Monitoring of benthic indicators of organic enrichment under aquaculture sites over mixed bottom substrates during a fish production cycle

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Abstract

Norway's sheltered coastal and fjord systems provide an ideal environment for marine fin-fish farming, a growing industry worldwide. However, fin-fish aquaculture is associated with several environmental issues, including the deposition of organic waste (uneaten feed and faeces) to the seafloor and the consequent impact on benthic communities. In Norway, a system of monitoring investigations is already in use for the assessment of benthic organic loading and community responses for soft-sediment environments. However, not uncommonly on the Norwegian coast, grab sampling is made difficult by the presence of mixed- and hard- bottom substrates. The aim of this study was to detect the presence of benthic visual indicators of organic enrichment (organic pellets, sulphur oxidizing bacteria-mats, opportunistic polychaete complexes (OPC), the lugworm *Arenicola marina*, polychaete tube aggregations, epifauna) through image analysis, and to determine the environmental factors affecting their distribution. Changes in the epifaunal community structure through time, and over different locations and habitats (soft-, mixed-, hard- bottom substrate), were also investigated. Images were collected beneath 3 fish cages (and a reference site) at a fin-fish farm site on the Western coast of Norway. A method was developed to characterize images and provide quantitative information on the ecological state under the farm throughout a fish production cycle. Through the use of the software Biigle 2.0, pictures were examined for surface area coverage of organic pellets and abundance of bacterial mats, OPC, *A. marina*, polychaete tube aggregations and epifauna. Results showed that the presence of organic material and bacterial mats was not indicative of a particular level of organic enrichment, as they were present throughout the whole fish production cycle. Benthic organisms such as the lugworm *A. marina* and aggregations of polychaete tubes were indicators of relatively low levels of organic enrichment and early stages of the production cycle. On the other hand, opportunistic polychaete complexes were indicators of relatively high levels of organic enrichment from the farm. Epifaunal community structure was considerably affected by the deposition of organic waste, as well by the sediment type, and taxa richness was considerably higher at reference sites (100 meters from farm cages). These results provide knowledge on the temporal impact of organic enrichment on benthic communities beneath fish farms over mixed- bottom substrates and highlight how the use of image characterization can improve the monitoring of benthic communities. The outcome of this study can contribute to the development of an environmental index to assess the ecological state around aquaculture farms placed over mixed- and hard- bottom areas.

KEYWORDS: Aquaculture – Organic waste – Benthic communities – Epifauna – Mixed substrates – Environmental index

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1. Introduction

1.1 Broad aquaculture overview

A growing global population has led to increasing demand for seafood (i.e. fish, shellfish), which represent a valuable source of high-quality protein. As a result, per capita consumption of seafood has grown up to 40% in the last 20 years. Marine aquaculture is increasingly gaining importance as an alternative to capture fisheries, partly driven by natural fisheries depletion (Goldburg and Naylor, 2005; Amberg and Hall, 2008). According to United Nations' Food and Agriculture Organization (FAO, 2016), inland and marine aquaculture production have increased from 6.2 million tons a year (in 1983) to 82.1 million tons in 2018. Currently, global fish aquaculture production equates to more than the fisheries biomass, with aquaculture reaching 46 per cent of the global fish production in 2016-2018 (FAO, 2020). In recent years, both inland and coastal/marine fed-aquaculture (fish-farming) has outgrown non-fed aquaculture (extractive species, e.g. molluscs, seaweed). Around 580 marine species are currently farmed all over the world and in the mariculture sector, salmon and shrimp farming have seen a relatively recent boom (Goldburg and Naylor, 2005). In particular, Atlantic salmon (*Salmo salar)* is a valuable and highly exploited commercial species. Wild stocks of this anadromous species have halved during the past decades (Hindar *et al*., 2011), whereas commercial farming has thrived. Fish farming has been especially flourishing in the North Atlantic (i.e. Norway, Scotland, Newfoundland) and regions of the eastern Pacific coast (i.e. British Columbia, Chile), where the temperate and sheltered conditions of coastal areas provide the most suitable conditions for the placement of salmon cages. In Norway, the production of Atlantic Salmon has experienced rapid growth. Consequently, the economic value of the industry has increased, together with the demand for new farm sites and the number of companies involved in the sector (Richardsen *et al., 2016;* Ellis and Tiller, 2019).

1.2 Aquaculture in Norway

In 2018, Norway was the 7th major aquaculture producer of fish, with 1354.9 thousand tonnes of finfish produced during the year (FAO, 2020). The extensive coast and fjord systems represent an ideal environment for aquaculture, providing sheltered conditions, steady water temperatures, and strong currents (Ellis and Tiller, 2019). Fish are bred in net cages, often anchored to the ocean bottom. Although farming of other species, such as Rainbow trout (*Oncorhynchus mykiss*), Halibut (*Hippoglossus hippoglossus*) and Char (*Salvelinus*), exists in the country, Atlantic salmon remains the most successful aquaculture species and one of the biggest export industries for the country, after oil and gas. The sector provides social and cultural benefits, such as the creation of a new market and employment opportunities, and thus has a major value at national level (Liu *et al.,* 2011). Nowadays, the sector accounts for more than 50 per cent of the world's production of farmed Atlantic salmon (*Statistics Norway, 2020*).

1.3 Environmental impact of aquaculture

Several environmental impacts have been associated with the increase of the finfish industry in Norway, such as fish escapements, sea-lice infections among farmed fish, and use of pesticides and chemicals (Forseth *et al.,* 2017; Mcginnity *et al*., 2003; Parsons *et al.,* 2020). One of the main environmental concerns is represented by the release of organic (in form of uneaten feed pellets and faeces) discharge (Goldburg and Naylor, 2005, Holmer *et al.,* 2005). If areas do not benefit from strong currents, these effluents do not get dispersed and particles sink to the seafloor (Goldburg *et al.,* 2001), creating a layer of sludge covering the sea bottom. An accumulation of flocculent material (faeces, feed pellets, sedimented organic matter; Salvo *et al.,* 2015) can represent a major change of substrate/habitat type for benthic communities, on both hard and soft bottom areas (Hamoutene *et al.,* 2016).

1.4 Impact of waste release

In Norway, salmon aquaculture is governed by the Ministry of Fisheries and Coastal Affairs (Liu *et al.,* 2011). The Ministry has so far implemented many regulations to reduce the impact of waste release and nutrient loading from fish cages through the control of production levels, optimization of fish-feed composition (e.g. to reduce the percentage of uneaten feed), site selection for fin-fish cages (e.g. where stronger currents can flush waste away), and of fallow periods between production cycles to allow seafloors to recover from organic loading. In Norway, a system of mandatory monitoring investigations (Hansen *et al.,* 2001; Anon, 2016) is used for the quantitative assessment of benthic organic loading and benthic community responses in soft sediment environments. Risk assessment of waste discharge and nutrient overload is based on a combination of research-based advice and compulsory monitoring (Taranger *et al*., 2014).

1.5 Indicators of organic enrichment

Organic enrichment from fish waste on the seafloor increases respiration rates and may lead to oxygen depletion, which favours the replacement of existent communities with benthic organisms characterized by high tolerance thresholds to organic enrichment (e.g., opportunist polychaetes (OPC), sulphur-oxidizing bacteria as *Beggiatoa* spp. (Wildish and Pohle, 2005; Verhoeven *et al.,* 2016)). Investigations and monitoring of hard substrates underneath cages have detected *Beggiatoa* spp.-bacterial mats, OPC and flocculent matter, which are considered the main visual indicators of benthic organic enrichment in Canadian waters (Fisheries and Ocean Canada, 2018; Hansen *et al*., 2011). White mats of sulphur-oxidizing bacteria as *Beggiatoa* spp. can be found in association with high levels of sulphide and were previously observed underneath or next to farms, over hard substrates (Hamoutene*,* 2014). OPC containing individuals from the genera *Vigtorinella* sp. and *Ophryotrocha* sp. had been reported to fully cover the hard substrate beneath fish cages and to feed on the organic waste from some Norwegian fish farms (Hansen *et al*., 2011; Eikje, 2013). According to previous studies, the percentage coverage of bacterial mats and OPC increases with decreasing distance from the cages indicating an association with farm enrichment (Hamoutene *et al.,* 2016).

Challenges related to monitoring non-soft substrates, where collecting samples is not feasible, have so far hindered the monitoring processes for mixed- and hard- bottom substrates (Keeley *et al*., 2021). Visual monitoring techniques, such as drop cameras surveys, allow to follow changes in the coverage of indicators of enrichment (DFO, 2013; Hamoutene *et al.,* 2014, 2016, 2018) but are challenged by the patchy nature and distribution of hard-bottom substrate organisms, often unable to indicate high levels of impact (Keeley *et al*., 2021). However, the isolation and analysis of eDNA sequences are progressively gaining importance in the characterization of hard-bottoms indicators of organic enrichment.

1.6 Integrated multitrophic aquaculture

Knowledge on the impact of organic overload from fin-fish aquaculture on the seabed and benthic communities has led to an interest in more sustainable aquacultural methods, such as integrated multitrophic aquaculture (IMTA) *(*Chopin *et al.,* 2008*;* Troell *et al.,* 2009*;* Hughes & Black, 2016; Jansen *et al.,* 2019*).* Within the IMTA practice, wastes from one species (in this case fin-fish) are recycled to become food and energy for another species, such as extractive species like mussels or seaweed (Troell *et al.,* 2003; Chopin *et al.,* 2008; Granada *et al.,* 2016). Given the considerable flux of organic particles to the seafloor from fin-fish farms, the use of benthic deposit-feeders in a benthic IMTA context has been studied by Filgueira *et al.* (2017) and Jansen *et al.* (2019). According to Torrisen *et al.* (2018), opportunistic polychaete systems that proliferate in areas with high organic matter levels are the most suitable candidates for benthic IMTA systems, as they can consume organic matter and mitigate the impact of waste sinking to the bottom underneath fish farms (Kinoshita *et al*., 2008; Brown *et al*., 2011; Nederlof *et al*., 2020).

1.7 Aims and objectives of the project

As part of a large Ocean Forest project, representing a collaboration between Lerøy and Bellona with the participation of the Institute of Marine Research (IMR), aquaculture by-products mitigation by polychaetes is under investigation in a Rainbow trout farm located around Austevoll, in Western Norway. The ability of polychaetes complexes (of the species *Ophryotrocha craigsmithi*) occupying (artificial) hard bottom substrates to mitigate the impact of organic co-products is evaluated, as well as by their growth, life-history traits, biomass and carbon turnover rates. To assess the succession and density of polychaetes, steel trays simulating rock substrates were placed underneath farms at 80 meters depth (Jansen *et al*., 2019). Through image collection and analysis, remote monitoring of the bottom was conducted to assess biomass and succession on the trays and the benthic state of the seafloor.

Within this framework, the present project thesis was designed. The presence on soft and hard substrate of indicators of benthic organic enrichment (presence of organic matter on the sediment, sulphur oxidizing bacteria forming mats, polychaete complexes (OPC), lugworm *Arenicola marina*, aggregations of polychaete tubes, and epifauna) was analysed through the images collected underneath fin-fish cages. Consequently, one of the main objectives of this project was to identify the main indicators of organic enrichment over mixed- and hard- bottom substrates. We expected bacterial mats, OPC and epifaunal species to provide a clear indication of aquaculture activity. Therefore, we examined the factors influencing the distribution and abundance of indicators of organic enrichment (IOE). We hypothesised habitat type (sandy, mixed or rocky substrate), changes in fish biomass and total particulate matter flux throughout the fish production cycle, and proximity to the farm to be the main factors to have an impact on benthic indicators of enrichment. Furthermore, we aimed at assessing changes in the epifaunal community structure over time, assuming the relation to habitat type and the proximity to the farm site to be the main factors driving community structure variations. Finally, we wanted to develop a methodology, based on software analysis, to characterize images based on the presence of visual indicators and to provide quantitative information on the benthic ecosystem state.

2. Materials and methods

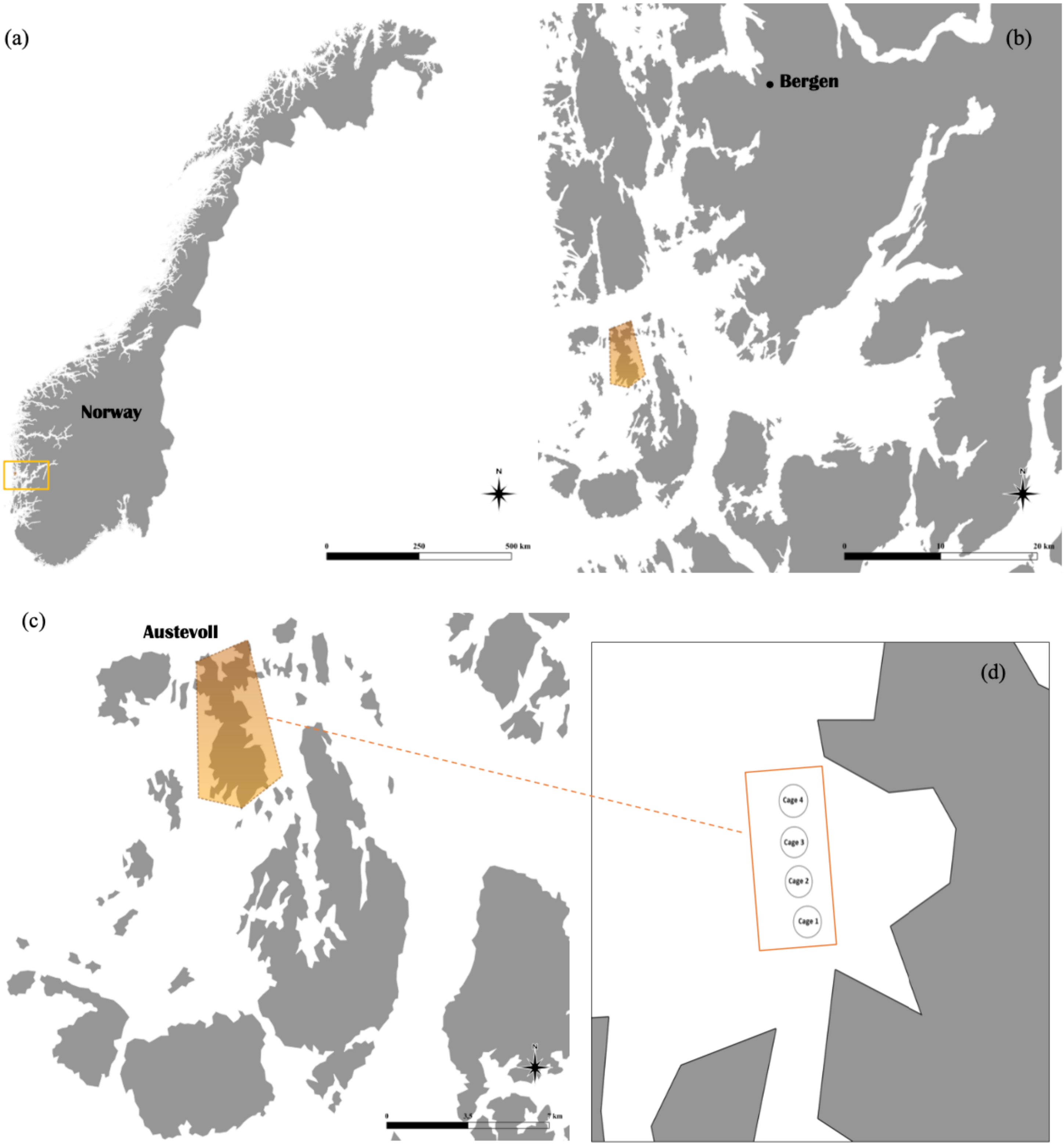
2.1 Site description

Drop camera surveys were conducted around a fish farm (60.138600 °N; 5.110533 °E) producing Rainbow trout (*Oncorhynchus mykiss*) near the northern coast of the island of Austevoll, western Norway (Fig. 1a, b). This farm site was established on June 18th, 1996 and was licenced to produce a maximum allowed biomass (MAB), at any time of production, of 3120 tonnes (from on-site production data). Farm cages were situated in an area of mixed-, hard- and soft- bottom substrate at a depth of approximately 80 meters. Here the main substrate types were identified as: 1) sandy, 2) patchy bedrock, and 3) broken rocks (Table 1). Fish were set out in 3 of the farm cages between the 23rd of August (Cage 4) and the 11th of September (Cage 2 and 3) 2020. At end of May 2021, approximately half the fish present in the net-pens was moved into 3 additional net-pens that were placed beside the 3 original ones. All fish was finally harvested in October/November 2021.

Table 1 | Survey sites characteristics and locations.

Site	Substrate characteristics	Depth (m)	Coordinates
Reference	Sandy, patchy bedrock, broken rocks	80-90	60 08.300 °N 5 06.426 °E
Cage 2	Sandy, patchy bedrock, broken rocks	90	60 08.297°N 5 06.703°E
Cage 3	Sandy, patchy bedrock	84	60 08.336°N 5 06.673°E
Cage 4	Sandy, patchy bedrock	84	60 08.379°N 5 06.649°E

Fig. 1 | (a) Overview map of Norway indicating the region along the Western coast of Norway, where the farm is located. (b) Island of Austevoll and (c) the specific location of the farm. (d) Set up of the farm with the 4 trout cages.



2.2 Experimental design

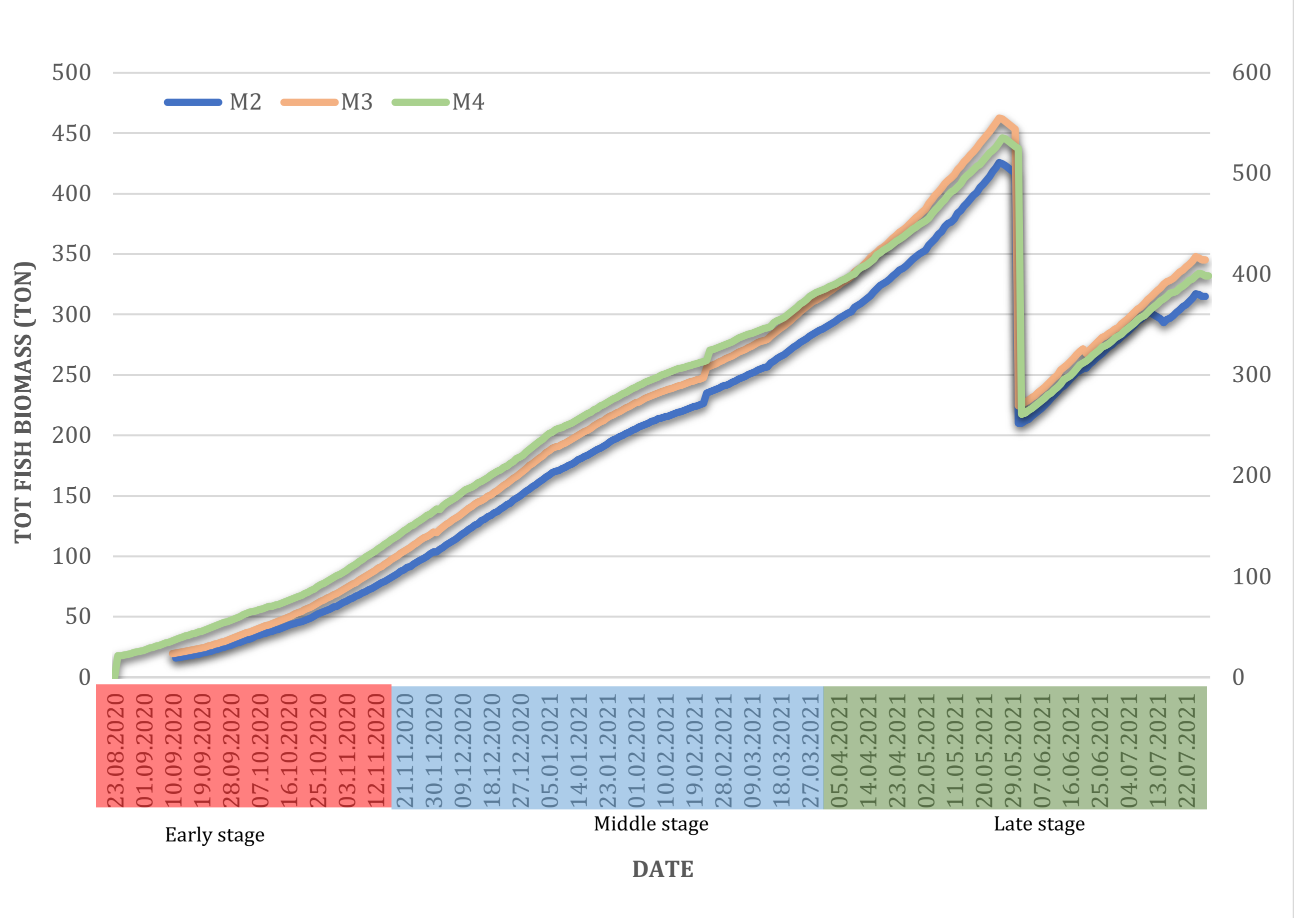
The presence of benthic visual indicators on the substrate beneath the farm cages was investigated. Surveys were conducted around three of the six farm cages: Cage 2, Cage 3, Cage 4 and one additional reference site situated 100 meters away from the net-pens (Fig.1d, Table 1). Images of the substrate underneath the farm were collected eleven times (approximately every 3-4 weeks), starting in September 2020 and ending in September 2021. The sampling points followed the production cycle of fish: 1) early stage, when fish were approximately 1 kg in weight and the amount of organic matter released was relatively low; 2) middle stage when organic products have sedimented for a prolonged period; 3) final stage, at the peak of production (Fig. 2a). At the outset of fish (late August), the total biomass of Rainbow trout in each net-pen was around 20 tons. The peak of production was around the end of May when the total amount of fish in the cages was around 500 tons (Fig 2a). Accordingly, the amount of feed used for each cage increased throughout the experimental period (Fig. 2b). By the end of July 2021, a total of 660 tons of feed were used for Cage 2, 722.5 tons for Cage 3 and 839 tons for Cage 4. Images of the substrate beneath fish cages were collected using drop camera surveys. Sediment traps were deployed to determine the total particulate material influx from the farm.

Table 2 | Sampling dates, stations and type of material used for monitoring.

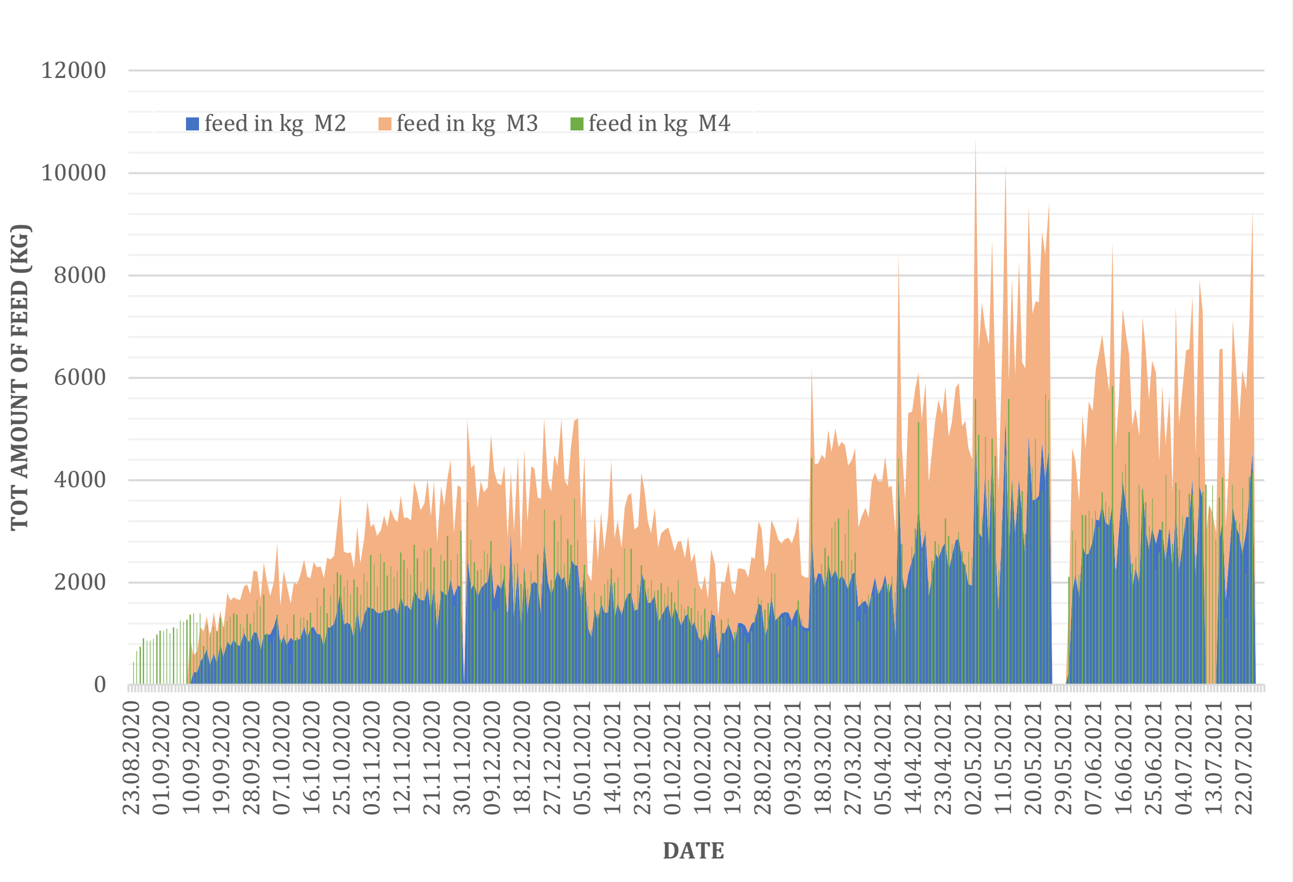
Date	**Station**	**N. of image stations at each location**
23.09.2020	M2, M3, M4 + Ref	M2= 10, M3= 11, M4= 9, Ref= 14
08.10.2020	M2, M3, M4 + Ref	M2= 5, M3= 17, M4= 9, Ref= 13
22.10.2020	M2, M3, M4 + Ref	M2= 17, M3= 15, M4= 16, Ref= 12
20.11.2020	M2, M4	M2= 15, M4= 6
20.01.2021	M2, M3, M4 + Ref	M2= 10, M3= 9, M4= 10, Ref= 11
01.02.2021	M2, M3, M4 + Ref	M2= 10, M3= 5, M4= 10, Ref= 12
16.02.2021	M2, M3, M4 + Ref	M2= 12, M3= 10, M4= 9, Ref= 11
05.03.2021	M2, M3, M4 + Ref	M2= 10, M3= 10, M4= 10, Ref= 11
23.04.2021	M2, M3, M4 + Ref	M2= 5, M3= 10, M4= 12, Ref= 10
03.06.2021	M2, M3, M4 + Ref	M2, M3, M4, Ref = 10
27.06.2021	M2, M3, M4 + Ref	M2, M3, M4, Ref = 10
04.08.2021	M2, M3, M4 + Ref	M2, M3, M4, Ref = 10
14.09.2021	M2, M3, M4 + Ref	M2, M3, M4, Ref = 10

Fig. 2 | Plots showing (a) the total biomass of fish (ton) for each net-pen (M2, M3, M4), (b) the amount of feed (kg) that was used for each cage from the outset in August 2020 to the end of July 2021. In plot (a), arrows indicate when images were collected. Dates in the x axes are highlighted according to the fish production cycle stage.

(a) Total biomass of fish (ton) for each net-pen



(b) Feed (kg) for each net-pen



2.4 Drop-camera surveys

To estimate the changes in the abundance and seafloor coverage of OPC, bacterial mats, fish waste (faeces and uneaten food) throughout the farm production cycle, a drop-camera system was used to collect digital stills images adjacent to fish cages and at a reference site. Camera surveys, besides sediment traps deployment, were conducted by Signe Svensson (IMR, Lerøy), within the framework of her PhD research.

Using a GoPro Hero 8 (f2.8, shutter speed 1/161s, ISO-666, focal length 3 mm), HD images of the seafloor at ca. 80 m depth were obtained. The camera was attached to a steel frame and dropped to the seafloor employing a 90 m long rope. One Keldan 4X video light (9000 lm) was placed next to the camera and angled to the centre of the photo frame to illuminate images at depth where natural light is low. Additionally, the steel frame was connected to a measuring frame with a scale bar marked every 1 cm which was placed in contact with the substrate to estimate surface area (Fig. 3). For each net-pen, images were taken every ca. 2 meters along an 18 meters long section of the cage, for a total of ca. 10 images per station. At each image station, 5 different frames were shot with 5 seconds between each frame (Fig. 4).

Fig. 3 | Diagram showing a drop-camera system.

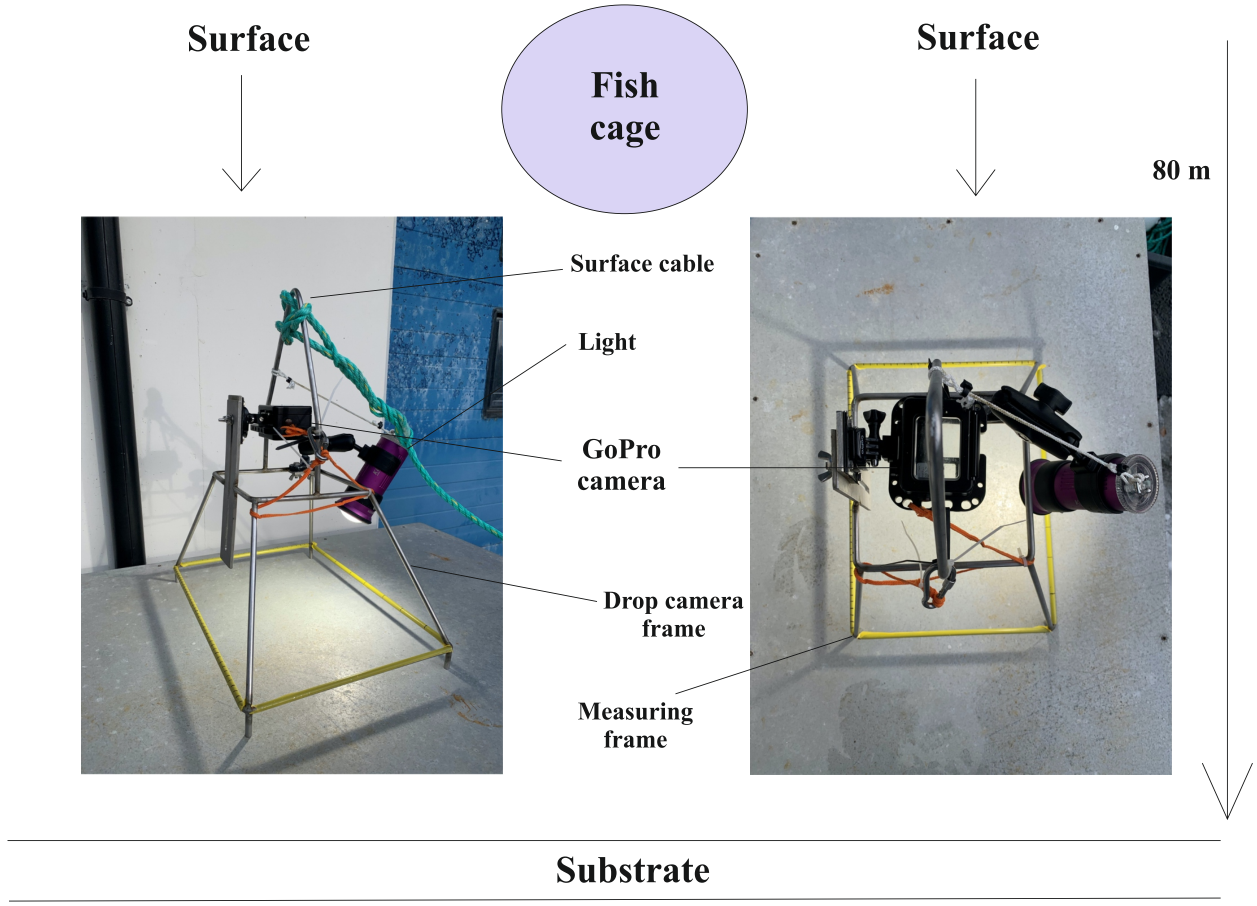
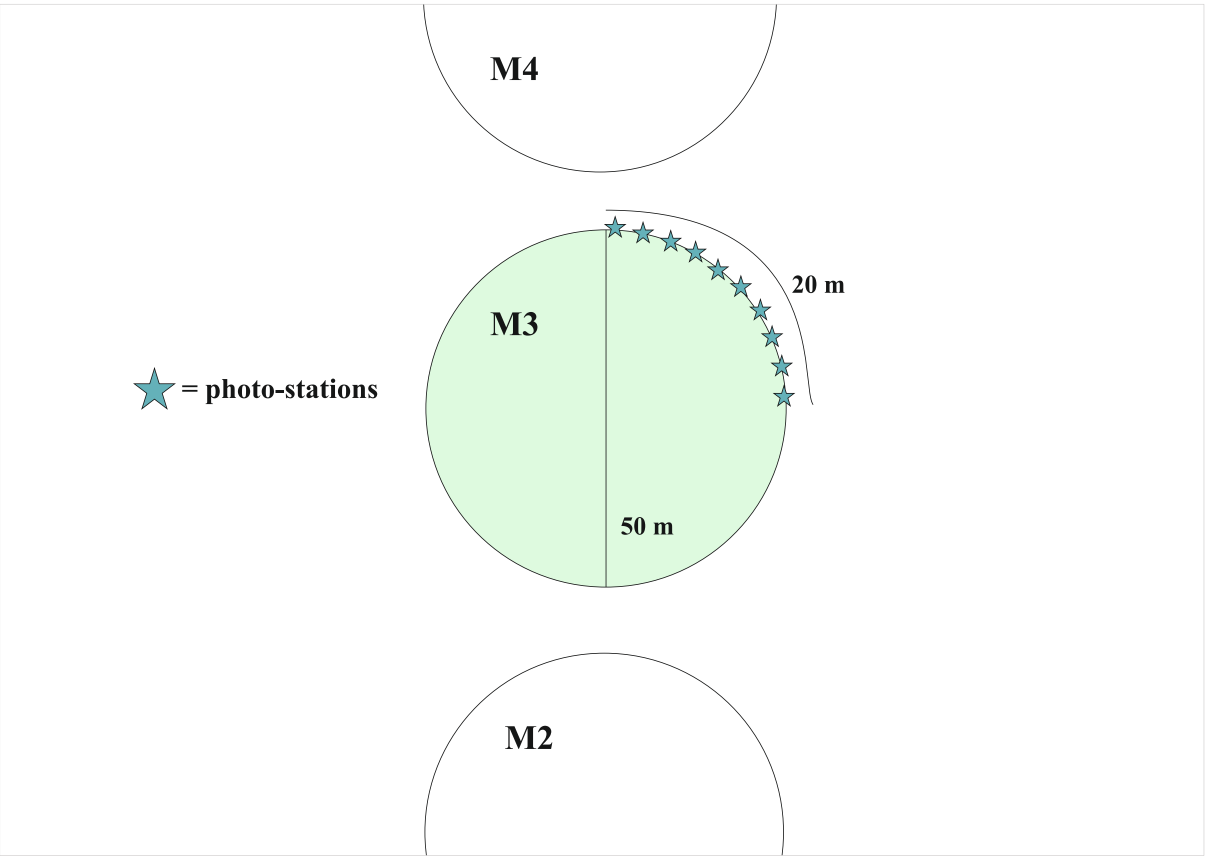


Fig. 4 | Diagram showing the set-up of photo-stations at each net pen in the farm.



2.5 Depositional flux measurements

To estimate the depositional flux of suspended particles to the seafloor, sediment traps were deployed next to each of the three study net pens. Each sediment trap rig consisted of three cylinders, each filled with 0,5 L of seawater enriched with 5 grams of NaCl and buffered with a 4% formalin solution. The high salinity seawater solution maintained the formalin solution at the bottom of the cylinder, preserving organic matter against decomposition.
Sediment traps collected suspended organic matter at ca. 6 meters above the seafloor. During the experiment, traps were deployed three times and for approximately 2 weeks each (Table 3).

Table 3 | Sediment traps dates and length of deployment.

Deployment date	N. days
24.09.2020	13
16.02.2021	13
16.04.2021	15

Each sediment rig (one for each net pen) was anchored to the bottom with 100 kg of weight. Traps were situated 6 meters above the seafloor (Fig. 5). Upon retrieval, suspended material in the cylinders was allowed to settle and excess water was removed. Finally, cylinders were sealed and brought to the lab for content analysis. Total particulate dry weight flux (g m^{-2} d^{-1}) was determined from material collected in the sediment traps by filtering a known volume of the suspended organic material on pre-weighed filters. Filters were oven-dried and reweighed to determine total particulate dry weight.

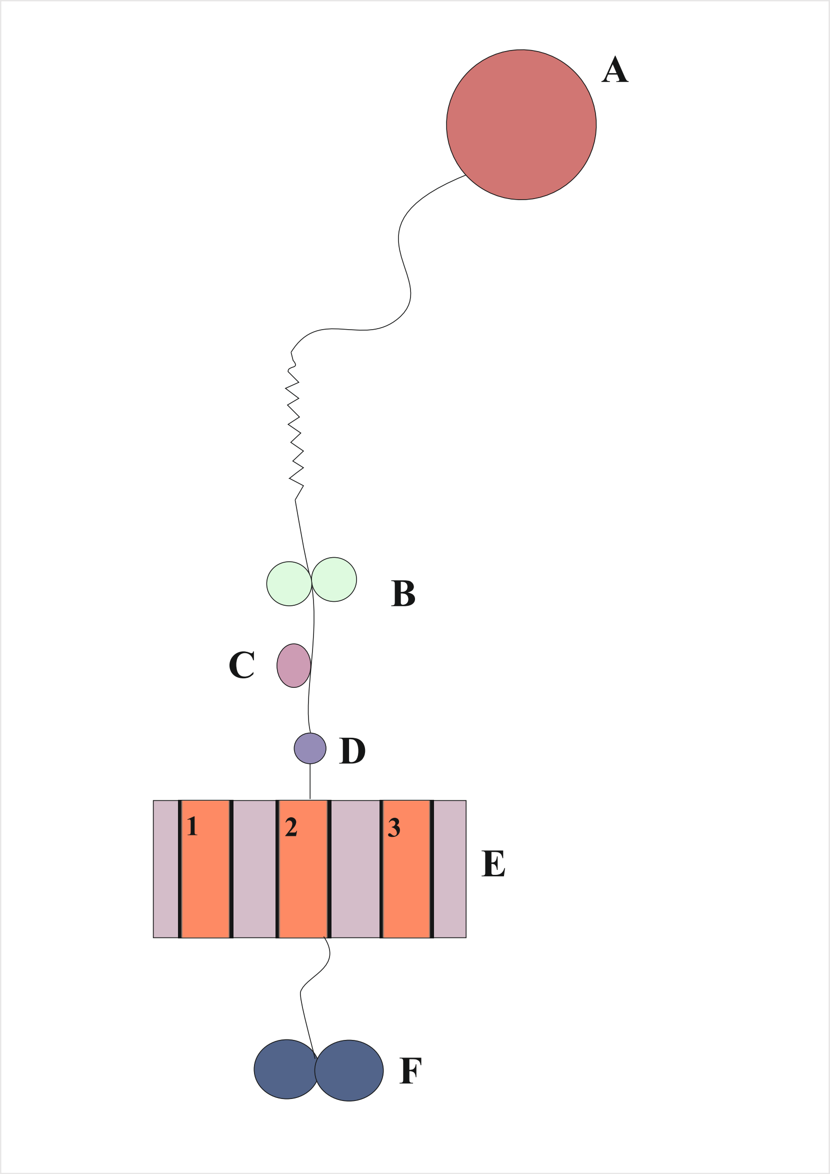
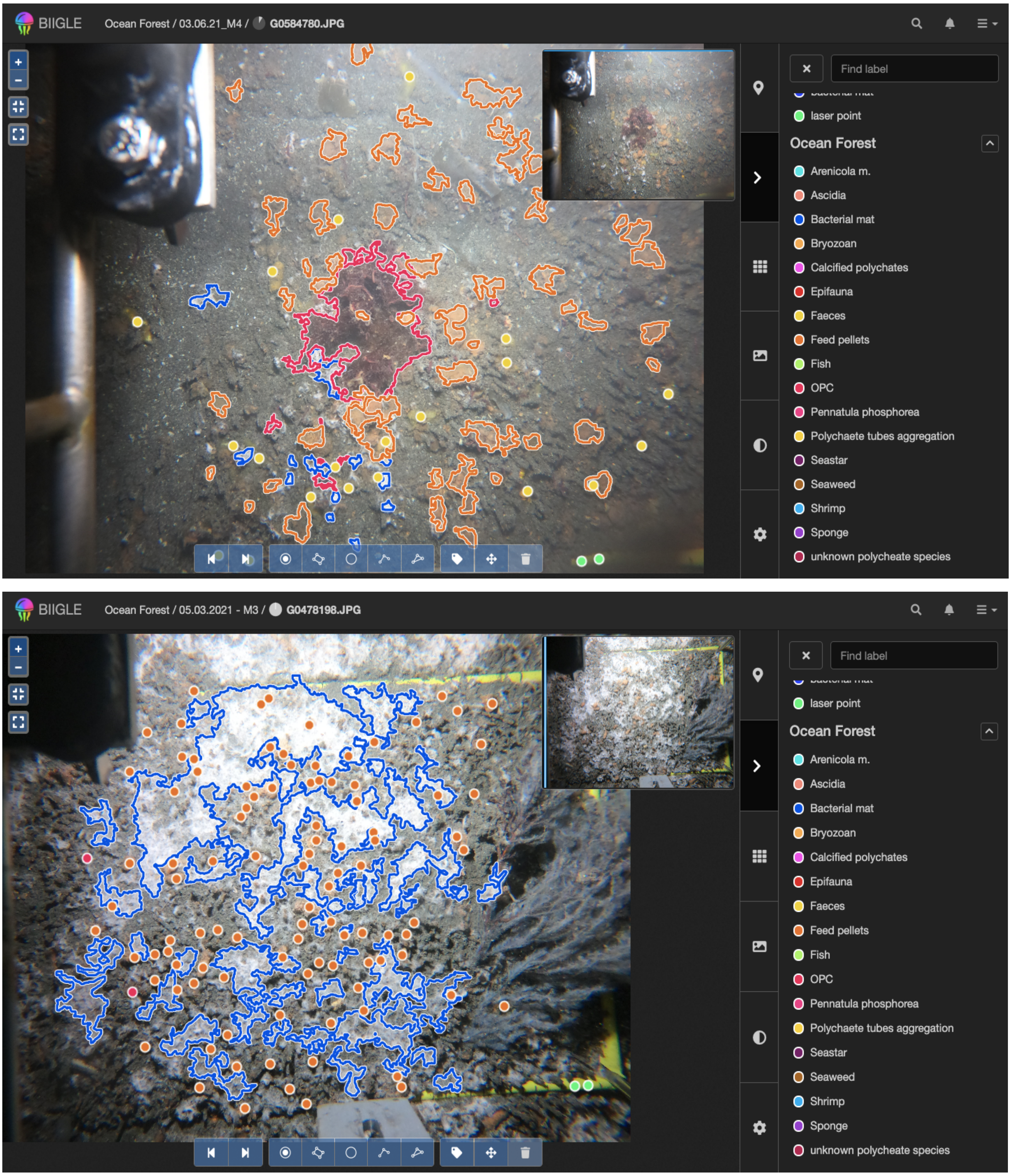


Fig. 5 | Representation of a sediment rigg. At surface, a float (A) attached to the cage keeps the trap suspended above the seafloor. Two large trawl floats (B), a release point (C) and a shackel (D) are attached to the suspension rope. The three cylinders (E-1,2,3) are placed inside a frame ca. 6 meters above the seafloor. At bottom, a 100 kg weight (F) anchors the rig to the sea floor.

2.6 Image annotation

The Bio-Image Indexing and Graphical Labelling Environment (BIIGLE 2.0) was used to annotate images for the presence of benthic indicators of organic enrichment and epifauna. From each sampling location, only one image was annotated for each image station, i.e. 10 images per location. A label tree was created to collate the labels used to annotate images (e.g. feed and faecal pellets, nematodes complexes, bacterial mats) (Fig. 6). Each label was characterized by a name and a colour. The annotation catalogue allowed all annotations within a label to be visualized together.

Fig. 6 | Example of images annotated using the image analysis software Biigle. Each benthic indicator is annotated with a different colour (e.g., orange for organic pellets, blue for bacterial mats, pink for polychaete complexes).



Visual indicators were annotated according to either total count or surface area coverage (Fig.7). Total counts were determined using the Biigle point annotation tools such as a point (consisting of a single coordinate), rectangle (consisting of four coordinates), and circle annotation (consisting of a centre point and a radius). To detect surface area coverage, the magic wand annotation tool was used. Such a tool allowed regions where pixels share similar colours to be detected and annotated by drawing be a polygon around them. To determine the pixel-to-centimetres ratio of the surface area, the laser point detection tool was used. Manual annotation of a few laser points (at least 4 images) was first required. For this, the scale bar (1 cm) from the measuring frame of the camera system was used (Fig. 3). The Biigle software was then able to automatically detect laser points for the rest of the images in the folder (each folder constituted by a sample location).

For sulphur oxidizing bacteria (presumably *Beggiatoa* spp.) mats, both surface area coverage and counts for abundance estimations were measured. Organic pellets (uneaten feed and faeces) were either counted when single pellets were easy to identify, or measured in size, in case they formed indiscernible aggregations. Eventually, count data was transformed into surface area data, later used for plots and statistical analysis. Opportunistic polychaete complexes (OPC) were both measured for surface area and counted for abundance. The indicators of enrichment as polychaete tubes aggregations (PTA) and the lugworm *Arenicola marina* were counted for abundance estimates. Other benthic organisms (e.g. tunicates, polychaetes, ascidians, fish, sponges), classified as epifauna, were identified to the lowest taxonomic level possible and counted. Complex-forming polychaetes, thought to be *Ophryothroca* spp. (not yet identified), were found during the last two sampling dates and counted. For plots and statistical analysis, count data of individuals per photo-station was used for all indicators of enrichment, except for organic pellets data.

At the end of the annotating process, image annotation reports were generated. For each annotated image, data on either the abundance (listing the abundance of an annotation label for each image) or area (listing the area covered by an annotation label) of each visual indicator were generated. Annotation data displayed in the reports was thus organized into Excel files and later used for statistical analyses.

2.7 Statistical analysis

2.7.1 Objective 1: To assess changes in visual enrichment indicator abundance and surface area coverage through time and indicator relationship with site location and habitat.

To delineate changes in the abundance and surface area coverage at the 3 farm cage locations (and reference site), each indicator of organic enrichment was plotted through time. 12 time points were considered for each location. Count values of bacterial mats, OPC*, A. marina*, PTA were plotted for abundance (N. individuals per photo-station) estimates. For organic pellets, total area (sqpx) coverage data was plotted. Indicators were plotted both separately and in combination with other indicators of enrichment (IOE) for comparison. To establish the relationship between time and abundance/surface area, a univariate analysis was carried out in R (R Core Team, 2021). In the negative binomial distribution model, time was the predictor variable (i.e., independent) and IOEs the dependent variables. Organic pellets data was Tukey-transformed prior to statistical analysis (Freeman and Tukey, 1950). Time was represented as dummy variable (from 1 to 12). The effect of time on indicators was considered separately. Significance of the predictor variable on abundance/coverage changes was given by p-values < 0.05. Univariate analysis was again performed to determine the relationship between the presence of each indicator and site location and substrate type (sand, broken rocks, patchy bedrock). All indicators were represented by count data, except for organic pellets (surface area). Site location (1: reference, 2, 3 and 4: cages) and habitat (1: sand, 2: broken rocks, 3: patchy bedrock) were transformed into dummy variables. The relationship between dependent variables (visual indicators) and predictor variables (location, habitat type) was explored through a negative binomial model. P values lower than 0.05 indicated a correlation between site location/habitat and indicators.

2.7.2 *Objective 2: To examine the effect of time, location, and habitat on epifaunal community structure.*

To test variations in the epifauna community (tunicate, polychaetes, bivalves, crustaceans, etc.) between time, location and habitat, a multivariate analysis was carried out in PRIMER-E version 7 (Clarke and Gorley, 2015). Epifauna abundance data was fourth-root-transformed prior to analysis. Through the Bray-Curtis similarity metrics, a resemblance matrix was built to compare community structure through time and in different locations/habitats. Non-metric MDS (nMDS) orientation plots were made to visualize the transformed data in a multi-dimensional space and recognize similarities/dissimilarities within the community structure. Permutational Multivariate Analysis of Variance (PERMANOVA) was run to recognize the factors (i.e., time, location, habitat) affecting community structure. A SIMPER analysis was carried out to identify the most important taxon for each time point, location, and habitat.

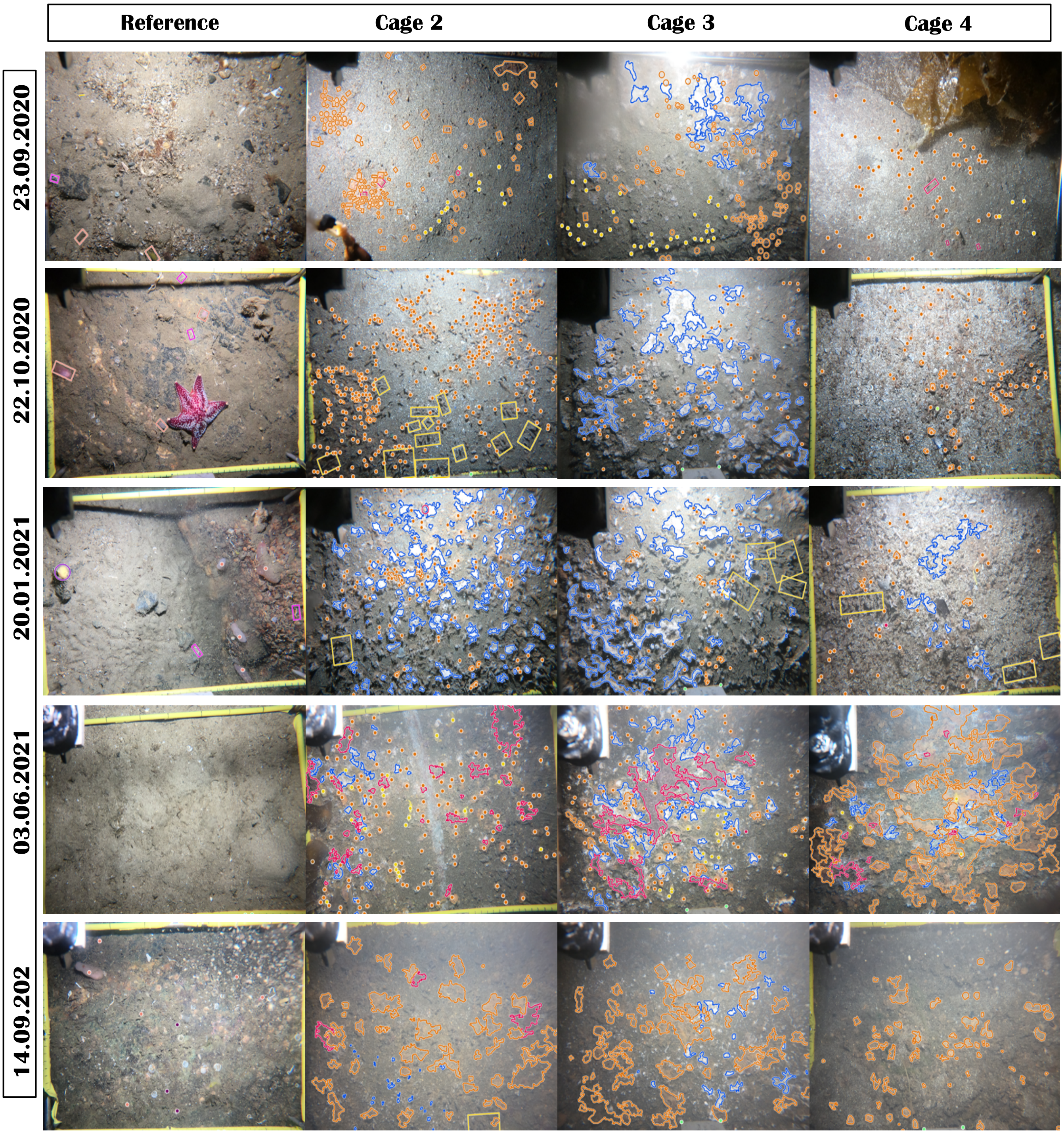


Fig. 7 | Images of the sea floor underneath farm cages. Each column represents a different location (Reference, Cage 2, 3, 4) and each row a different date. Indicators of organic enrichment are highlighted by colours, as annotated using the Biigle software. Blue shapes represent *Beggiatoa* spp., orange shapes are for organic pellets, pink shapes highlight OPC complexes, and yellow shapes are for polychaete tubes aggregations.

3. Results

3.1 Environmental site conditions: sedimentation, farm production rates, and current velocities

Total particulate matter (TPM) (g m^2 d^{-1}) flux rates were 10 times higher around cage 3 (data is missing for the other 2 cages) compared to the reference station in September 2020, and 40 times higher around the three cages (average) than the reference site in April 2021 (Table 4). TPM flux rate increased throughout the surveying period around all three farm cages, whereas flux values remained similar at reference sites (~ 2 ± 0.2 g m^2 d^{-1}). Feed (kg) and fish biomass (tons) data followed a similar trend and showed an overall increase throughout the survey time (Fig. 8). Velocity measurements were carried out in the upper 15 meters of the water column where average velocities were 8 and 6 cm/s at 5- and 15-meters water depth, respectively (MOM B reports).

Table 4 | Table showing the total particulate matter (TPM) flux (g m^2 d^{-1}) and particulate organic matter (POM) flux (m^2 d^{-1}) around farm cages and reference sites at three different dates during the survey. Data was provided by Signe Svensson (IMR).

Date	**Site**	**Mean g TPM m^2d^{-1}(± S.E.)**	**g POM m^2d^{-1}(± S.E.)**
24.09.2020	Cage 3	22.90 ± 5.84	18.7 ± 5.22
24.09.2020	Reference site	2.52 ± 0.216	0.908 ± 0.0548
16.02.2021	Cage 2	26.86 ± 0.516	19.7 ± 2.06
16.02.2021	Cage 3	19.98 ± 3.58	13.3 ± 1.44
16.02.2021	Cage 4	35.45 ± 10.8	11.3 ± 1.21
16.02.2021	Reference site	1.14 ± 0.124	0.396 ± 0.0074
16.04.2021	Cage 2	102.81 ± 34.0	53.9 ± 3.15
16.04.2021	Cage 3	78.61 ± 2.90	50.3 ± 7.41
16.04.2021	Cage 4	66.84 ± 13.0	43.6 ± 13.1
16.04.2021	Reference site	2.55 ± 0.248	1.19 ± 0.0179

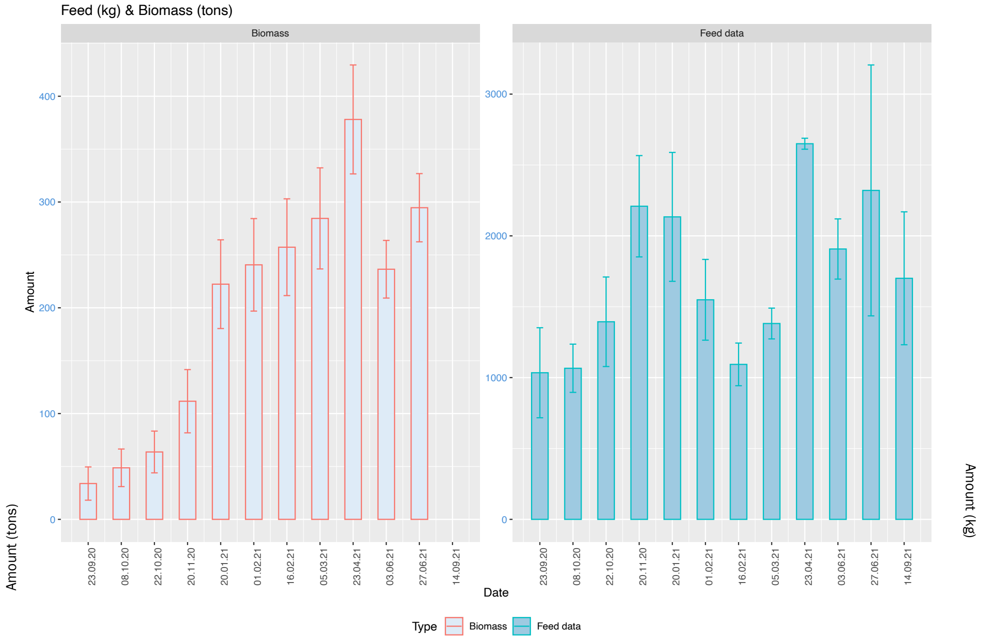


Fig. 8 | Boxplot of production data, shown in feed (kg) and biomass (tons) data. Average data and standard error lines are represented for Cage 2, 3 and 4, through time.

3.2 Changes in benthic enrichment indicators through the farm production cycle

The negative binomial model examined the effect of time (as a proxy of production cycle and TPM flux rates), location and habitat on the abundance and surface area coverage of benthic indicators (Table 5).

Organic pellets and bacterial mats – The extent of organic pellets surface area was not explained by time as a predictor variable and flocculent matter (i.e., organic pellets) covered a significant proportion of images throughout the production period (Table 5, Fig.9). Trends of changes in coverage per photo-station differ for each farm cage, with for instance an increase through time at cage 4, and two peaks (October 2020 and June 2021) at cage 2 (Fig. 9, Table 5). No organic pellets were found at reference sites, which leads to a significant effect of location. Seemingly, count data of *Beggiatoa* spp. mats showed a peak between January and May 2021 under cage 2 (between 770 and 330 ind. per photo-station), whereas no peak was detected underneath cage 3 and overall abundances were lower under cage 4 (Fig.9). Presence of *Beggiatoa*-like mats was not observed at the reference station, which also explained the significant effect of location (Fig. 9, Table 5). For both organic pellets and bacterial mats, no clear pattern was detected overtime during the production cycle.

Table 5 | Negative binomial model results for the effect of time, location, and habitat on the abundance and surface coverage per photo-station of IOE. Significant P values ($p < 0.01$) are highlighted in bold. Standard errors are shown between brackets.

	PELLETS	**BACTERIAL MATS**	**POLYCHAETE COMPLEXES**	**ARENICOLA MARINA**	**POL. TUBES AGGR.**	**EPIFAUNA**
TIME	0.52741 (0.35)	0.65661 (0.09)	**0.0120** (0.08)	**0.00198** (0.10)	**0.00384** (0.08)	0.100 (0.07)
LOCATION	**0.00246** (0.12)	**0.00619** (0.30)	**0.0253** (0.26)	0.24960 (0.28)	0.25883 (0.27)	**2.46e^{-05}** (0.24)
HABITAT	0.79956 (0.17)	0.33026 (0.42)	0.3654 (0.35)	0.78053 (0.53)	0.72487 (0.37)	0.535 (0.30)

Opportunistic polychaete complexes – Changes in filamentous OPC abundance were significantly affected by survey time (p-value = 0.01) (Table 5). The abundance of this indicator of enrichment increased over time in all three locations, except for the reference site where almost none were observed. Peaks in filamentous OPC abundance to > 200 individuals per photo-station were reached in June 2021 underneath all three farm cages (Fig.10). The location variable also had a significant effect on OPC abundance (p-value = 0.01; Table 5). Another species of complex-forming polychaetes, classified as *Ophryotrocha* sp. complexes (OPC) by visual identification, showed up late in the production cycle and were observed in August and September 2021 beneath fish cages (Fig.10), reaching a peak of 82 OPC per photo-station under cage 4 in August 2021.

Arenicola marina and polychaete tubes aggregations – Univariate analysis showed that time was a significant explanatory factor for *Arenicola marina* and PTA abundances (p-value < 0.001). Overall, the abundance of these two IOE declined in abundance through time. The presence of *A.marina* was not detected after the month of June 2021 in any cages (Fig. 9). At cage 4, a peak in *A.marina* abundance was observed in September 2020 (12 individuals per photo-station). PTA went from around 400 individuals for photo-station between September and November 2020 to less than 10 individuals in September 202, in both cages 2 and 3 (Fig.9). Both indicators were only detected at cages sites, and not at reference sites.

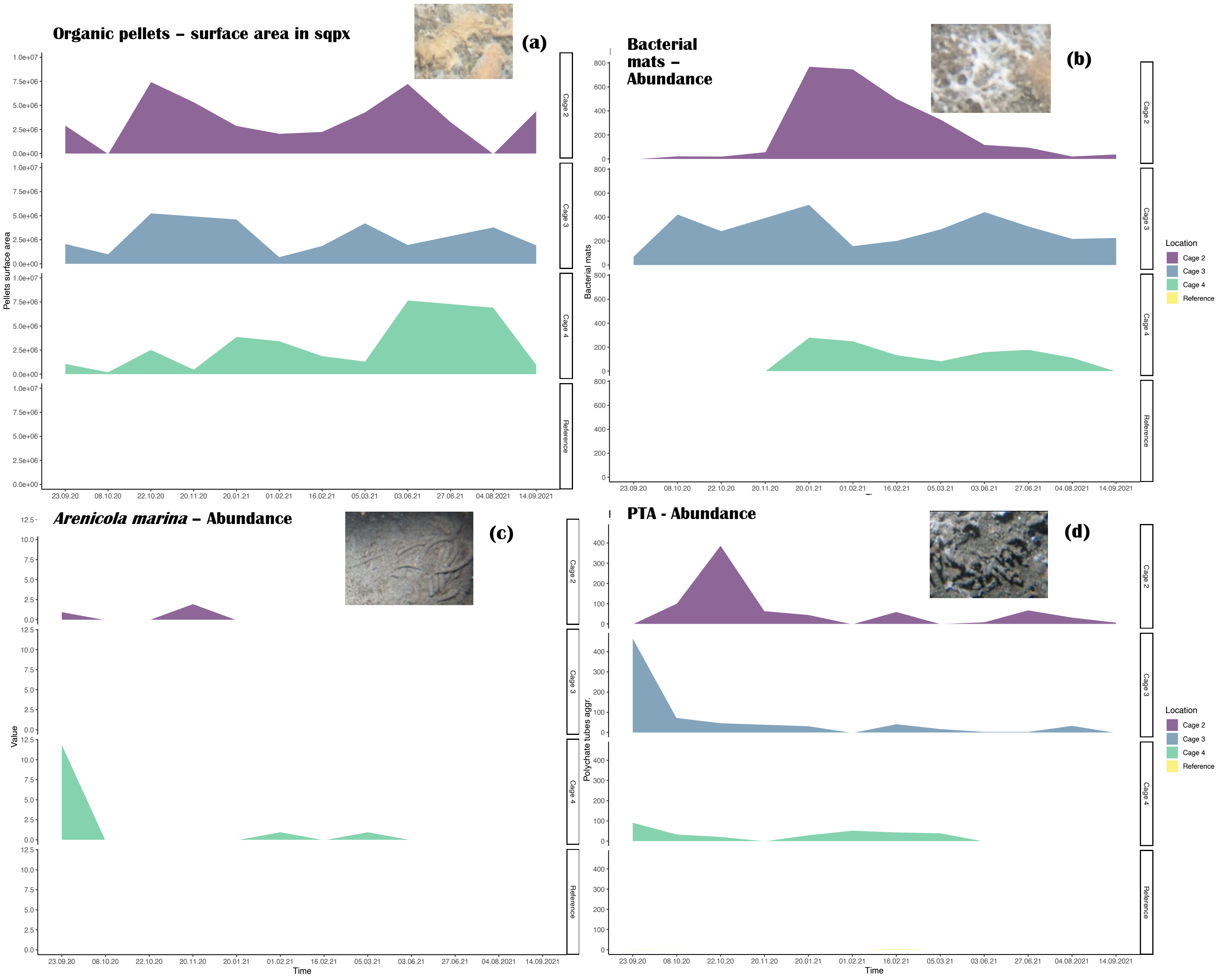


Fig. 9 | Plots showing the surface area coverage (sqpx) of organic material (a), *Beggiatoa* spp. mats (b), Arenicola marina (c), and polychaete tubes aggregations (c), at the 4 different locations (reference site and cage 2, 3, 4), from September 2020 to September 2021.

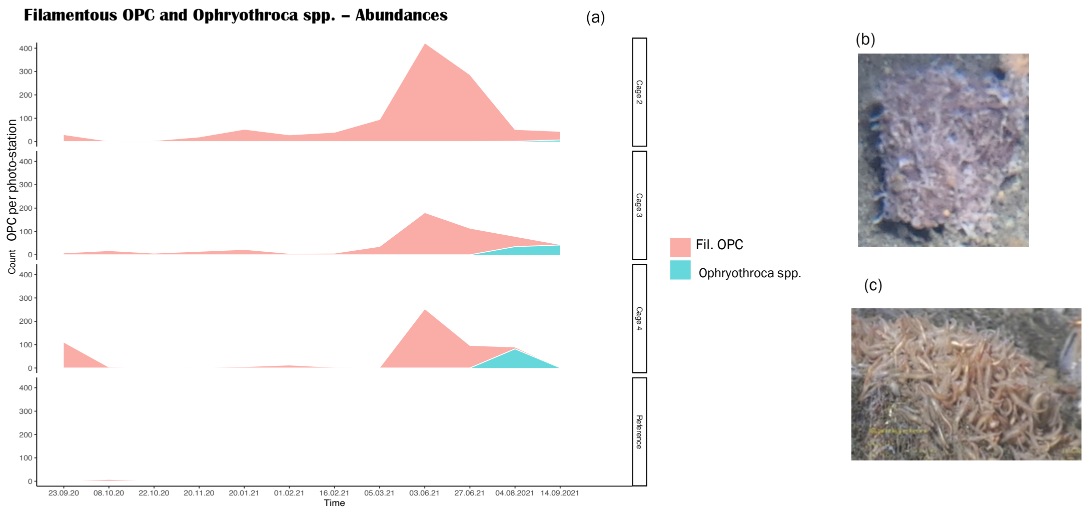


Fig.10 | (a) Plot showing the abundances of filamentous OPC (b) and Ophryothroca spp. complexes (c) at the four different locations (Reference Site, Cage 2, 3, 4), from September 2020 to September 2021.

3.3 Epifauna community structure

Throughout the whole sampling period, epifauna abundance at the reference site was significantly higher (N= 0-90 ind. per photo-station) compared to any of the three farm locations. Mean epifauna abundances at the reference site were seemingly higher over hard bottom/rocky substrates (15 ± 91 ind.) compared to soft/sandy substrates (0 ind.; Fig.11). However, univariate analysis revealed habitat type to be not significant (Table 5).

Multivariate analyses (PERMANOVA) were carried out for the epifaunal communities at taxa level. Results revealed that time did not represent an explanatory factor for variation in community composition. No significant change in epifauna community composition over the production cycle was shown at farm sites or reference sites. Whereas epifauna community structure, primarily at the reference site, was influenced by different substrate types (F_3= 3.33, p = 0.004; Table 6).

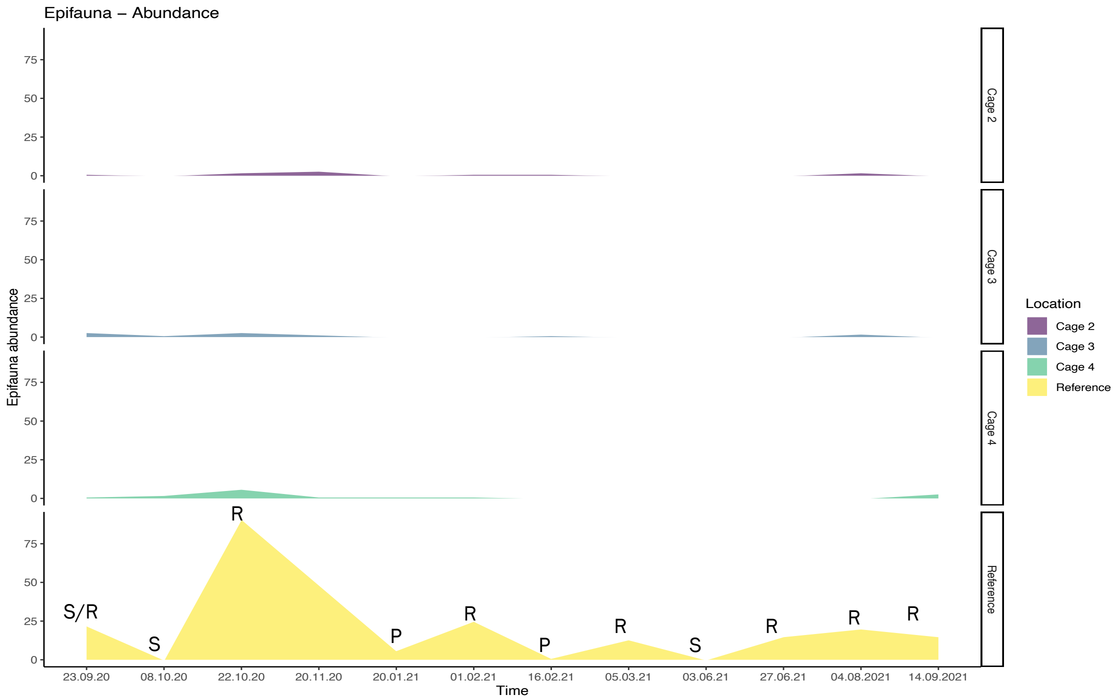


Fig. 11 | Plot showing the overall abundance of epifaunal individuals at the four different locations (Reference site, Cage 2, 3, 4), from September 2020 to September 2021. Above the abundance curve at the Reference station, letters represent the type of substrate for the date on the y-axis below: sandy (S), patchy bedrock (P), broken rocks (R).

Table 6 | PERMANOVA table of results.

SOURCE	DF	SS	MS	PSEUDO-F	P(PERM)	UNIQUE PERMS
LO	3	4439.2	1479.7	1.7304	0.097	998
HA	2	5711.7	2855.8	3.3395	0.004	999
LOXHA	3	2253.2	751.07	0.87827	0.548	999
RES	37	31641	855.16			
TOTAL	45	51671				

The taxonomic groups most frequently found at both farm locations and the reference site were polychaetes, tunicate, crustaceans, asteroidean and porifera. As revealed by SIMPER analysis, the dominating taxon in most stations was tunicate, contributing up to 80% to the total epifaunal community at reference sites, and 72% at farm location 4. At cage 2, crustaceans contributed to 70% of the whole community, and polychaetes 65% at cage 3. The average abundance of the dominating epifaunal group (tunicate) at the reference station was considerably higher compared to all farm sites (0.3; Table 7). The greatest dissimilarity was between cage 2 and cage 4 (95.2%) and cage 2 and cage 3 (94.4%), while the lowest was between the reference site and cage 3 (84.2%).

Polychaetes were the most common epifaunal group in sandy habitats, constituting 55% of the whole community, followed by tunicates (34%). On hard substrates (i.e., patchy bedrock, broken rocks), tunicates were the dominant taxon and on broken rocks habitats, tunicates reached an average abundance of 2.18 individuals per photo-station and contributed to 80% of the epifaunal community (Table 8). Non-metric MDS plots revealed higher levels of clustering for broken rocks communities compared to sandy and patchy bedrock habitats (Fig. 12). As shown in the overlay in Fig. 14, the main taxa contributing to the epifaunal community composition are tunicates and polychaetes, in line with the results from PERMANOVA and SIMPER analyses. On average, the dissimilarity between the structure of epifaunal communities on soft and hard substrates was around 88%.

Table 7 | SIMPER table of results for the predictor variable location.

	TAXON	AV. ABUNDANCE	AV. SIMILARITY	SIM/SD	CONTRIB%
SANDY	Polychaetes	0.22	3.39	0.21	55.26
	Tunicate	0.25	2.20	0.21	33.80
BROKEN ROCKS	Tunicate	2.18	41.99	4.04	79.95
PATCHY BEDROCK	Tunicate	0.55	14.96	0.50	75.44

Table 8 | SIMPER table of results for the predictor variable habitat.

	TAXON	AV. ABUNDANCE	AV. SIMILARITY	SIM/SD	CONTRIB%
REFERENCE	Tunicate	1.61	29.03	1.25	79.29%
CAGE 2	Crustaceans	0.25	3.83	0.23	70.95%
CAGE 3	Polychaetes	0.38	8.30	0.36	65.32%
	Tunicate	0.27	3.20	0.25	25.17%
CAGE 4	Tunicate	0.36	6.17	0.34	72.16%

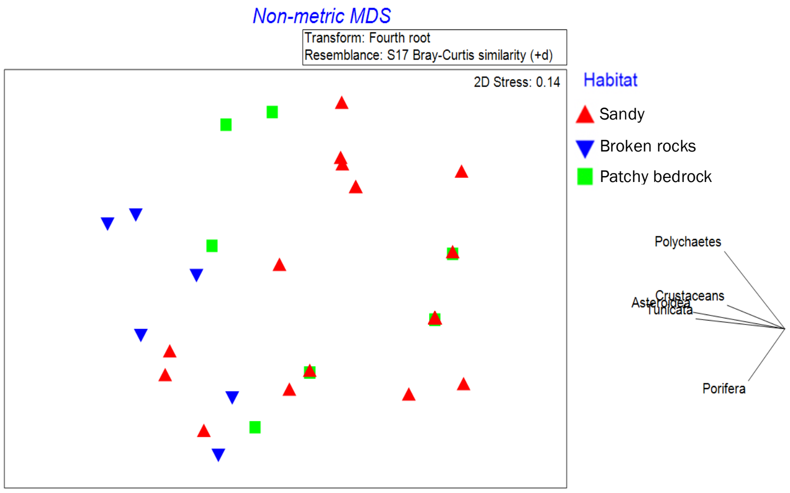


Fig. 12 | Multidimensional scaling (nMDS) plot of epifaunal community. The MDS coloured points represent community structure for the three different habitats (sandy, broken rocks, patchy bedrock) after fourth root transformation. Overlay represents the key epifauna taxa responsible for community structure in the 'broken rocks' habitat.

4. Discussion

4.1. Summary of results

Changes in abundance (i.e., count of individuals per photo-stations) and surface area coverage of indicators of organic enrichment were assessed during a Rainbow trout production cycle, from September 2020 to September 2021, under three fish cages and a reference site at > 100 meters distance from the farm. The results we found showed a clear impact of aquaculture activities on benthic indicators of enrichment. Significant coverage of flocculent matter (uneaten feed and faeces) was present in images from under all three cages throughout the whole fish production cycle. The absence of organic pellets at reference sites, and the 10 to 40 times higher sedimentation rates around the farm, indicated that flocculent matter consisted of fish waste. Furthermore, the presence of bacterial mats under all fish cages, and their absence at reference sites, conveyed the previously observed relationship between benthic organic pollution and the presence of sulphide oxidizing bacteria under aquaculture sites (Preisler *et al.*, 2007). While organic pellets and bacterial mats were found throughout the whole production cycle, other benthic indicators of organic enrichment were either present during the early stages of production (*Arenicola marina*, polychaete tube aggregations) or during the final stages (opportunistic polychaete complexes). Abundances of epifaunal species were significantly higher at the reference site than beneath all three fish cages, showing how epifaunal communities were negatively impacted by benthic pollution associated with the presence of fish waste on the seafloor. Epifauna community structure was also strongly influenced by substrate type (soft, hard), with significantly higher abundances found on hard bottom substrates.

4.2 Impact of organic enrichment on benthic visual indicators

4.2.1 Visual indicators of enrichment present throughout the production cycle

Organic pellets – Fish faecal pellets and uneaten food were observed in images under the three farm cages. Sampling at reference sites did not detect organic enrichment, revealing a correlation between aquaculture activities and the enhancement of flocculent material on the seafloor, in line with the higher TPM (g m^2 d^{-1}) flux rates detected around farm cages (compared to reference sites). However, the surface area coverage (sqpx) of pellets did not increase alongside fish biomass, as we expected, but instead fluctuated throughout the whole sampling period. Fish relocation events, variations in feeding rates, dispersion of pellets in the water column, and flushing by bottom water currents can explain variation in trends in bottom coverage of faecal and feed pellets underneath the three cages. As revealed by Hamoutene *et al.* (2016), the presence of organic pellets is not characteristic of a particular phase of organic enrichment during a fish production cycle. However, their distribution provides an indication of the deposition zones under cages. The discrepancy in peaks and troughs between cages has previously been related to hydrodynamic and flushing dynamics along the water column and at the seafloor, removing and distributing fish waste (Alongi, 1996; Sarà *et al.*, 2006). By changing the substrate type (especially on hard-bottom habitats), the extent of flocculent matter on the seafloor has a drastic impact on the presence and distribution of other benthic IOE (Hamoutene *et al.*, 2016).

Sulphur oxidizing bacteria – Similarly to faecal and feed pellets, bacterial mats were found during the whole production cycle, at all farm locations, both on soft and hard substrates. At reference sites, mats were not detected, showing the positive correlation of this benthic visual indicator with organic deposition from fish cages. The coverage of sulphur oxidizing mats beneath aquaculture sites over hard substrates was previously characterised by Hamoutene *et al.* (2013). In our study, the presence of bacterial mats was detected both over hard and soft sediments. Lack of a clear pattern in the temporal distribution of bacterial mats around farm sites was previously recorded by Salvo *et al.* (2017) in Newfoundland, Canada.

Images taken under the farm displayed some variation in the overall abundance of bacterial mats between the three cages. As the distribution of bacteria does not follow the ones of other indicators of

enrichment (organic pellets, nematodes, etc.), and as fish biomass was relatively similar in all three farm sites, the reason behind the difference between cages could be related to hydrodynamics.
Different water current patterns and flushing dynamics can be present beneath different cages, and throughout the production cycle. Environmental factors, such as sulphide concentration within the sediment, have been used as an explanation for bacterial mats distribution on sandy substrates by Hamoutene *et al.* (2014). On hard substrates, *Beggiatoa* spp. can exploit the oxygen-sulphide interface created by the layer of organic matter. A patchy distribution of organic matter on the sediment or hard bottom can thus result in a patchy distribution of bacterial mats.

4.2.2 Late indicators of enrichment

Filamentous OPC –Polychaete complexes showed a positive correlation with the increased time span of fish production and higher fish biomass. OPC peaked in June 2021 under all three farm cages, reaching up to > 400 ind. per photo-station at cage 2. Due to morphological assets (e.g., small size, direct benthic recruitment, etc.), meiofaunal species such as polychaetes have been previously shown to be highly sensitive to environmental pollution, as they display quick responses to increases in organic overload (Higgins and Thiel, 1988; Coull and Chandler, 1992; Sutherland *et al.*, 2007). The reliance on organic material for their nutrients and energy requirements can explain the presence of OPC beneath cages, especially when organic overloading occurs. OPC can represent an important factor to help determine benthic organic conditions. However, the exact polychaete species constituting the complexes we found under the study site has yet to be identified.

Ophryotrocha sp. complexes – By the final stage of our surveying period (August and September 2021), another polychaete species was detected underneath farm cages. These individuals were found in high abundances, particularly underneath stations with high amounts of organic matter, and seemed to form tight aggregations. Like the polychaete complexes previously encountered (i.e., filamentous OPC), this polychaete appeared during the latest farm production stages and increased with enhanced organic material flux, allegedly associated with organic flux to the bottom. From visual identification, we hypothesised this polychaete to be *Ophryothroca* sp., complex-forming opportunistic polychaetes often encountered in aquaculture sites and considered an important indicator of organic enrichment (Hansen *et al.*, 2011; Eijke *et al.*, 2013; Hamoutene *et al.*, 2015; Jansen *et al.*, 2019). Morphological and molecular species identification through laboratory analysis is still ongoing. The appearance of these opportunistic polychaete complexes can be favourable, as they are capable of ingesting organic matter and hold a high bio-mitigation potential (Kinoshita *et al.*, 2008; Brown *et al.*, 2011; Nederlof *et al.*, 2020). In previous research, conducted by Jansen *et al.* (2019), these polychaetes were seen rapidly colonizing hard substrates covered by a layer of flocculent matter. Therefore, their presence and abundance beneath cages are valuable for mitigation processes and hold high potential within IMTA frameworks.

4.2.3 Early indicators of enrichment

Arenicola marina and polychaete tubes aggregations – The lugworm *A. marina* was found at the beginning of the production cycle and subsequently decreased in abundance throughout the survey time. *A. marina* is an important 'ecosystem engineer' (Riisgård and Banta, 1998), able to metabolize organic carbon and re-oxygenate sediments (Volkenborn *et al.*, 2007; Wendelboe *et al.*, 2013), and thus has often been found to be present in high abundances beneath and around fish farms in both sandy/mud substrates (Keeley *et al.*, 2020), and hard bottom habitats (Dunlop *et al.*, 2021). Using waste from the farm as food source, the presence of this lugworm is thought to be beneficial for mitigating the overload of organic matter and anoxic conditions on the seabed (Keeley *et al.*, 2020). The ability of *A.marina* to build burrows in the sediment up to 40 cm deep (Kristensen, 2001) might make this species difficult to detect through video monitoring.
Seemingly, abundances of PTA decreased through time after an initial peak, particularly under cage 2 (in October 2020) and cage 3 (in September 2020). As with bacterial mats, the difference between cages might be given by the different hydrodynamics under each cage.

The ability of these taxa to colonize high sedimentation areas might be given by the adoption of deposit-feeding mechanisms. The bottom deposition of organic pellets not only allows them to exploit higher amounts of organic matter as food but also covers the seafloor of finer sediments, easier for these taxa to feed on (Dunlop *et al.*, 2021). The disappearance of these indicators after the first stages of the production indicates the presence of a threshold when it comes to the level of organic overload they can tolerate.

4.2.4 Epifaunal community

Beneath fish cages, epifaunal species were very low in abundance and did not show any temporal pattern. However, epifaunal communities at reference sites exhibited a significantly greater richness. Being highly reliant on bottom surfaces, and because of their feeding mechanisms (e.g., filter-feeding) and diets based on particulate material, epifaunal taxa are particularly vulnerable to suspension and deposition of organic material (Trannum *et al.*, 2019). These results align with the findings from Dunlop *et al.* (2021) on aquaculture sites near the coast of Northern Norway. There, some deposit-feeding species in the epibenthic communities were negatively affected by fish waste release and thus their abundances, as well as biodiversity, decreased underneath and around salmon farms.

Far from aquaculture activities, epifauna showed fluctuations through time. However, the pattern seemed to be related to habitat type, rather than to survey time. Abundances were the highest on broken rocks bottom substrates and the lowest on sandy sediments. Mixed- and hard- bottom habitats, with their high structural complexity, have been previously linked to an increase in species richness, compared to soft substrates (Howell *et al.,* 2016). Our findings regarding the association of enhanced epifaunal richness with hard substrates are in line with previous results from the studies of Dunlop *et al.* (2020) on hard bottom substrates in Northern Norway.

4.3 Use of indicators of organic enrichment for monitoring systems

In Norway, a mandatory monitoring system (Norwegian Standard NS9410:2016) is used for the evaluation of the impact of organic waste from salmon farming (Hansen *et al.*, 2001; Anon, 2016). The monitoring investigations are periodically conducted in the vicinity of fish farms and are based on the quantitative assessment of organic overload from fish cages, qualitative analysis of chemical parameters such as pH, and presence/absence of benthic macrofauna (Taranger *et al.*, 2014; Anon, 2016). Thresholds of benthic response were set to determine the level of impact of farms, categorized into four levels (1- low impact, 2- medium impact, 3- high impact-organic loading, 4-organic overloading). However, the system is still limited to thresholds developed for soft-bottom habitats and communities. Further information on the impact of aquaculture on mixed- and hard-bottom habitats, and on the benthic communities associated to these substrates, should be gathered for implementation into the monitoring system or the creation of a new hard substrates-specific monitoring tool.

Multiple studies, mainly conducted in fish farming-impacted regions such as the Western coast of Norway and Western Canada, highlighted the necessity to acquire better knowledge on hard-bottom benthic communities, especially regarding their distribution under fish farms, sensitivity to organic overloading, and overall ecological value (Hamoutene *et al.*, 2014, 2015; Keeley *et al.*, 2015; Armstrong *et al.*, 2020; Dunlop *et al.*, 2021). Site-specific factors such as substrate composition, hydrodynamics, temperature, depth profile, and the morphological features of endemic benthic organisms (e.g., sensitivity to organic enrichment) are to be considered alongside organic matter deposition (Lin and Bailey-Brock, 2008; Macleod *et al.*, 2006; Macleod *et al.*, 2007). The combination of these factors can make the correlation between waste deposition and the presence and/or abundance of indicators difficult to interpret (Armstrong *et al.*, 2020).

According to our results, the presence of *Beggiatoa* spp. mats and organic pellets can hardly be used for the establishment of monitoring thresholds, as these indicators were present during the whole fish production cycle. On soft bottoms, indicators of enrichment as *A.marina* and tube-forming polychaetes can be indicative of low levels of enrichment and reflect decent levels of environmental pollution from farms. The presence of OPC can instead reveal high levels of organic loading and a significant impact of aquaculture activities on soft-, mixed- and hard- bottom substrates.

Within this context, video and image monitoring represent a viable solution to collect information over hard substrates benthic communities, as grab sampling is hindered by the nature of the substrate (Hamoutene *et al*., 2015). The use of the software Biigle 2.0 for analysing images and characterizing benthic communities would make the evaluation quicker and accessible, as data on presence, distribution, abundance, and surface area coverage is readily provided by the software to the user.

Such results and methodology hold high potential within the ongoing process of developing a substrate-specific monitoring system, where benthos responses to organic enrichment, visually characterized by changes in distribution and abundance/surface coverage, are used to evaluate and make informed decisions regarding the activity of fish farms located over mixed- and rock- bottom habitats (Hansen *et al*., 2011; Brennan, 2018).

4.4 Limitations

Other than scientific gaps in the knowledge of distribution and functioning of some benthic taxa, the use of this methodology, based on image analysis, entails other limitations and challenges. Some of these were encountered during this study.

Relocation of fish happened several times during the survey period. Trout were occasionally moved from their original cage to a fourth cage for short periods (e.g., during sea-lice treatments). Furthermore, half of the fish present in the three farm cages were moved in May 2021 to three new cages, located next to the old ones. These relocation events could affect the amount of waste deposited on the seafloor, misleading us in the full understanding of benthic responses.

The amount of feed (kg) given to fish was not standardized but followed the health conditions of the fish, and subsequent treatments. Therefore, an increase of biomass (tons) through the production cycle did not always relate to an increase in feed (kg) levels.

In our study, current velocity and temperature measurements were not available in sufficient resolution for statistical analysis and thus were not considered as explanatory variables. However, previous studies (e.g., Hamoutene *et al.,* 2016; Dunlop *et al*., 2021) described the importance of these abiotic factors in changes in benthic community distribution and abundances. Spatial measurements of current velocity at the seabed are necessary to detect the difference in potential erosion of sediment and the distribution of organic waste.

When the layer of flocculent matter on the seafloor was considerably thick, the camera rig would often sink into the sediment. This was particularly in the case of sandy substrates. For this reason, the distance between camera and seafloor was smaller compared to standard images, and an overestimation of the total surface area coverage (sqpx) was likely. On the other hand, surveys conducted over hard bottom substrates were often challenged by the camera frame sliding on rocks. The development of an improved camera system, where the frame does not get in contact with the seafloor, is necessary.

High concentrations and/or thickness of bacterial mats can obstruct the proper identification of some species, which could be placed below the mat, and lead to underestimations of the abundance of OPC and nematodes complexes (Hamoutene *et al*., 2015).

In line with results from previous studies, we consider bacterial mats suitable indicators of organic enrichments, but they provide limited information regarding the benthic ecologic state. According to Knight *et al.* (2021), characterizing bacterial community composition can be more suitable for the evaluation of benthic conditions of hard-bottom substrates. This can be resolved using eDNA sequencing procedures.

At the time of our study, complex-forming polychaete species were only visually identified, while molecular and morphological identification through laboratory analysis was still to be performed and required to confirm the results of the study.

Variations in indicators of enrichment distributions under the three different cages, likely determined by current and temperature patterns, highlight the importance of future research to create a sampling design embracing all the environmental conditions at the site.

Finally, extending research over a longer period and applying the method to farm sites with different geographical profiles and layouts, are necessary to determine the validity of our results and to develop an overarching monitoring tool for mixed- and hard- bottom substrates.

5. Conclusions

During this study, we observed the impact fish farming and waste release have on benthic communities inhabiting the soft-, mixed- and hard- bottom substrates beneath fish cages. Contrasting responses, in time and extent, were detected between different benthic indicators of organic enrichment. Images collection and the use of an image characterization software can improve and fasten the monitoring process, especially where grab sampling is hindered by the presence of hard substrates. Characterizing the response of different taxa on mixed- and hard- bottom substrates allows for the introduction of thresholds for the monitoring of these benthic communities, currently lacking within the soft sediments-based monitoring system in Norway. Eventually, gathering more knowledge on benthic communities' dynamics, combined with the application of new monitoring methodologies, can improve the monitoring process and support the development of an environmental index to assess ecological state around aquaculture farms in mixed- and hard- bottom areas.

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