., OLENIN, S., REISE, K. & YSEBAERT, T. 2009. Ecosystem engineering and biodiversity in coastal sediments: posing hypotheses. *Helgoland Marine Research,* 63**,** 95-106.

BROWN, S., HANDLEY, S., MICHAEL, K. & SCHIEL, D. 2010. Annual pattern of brooding and settlement in a population of the flat oyster Ostrea chilensis from central New Zealand. *New Zealand journal of geology and geophysics.,* 44**,** 217-227.

BUDELMANN, B.-U. 1988. Morphological Diversity of Equilibrium Receptor Systems in Aquatic Invertebrates. *In:* ATEMA, J., FAY, R. R., POPPER, A. N. & TAVOLGA, W. N. (eds.) *Sensory Biology of Aquatic Animals.* NY: Springer, New York.

BUHL-MORTENSEN, L., VANREUSEL, A., GOODAY, A. J., LEVIN, L. A., PRIEDE, I. G., BUHL-MORTENSEN, P. L., GHEERARDYN, H., KING, N. J. & RAES, M. 2010. Biological structures as a source of habitat heterogeneity and biodiversity on the deep ocean margins Biological structures and biodiversity. *Marine Ecology,* 31**,** 21-50.

CAMERON, R. A. & HINEGARDNER, R. T. 1974. Initiation of Metamorphosis in Laboratory Cultured Sea Urchins. *Biological Bulletin,* 146**,** 335-342.

CAMPBELL, A. H., MERITT, D. W., FRANKLIN, R. B., BOONE, E. L., NICELY, C. T. & BROWN, B. L. 2011. Effects of age and composition of field-produced biofilms on oyster larval setting. *Biofouling,* 27**,** 255-265.

CHRISTIANEN, M. J. A., LENGKEEK, W., BERGSMA, J. H., COOLEN, J. W. P., DIDDEREN, K., DORENBOSCH, M., DRIESSEN, F. M. F., KAMERMANS, P., REUCHLIN-HUGENHOLTZ, E., SAS, H., SMAAL, A., VAN DEN WIJNGAARD, K. A. & VAN DER HAVE, T. M. 2018. Return of the native facilitated by the invasive? Population composition, substrate preferences and epibenthic species richness of a recently discovered shellfish reef with native European flat oysters (Ostrea edulis) in the North Sea. *Marine Biology Research,* 14**,** 590-597.

COLE, H. A. & JONES, E. W. K. 1939. Some observations and experiments on the setting behaviour of larvae of *Ostrea edulis*. *ICES Journal of Marine Science,* 14**,** 86-105.

COOLEN, J. W. P. 2017. *North Sea reefs, benthic biodiversity of artificial and rocky reefs in the southern North Sea.* Wageningen University.

COON, S. L., WALCH, M., FITT, W. K., WEINER, R. M. & BONAR, D. B. 1990. Ammonia Induces Settlement Behavior in Oyster Larvae. *Biological Bulletin,* 179**,** 297-303.

CRANFIELD, H. J. 1973. Observations on the behaviour of the pediveliger of Ostrea edulis during attachment and cementing. *Marine Biology,* 22**,** 203-209.

CRIMALDI, J. P., THOMPSON, J. K., ROSMAN, J. H., LOWE, R. J. & KOSEFF, J. R. 2002. Hydrodynamics of larval settlement: The influence of turbulent stress events at potential recruitment sites. *Limnology and Oceanography,* 47**,** 1137-1151.

CULLOTY, S. C. & MULCAHY, M. 2007. Bonamia ostreae in the Native Oyster Ostrea edulis. A review. *Marine Envirnment and health series.* University of Cork.

DAVIS, H. C. & CALABRESE, A. 1969. Survival and Growth of Larvae of the European Oyster (Ostrea edulis L.) at Different Temperatures. *Biological Bulletin,* 136**,** 193-199.

DE BRITO-SIMITH, D. J., ABRUNHOSA, F. A. & DIELE, K. 2017. Metamorphosis of the edible mangrove crab Ucides cordatus (Ucididae) in response to benthic microbial biofilms. *Journal of Experimental Marine Biology and Ecology,* 492**,** 132-140.
ECONCRETE. 2019. *ECOncrete* [Online]. Available: https://econcretetech.com/ [Accessed January 14 2020].
ENGELSMA, M. Y., KERKHOFF, S., ROOZENBURG, I., HAENEN, O. L. M., VAN GOOL, A., SISTERMANS, W., WIJNHOVEN, S. & HUMMEL, H. 2010. Epidemiology of Bonamia ostreae infecting European flat oysters Ostrea edulis from Lake Grevelingen, The Netherlands. *Marine Ecology Progress Series,* 409**,** 131-142.
FITT, W. K., COON, S. L., WALCH, M., WEINER, R. M., COLWELL, R. R. & BONAR, D. B. 1990. Settlement behavior and metamorphosis of oyster larvae (*Crassostrea gigas*) in response to bacterial supernatants. *Marine Biology : International Journal on Life in Oceans and Coastal Waters,* 106**,** 389-394.
GALTSOFF, P. S. 1964. The American oyster Crassostrea virginica Gmelin. *Fishery Bulletin,* 64**,** 1-480.
GERCKEN, J. & SCHMIDT, A. 2014. Current Status of the European Oyster (*Ostrea edulis*) and Possibilities for Restoration in the German North Sea 2014. Bonn, Germany: Bundesamt für Naturschutz.
GRABOWSKI, J. H. & PETERSON, C. H. 2007. Restoring oyster reefs to recover ecosystem services. *Theoretical Ecology Series,* 4**,** 281-298.
HOLIDAY, J. E., ALLAN, G. L. & FRANCES, J. 1991. Cold storage effects on setting of larvae of the Sydney rock oyster, Saccostrea commercialis, and the Pacific oyster, Crassostrea gigas. *Aquaculture,* 92**,** 179-185.
HOLMES, G. 2008. Estimating three-dimensional surface areas on coral reefs. *Journal of Experimental Marine Biology and Ecology,* 365**,** 67-73.
IENM 2014. North Sea 2050 Spatial Agenda. Rijksoverheid, Ministry of Infrastructure and the Environment.
JENSEN, R. A. & MORSE, D. E. 1990. Chemically induced metamorphosis of polychaete larvae in both the laboratory and ocean environment. *Journal of Chemical Ecology,* 16**,** 911-930.
JONATHAN, H. G., ROBERT, D. B., ROBERT, F. C., ANDREW, G. K., JAMES, J. O., CHARLES, H. P., MICHAEL, F. P., SEAN, P. P. & ASHLEY, R. S. 2012. Economic Valuation of Ecosystem Services Provided by Oyster Reefs. *BioScience,* 62**,** 900-909.
KAMERMANS, P., BRUMMELHUIS, E., POELMAN, J., VAN GOOL, A. & TROOST, K. 2004. *Onderzoek naar verbetering broedvangst oesters,* IJmuiden [etc.], Nederlands Instituut voor Visserij Onderzoek (RIVO).
KAMERMANS, P., WALLES, B., KRAAN, M., VAN DUREN, L. A., KLEISSEN, F., VAN DER HAVE, T. M., SMAAL, A. C. & POELMAN, M. 2018. Offshore Wind Farms as Potential Locations for Flat Oyster (Ostrea edulis) Restoration in the Dutch North Sea. *Sustainability,* 10.
KAMP, H. G. J. 2016. Kavelbesluit V (innovatiekavel) windenergiegebied Borssele. *In:* ZAKEN, M. V. E. (ed.).
KENNEDY, R. J. & ROBERTS, D. 1999. A survey of the current status of the flat oyster Ostrea edulis in Strangford Lough, Northern Ireland, with a view to the restoration of its oyster beds. *Biology and Environment,* 99**,** 79-88.
KNIGHTS, A. M. & WALTERS, K. 2010. Recruit-recruit interactions, density-dependent processes and population persistence in the eastern oyster Crassostrea virginica. *Marine Ecology Progress Series,* 404**,** 79-90.
KOEHL, M. A. R. 2007. Mini review: Hydrodynamics of larval settlement into fouling communities. *Biofouling,* 23**,** 357-368.
KORRINGA, P. 1946. The decline of natural oyster beds. *Basteria,* 10**,** 36-41.
KORRINGA, P. 1952. Recent Advances in Oyster Biology. *The Quarterly Review of Biology,* 27**,** 266-308.
KORRINGA, P. 1976. *Farming the Cupped Oysters of the Genus Crassostrea: A Multidisciplinary Treatise*, Elsevier Scientific Publishing Company.
LILLIS, A., EGGLESTON, D. B., BOHNENSTIEHL, D. R. & THRUSH, S. 2013. Oyster Larvae Settle in Response to Habitat-Associated Underwater Sounds. *PLoS ONE,* 8**,** e79337.
LOTZE, H. K., JEREMY, B. C. J., LENIHAN, H. S., BOURQUE, B. J., BRADBURY, R. H., COOKE, R. G., KAY, M. C., KIDWELL, S. M., KIRBY, M. X. & PETERSON, C. H. 2006. Depletion, Degradation, and Recovery Potential of Estuaries and Coastal Seas. *Science,* 312**,** 1806-1809.
MAATHUIS, M. A. M., COOLEN, J. W. P., VAN DER HAVE, T. M. & KAMERMANS, P. 2020. Factors determining the timing of swarming of European flat oyster (*Ostrea edulis* L.) larvae in the Dutch Delta area: Implications for flat oyster restoration. *Journal of Sea Research,* 156.
MACDONALD, J., FREER, A. & CUSACK, M. 2010. Attachment of oysters to natural substrata by biologically induced marine carbonate cement. *Marine biology.,* 157**,** 2087.
MACKENZIE, C. L. 1970. Oyster culture in Long Island Sound 1966-69. U.S. Department of the interior Fish and Wildlife Service.
MACKIE, G. O., SINGLA, C. L. & THIRIOTQUIEVREUX, C. 1976. Nervous Control of Ciliary Activity in Gastropod Larvae. *Biological Bulletin,* 151**,** 182-199.

MARSHALL, R. D. & DUNHAM, A. 2013. Effects of culture media and stocking density on biofouling, shell shape, growth, and survival of the Pacific oyster (Crassostrea gigas) and the Manila clam (Venerupis philippinarum) in suspended culture. *Aquaculture,* 406-407**,** 68-78.
MASCARÓ, M. & SEED, R. 2001a. Choice of prey size and species in Carcinus maenas (L.) feeding on four bivalves of contrasting shell morphology. *Hydrobiologia : The International Journal of Aquatic Sciences,* 449**,** 159-170.
MASCARÓ, M. & SEED, R. 2001b. Foraging behavior of juvenile *Carcinus maenas* (L.) and *Cancer pagurus* L. *Marine Biology : International Journal on Life in Oceans and Coastal Waters,* 139**,** 1135-1145.
MESIAS-GANSBILLER, C., SILVA, A., MANEIRO, V., PAZOS, A., SANCHEZ, J. L. & PEREZ-PARALLE, M. L. 2013. Effects of chemical cues on larval settlement of the flat oyster (Ostrea edulis L.): A hatchery approach. *Aquaculture,* 376**,** 85-89.
MICHAELIS, R., HASS, H. C., MIELCK, F., PAPENMEIER, S., SANDER, L., EBBE, B., GUTOW, L. & WILTSHIRE, K. H. 2019. Hard-substrate habitats in the German Bight (South-Eastern North Sea) observed using drift videos. *Journal of Sea Research,* 144**,** 78-84.
MÖBIUS, K. A. 1877. *Die Auster und die Austernwirthschaft,* Berlin, Verlag von Wiegandt, Hemple & Parey.
NALESSO, R. C., PARESQUE, K., PIUMBINI, P. P., TONINI, J.-P. R., ALMEIDA, L. G. & NÍCKEL, V. M. 2008. Oyster spat recruitment in Espírito Santo State, Brazil, using recycled materials. *Brazilian Journal of Oceanography* [Online], 56.
NAUMANN, M. S., NIGGL, W., LAFORSCH, C., GLASER, C. & WILD, C. 2009. Coral surface area quantification-evaluation of established techniques by comparison with computer tomography. *Coral Reefs : Journal of the International Society for Reef Studies,* 28**,** 109-117.
NELL, J. A. & HOLLIDAY, J. E. 1988. Effects of salinity on the growth and survival of Sydney rock oyster (Saccostrea commercialis) and Pacific oyster (Crassostrea gigas) larvae and spat. *Aquaculture,* 68**,** 39-44.
NESTLERODE, J. A., LUCKENBACH, M. W., RSQUO & BEIRN, F. X. 2007. Settlement and Survival of the Oyster Crassostrea virginica on Created Oyster Reef Habitats in Chesapeake Bay. *Restoration Ecology,* 15**,** 273-283.
Ó FOIGHIL, D. & TAYLOR, D. J. 2000. Evolution of Parental Care and Ovulation Behavior in Oysters. *Molecular Phylogenetics and Evolution,* 15**,** 301-313.
OORD, V. 2018. Project plan Borssele V - Ecoscour.
OSPAR 2008. Case reports for the OSPAR list of threatened and/or declining species and habitats. *Biodiversity Series.* London, UK.
PAWLIK, J. & HADFIELD, M. G. 1990. A symposium on chemical factors that influence the settlement and metamorphosis of marine invertebrate larvae: introduction and perspective. *Bulletin of Marine Science,* 46**,** 450-454.
POGODA, B., BROWN, J., HANCOCK, B., PRESTON, J., POUVREAU, S., KAMERMANS, P., SANDERSON, W. & NORDHEIM, V. H. 2019. The Native Oyster Restoration Alliance (NORA) and the Berlin Oyster Recommendation: bringing back a key ecosystem engineer by developing and supporting best practice in Europe. *Aquatic Living Resources,* 32.
RIGTER, J., BEKENDAM, J. W. & DONSELAAR, K. 2019. *Ecologische verrijking van breuksteenconstructies.* BSc, Hogeschool van Arnhem en Nijmegen.
ROBERT, R., VIGNIER, J. & PETTON, B. 2017. Influence of feeding regime and temperature on development and settlement of oyster Ostrea edulis (Linnaeus, 1758) larvae. *Aquaculture Research,* 48**,** 4756-4773.
RODRIGUEZ-PEREZ, A., JAMES, M., DONNAN, D. W., HENRY, T. B., MOLLER, L. F. & SANDERSON, W. G. 2019. Conservation and restoration of a keystone species: Understanding the settlement preferences of the European oyster (Ostrea edulis). *Mar Pollut Bull,* 138**,** 312-321.
SAS, H., DEDEN, B., REUCHLIN, E., KAMERMANS, P., ZU ERMGASSEN, P., POGODA, B., HELMER, L., LOWN, A., ZWERSCHKE, N. & PRESTON, J. in prep. Bonamia infection in flat oysters (Ostrea edulis) in relation to European restoration projects.
SAS, H., KAMERMANS, P., HAVE, T. M. V. D., CHRISTIANEN, M. J. A., COOLEN, J. W. P., LENGKEEK, W., DIDDEREN, K., DRIESSEN, F., BERGSMA, J., DALEN, P., GOOL, A. V., POOL, J. V. D. & WEIDE, B. E. V. D. 2018. Shellfish reef restoration pilots : Voordelta The Netherlands : annual report 2017. Wageningen: Wageningen University & Research.
SAWUSDEE, A., JENSEN, A. C., COLLINS, K. J. & HAUTON, C. 2015. Improvements in the physiological performance of European flat oysters Ostrea edulis (Linnaeus, 1758) cultured on elevated reef structures: Implications for oyster restoration. *Aquaculture,* 444**,** 41-48.
SHUMWAY, S. 2011. Shellfish aquaculture and the environment. Ames, Iowa: Wiley-Blackwell.
SMAAL, A., KAMERMANS, P., KLEISSEN, F., DUREN, L. V. & HAVE, T. V. D. 2017. Flat oysters on offshore wind farms : opportunities for the development of flat oyster populations on existing and planned wind farms in the Dutch section of the North Sea. Yerseke: Wageningen Marine Research.

SMAAL, A. C., KAMERMANS, P., HAVE, T. M. V. D., ENGELSMA, M. & SAS, H. J. W. 2015. Feasibility of Flat Oyster (Ostrea edulis L.) restoration in the Dutch part of the North Sea. IJmuiden: IMARES Wageningen UR.

SMYTH, D., KREGTING, L., ELSÄßER, B. R., KENNEDY, R. & ROBERTS, D. 2016. Using particle dispersal models to assist in the conservation and recovery of the overexploited native oyster (Ostrea edulis) in an enclosed sea lough. *Journal of Sea Research,* 108**,** 50-59.

SMYTH, D. & ROBERTS, D. 2010. The European oyster (*Ostrea edulis*) and its epibiotic succession. *Hydrobiologia : The International Journal of Aquatic Sciences,* 655**,** 25-36.

SPENCER, B. E. & GOUGH, C. J. 1978. The growth and survival of experimental batches of hatchery-reared spat of Ostrea edulis L. and Crassostrea gigas thunberg, using different methods of tray cultivation. *Aquaculture,* 13**,** 293-312.

STEVEN, B. S., SEAN, P. P., KENNETH, L. H. & DOROTHY, B. 2011. Oyster Reefs as Natural Breakwaters Mitigate Shoreline Loss and Facilitate Fisheries. *PLOS ONE* [Online], 6.

STIMSON, J. & KINZIE, R. A. 1991. The temporal pattern and rate of release of zooxanthellae from the reef coral Pocillopora damicornis (Linnaeus) under nitrogen-enrichment and control conditions. *Journal of Experimental Marine Biology and Ecology,* 153**,** 63-74.

THEUERKAUF, S. J., BURKE, R. P. & LIPCIUS, R. N. 2015. Settlement, Growth, and Survival of Eastern Oysters on Alternative Reef Substrates. *Journal of Shellfish Research,* 34**,** 241-250.

THURSTAN, R. H., HAWKINS, J. P., RABY, L. & ROBERTS, C. M. 2013. Oyster (*Ostrea edulis*) extirpation and ecosystem transformation in the Firth of Forth, Scotland. *Journal for Nature Conservation,* 21**,** 253-261.

TROOST, K. 2010. Causes and effects of a highly successful marine invasion: Case-study of the introduced Pacific oyster Crassostrea gigas in continental NW European estuaries. *Journal of Sea Research,* 64**,** 145-165.

UNABIA, C. R. C. & HADFIELD, M. G. 1999. Role of bacteria in larval settlement and metamorphosis of the polychaete Hydroides elegans. *Marine Biology,* 133**,** 55-64.

UTTING, S. D. 1988. The growth and survival of hatchery-reared Ostrea edulis L. spat in relation to environmental conditions at the on-growing site. *Aquaculture,* 69**,** 27-38.

VAN DEN BRINK, A. 2012. OYSTERECOVER: The efficency of different types of oyster spat collectors for *Ostrea edulis*. Yerseke: Wageningen Marine Research.

VAN MOORSEL, G. W. N. M. 1993. *Long-term recovery of geomorphology and population development of large molluscs after gravel extraction at the Klaverbank (North Sea),* Culemborg, Bureau Waardenburg.

VEAL, C. J., HOLMES, G., HOEGH-GULDBERG, O., NUNEZ, M. & OSBORN, J. 2010. A comparative study of methods for surface area and three dimensional shape measurement of coral skeletons. *Limnology and Oceanography: Methods,* 8**,** 241-253.

VEENSTRA, H. J. 1969. Gravels of Southern North Sea. *Marine Geology,* 7**,** 449-&.

WEERMAN, E., ERIKSSON, B. K., OLFF, H. & VAN DER HEIDE, T. 2014. Predation by native brown shrimp on invasive Pacific oyster spat. *Journal of Sea Research,* 85**,** 126-130.

WHITEHOUSE, R. J., HARRIS, J. M., SUTHERLAND, J. & REES, J. 2011. The nature of scour development and scour protection at offshore windfarm foundations. *Marine pollution bulletin,* 62**,** 73-88.

WHITMAN, E. R. & REIDENBACH, M. A. 2012. Benthic flow environments affect recruitment of Crassostrea virginica larvae to an intertidal oyster reef. *Marine Ecology Progress Series,* 463**,** 177-191.

WILSON, J. C. & ELLIOTT, M. 2009. The habitat-creation potential of offshore wind farms. *Wind Energy,* 12**,** 203-212.

Justification

Report C063/20
Project Number: 4313100095

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: Oscar Bos
Researcher

Signature:

Date: 08-07-2020

Approved: Jakob Asjes
Manager integration

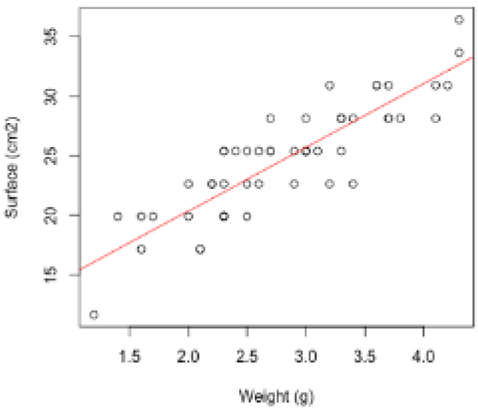
Signature:

Date: 08-07-2020

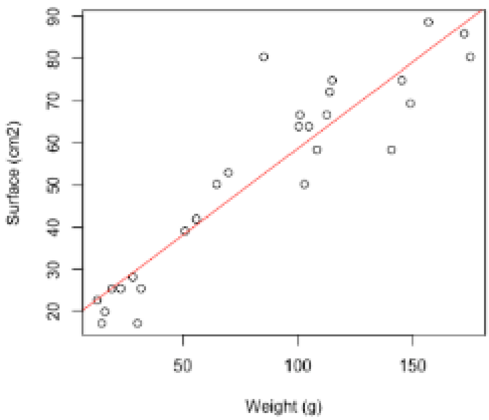
Annex 1 Wax dipping

This appendix gives an overview of the number of pieces that were dipped in wax for each substrate and the resulting R^2 of the relation between the weight of the substrate and the three-dimensional surface area as calculated using the conversion factor in **equation x** and **figure x**. All figures show the three-dimensional surface in cm^2 plotted against the substrate weight. Above the figures the R^2 of this relation is displayed and N shows the number of pieces of substrate dipped in wax for all the different types of substrates.

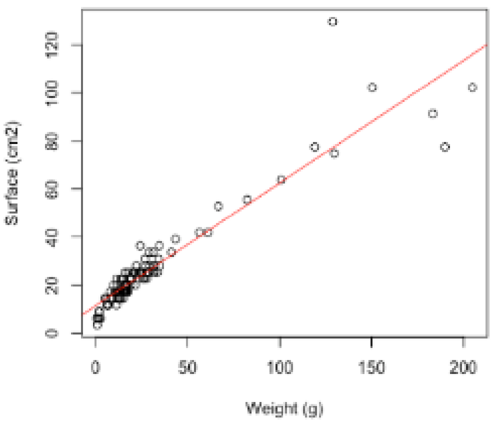
Mussel shells N= 56 R^2 = 0.7569024



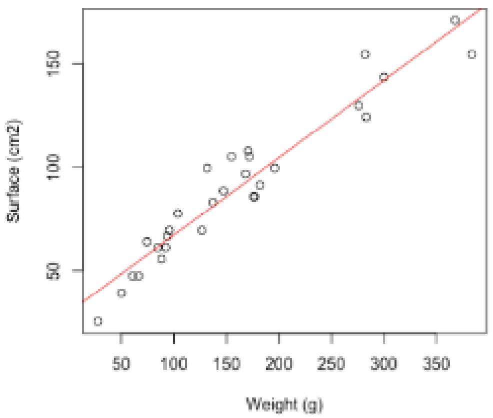
Granite N= 27 R^2 = 0.8565559



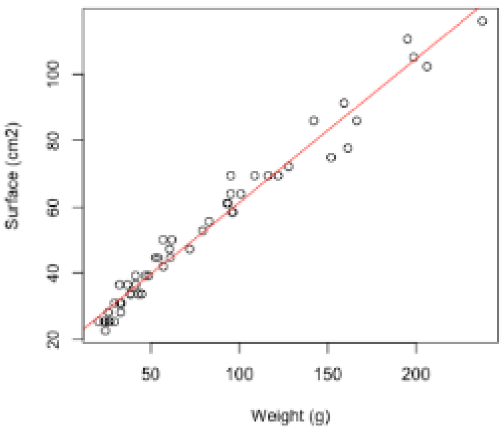
Sandstone N= 114 R^2 = 0.8803807



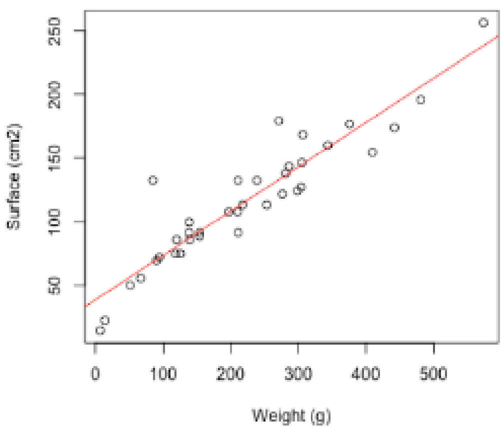
Silex N= 29 R^2 = 0.9229932



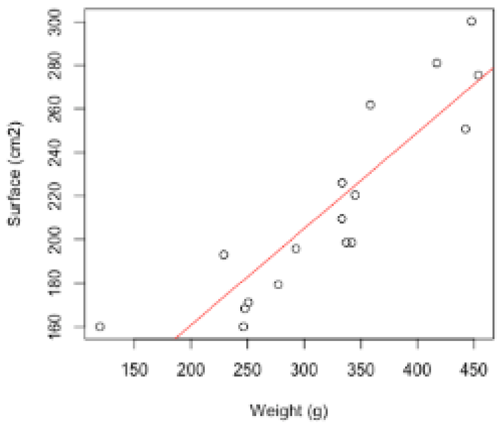
Marble N= 52 R^2 = 0.9714775



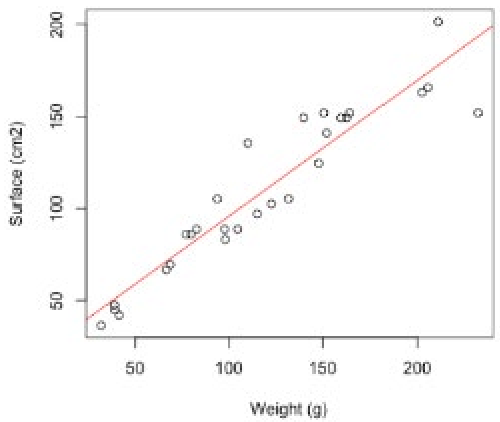
Concrete N= 36 R^2 = 0.861008



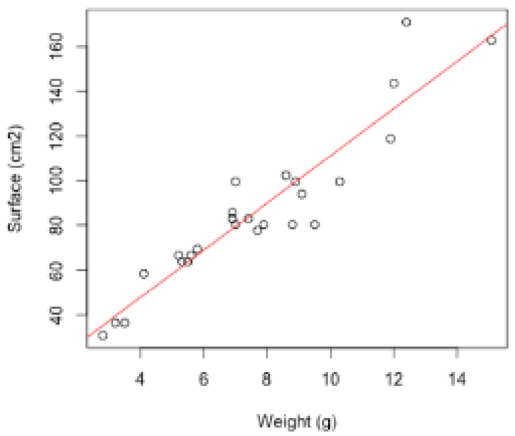
ECOncrete N= 17 R^2 = 0.7888868



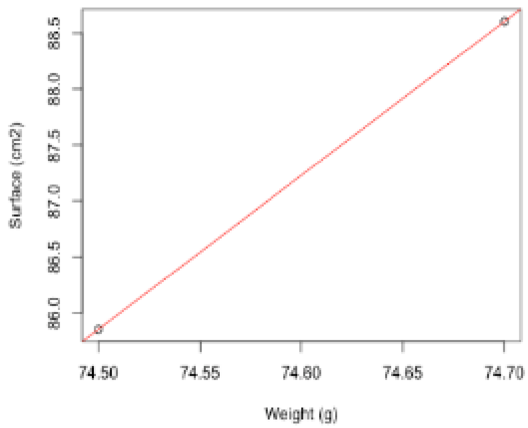
Rooftile N= 28 R^2 = 0.8842783



BESE N= 27 R^2 = 0.8759576



Steel N= 2 R^2 = 1



Annex 2 Tukey result tables

This appendix gives all the values for the Tukey post-hoc tests that were performed to test the differences in settlement success between the different types of substrates. The four different measures for settlement success are listed for the three locations of the field experiment. From left to right all the result tables display the type of substrate, the mean value (emmean: estimated marginal mean), the standard error, the degrees of freedom, the **xx** (asymp. LCL: asymptotic lower control limit)**, the yy** (asymp. UCL: asymptotic upper control limit), and the Tukey post-hoc group.

Total spat

New Quay

type	emmean	SE	df	asymp.LCL	asymp.UCL	group
bese	0.693	4.737	Inf	-8.591	9.98	abc
steel	2.747	1.517	Inf	-0.226	5.72	abc
roof tile	4.067	0.784	Inf	2.531	5.60	a
sandstone	5.729	0.342	Inf	5.059	6.40	ab
silex	6.129	0.280	Inf	5.581	6.68	abc
concrete	6.388	0.246	Inf	5.906	6.87	abc
mussel w	6.500	0.212	Inf	6.084	6.92	abc
econcrete	6.533	0.229	Inf	6.085	6.98	abc
mussel	6.884	0.192	Inf	6.508	7.26	bc
granite	7.022	0.179	Inf	6.671	7.37	c

Tralee

type	emmean	SE	df	asymp.LCL	asymp.UCL	group
bese	-15.303	2196.747	Inf	-4320.85	4290.24	abc
steel	0.288	1.925	Inf	-3.49	4.06	abc
sandstone	4.457	0.131	Inf	4.20	4.71	a
econcrete	4.491	0.204	Inf	4.09	4.89	ab
roof tile	4.670	0.215	Inf	4.25	5.09	abc
silex	4.686	0.165	Inf	4.36	5.01	abc
mussel	5.014	0.222	Inf	4.58	5.45	abc
concrete	5.083	0.152	Inf	4.79	5.38	bc
granite	5.332	0.134	Inf	5.07	5.59	c

Grevelingen

type	emmean	SE	df	asymp.LCL	asymp.UCL	group
steel	-16.303	1.62e+03	Inf	-3184.989	3152.38	ab
bese	-0.223	8.59e-01	Inf	-1.907	1.46	a
roof tile	1.335	3.94e-01	Inf	0.562	2.11	a
concrete	1.335	3.94e-01	Inf	0.562	2.11	a
econcrete	1.569	3.51e-01	Inf	0.881	2.26	a
silex	2.197	2.34e-01	Inf	1.739	2.66	a
sandstone	2.197	2.34e-01	Inf	1.739	2.66	a
marble	2.219	2.53e-01	Inf	1.723	2.72	a
granite	2.534	2.16e-01	Inf	2.109	2.96	a
mussel	3.376	9.17e-02	Inf	3.196	3.56	b
mussel w	3.409	1.56e-01	Inf	3.103	3.72	b

Spat per kg substrate

New Quay

Type	emmean	SE	df	asymp.LCL	asymp.UCL	group
steel	2.33	1.419	Inf	-0.450	5.11	ab
bese	2.59	1.394	Inf	-0.142	5.32	ab
roof tile	2.87	1.084	Inf	0.746	4.99	a
sandstone	4.48	0.484	Inf	3.534	5.43	a
silex	4.78	0.417	Inf	3.965	5.60	a
concrete	4.89	0.394	Inf	4.121	5.67	a
econcrete	5.38	0.308	Inf	4.780	5.99	a
granite	5.58	0.279	Inf	5.036	6.13	a
mussel w	6.61	0.152	Inf	6.312	6.91	bc
mussel	7.19	0.125	Inf	6.940	7.43	c

Tralee

Type	emmean	SE	df	asymp.LCL	asymp.UCL	group
bese	-16.303	2567.368	Inf	-5048.25	5015.65	ab
steel	-0.163	1.710	Inf	-3.51	3.19	ab
sandstone	3.180	0.176	Inf	2.83	3.52	a
silex	3.192	0.247	Inf	2.71	3.68	a
econcrete	3.300	0.262	Inf	2.79	3.81	a
roof tile	3.417	0.285	Inf	2.86	3.98	a
concrete	3.544	0.232	Inf	3.09	4.00	a
granite	3.862	0.198	Inf	3.47	4.25	a
mussel	4.983	0.160	Inf	4.67	5.30	b

Grevelingen

Type	emmean	SE	df	asymp.LCL	asymp.UCL	group
Steel	-17.303	2.28e+03	Inf	-4489.861	4455.26	ab
concrete	-0.174	7.18e-01	Inf	-1.580	1.23	a
roof tile	0.107	6.24e-01	Inf	-1.115	1.33	a
econcrete	0.346	5.53e-01	Inf	-0.739	1.43	a
bese	0.575	4.93e-01	Inf	-0.392	1.54	a
silex	0.813	4.00e-01	Inf	0.028	1.60	a
sandstone	0.847	3.93e-01	Inf	0.076	1.62	a
granite	0.974	4.04e-01	Inf	0.181	1.77	a
marble	1.175	3.66e-01	Inf	0.458	1.89	a
mussel w	3.076	1.58e-01	Inf	2.766	3.39	b
mussel	3.354	7.94e-02	Inf	3.199	3.51	b

Spat per cm^2 substrate

New Quay

type	emmean	SE	df	asymp.LCL	asymp.UCL	group
bese	-6.75	3.478	Inf	-13.57	0.0633	abc
steel	-4.73	1.133	Inf	-6.95	-2.5118	ab
rooftile	-4.00	0.786	Inf	-5.54	-2.4609	a
sandstone	-2.87	0.446	Inf	-3.74	-1.9943	a
mussel w	-2.50	0.338	Inf	-3.16	-1.8334	a
mussel	-1.92	0.278	Inf	-2.47	-1.3769	abc
silex	-1.65	0.243	Inf	-2.13	-1.1762	abc
concrete	-1.61	0.237	Inf	-2.07	-1.1408	abc
econcrete	-1.16	0.190	Inf	-1.54	-0.7911	bc
granite	-1.06	0.181	Inf	-1.41	-0.7051	c

Tralee

type	emmean	SE	df	asymp.LCL	asymp.UCL	group
bese	-23.30	1561.908	Inf	-3084.59	3037.98	abcd
steel	-7.23	1.074	Inf	-9.33	-5.12	a
sandstone	-4.17	0.128	Inf	-4.42	-3.92	a
mussel	-4.12	0.279	Inf	-4.67	-3.58	ab
roof tile	-3.46	0.163	Inf	-3.77	-3.14	bc
econcrete	-3.25	0.127	Inf	-3.50	-3.00	bcd
silex	-3.24	0.113	Inf	-3.46	-3.02	bcd
concrete	-2.96	0.110	Inf	-3.17	-2.74	cd
granite	-2.78	0.101	Inf	-2.98	-2.58	d

Grevelingen

type	emmean	SE	df	asymp.LCL	asymp.UCL	group
steel	-23.30	779.271	Inf	-1550.65	1504.04	abc
bese	-8.77	0.897	Inf	-10.52	-7.01	a
roof tile	-6.77	0.330	Inf	-7.41	-6.12	ab
concrete	-6.67	0.315	Inf	-7.29	-6.06	ab
sandstone	-6.51	0.264	Inf	-7.02	-5.99	ab
econcrete	-6.20	0.249	Inf	-6.69	-5.72	abc
mussel w	-6.03	0.255	Inf	-6.53	-5.53	abc
mussel	-5.75	0.128	Inf	-6.00	-5.50	bc
granite	-5.67	0.190	Inf	-6.04	-5.30	bc
silex	-5.62	0.170	Inf	-5.95	-5.29	bc
marble	-5.47	0.173	Inf	-5.81	-5.13	c

Fraction living spat

New Quay

type	emmean	SE	df.	asymp.LCL	asymp.UCL	group
steel	0.58	0.736	Inf	-0.863	2.02	abc
econcrete	1.03	0.121	Inf	0.794	1.27	a
concrete	1.11	0.132	Inf	0.846	1.3	a
sandstone	1.51	0.207	Inf	1.107	1.92	ab
silex	1.60	0.174	Inf	1.260	1.94	ab
granite	1.77	0.118	Inf	1.537	2.00	b
bese	1.79	2.382	Inf	-2.876	6.46	abc
roof tile	1.96	0.555	Inf	0.874	3.05	abc
mussel	2.28	0.154	Inf	1.977	2.58	bc
mussel w	2.62	0.197	Inf	2.238	3.01	c

Tralee

type	emmean	SE	df	asymp.LCL	asymp.UCL	group
steel	1.10	1.638	Inf	-2.11	4.31	a
sandstone	2.77	0.205	Inf	2.37	3.17	a
econcrete	3.20	0.387	Inf	2.44	3.96	a
granite	3.47	0.288	Inf	2.90	4.03	a
concrete	3.61	0.349	Inf	2.93	4.29	a
silex	3.88	0.432	Inf	3.03	4.72	a
mussel	4.60	0.823	Inf	2.99	6.21	a
roof tile	4.66	0.823	Inf	3.05	6.27	a

Grevelingen

type	emmean	SE	df	asymp.LCL	asymp.UCL	group
bese	1.10	1.003	Inf	-0.867	3.06	ab
sandstone	1.90	0.352	Inf	1.214	2.59	a
silex	1.90	0.352	Inf	1.214	2.59	a
econcrete	2.40	0.641	Inf	1.141	3.65	ab
concrete	2.89	0.892	Inf	1.141	4.64	ab
marble	3.09	0.628	Inf	1.860	4.32	ab
mussel	4.46	0.437	Inf	3.607	5.32	b
mussel w	4.79	0.872	Inf	3.078	6.50	ab
roof tile	20.33	3132.133	Inf	-6118.543	6159.19	ab
granite	21.25	2729.011	Inf	-5327.515	5370.01	ab

Wageningen Marine Research
T +31 (0)317 48 09 00
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

With knowledge, independent scientific research and advice, **Wageningen Marine Research** substantially contributes to more sustainable and more careful management, use and protection of natural riches in marine, coastal and freshwater areas.

Wageningen Marine Research is part of Wageningen University & Research. Wageningen University & Research is the collaboration between Wageningen University and the Wageningen Research Foundation and its mission is: 'To explore the potential for improving the quality of life'