Understanding scales of density-dependence to improve the use of resources in benthic mussel aquaculture

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Abstract

Shellfish aquaculture is considered a sustainable way to help meet rising protein demands worldwide. In shallow coastal dynamic ecosystems mussels can be cultivated directly on the seabed, however this method has a low return as mussels exposed to natural environments risk dislodgment, high predation rates, sedimentation and competition. The formation of spatial patterns in natural mussel beds, that result in 'organized patchiness', is thought to be an adaptive mechanism to reduce population losses. The driver and effects of this patterning need to be disentangled at multiple spatial scales in which patterns are observed. With a field experiment we aimed to understand how small-scale density (actual cover) and patterning (perimeter : area ratio of clumps and number of mussel layers) can be altered by manipulating large scale density (re-laying biomass), that farmers could control during seeding activity. Within this study we considered the interplay between environmental conditions (manipulating flow rate with the use of large mesh cages) and density for pattern development and persistence, and the repercussions of this on mussel productivity (growth and condition). We further investigated local scale

processes, such as the role of within-clump biological activity (biodeposition), that may be a predictor for the larger scale observations of losses and persistence relative to density. We found that manipulating density by controlling seeding biomass from boats is not an accurate predictor of actual seabed density and resulting patterning. The growth and condition of the mussels was only influenced by local scale effects, resulting in high 'within clumps' variation. Aiming for an intermediate density to avoid both excessive fragmentation and excessive layering may be viewed as an optimal strategy to maximise returns, but we encourage the incorporation of the hierarchy of multiple scales of density in future studies of patterning that will allow the inclusion of these effects in a model of growth and productivity.

Keywords bottom-culture; hydrodynamic; *Mytilus edulis*; patches; spatial patterning

1. Introduction

Shellfish culture is considered a sustainable option to answer the issue of meeting rising food demands. Most commercial shellfish species are fast growing, can be farmed at relatively high densities and have no needs for additional feed (Cranford et al., 2003). Moreover, shellfish can contribute to maintaining essential ecosystem functions, such as nutrient cycling, and could be considered an integral part of carbon storing, fitting both into blue carbon and blue growth sustainability goals (Shumway et al., 2003; Smaal et al., 2019).

Mussels are a commonly cultivated shellfish group, comprising of many different species currently cultivated for consumption (e.g. *Mytilus edulis*, *Mytilus galloprovincialis* and *Perna perna*), in many areas worldwide (Lutz et al., 1991). Distinct methods of cultivation are employed, with the choice based upon the type of environmental conditions present in the specific area (Lutz et al., 1991). Cultivation can take place off-bottom (e.g. offshore cultivation on ropes, seeding in socks, on 'bouchots') or on bottom (directly seeding the seabed).

Bottom culture is commonly employed in shallow dynamic coastal ecosystems. In bottom culture however, the mussels are exposed to natural environmental variability (Ysebaert et al., 2009) similar to that experienced by natural mussel beds. This means not only disparities in food

availability but risk of dislodgement, particularly in high energy habitats, predation and biofouling, in areas of 'low to intermediate' hydrodynamic stress (Capelle et al., 2016a; Murray et al., 2008; South et al., 2019). One of the main issues with this type of culture is a low return per biomass (RBP), due to high losses occurring shortly after seeding (Capelle et al., 2016b). This implies a poor use of the two limiting resources: mussel seeds and seabed space to dedicate to culturing (Dolmer et al., 2012; Kamermans and Capelle, 2018; Sanchez-Jerez et al., 2016). Optimization is thus needed to make this practice more sustainable.

One of the key aspects to address in order to maximise returns are the post-seeding losses, which is the greatest loss in the production cycle (up to 50%, (Capelle, 2017)). Pinpointing the key drivers of this is an essential step to find potential mitigation solutions. On one hand, previous studies identified dislodgement as one of the main issues for isolated mussels or small, light, clumps (Capelle et al., 2019; Denny, 1987), followed by predation (Dolmer, 1998). On the other hand, competition for food and space can be a cause of losses when extensive mussel cover is present, and a field survey showed that after a winter season areas of continuous mussel cover were also lost within a culture plot (Bertolini et al., 2019). Thus it is evident that density plays a big part in bed persistence (Capelle et al., 2016b, 2014) – but observations of culture plots show that real density can be very heterogeneous over the scale of the plot (Capelle, 2017). The current seeding technique for on-bottom cultivation, for example in the Netherlands, involves re-laying mussels from a boat that moves in a circular manner around the specified area of the plot leased by the farmer, according to the farmer's experience. The outcome of this is a patchy mosaic, of high and low mussel densities (Capelle et al., 2016b; Dolmer et al., 2012). Thus both processes of loss at low density from disturbance and loss at high density from competition are likely to occur (Gascoigne et al., 2005).

To avoid these losses, results from experimental manipulation suggested that spreading would help to obtain 'medium' patch density that were suggested to be optimal in terms of persistence (Capelle et al., 2014). However, achieving this 'spread-out' distribution from current seeding methods can be challenging in practice. Some possible ways to achieve this are to reduce overall plot-scale seeding density (Capelle et al., 2016b). As mussels beds in nature are a self-organised system, density at the larger scale can drive self-organisation processes (e.g. Liu et al. 2016) and modify small scale density effects via the resulting patterning. However, also

'within-pattern' effects are observed in mussel patches, more commonly thought of as 'edge-effects', resulting, for example, in larger mussels at the edges compared to middle of patches (Okamura, 1986).

With this study we aimed to understand how small scale density (patterning) was influenced by large scale density (seeding biomass) manipulated by farmers during boat operations. Further we aimed to assess the interplay between environmental conditions (flow rates) and initial density in patterning and persistence, as well as mussel health and productivity. We also investigated local scale processes happening within the formed clumps to gather an insight into pattern formation and persistence mechanisms.

2. Methods

2.1 Site and experiment set up

The experiment was conducted on a mussel culture plot located in the Eastern Scheldt, the Netherlands (N 51° 37.647', E 4° 03.421'; Figure 1) in autumn-winter 2018-2019. The plot was 0.04 km^2. Mussel seeds, *Mytilus edulis*, (25 tonnes, measured starting seed measured from 600 mussels: 2.6 ± 0.7 cm) were collected from natural seed beds in Doove Balg, Wadden sea, the Netherlands and were then immediately transported to our site in a temperature controlled truck overnight, following the standard procedures used in local mussel farming. Seeding took place on October 15th, 2018. Mussels were re-laid in bands (length 100 m , width 3.5 m) parallel to the coastline (Fig. 1) and perpendicular to the expected main flow on the plot, following observations of natural bands (van de Koppel et al., 2005).

To address our hypotheses, mussels were seeded in three different initial densities (low (3 kg/m^2), medium (6 kg/m^2) and high (9 kg/m^2)), spread over twelve bands with 4 bands per density laid parallel to the shoreline, with at least 3.5 m of empty space in between bands (Fig. 1). Bands were assigned to either low, medium or high density in a semi-randomised design to ensure that all bands types would be present at all distances from the shore. Only one high density band was erroneously laid out of place (see Fig. 1). To manipulate flow, eight metal cages lined with plastic mesh (Ø ≈ 3 meters, height = 1 meter) were placed on the following day (October 16 th 2018) randomly on top of each band. Of these, 4 were lined with a wide plastic

mesh (Ø of holes = 2.5 centimetres) found in preliminary flume trials to allow a similar flow through as the background flow levels while 4 were lined with a fine plastic mesh (Ø of holes = 0.3 centimetres), which was found in preliminary trials to reduce flow rates by >50%. Control plots were also identified outside of the cages, however due to some extreme low tides leaving the whole plot uncovered, most of the uncaged mussels were eaten by birds (mostly seagulls and oystercatchers) and therefore all control plots were lost within the first month of sampling. The cages were anchored into the sediment with 3 to 4 metal anchors (metal rods with a bent end). For the duration of the experiment the water heights were between +200 Normaal Amsterdams Peil (NAP, in cm) with high tide and were -200 NAP with low tides. This made the plot accessible by foot for maintenance of the cages and the collection of data with low tide.

2.2 Environmental differences between the meshes

Environmental parameters were measured inside one of the fine and one of the wide meshes to check for effects of the mesh. The two cages were located in the same side of the plot to avoid confounding mesh with plot heterogeneity. Chlorophyll-a was measured with dataloggers sampling every 30 minutes (JFE Advantech, ACL W2- USB from October 31st to December 1st 2018). Hydrodynamic measurements were taken over one week in February (6th to 12th) using an ADCP, placed 1m over the seabed and measuring currents at 10 cm intervals in the space overlying the mussel bed. The time-lapse of images used for patterning (see below for sampling design) were also used to quantify the number of predators (crabs, starfish or birds) inside the cages. The time-lapse design allows for better predator quantification by enhancing chances of predator visibility.

2.3 Pattern development

The data for cover and patterning was collected with GoPros Hero 4 (initially 30 but the number declined over the experiment) attached to the side of the cages. Cameras were angled as much as possible to obtain a top down view while capturing most of the cage (see Fig. 1A). Every 2 weeks (from 6th November 2018 until 13th Feb 2019) all of the available cameras were placed on a random cage, ensuring an even spread of cameras around the plot at each deployment. Sampling was done using a randomised design across the plot but ensuring that all cages were sampled once before sampling them all for a second time. This resulted in all cages sampled on two occurrences, two months apart (i.e. first sample in November, second in January). Time

lapse (one photo every minute) was chosen as a methodology to allow the system to be set up at low tide while allowing to see the area when undisturbed and underwater. From the series, one random picture was selected to investigate pattern development. This picture was analysed with ImageJ (Bourne and Bourne, 2010) for the percentage cover and the perimeter : area ratio. First the area of the image containing the seabed part enclosed in the cage (see Fig. 1 B) was selected with the freehand selection tool for the overall area of the cage (measured with the '*analyse*' tool, in pixels). Then the area of the same image containing mussels were selected with the freehand selection tool and the '*analyse'* tool was used again to calculate area and perimeter (in pixels). For the percentage cover the area of containing mussels was divided by the total area of the cage. To understand the level of patterning of the mussels inside the cage, the perimeter : area ratio was used, calculated by dividing the total measured perimeter of the mussels by the total measured area of the mussels. A greater perimeter : area ratio would be an indicator of a greater degree of patterning.

2.4 Mussel health and productivity

The sampling for mussel size, layering and the condition index was done by taking core samples. Cores were taken over the experiment every two weeks from the same cages where cameras were placed, thus sampling each cage twice. Cores consisted of a hollow PVC tube (Ø ≈ 6cm) that was pressed down on a patch of mussels (with 100% cover) inside a cage and then creating a vacuum by closing the other end. The mussels could then be lifted from the patch and put in labelled zip lock bags and transported in the laboratory for processing.

Every mussel in the core was measured using a digital calliper for individual length, width and height to monitor both growth, here intended as mussel size change from the previous period, and number of mussels within a core. Then three (3) random mussels per core were taken to measure the condition index. The condition index was then calculated with the ash free weight (mg) and the shell length (cm) with the formula: ash free dry weight / shell length^3.

The number of mussels in the cores and their sizes (length, width) were used to calculate the area of mussels in the core. This was then used to calculate the number of layers as the area occupied by the mussels divided by area of the core (Filgueira et al., 2008). The number of layers can be a useful additional metric to understand whether changes in patterning observed from images are driven by clumping or losses.

The number of mussels in the cores and the percentage cover of each cage were also used to estimate the effective number of mussels in the cages (multiplying number of mussels per area in each cage by the cover in each cage) . The average weight of one mussel was then multiplied by the number of mussels to estimate production in terms of biomass useful for production (kg/m^2).

2.5 Within pattern dynamics

To address changes at local scale (within patterns), sampling was done within two of the high density cages that developed clear patterns, one lined with fine mesh and one lined with the wide mesh, located in the same area of the plot to avoid confounding potential environmental effects. Within the cage, 10 random mussels were collected from the top-most layer and 10 random individuals from the edge of the bottom-most layer. Mussels were briefly cleaned of any external sediment and left in individual containers containing filtered seawater for c.ca twelve hours in a temperature controlled room, after which the biodeposits were collected to gather insights into mussels feeding status. Water containing biodeposits was filtered on pre weighed filters (GF/F filter 47mm, Whatman), and dry weights were obtained by drying at 60°C until constant weight of filters was obtained (c.ca 24 h). Filters were then burnt at 500 °C for 4 hrs, before weighing them again. This weight was subtracted from the original dry weight to obtain percentage of organic matter. Mussel length and condition index were also measured at the end of the twelve hours as described above. Control filters were also used to address any potential issues of weight change while drying and burning.

In February 2019, at the end of the experimental period, Sediment analysis was also conducted to address small scale processes. In three wide mesh cages and three fine mesh at different densities. In each cage, 3 sediment samples from below mussels, three from the edge and three from an empty space (10 cm away from the edge of the mussels) were taken using a cut syringe (30 ml). Sediment granulometry, and organic matter (C and N) analysis were then performed on the sediments at the NIOZ analytical laboratories in Yerseke.

2.6 Statistical analyses

All statistical tests were conducted in R (R Development Core Team, 2015).

2.6.1 Environmental differences between the meshes

To analyse the effect of the two mesh types on flow speeds, chlorophyll-a and turbidity, linear models were used with mesh type (two levels: 'wide', 'fine') as a fixed factor. The differences in average number of predators were analysed with a linear mixed model (*lme* from the *nlme* package), to account for repeated measures over the same cage. Mesh type (two levels: 'wide', 'fine'), density (three levels: '3 kg/m^2', '6 kg m^2' , '9 kg m^2') and sampling occurrence (two levels: 'first' or 'second' time for each cage) were the fixed effects (Table 1).

2.6.2 Patterning

Before proceeding with separate statistical analyses, two-way correlations between the three patterning variables (percentage cover, perimeter area and number of layers) were considered using Pearson's correlations. To account for repeated measures over the same cage, linear mixed models (*lme* in the *nlme* package) were used in the analyses investigating all aspects related to patterning and mussel health (see Table 1). The random part of the model had a nested structure with mesh type nested within density and nested within cage. The fixed effects were sampling occurrence (two levels: 'first' or 'second' time for each cage), density (three levels: '3 kg', '6 kg' , '9 kg'), mesh (two levels: 'wide' and 'fine') and the interactions between density and occurrence and between density and mesh. The effects of this fixed and random structure were analysed firstly on the resulting percentage cover, which was then secondly used as an additive covariate for analyses on perimeter : area ratio. Perimeter : area ratio was then added to percentage cover as an additive covariate when the number of layers was investigated and was used alone as additive covariate for the effective biomass (see Table 1).

2.6.3 Mussel health and productivity

To address the relationships between mussel health parameters (average length and condition index) and patterning, we used percentage cover, perimeter : area and layers as covariates, with mesh (two levels: 'wide' and 'fine') and sampling occurrence (two levels: 'first' or 'second' time for each cage) as fixed factors. The mixed models included a random structure with mesh nested within cage. Initial model included all fixed and covariates and all possible two ways interactions. A backwards step model selection based on AIC values was then taken to simplify the models. The final model for size included percentage cover, layer, mesh and sampling

occurrence and the interaction between percentage cover and occurrence, layer and occurrence and mesh and occurrence, whereas the final model condition index included percentage cover, mesh and sampling occurrence and the interaction between percentage cover and mesh (see Table 1).

2.6.4. Within pattern dynamics

For 'within pattern' effects on mussels health we used linear models with position (two levels: 'top', 'bottom'), mesh type (two levels: 'wide', 'fine') and their interaction on the total amount of biodeposits, percentage of organic matter of biodeposits, condition index and size of mussels. We also conducted a Pearson correlation between organic content of deposit and mussel condition index.

To address how the median sediment grain size and the organic carbon content changed with mussel presence in the different mesh types, we used a linear mixed model with mesh nested within cage as random structure, position (three levels: 'under, 'edge', 'far'), mesh type (two levels: 'wide', 'fine') and their interaction as fixed factor and perimeter : area, percentage mussel cover and number of layers as additive covariates.

All models were validated by visual analysis of residual plots (qq plots, residuals vs fitted values, constant leverage and autocorrelation of residuals). To obtain overall factor significance, Anova tables were made with the *car* package, with type III sums of squares for models involving interactions or type II for models including only additive terms. When terms were significant, Tukey adjusted comparisons of estimated marginal means, or estimated marginal trends when one variable was continuous, were conducted with the package *emmeans* (Lenth, 2016).

3 Results

3.1 Environmental differences between the meshes

There was a significant decrease in flow velocity ($F_{1, 7248} = 30.9$, $p < 0.001$), *chl-a* ($F_{1, 60029} = 32061$, $p < 0.001$) and turbidity ($F_{1, 60029} = 25288$ $p < 0.001$) in the fine mesh compared to the wide mesh (Fig. 2). The only predators observed within the cages were crabs. There was only a tendency ($X^2 = 3.5$, $p = 0.06$, Fig. 3) for fine mesh to have less crabs (average 0.5 ± 0.4) than

wide mesh (1.5 ± 0.3), but there was a decrease in crabs over time ($X^2 = 9.5$ $p < 0.01$, first occurrence, 1.6 ±0.3 ; second 0.4 ± 0.3, Fig. 3).

3.2 Patterning

Correlations between patterning covariates (cover, p:a and number of layers) were overall relatively weak (< 0.5). Perimeter : area of patches was negatively correlated with percentage cover (Pearson= -0.42) and number of layers (Pearson = -0.11). A positive correlation was found for percentage cover and number of layers (Pearson= 0.32).

Cover was significantly influenced by initial density. The cover at 3 kg/m^2 was 21.9 (± 6.4) % which was significantly lower ($X^2 = 15.23$, $p < 0.001$) than the cover when mussels were re-laid at 9 kg/m^2 (48.9 ± 4.8). This was the same at both sampling times (sampling occurrence * initial density : $p > 0.05$, sampling occurrence: $p > 0.05$, Fig.4)

Perimeter : area was also significantly influenced by initial density ($X^2 = 9.9$, $p < 0.01$, Fig. 4), with more fragmented mussels in the 3 kg/m^2 (p:a 0.04 ± 0.006) compared to the 6 kg/m^2 (0.016 ± 0.005), and decreased with increasing percentage cover ($X^2 = 5.7$, $p < 0.05$, Fig 4). There was also a tendency for sampling occurrence to change the ratios, rendering them more similar to each other on the second sampling occasion ($X^2 = 3.5$, $p = 0.06$, Fig. 4).

The number of layers was only different between the three 'initial density' treatments ($X^2 = 6.7$, $p < 0.05$, Fig. 4), with less layers in the 6 kg/m^2 treatment (2.5 ± 0.2) compared to the 3 kg/m^2 (2.9 ± 0.3) and 9 kg/m^2 (3 ± 0.2), and only trended towards a decrease between the second and first sampling occurrence ($X^2 = 3.3$, $p = 0.66$, Fig. 4). There was no significant effect of covariates, which only trended towards a significant effect (increasing with percentage cover: $X^2 = 3.3$, $p = 0.065$, Fig. 4)

3.3 Mussel health and productivity

Effective obtained mussel biomass (expressed as estimated kg / m^2) depended on initial relaying density ($X^2 = 10.8$ $p < 0.001$, Fig. 5). The mean obtained biomass at the 9 kg / m^2 treatment was 7.1(± 1) kg / m^2 , significantly greater than those obtained when mussels were initially relayed at either 3 kg / m^2 (biomass obtained: 3.46 ± 1.3 kg / m^2), or 6 kg / m^2 (biomass obtained: 3 ± 1.2 kg / m^2). The differences were also driven by perimeter: area (X^2

=4.8, $p < 0.05$, Fig. 5), while there were no influence of sampling occurrence or interactions, for any of the three 'initial density' treatments.

During the experiment, no shell growth was observed. Condition index at all observations showed an interaction between mesh and percentage cover (X^2 =6.8 $p < 0.01$, Fig. 6), with a slight negative influence of percentage cover on condition in the fine mesh (trend: - 0.005) and a slightly positive influence in the wide mesh treatment (trend 0.004). Overall, there was decrease in condition index at the second (1.9 ± 0.08) compared to the first (2.5 ± 0.06) sampling occurrence (X^2 =32.6 $p < 0.001$, Fig. 6), and an overall effect of mesh ($X^2 = 4.6$, $p < 0.05$, Fig. 6).

3.4 Within patterns dynamics

When local scales were considered, mussels at the top of the patches had a better condition than mussels at the bottom ($F_{1,76} = 6.9$, $p < 0.05$, Fig.7), and mussels in the wide mesh had overall greater condition than those in the fine ($F_{1,76} = 5.3$, $p < 0.05$, Fig.7). In the wide mesh, biodeposits released from mussels that were at the top were heavier than those from mussels at the bottom, while at in the fine mesh there were no effects (mesh*position $F_{1,76} = 18.9$, $p < 0.001$, Fig.7). Biodeposits from mussels in the wide mesh contained significant greater proportion of organic matter (mesh: $F_{1,76} = 8.7$, $p < 0.01$, Fig 7). There was a slight positive correlation between the percentage organic matter in the biodeposits and mussels condition index (Pearson correlation: 0.24, Fig.8).

When analysing the median sediment particle size, there was a three-way interaction between mesh, percentage cover and position (X^2 =10.8 $p < 0.05$), showing the greatest decrease in sediment particle size under and at the edge of the mussels in both mesh types with increasing percentage cover, disappearing when moving to the 'far' spot in the wide mesh while persisting in the fine mesh (Fig.9). For the organic carbon content of the sediments, there was the same three-way interaction between mesh, percentage cover and position (X^2 =11.3 $p < 0.05$), showing the greatest increase in organic carbon content under and at the edge of the mussels in the fine mesh, with increasing percentage cover, disappearing when moving to the 'far' spot, away from mussels, for both mesh types (Fig.9).

4.Discusssion

With this study we investigated how small-scale density and patterning changed by manipulating large scale density (as seeding biomass). We found that manipulating the density at which mussels are seeded did not determine the actual observed patch scale density on the seabed. Only at the lowest seeding density were different patterns, clumps with more edge areas, discernible. Moreover, the health of the mussels did not depend on the large scale density (manipulated here) but was only influenced by layering (top vs bottom) and changes to flow conditions.

4.1 Patterns development

There was a marked difference between patterns developed at the low initial density and both those developed at medium and high initial densities. At low density there was increased fragmentation, resulting in patches of greater perimeter : area ratio even in patches of similar percentage cover, suggesting that seeding with bands of such low density is not recommended. Increased fragmentation can lead to greater losses, from predation and dislodgement (Capelle et al., 2019; Dolmer, 1998). Seeding in bands of medium density (6 kg/m^2) resulted in the least heterogeneity in terms of observed percentage cover, with intermediate values that can be considered high enough to avoid major losses. In terms of mussel layering, it did not increase between the two time points despite the decrease in cover, although there was also not a significant decrease that may indicative of high mortalities from self-thinning processes (Lachance-Bernard et al., 2010) or predation. Layers were significantly influenced by initial density, as to be expected given our methodology of repeatedly seeding the same spot to obtain the medium and high density treatments.

4.2 Effects on mussels health and productivity

During our experimental period, which was winter, representative of the initial months after autumn seed fisheries, mussels' shell growth was not observed. The winter period may have had a significant influence on mussels conditions, due to limited food availability and low temperatures typical from this time of the year, known factors to influence condition (Gallardi et al., 2017; Hawkins and Bayne, 1985). There was a some evidence of competitive effects, with mussels at higher covers displaying reduced fitness, particularly in the scenarios where flow was reduced by the fine mesh (McQuaid and Mostert 2010). This implies that choosing appropriate

seeding methods may depend upon background conditions at cultivation sites. Moreover, when we zoomed in the condition at the smallest 'within patterns' scale, in both cage types the mussels at the top were found to have better condition than the mussels at the bottom.

Our experiment was only 4 months in duration, over the coldest and least productive months (Smaal and Haas, 1997), thus perhaps a prolonged time would have shown further effects of the mesh. According to previous knowledge 'When seeded in autumn, loss is compensated by growth in the following spring' (Capelle 2016), thus it would be important to consider the variation in condition found within patches, even in autumn, in order to maximize the growth potential in the following year. Following mussels at this scale over a longer period should be done in order to gather a better idea of the variability in production outcome, so that it can be incorporated in models of mussels production and avoid assumptions of homogeneity at the small scales.

It is therefore suggested that models of mussels production may consider different scales of density, and how they can interplay. While the effects of multiple scales of patterning have so far considered its role for ecosystem functioning (e.g. edge effects and fragmentation Paquette et al. 2019), there is limited knowledge on patterning effects on the health of the habitat formation itself (e.g. Hunt and Scheibling 2001). Some more mechanistic and fundamental studies are needed to move forward – for example to gather more insights into reasons for greater conditions at the top of clumps. While previous model assumptions considered only increased favourable hydrodynamics to obtain greater food delivery (Liu et al., 2014), it could be useful to address whether other feedbacks, such as increased risks of suffocation from neighbour faeces and pseudo-faeces in mussels located closer to the bottom, play a part. We found that mussel cover influenced one of the small-scale processes, namely the quantity of biodeposit produced and their organic content, that might be responsible for the differences in condition index of the mussels. Mussels at the bottom produced a greater volume of biodeposits which contained less organic material, suggestive of a lower food quality (food with greater inorganic material) available (Jade et al., 2014; Zúñiga et al., 2014).

It is important to consider environmental conditions of the culture plot when deciding on seeding methodologies. Environmental context is found to be an important driver of sessile species dynamics (Hunt and Scheibling 2001; Petes et al. 2008; O'Connor and Donohue 2013, Bertolini

et al., 2020). McQuaid and Mostert (2010), suggested that bottom-up and top-down processes can interact at multiple spatial scales and found that effect on condition index were likely driven by differences in food supply created by modifying local flow rates. We here add to the body of evidence that local flow rates are essential component of individuals health and suggest that mussel cultures in less dynamic systems would benefit more from spreading compared to those in more dynamic areas which may benefit more from being at greater density to avoid dislodgements.

Before concluding that modifying density at seeding is not the only solution to obtain more productive mussel patches, it is important to consider the relatively short duration of the experiment, conducted in the winter, less productive, months. Moreover, predation was not taken into account: our experiment did not allow for the bird predation characteristic of these systems (Nehls et al., 1997). Bird predation was observed outside of our cages, resulting in a near total loss of mussels, particularly in the shallow areas, while due to the winter months other types of predation were not significantly impacting, with only a few crabs being present.

Despite these limitations, this study suggests that multiple scales should be incorporated into production models for aquaculture. Patterning in nature is of a hierarchical nature, with a nested system of patterns within patterns (Commito et al., 2006; Lawrie and McQuaid, 2001; Liu et al., 2014; van de Koppel et al., 2005). This is also the case for mussels density on culture plots. Moreover, previous studies suggested that large scale bands emerge from small scale processes of aggregation leading to increased survival and thus greater, persisting density (Liu et al., 2014), and recruitment success (McQuaid and Mostert, 2010b), whereas other studies found that relaying density could predict patterning (Capelle et al., 2014). The present study shows that only extreme differences in relaying density (e.g. using an extremely low of 3 kg/m^2) result in a different pattern formation, but that processes happening at the smallest scale, thus within a patch, may be the ones driving individual health and resulting in greater productivity.

4.3 Conclusions

The only large-scale effect that can be harnessed in practice is that the lowest seeded density should be generally avoided as it caused an initial fragmentation, followed by greater overall losses. Condition of individuals did not depend upon the initial relaying density (large scale), but only declined with greater cover and ‘within patterns’. To avoid small-scale heterogeneity

influence on the condition of individuals, it could be suggested to move individuals around more, implementing some extra 're-spreading' over certain period. This will need further research to ensure the benefits of competition avoidance will not be undermined by the energy needs for reattachment. Farmers may benefit from employing strategies that avoid competition between individuals by allowing density high enough to maximise the facilitative effects of aggregation while minimising the number of layers created. This study adds to the body of evidence that suggests a benefit of medium density spreading. We also suggest this trade-off avoidance may be attained by encouraging methods that will avoid burial from the biodeposition activity of other mussels. Previous studies have found the addition of a shell substratum to be useful in avoiding competition and ensuring some protection from other stressors (Bertolini et al., 2018, 2017; Capelle et al., 2019). In conclusion, we suggest that the three dimensional aspects of patterns should be studied in more detail, and that in order to optimise culturing, trade-offs between competition and facilitation should be done on a site-by-site assessment, based on flow conditions, in order to decide on the optimal relaying density.

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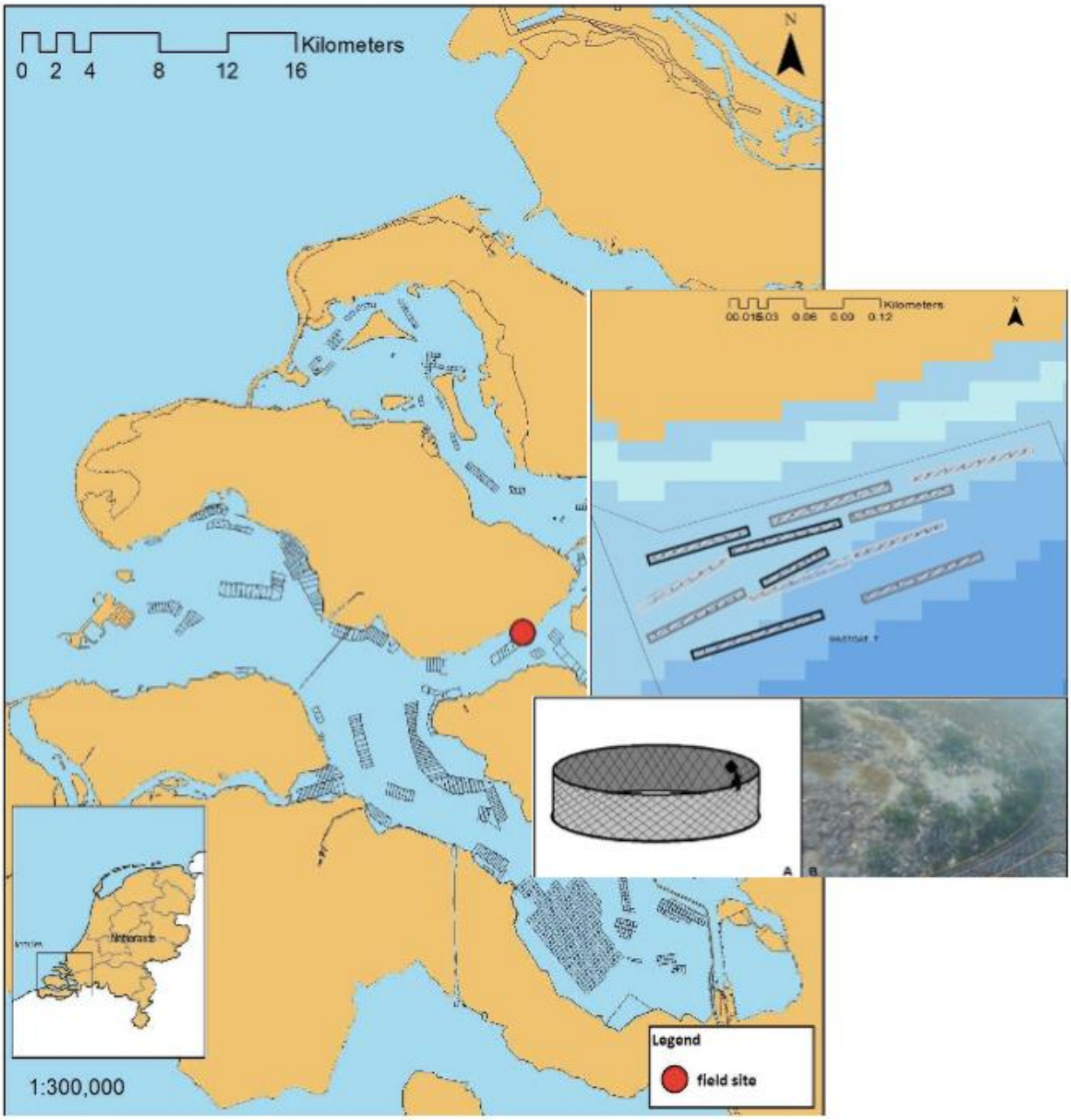
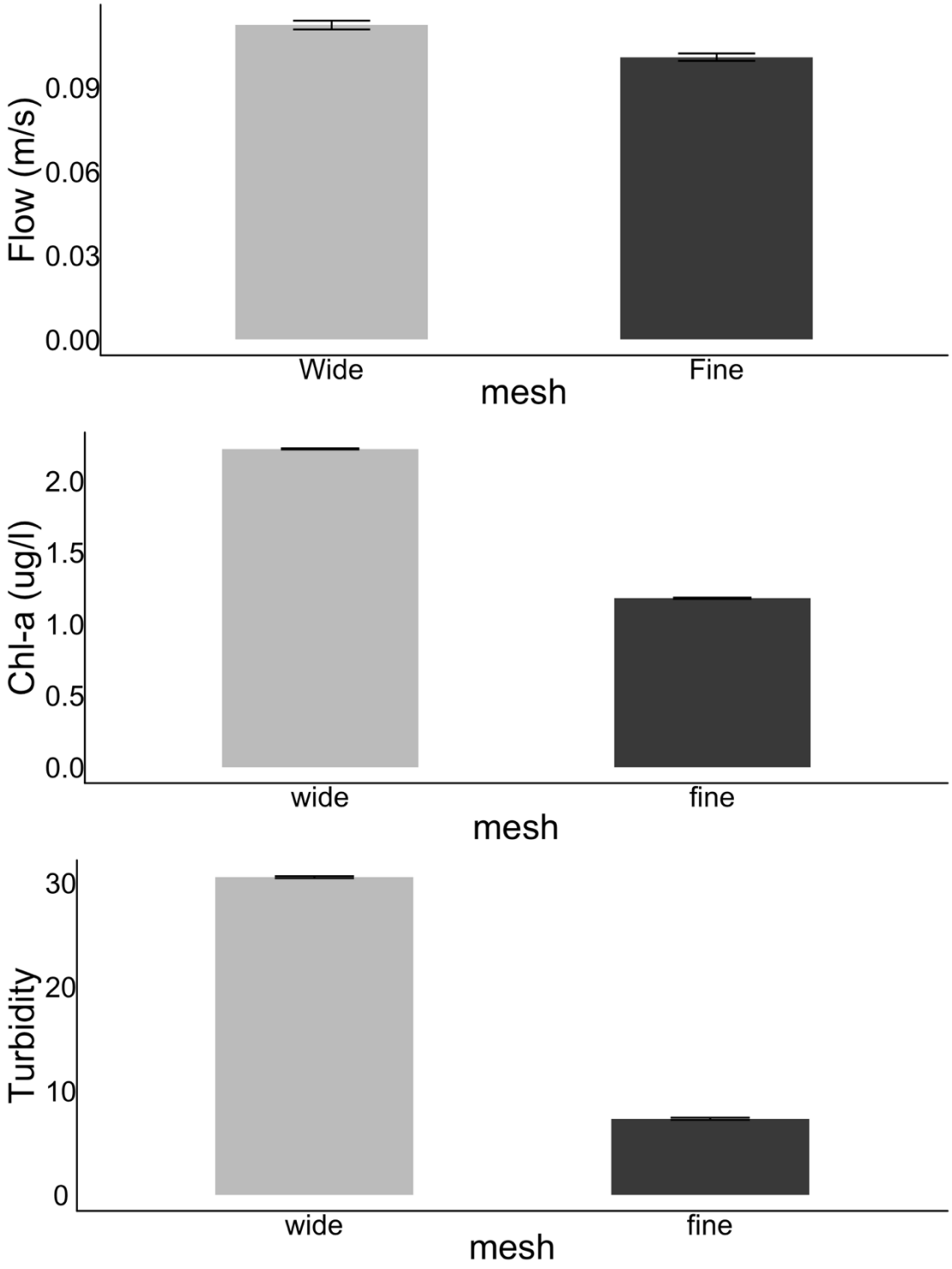


552

553 Fig.1 Location of field site and disposition of bands (taken from GPS coordinates). Light grey is
554 low density, grey is medium density and black is high density. Circles represent cages. Insert
555 represent methods for determination of patterning showing (A) camera placing, (B) example of
556 image.



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559 **Fig. 2** Estimated means and standard error of flow above the mussel beds, chlorophyll-a and
560 turbidity as measured inside cages with either wide (grey) or fine (black) mesh.

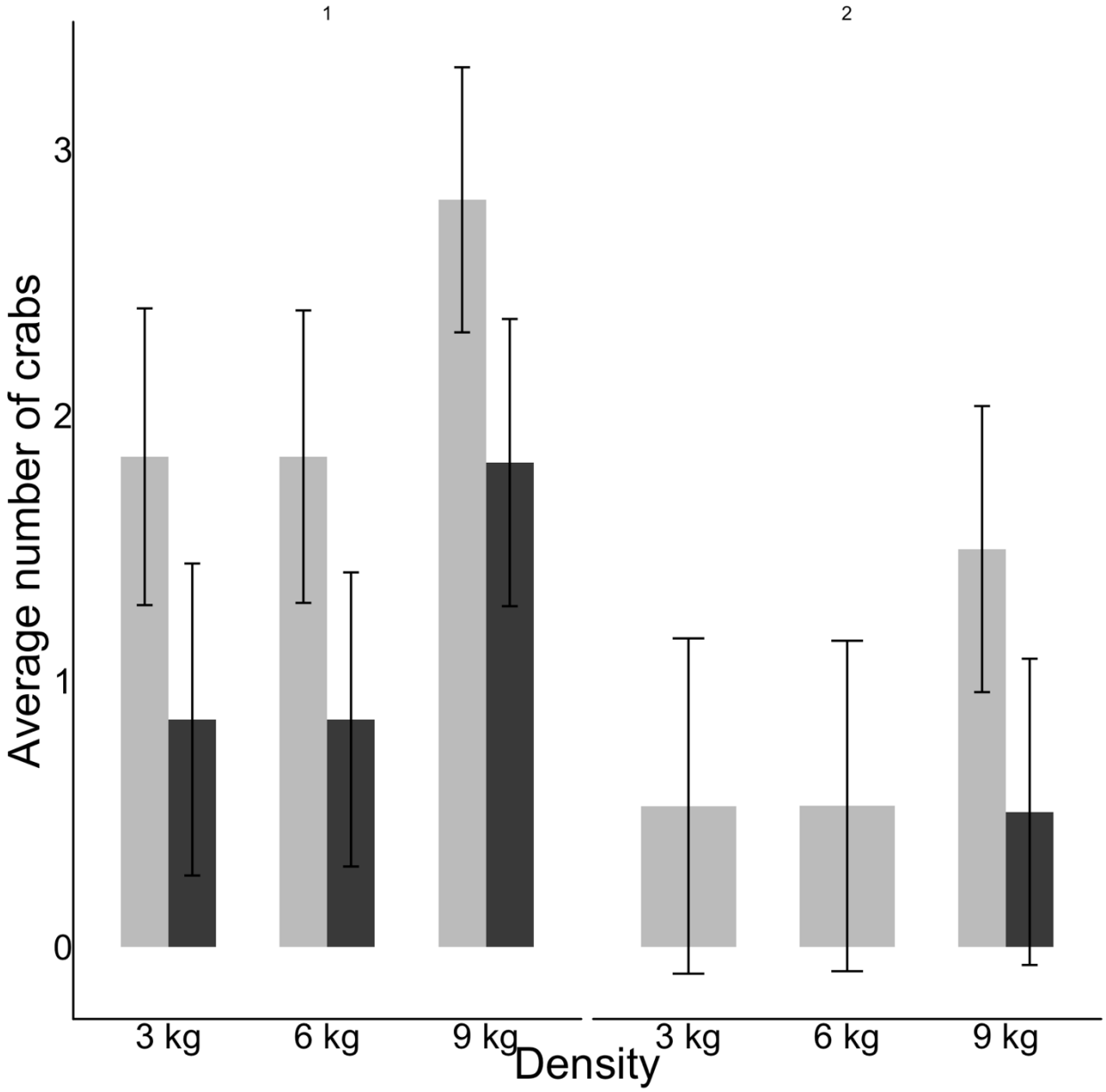
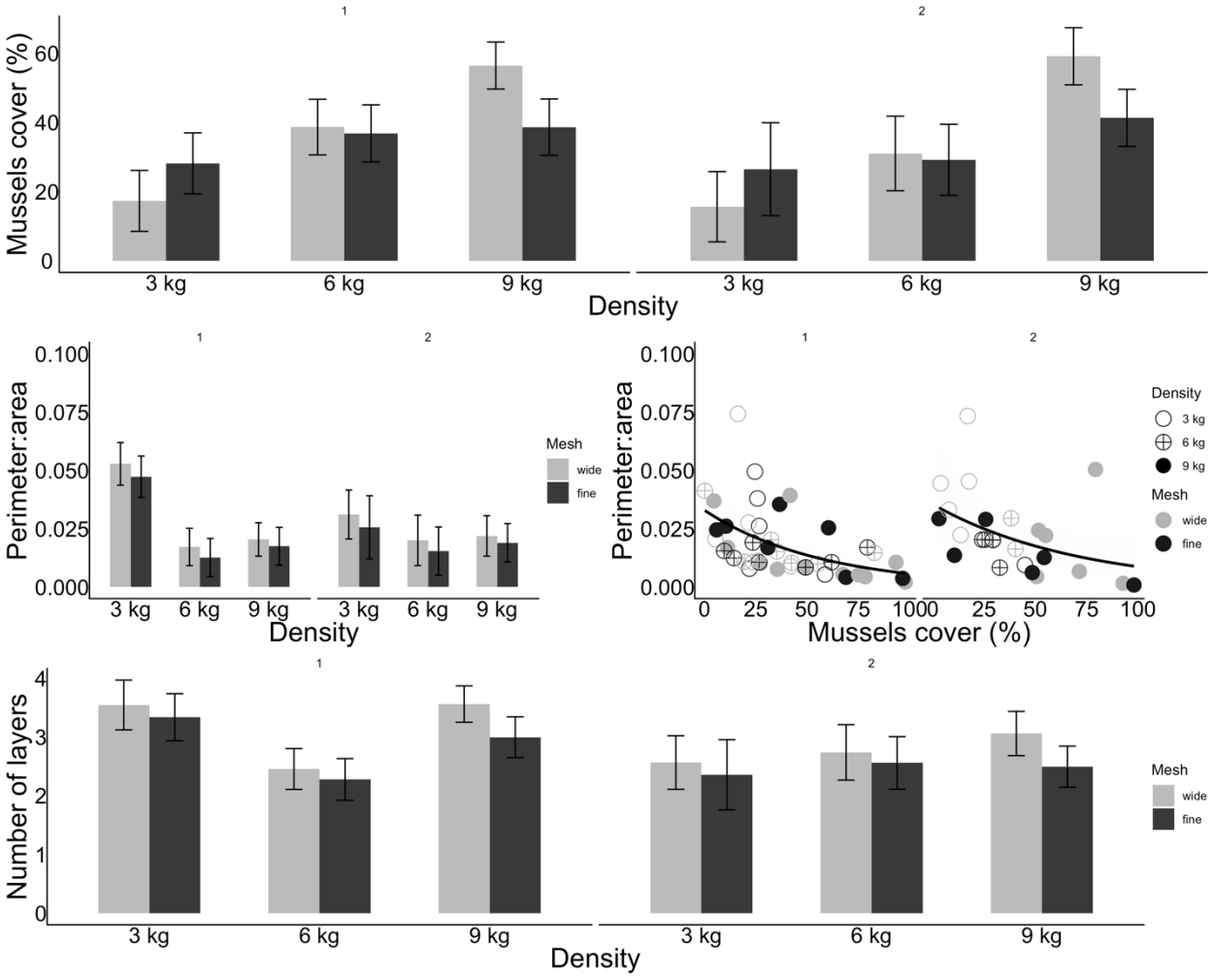


Fig 3. Estimated means and standard errors of number of crabs in the different density treatments, in cages with the two mesh sizes (grey: wide mesh; black: fine mesh) at the two sampling occurrences



568

569 **Fig 4 –** Aspects of patterning. Bar plot showing estimated means and standard errors of top:
570 mussel cover, middle: perimeter: area, and bottom: number of layers for the three initial density
571 treatments and two mesh types (grey: wide; black: fine) at the two sampling occurrences.
572 Scatterplot shows how perimeter: area changes across mussel cover (open dots, dotted line: 3
573 kg/m^2; crossed dots. dashed line: 6 kg/m^2; full dots, solid line: 9 kg/m^2; colour represent mesh
574 type grey: wide, black: fine).

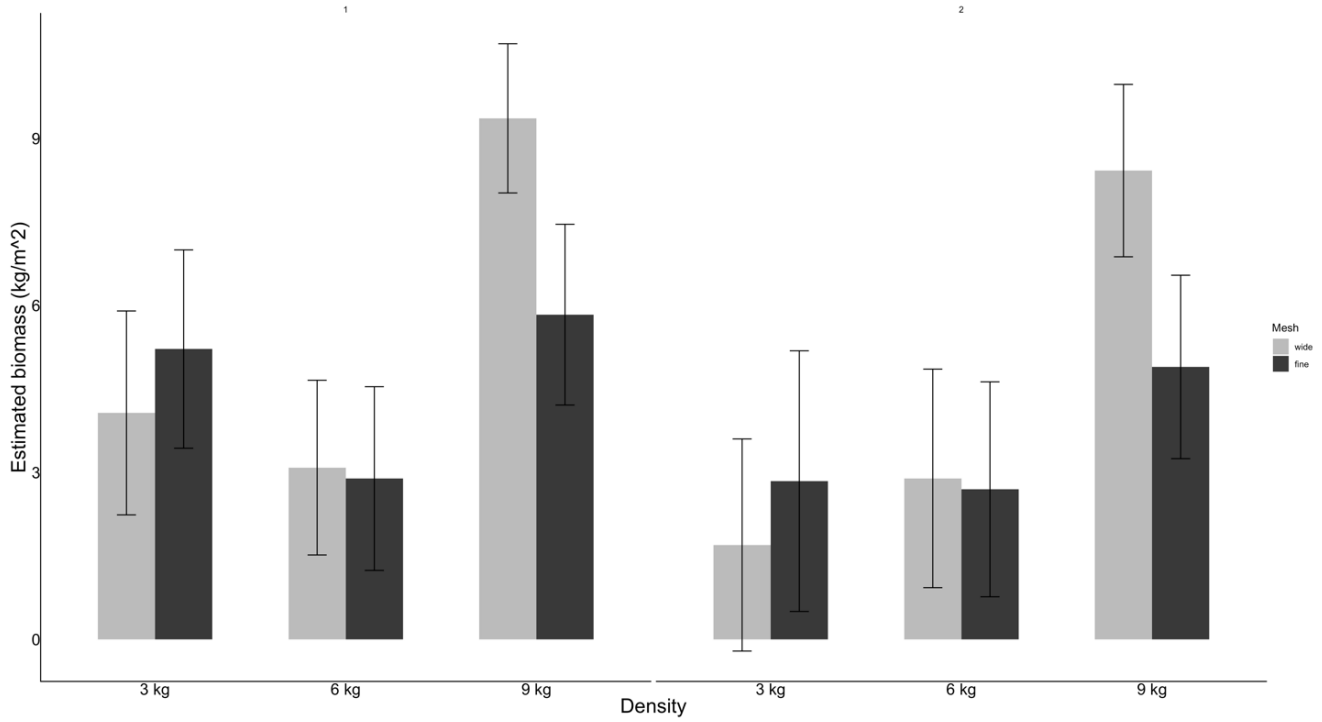


Fig.5 – Bar plot showing means and standard error of estimated mussels biomass at different initial relaying density (open dots, dotted line: 3 kg/m^2; crossed dots. dashed line: 6 kg/m^2; full dots, solid line: 9 kg/m^2) and mesh types (grey: wide; black: fine) for the two sampling occurrences.

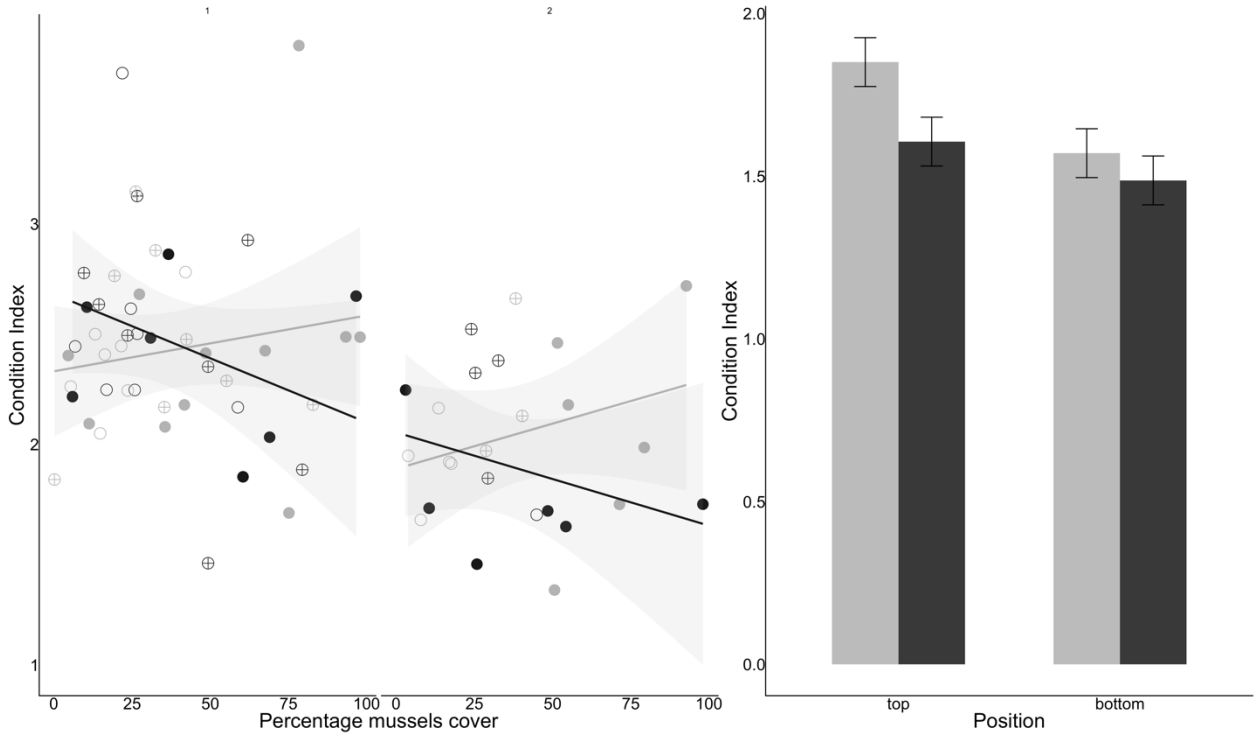
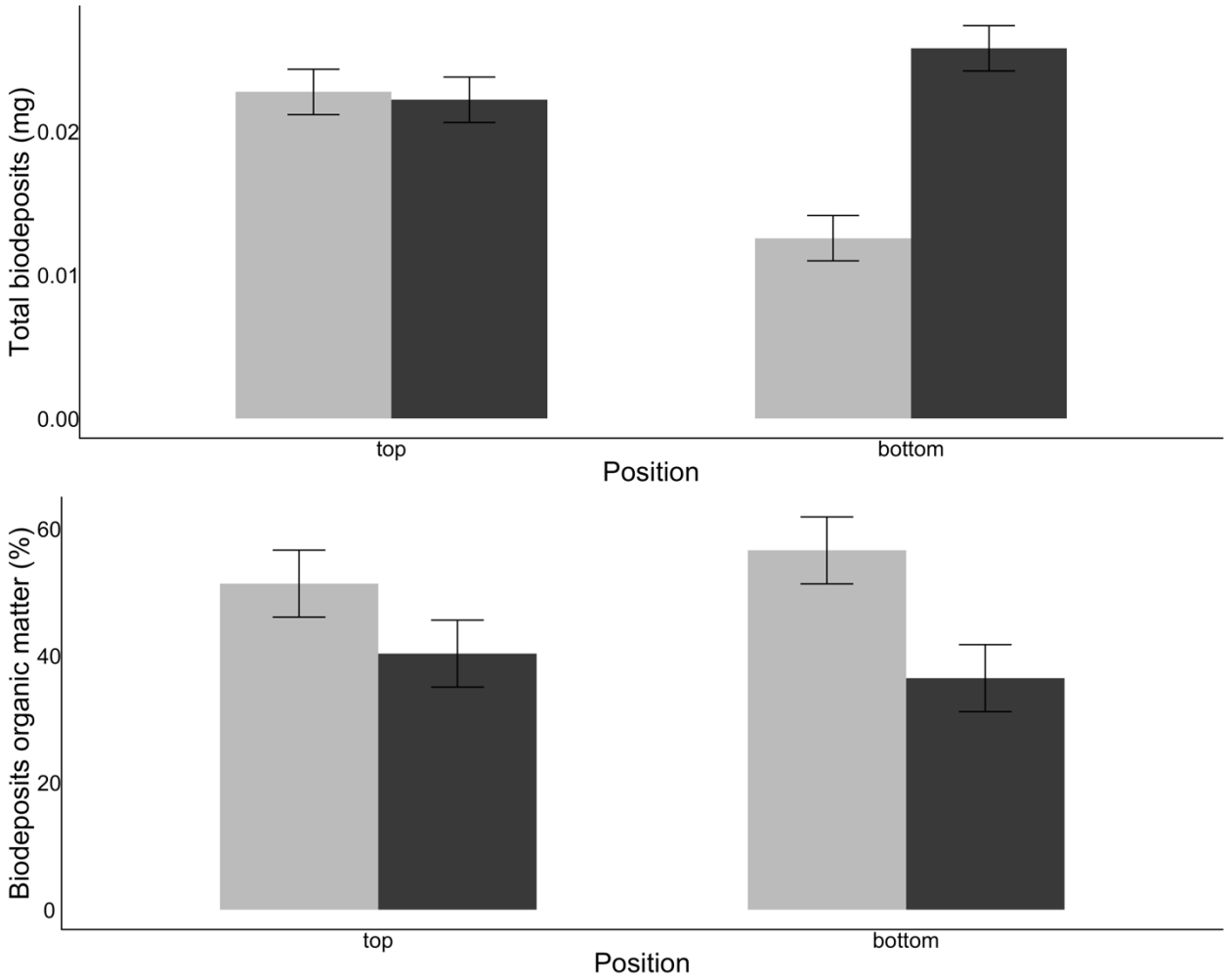


Fig 6 Scatterplot showing the condition index of mussels in cages with different percentage covers, at different initial relaying density (open dots: 3 kg/m^2; crossed dots. 6 kg/m^2; full dots: 9 kg/m^2) and mesh types (grey: wide; black: fine). The solid lines are mesh type effect (grey: wide, black: fine). Bar plot showing means and standard error of the condition index of mussels at the top and bottom of clumps in cages with different mesh types (grey: wide, black: fine).



590

591 **Fig. 7**

592 Bar plot showing means and standard error of total biodeposit and percent of organic matter in
593 the biodeposit released from mussels collected at the top and bottom of clumps in cages with
594 different mesh types (grey: wide, black: fine).

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596

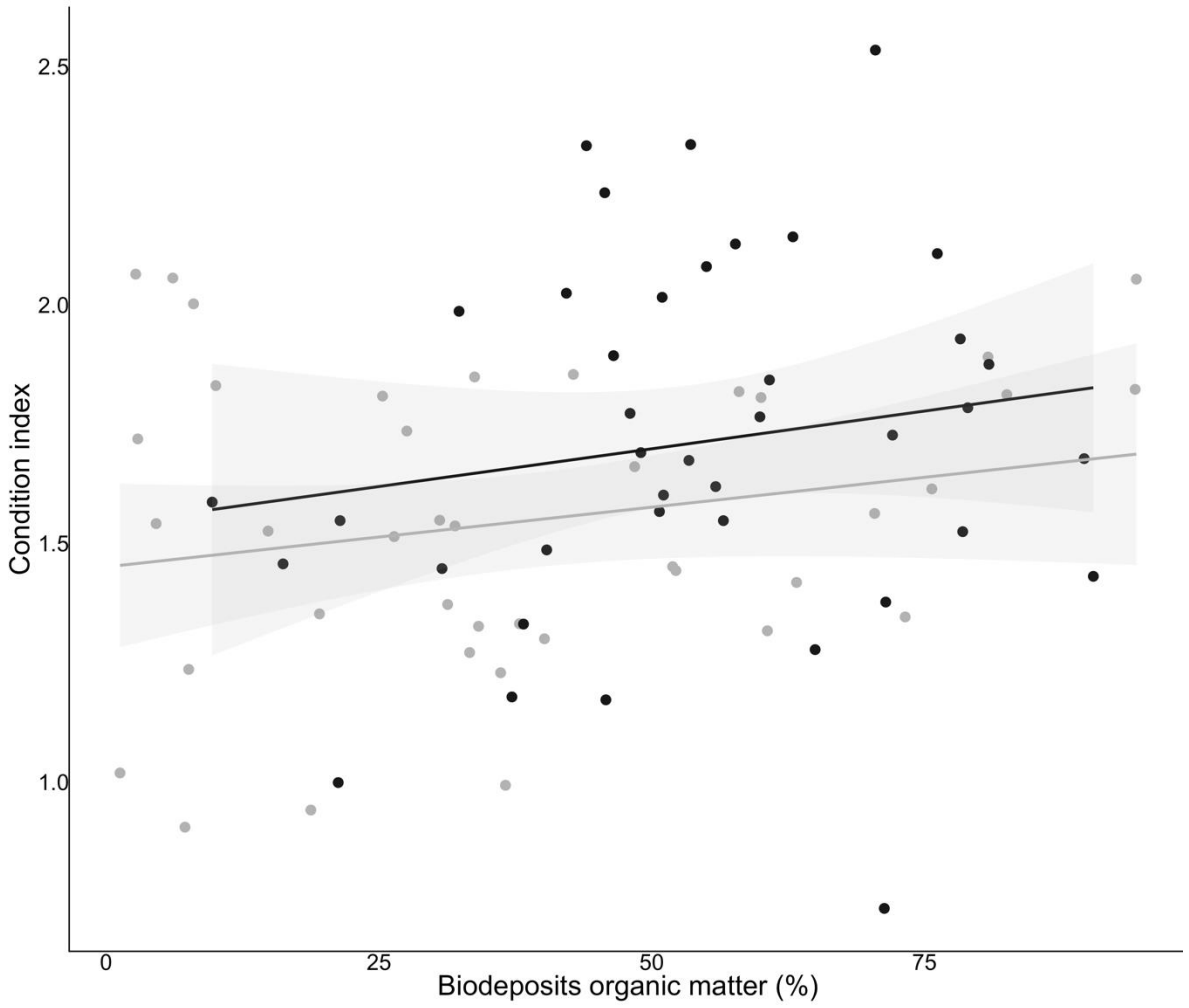

Fig 8: Correlation between percentage organic matter found in mussel biodeposits and mussels condition index for both mesh types (grey: wide, black: fine). R^2= 0.24 , no significant differences.

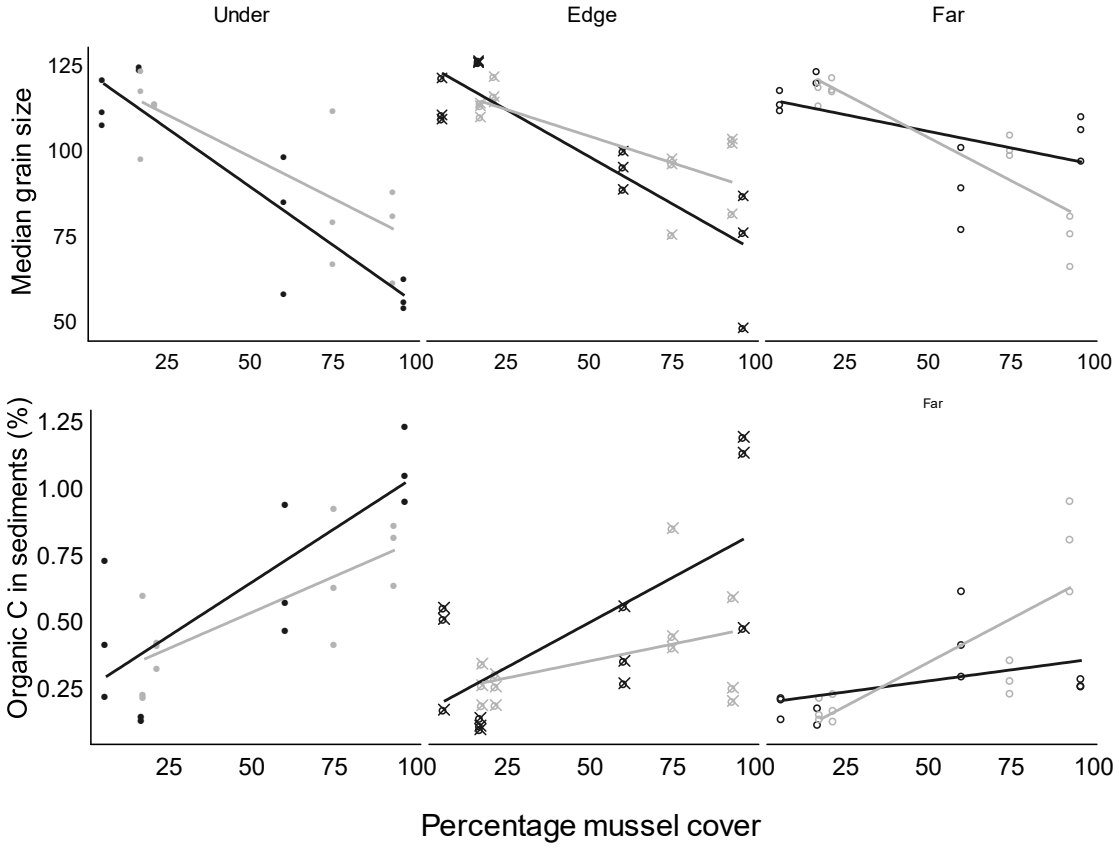

Fig 9 Sediment properties (median grain size and percentage of organic carbon) under the mussel patches (under, filled circles), at the edge of the patch (edge, crossed circles), and in area without mussels (far, empty circles) in cages of differ mussel cover with different mesh types (grey: wide, black: fine).

615

616 Table 1. List of models used. Model, covariate used, type of covariates as additive (ADD) or
617 interactive (INT), fixed structure and random structure.

Parameter	Model	Covariate	Type of covariate	Fixed	Random
Velocity	lm	n/a	n/a	Mesh	n/a
Chl-a	lm	n/a	n/a	Mesh	n/a
Turbidity	lm	n/a	n/a	Mesh	n/a
Number of crabs	lme	n/a	n/a	Density + Mesh + Occurrence	1|cage/density/mesh
Percentage cover	lme	n/a	n/a	Density + Occurr + Mesh + Density : Occurr + Density : Mesh	1|cage/density/mesh
Perimeter: area	lme	Percentage cover	ADD	Density + Occurr + Mesh + Density: Occurr + Density: Mesh	1|cage/density/mesh
Layer	lme	Percentage cover, perimeter: area	ADD	Density + Occurr + Mesh + Density : Occurr + Density : Mesh	1|cage/density/mesh
Effective Kg	lme	Perimeter: area	ADD	Density + Occurr + Mesh + Density : Occurr + Density : Mesh	1|cage/density/mesh
Mussel length	lme	Layer, percentage cover	INT	Mesh + Occurr + Mesh: Occurr	1|cage/mesh
Condition index (large scale)	lme	P cover	INT	Occurr + Mesh	1|cage/ mesh

Condition index (within pattern)	lm	n/a	n/a	Location*Mesh	n/a
Dry weight of biodeposit	lm	n/a	n/a	Location*Mesh	n/a
Organic matter of biodeposits	lm	n/a	n/a	Location*Mesh	n/a
Median grain size sediments	lme	P:a, p cover,	ADD, INT	Position + mesh + Position: mesh	1|cage/mesh
Organic carbon sediment	lme	P: a, p cover,	ADD, INT	Position + mesh + Position: mesh	1|cage/mesh

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