Studying the waste recycling potential of naturally occurring opportunistic polychaetes on benthic trays under a Norwegian fish farm

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Master of Science in Aquaculture

By

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Abstract

With an increasing salmon industry comes an increasing amount of waste released into the environment, impacting benthic environments in particular. This study investigated the potential of utilizing naturally occurring polychaetes to recycle fish waste on benthic trays placed on the seabed under a Norwegian fish farm. The four-week field study investigated the polychaete complexes under a deep (80-200m) salmon farm, identifying species composition and estimating their abundance and biomass. Video surveys were taken during the deployment period to document *in situ* epi-faunal polychaete development and spatial variation. Dominant species were then brought to the lab for physiological experiments to determine respiration rates, oxygen/nitrogen ratios and Respiratory Quotients (RQ). The lab and field data were used to calculate approximate carbon turnover rates for the dominant species.

Capitella spp and *M.fuliginsosus* were found to colonize trays in low abundances, while *Ophryotrocha* spp was found to be the most abundant with rapid colonization rates (<6 days) in higher waste fall areas. Higher polychaete abundances were seen in areas with higher amounts of fish waste. Physiological experiments found all polychaetes were metabolizing a macronutrient source resembling a protein or lipid source and at the same metabolic rate. The RQ values found for *Capitella* spp (0.23), *M.fuliginosus* (0.31) and *Ophryotrocha* spp (0.25) were below the conventional range (0.6-1) and subsequent carbon turnover rate estimates were low for all species. *Ophryotrocha* spp were found to have the highest carbon turnover rate (2.54mg C/day/g AFDW). In order to decompose 20% of the POC coming down the biomass of Ophryotrocha would have to be 4 times that found in this study. It was concluded that the waste recycling potential of *Ophryotrocha* species in particular is worthy of further investigation.

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1 Introduction

1.1 Norwegian aquaculture in a world context

Aquaculture is steadily increasing and set to continue both worldwide and in Norway (FAO, 2016). The increasing global population and plateauing of wild fish stocks further ensures the growth of this already huge industry (Duarte et al, 2009). Norway is heavily invested in this aquaculture surge, especially that in marine coastal environment. In 2014, Norway had the highest global production of coastal aqua cultured fish (includes molluscs and crustaceans), ahead even of China. The finfish aquaculture production is of huge importance economically to the country as well, being the 2nd biggest production export after oil and a value of 64 billion in 2015/2016 (SSB, 2017). The majority of Norwegian aquaculture is the farming of salmonids, specifically Atlantic salmon (*Salmo salar*) (SSB, 2017). It takes around 18 months to grow a salmon to a suitable weight in large open net pens, out in the fjords and along the Norwegian coast. These open net pens are large (157m circumference) nets, generally 8-12 to a farm that are secured either in a steel rectangular frame or by underwater moorings (Fig.1).

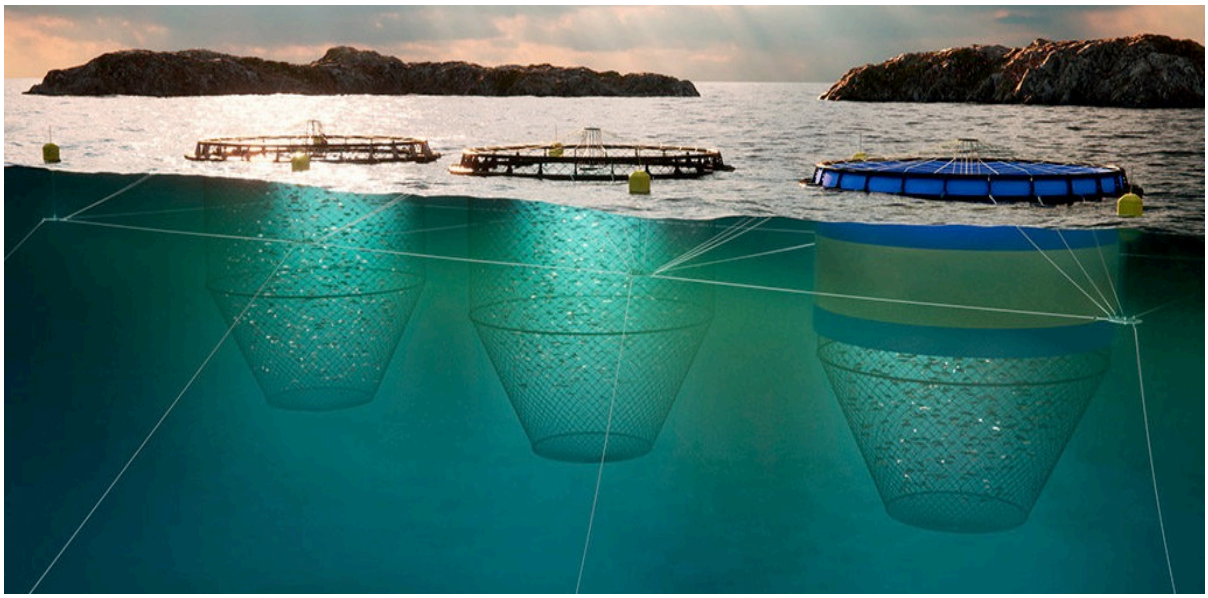

Figure.1. An example of 3 Norwegian open net pens with underwater moorings. (image from Lerøy)

They are designed to maximize water exchange with the surrounding seawater to replenish oxygen levels and remove waste products. Waste from a fish farm includes both a dissolved portion, such as ammonia and phosphate, and a particulate portion containing fish faeces and excess feed pellets.

The dissolved waste is washed out and away with surface currents while the particulate waste sinks down to the bottom, settling under or near the cages, depending on depth and current velocities (Valdemarsen et al, 2012; Bannister et al, 2014). Originally, fish farms were placed in highly sheltered areas in the fjords with limited water flow and often shallow depths. Waste build up under the cages became a serious issue with high concentrations of methane, CO2 and hydrogen sulphide in the surface sediments (Hansen et al, 1991; Samuelsen et al, 1988). In response, salmonid farms moved to deeper (50-300m) and more exposed sites in the fjords (Taranger et al, 2014). The result was greater waste dispersal due to higher current velocities and greater depths under the cages (50-300m) (Kutti et al, 2007a; Taranger et al, 2014 and Bannister et al, 2016). Since then, Norway's industry has continued to increase, producing over 1.3 million tonnes of salmonids in 2015/2016 and between 3000 and 5000 tonnes per farm per 18 month production cycle (Taranger et al, 2014 and SSB, 2017). This has led to an increase of fish waste, potentially leading to increased accumulation under and around the farm.

1.2 Environmental impacts of organic effluents

Particulate Organic Matter (POM) once settled on the bottom can then be re-suspended, decomposed by bacteria, eaten by invertebrates or all of the above. The current not only disperses waste as it settles down but also provides oxygen to the sediment, critical for aerobic waste decomposition. Despite many well-flushed sites and large waste dispersal areas (up to 1km), anoxic conditions and reduced benthic fauna occur under some farms (Kutti et al, 2007b; Valdermarsen et al, 2012 and Sweetman et al, 2014). This is a problem for both the opportunistic macrofuna, mainly polychaetes that colonize the organically enriched sediments (consisting of faeces and feed pellets) and for aerobic microbes, where enough oxygen must be available for the break down of waste into energy. If there is enough oxygen and input of waste, opportunistic polychaetes can thrive with rapid colonization of the area (Kinoshita et al, 2008 and Chareonpanich et al, 1994). This can result in significant decomposition of POM and remediation of the soft bottom environment (Heilskov and Holmer, 2001; Kinoshita et al, 2008). If, however, the POM input rate exceeds the available oxygen, then an increase in anaerobic mineralization will occur. Anaerobic microbes may use sulphate instead of oxygen for metabolism, releasing hydrogen sulphide as waste, while others can produce methane (Holmer and Kristensen, 1992). High levels of hydrogen sulphide may hamper mineralization efficiency and the ability for macrofauna to recolonize (Valdemarsen et al, 2012 and Hargrave et al, 2008). If conditions do not improve, farms can face a reduction in maximum allowable biomass (MTB) or temporary closure until conditions improve.

In Norway, there is a compulsory 2 month fallowing period at the end of each production cycle that aids in benthic recovery. This benthic recovery time is especially important if conditions under the farm had exceeded their capacity during production and dropped into anoxic, even azoic (no life) conditions. Norwegian fish farms are subject to systematic monitoring using the Norwegian Standard NS9410 (Standard Norge, 2016). The more benthic impact under the farms, the more often the monitoring is performed (Hansen et al, 2001). The benthic condition is then reported back to the Fisheries Directorate and if bad conditions persist the authorities take action. The monitoring system is specifically for soft bottom habitats, as grab samples do not work on hard rock. Currently there is no established monitoring system for hard bottom habitats (bedrock, boulders and cliffs). The few investigations that have been done in Norway (Hansen et al, 2011 and Eikje, 2013) have shown that waste does accumulate on these hard substrates. They have also found, that similar to the soft bottom habitats, extensive assemblies of opportunistic polychaetes, (*Ophryotrocha* spp, *Vigtorniella* spp) occur in these environments, in association with the waste. Similar findings have been shown under Canadian fish farms where an *Ophryotrocha* genus also dominates many hard bottom areas (>40m and <5^{o}C), often in association with *Beggiatoa* bacterium (Hamoutene, 2014; Salvo et al, 2015 and 2017). These bacterium are generally found in the transition between hypoxic and oxic enviroments (Teske and Nelson, 2006). Though *Ophryotrocha* spp are not exclusively found on hard bottom, their extensive mucus structures allow them to inhabit areas unavailable to their sediment dwelling counterparts and appear in areas of low current and temperature (Eikje, 2013; Valdemarsen et al, 2015 and Hamoutene et al, 2016). Hard bottom impacts from fish waste are still being researched (Bannister et al, in prep) and monitoring systems in Norway are under development (Hansen pers. com). The potential of utilizing both soft and hard bottom occurring polychaetes in fish waste recycling has begun to be explored world wide and in Norway.

1.3 Opportunistic Polychaetes and Waste Recycling

Opportunistic polychaetes are found in often high abundance in organically enriched marine environments (Dauvin et al, 2017, Valdemarsen et al, 2015). These polychaetes occur in a range of environments, and the soft bottom/sediment dwellers such as *Capitella* spp, *Hediste (Nereis) diversicolour, Nerein neries* and *Malacoceros fuliginosus,* and are generally common and well researched (Heilskov and Holmer, 2001; Kristensen, 1989 and Hall and Frid, 1995).

Most research states that opportunistic polychaetes are indicator species for polluted environments (Pearson and Rosenberg, 1978 and Giangrande et al, 2005 and Dean, 2008). There have, however,

been studies into their responses to metals (Hall and Frid, 1995), medicines (Linke-Gamenick et al, 2000), their population dynamics (Marsh and Tenore, 1990 and Ramskov and Forbes, 2008) and general life history (Mendez, 2016). With rising public concern about marine polluted environments, such as oil spills, sewerage outlets and fish farm waste, there has been further research in utilizing them for waste recycling and bioremediations (Heilskov et al, 2006; Ito et al, 2016 and Fang et al, 2017). The bioremediation investigations have included numerous lab studies (Chareonpanich et al, 1994, Holmer et al, 1997; Banta et al, 1999; Ito et al, 2016 and Fang et al 2017) but few studies are large scale experiments (Kinsohita et al, 2008). Generally, the sediment re-working and burrows made by the polychaetes are efficient bioturbation methods, promoting survival of aerobic microbes. Research into the waste decomposition potential of polychaetes on hard bottom, such as *Ophryotrocha* spp, in Norway is limited to preliminary investigations (Eikje (master thesis), 2013; Nederlof et al, in prep; Fang et al, in prep). Salvo et al (2015a and 2015b) has studied trophic analysis and fatty acid composition on the Canadian *Ophryotrocha* species (*O.cyclops*). Both countries are also considering them as indicator species for hard bottom monitoring of fish farm impacts (Bannister et al, in prep and Hamoutene et al, 2016).

One of the main advantages with polychaete waste recycling is that biomass is produced in conjunction with the CO_2 and water. The biomass produced from the waste can then be utilized for other purposes, such as animal feed; this is the concept of an Integrated Multi Trophic Aquaculture (IMTA) system. These systems often contain a finfish species then 1 or 2 lower trophic level species that consume and convert the waste to harvestable biomass. Excess particulate and dissolved waste can then be removed for the gain of growing another harvestable species, potentially yielding more profit for the farmer. However, before IMTA systems can be implemented an understanding of the organism carbon turnover, the environment and harvest techniques must first be explored.

1.4 Calculating Carbon Turnover

A carbon turnover calculation for an organism provides a rough estimate of the amount of carbon (food) they require as well as the amount of carbon that would be recycled from the environment. In order to calculate a carbon turnover/consumption capacity of an organism, the metabolic fuel source (carbohydrates, proteins or lipids) and respirational rate is required. This can be found one of two ways either by measuring the difference in nutrient content of food going in compared to excreted fecal matter (difficult with small organisms) or finding the Respiratory Quotient (RQ).

The RQ is the ratio between carbon dioxide produced and oxygen consumed (CO_2/O_2) and indicates the macronutrient source being used during aerobic cellular respiration (energy production) (Richardson, 1929). It is based upon the fact that certain amounts of CO_2 are produced to the O_2 needed to metabolize either the carbohydrate, protein or lipid source. RQ values generally fall in a range between 0.7 (lipid catabolism), 0.9 (protein catabolism) and 1 (carbohydrate catabolism), but can also vary with food source.

The RQ can be influenced by many factors, such as organism behavior, life stage, starvation and environmental conditions (Richardson, 1929). Measuring nitrogen excretion, synonymous with protein catabolism, and comparing this to oxygen uptake indicates the degree of protein involved with metabolism.

By measuring metabolic rates of CO2 production, O2 uptake and total nitrogen excretion, an insight into the physiological energetics of the polychaetes can be gained, fundamental in estimating the amount of waste they can potentially recycle.

This study is part of the larger Ocean Forest project, a collaboration between, Lerøy, Bellona, and the Institute of Marine Research. The larger subproject is investigating cultivating polychaetes on elevated trays under the fish farm to both recycle waste and produce a harvestable biomass, to be potentially utilized in fish feed.

1.5 Aims

The main of aim of this Masters project is to investigate the waste recycling potential of naturally occurring polychaetes on benthic trays under a mixed bottom (soft and hard substrate) Norwegian fish farm. This will be done by field and lab studies; 1) Field; describe colonization patterns of trays by *Ophryotrocha* spp and other opportunistic polychaetes by use of quantitative and qualitative field methods. The effect of two different tray substrate types on collected polychaete abundance will also be tested. 2) Lab; Metabolic rates for oxygen consumption, nitrogen excretion and carbon dioxide production, as well as oxygen/nitrogen ratios and Respiratory Quotients (RQ) will be determined for the dominant polychaete species collected from trays. 3) A carbon turnover estimate will then be found for the polychaete species and abundances found on benthic trays and related back to organic matter (OM) input to the benthic environment.

2 Materials and Methods

2.1 Study Site

This study was performed at Ocean Forest salmon farm (more information visit http://bellona.org/projects/ocean-forest), located in Hjeltefjorden, north west of Bergen, Norway (60^{o} 30.53´N, 4^{o} 55.96´E) (Fig.2). The Hjeltefjorden is a sill fjord with a relatively wide and deep mouth and frequent deep water flushing. The farm is situated over a trench running NNE to SSE, with depths varying between ~80m-200m. The bottom substrate is mixed, with soft sandy bottoms and intermittent boulders to steep rock cliffs lining the trench walls. The Ocean Forest fish farm has been functional on the site for 4 years, producing ~16,000 tonnes of salmon in that time. The farm, covers 0.077km^2 with 12 parallel circular open net pens, though only eight pens were stocked during the study period with a total biomass of 4400 tonnes of salmon and larger fish in cage 6 (Fig.3). Each net pen is 55m deep with a 157m circumference and moored in multiple points, quite standard for Norwegian net pens. The external frame allows for some movement of the pens in winds and currents. The farm was fallowed from July 2016 to March 2017 (8months) with first stock of 194g smolts in March 2017. Throughout the study period fish were fed everyday and had a feed conversion ratio of 1.18 with no reported sickness or feed spills. Furthermore, cage 8 was moved south one spot one week prior to sampling (Fig. 3).

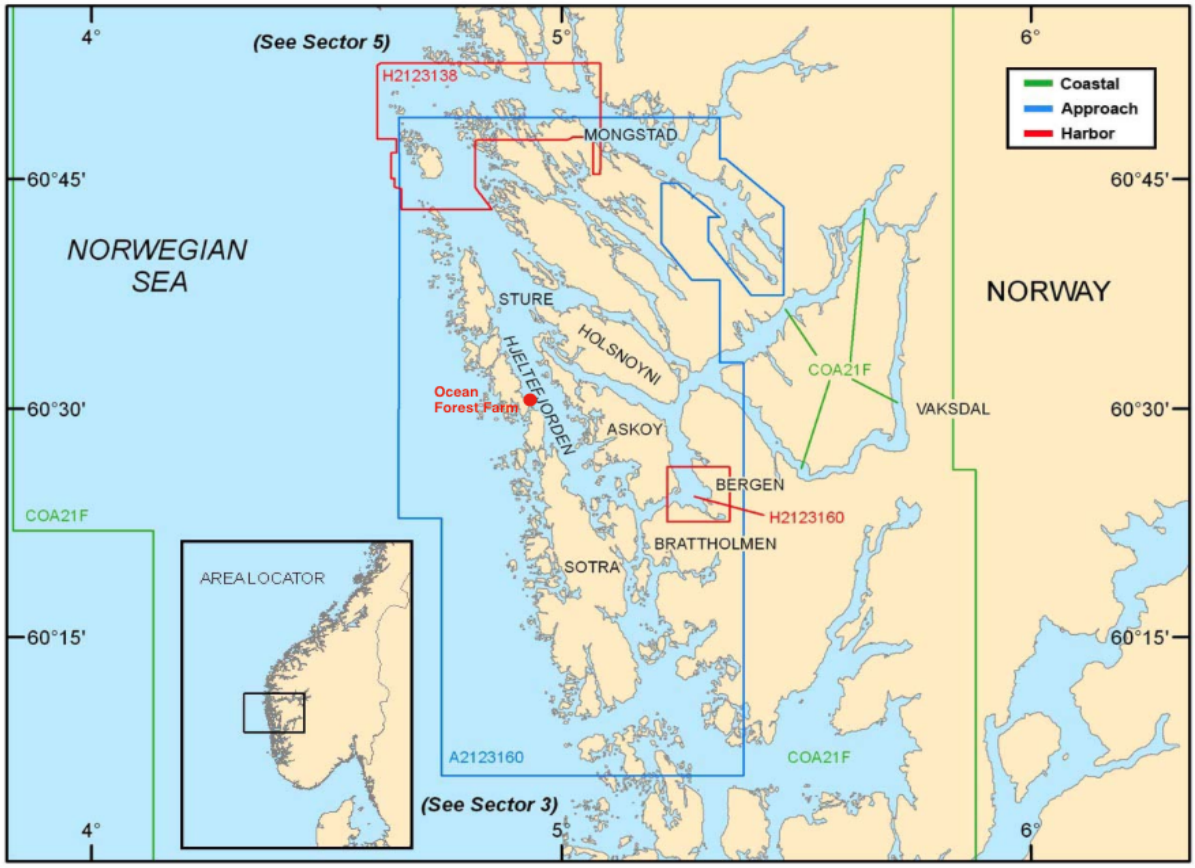

Figure.2. Map of Norway and Hordaland region with the Ocean Forest fish farm (study site) marked in red. Original Image from; https://msi.nga.mil/MSISiteContent/StaticFiles/NAV_PUBS/SD/Pub182/Pub182bk.pdf

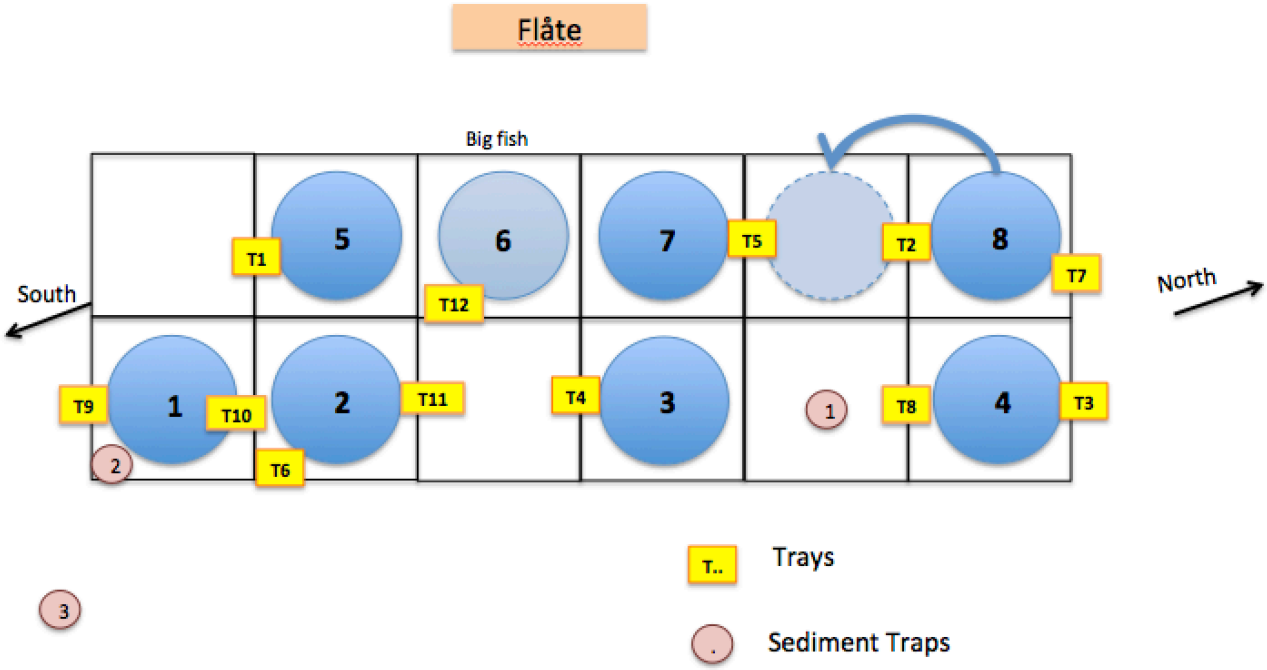

Figure.3. The Ocean Forest fish farm cage positions during the study period (September 2017) with the locations of cultivation trays (yellow boxes) attached to the net pens (blue circles). 3 of the 4 sediment trap positions also marked, the 4th trap and current meter were ~1km further north.

2.2 Field Materials and Methods

2.2.1 Current and CTD

A current meter was placed 800m north of the farm (60° 31.0351´N, 4° 55.4442´E) on the 7th of September and remained there for one month at a depth of 180m. It was placed for an indication of current direction and strength at depth. A CTD profile was also performed on the 26th of September at the north and south end of the farm at ~200m and ~80m depth, respectively.

2.2.2 Sedimentation

For the duration of the four week field study, four sediment traps with duplicate cores (inner diameter 9.6cm) were placed in and around the Ocean Forest farm, with one reference location (#4) (Table 1) for measurement of organic waste from the farm. Each core had an aspect ratio of 6 (height of core/diameter) and 80cm between them on each trap in accordance with Wassmann and Heiskanen, (1988). Traps were placed ~10m above the bottom, to minimize re-suspension, and kept in place with a ~70kg weight and bouy on the surface. Before deployment all cores were filled with seawater and 500ml of 4% formalin was added to the bottom; preventing decomposition of organic material (OM), as described in Kutti et al (2007a).

Upon retrieval after 20/22 days of deployment, sediment cores were removed from the trap, corked and transported to the lab, where they were stored until analysis. Sediment trap #2 had one core missing when retrieved and had gotten caught on a chain during deployment,

Table 1. Sediment Trap i.d, Position, Depth and Number of Days Deployed at Ocean Forest Farm

Sed. Trap Number	Position	Depth	No. Days Deployed
1	60°30.6345`N 4° 55.9544´E	160	22 days
2	60°30.4336`N 4° 55.9309´E	100	20 days
3	60°30.3587`N 4° 56.0703´E	120	22 days
4	60°31.2682`N 4° 55.3119´E (ref)	160	22 days

In the lab, excess water was removed by slow pouring until the organic material was seen. If > 1litre remained, the core was left to settle then re poured until volume was ~1litre, then, transferred to a 1 litre flask. The cores were rinsed with distilled water to ensure all particles were collected. Subsamples were taken using a 50ml Kip (aka bird) pipette, material was shaken into suspension to assume homogeneity. Three subsamples were taken per core (N=21) and filtered through pre-burnt and weighed filters (Whatman GF/F). To remove salt, 25mL of distilled water was added which then took ~2hours to pump through as there was a lot of material. Due to the amount of material, filtration cups were scraped to remove any stuck matter and added to the filter papers. To determine Total Particulate Matter (TPM) filter papers were dried at 60^{o}C for 24 hours then weighed (DW). For Particulate Organic Matter (POM) filters were burnt at 450 ^{o}C for 4hours and placed in an exicator for an hour before weighing. Sedimentation lab work was performed by lab technicians though, I was present for the majority of the analysis.

To calculate POM sedimentation rate (g m^{-2} d^{-1}) ash weight was subtracted from DW, for weight of organics per subsample. These were then adjusted to up scale for organic weight per litre (therefore whole sediment sample), before an average taken for each sediment core. The per day rate of POM fall into sediment cores was then found before up scaling to the fall rate per m^2 by dividing by sediment trap inner area.

2.2.3 Cultivation Trays

The presence and development of opportunistic polychaetes were studied by using novel cultivation trays with two different substrate types. The basic tray design has been used previously in the project to collect *Ophryotrocha* spp from hard bottom habitats under a fish farm in Hardanger fjord (Fang et al, in prep; Nederlof, in prep) and it was known that polychaetes are washed off during tray retrieval. The *Ophryotrocha* spp, especially, on trays were, therefore measured by *in situ* video investigations to monitor development and see coverage before most would be lost when retrieving the trays.

The actual trays were used as a medium to attach substrates in order to collect samples of deep benthic polychaetes, especially *Ophryotrocha* spp. A total of 12 trays were made and placed underneath the salmon farm; six trays previously used in pilots, consisting of scallop trays in a steel frame (T7-T12) (Fig.4) and six newly constructed aluminum trays (T1-T6) (Fig.6).

In order to optimize future tray design, 2 different types of substrate were attached to the trays. The first, was a blue/grey plastic doormat with a 20mm blade length (article no.375022 by AstroTurf, from Jula) (Blue) (Fig.5b) and a green artificial grass with a 40mm blade length (Slide Pro kunstgress,

from UniSport) (Green) (Fig.5a) The Blue substrate had been used (with the steel framed trays) in pilot studies to collect polychaetes off deep, hard bottom substrates. The Green, being trialed for the first time, was selected for its longer blade length with the aim of further limiting polychaete wash off from the trays. Each of the 12 trays had a total of $1m^2$ of substrate coverage, $0.5m^2$ of Blue and $0.5m^2$ of Green; they were attached with zip ties to the tray base with at least a ~2cm gap around all edges to allow for some water drainage.

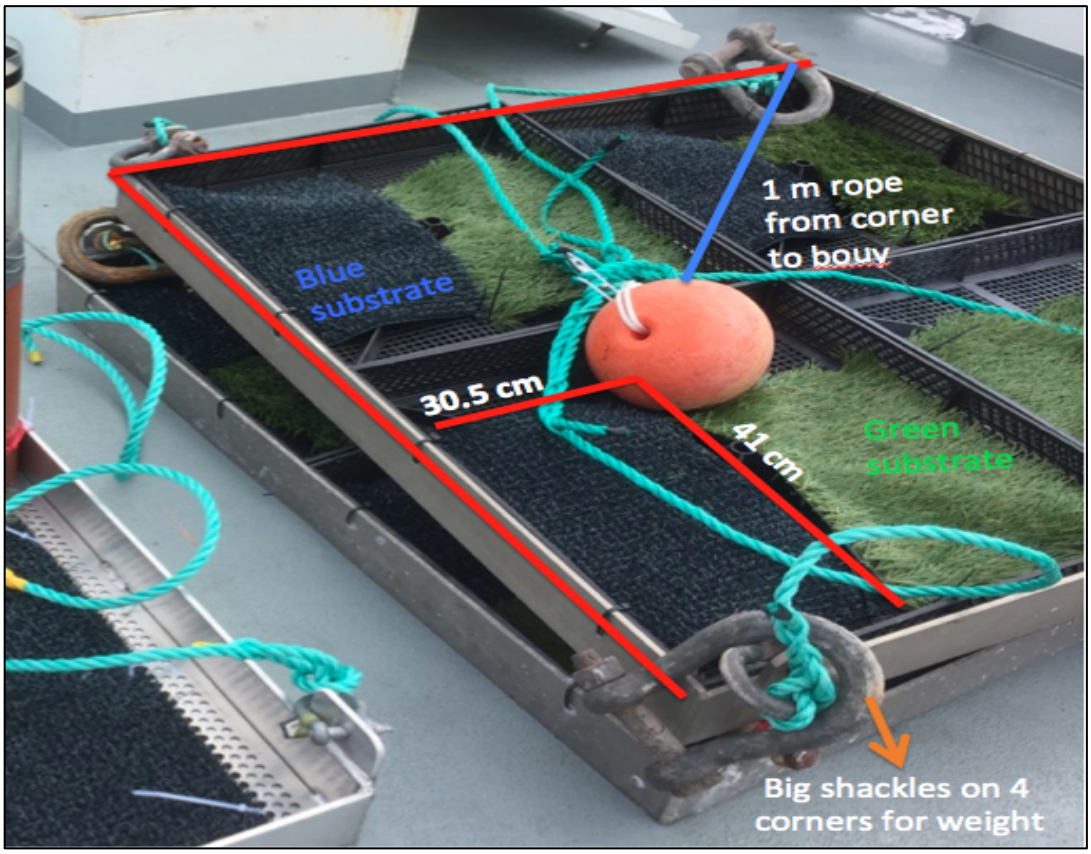

Figure.4. The cultivation trays (T7-T8), with a steel frame and x4 scallop trays within. The two different substrates are marked, 'Blue' and 'Green'. Blue line = the rope length on each of the 4 corners to the buoy. Redlines = the tray dimensions in cm.

Figure.5. The Green (A) and Blue (B) substrate used to help cultivate and retain opportunistic polychaetes on benthic trays under a Norwegian fish farm

Small (20cm) sediment cores (plankton containers) were attached to 6 trays (T1-T6) to gain insight to the amount of organic matter (OM) washing off substrates upon retrieval (Fig.6). Taller (60cm height) sediment cores were attached to 5 of those trays (T1-T5) to measure the amount of OM falling on the substrates. Due to their height, the amount of re-suspended OM from the trays and sediment would be a lot less than that going into the 20cm cores. The T7-T12 trays did not have any additions, as tray structure did not allow a secure attachment.

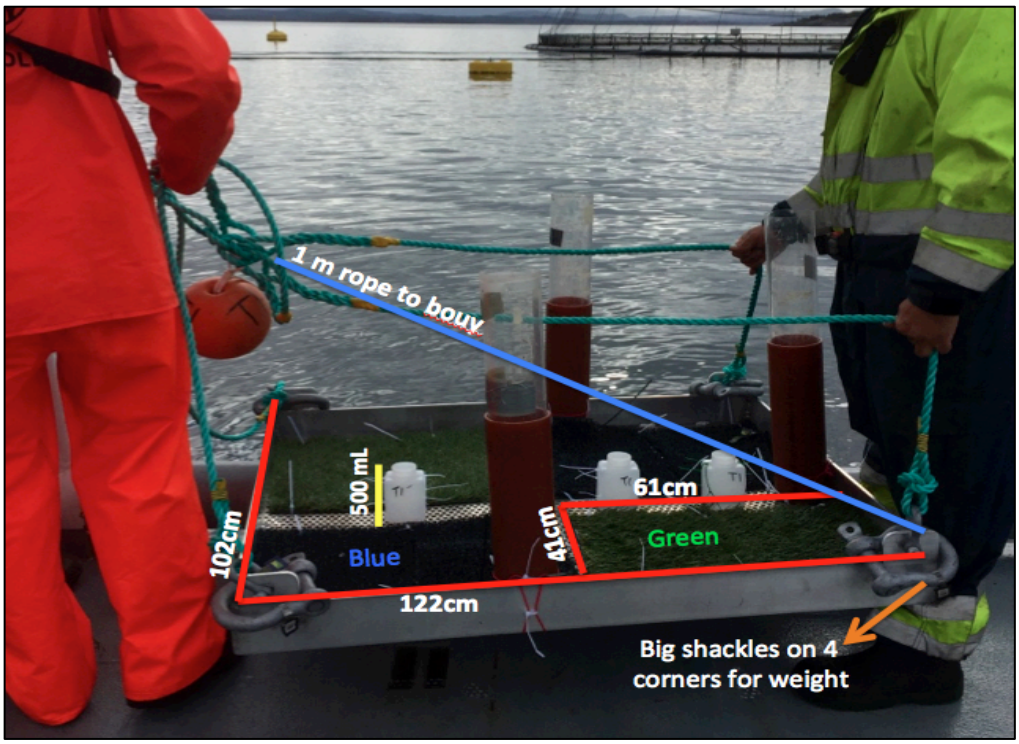

Figure.6. Aluminum cultivation tray used for polychaete sample collection. The two different substrates are marked, 'Blue' and 'Green'. Yellow line marking 20cm sediment core height/volume and brown tall structures the 60cm cores (x3 on 5 trays). Blue line = the rope length on each of the 4 corners to the bouy. Redlines = the tray dimensions in cm.

Tray deployment occurred over 2 days in total with 1 week in between, due to delousing at some cages. The first deployment was on 01.09.2017 with 7 trays, T1, T2, T3, T4, T5, T7 and T8, the second was on 08.09.2017 with 5 trays, T6, T9, T10, T11 and T12 (Table 2). The tray deployment time appeared to have little effect on polychaete abundances with all 6 of the most northerly positioned trays being deployed one week prior to those down current. Trays were placed mostly on the south and north side of the net pens, due to farm requirements, with a depth varying between 80-200m.

Table 2. Dates of Tray Deployment and subsequent Filming and Sampling. A preliminary video investigation was taken in June prior to the study period.

	Tray Number											
September Days	**T1**	**T7**	**T8**	**T5**	**T3**	**T4**	**T2**	**T9**	**T11**	**T12**	**T6**	**T10**
1												
…												
8												
…												
14												
…												
25												
26												
27												
28												
Total Days Deployed	**24**	**24**	**24**	**25**	**26**	**26**	**27**	**17**	**17**	**18**	**20**	**20**

Key
Deployed
Filmed
Sampled

2.2.4 Video Surveys

Video surveys were taken with a Go Pro 5, using a live HD camera for guidance to maximize Go Pro footage quality, while light was provided with a Keldan underwater video light on low setting. These were all attached to a small plastic frame, and lowered to the bottom by the live camera cable; Movement was only by pulling or releasing this cable.

An initial video investigation of the benthic environment was taken one to two months into the main production cycle, June 2017, before trays were deployed. One day was used to take drop down video surveys around the farm to observe polychaete colonies, specifically *Ophryotrocha* spp, as well as topography and fish waste levels.

After tray deployment video surveys were performed as a non-invasive method to both monitor development over time but also to observe polychaete complexes *in situ*. Videos provided snapshots of the polychaete and waste coverage on the trays and substrates. The aim was to film all trays at least twice, once part way through deployment and then again just prior to retrieving the trays. However, due to weather, steep rock walls and a flooded live camera, only 5 trays could be filmed twice (Table 2).

2.2.4.1 Video Analysis

For both temporal and spatial analysis, screenshots of the film were taken and used as the basis for categorizing waste and polychaete coverage on the substrates (Table 3). From the 17 films taken during the surveys only one film, from T11, was excluded from the analysis as the tray was vertical and no substrate visible.

Table 3; Groups relating to coverage percent used for film analysis to estimate the amount of waste and polychaete cover on substrates attached to benthic cultivation trays under a Norwegian fish farm

Group	Coverage Percent
1	<10%
2	10-40%
3	40-70%
4	70-100%

Depending on film quality, ~3 snapshots from each of the 16 films were used to first group substrate coverage as a whole into 1 of the 4 groups (Table 3). The groups are based on a percent range of waste/polychaete coverage as the analysis had to be made by human eye due to the sometimes-poor light and film quality. Furthermore, substrate coverage estimates were only made from the Green substrate as the Blue substrate was often indiscernible from the waste cover. To reduce subjectivity, an expert panel of nine people also individually analyzed the snapshots (appendix, Table 1).

To then categorize substrate coverage into waste and polychaete groups the same snapshots were further analyzed by human eye. Fish fecal and feed pellets and partially floating sediments were categorized as Waste (Fig.7a) Polychaetes, identified as mainly *Ophryotrocha* spp (Eikje, 2013) (Fig,7b). Mucus structures without polychaetes were classed under Waste. Due to the small difference in groupings made by the nine people and the time and knowledge required, this further break down of coverage categories were not analyzed by more people.

For the spatial comparison of *in situ* polychaete abundances, coverage estimates/groups from film taken just before sampling were used, except for T1 and T9, where film from 10 days prior was used. The temporal comparison of waste and polychaete development used the estimates from the trays filmed twice, T2, T4, T6, T8 and T10 (n=10).

Figure.7 Video survey snapshot of fish waste containing fecal waste and feed pellets (A) and polychaete cover, mainly Ophryotrocha spp (B) on the Green substrate attached to benthic cultivation trays, deployed for 4 weeks under a Norwegian fish farm in September, 2017.

2.2.5 Polychaete Sample Processing and Analysis

Polychaete abundance and biomass per m^2 and species diversity were calculated for each substrate type on each of the 12 trays.

2.2.6 Polychaete and Waste Sample Collection

Trays were left for 21 ±4 days before being retrieved for sample collection, over 4 consecutive days in late September. All 12 trays were slowly and smoothly brought to the surface using a boat with a nook. The substrates were removed and separated into Blue and Green washing tubs. Sediment cores and plankton containers were also removed, sealed and stored for transport back to Bergen. Substrates were washed with small vigorous shakes at the waters surface. For infaunal polychaetes, folding the substrate outwards and so exposing them beforehand was found to be quite effective. The polychaetes from one substrate type were then poured gently from the tub onto a sieve (0.5mm) collected in a bucket. There were some smaller polychaetes washed through, however, a smaller mesh size became quickly blocked with waste and mucus. A subsample was taken then poured into a photo tray and a picture taken to count the number of polychaetes per sub sample (100-300ml).

2.2.6.1 Species Identification

Main species groups were identified in the field and further identification was provided by taxonimst (Dahlgren, Uni Research) from pictures of live sedated specimens, using magnesium chloride. Minority polychaete species and other invertebrate species found were noted in comments.

The polychaete species were then counted from both the photo tray and bucket and when numerous enough, ~10 individuals, samples were collected for species identification. For the dominant polychaete species, samples of 30 individuals were collected and pooled for individual biomass (number/total weight) then frozen until further analysis. If less than 30 individuals of one species were present then no sample was collected. On the last sampling day the remaining polychaetes were kept alive and transferred to the lab for metabolic experiments.

2.2.6.2 Abundance

An abundance per m^2 estimate for the dominant genera was calculated by using ImageJ (Schneider et al, 2012) to count individual polychaetes for each substrate from the photo trays. Where counting was difficult (large quantities of polychaetes or waste) photos were counted twice and an average taken if there was a discrepancy of >10 polychaetes. The abundance estimate for the substrates was then found by dividing count by subsample volume and adjusting for bucket (total) volume and then multiplying by 2; as each substrate type had an area of $0.5m^2$. For the tray abundance estimates, the two substrate densities were added together instead of multiplying them by 2. In total there was x23 abundance m^2 estimates, (Green n=12, Blue n=11) with missing data from the Blue substrate on the T7 tray. This tray was excluded for the substrate comparison as to compare the densities of polychaetes on the two substrate types, the same area/number of substrates is needed. The trays in the substrate analysis were viewed simply as replicates, whereas, the tray and species comparison looked at trays as a whole. In this case the average difference between Blue and Green substrates for both trays and species was used to extrapolate an estimate for the T7 Blue substrate.

2.2.6.3 Biomass

Biomass samples were only collected for the dominant polychaete genera, and only if their total number was >30. Biomass samples were not washed in the field nor in the lab due to time and degradation of tissue from being frozen; meaning the subsequent dry weights contain salt particles and only ash free dry weights can be used biomass estimates.

In the lab, polychaetes were dried at 60°C for 4 days then placed directly into an exicator for an hour before being weighed for Dry Weight (DW). A combustion oven was then used to burn the samples at 450°C for 6 hours. Samples were taken out at ~88°C and immediately placed in an exicator for an hour before being weighed for the Ash Weight (AW). Ash Free Dry Weight (AFDW) was calculated by subtracting AW from DW, this was then divided by the number of polychaetes for the individual AFDW. For the final biomass m^2 estimate the average individual AFDW for each genus was multiplied by the tray abundance (Green + Blue substrates).

2.3 Polychaete Metabolic Rates

The three most dominant polychaete species collected from trays on the last days of sampling were used for metabolic measurements in the lab to estimate carbon turnover. In order to do this, polychaete oxygen uptake, nitrogen excretion and carbon dioxide production were measured in controlled incubation experiments (equipment and details described below). The RQ and O/N ratio were then found to determine the main macronutrient being metabolized. Once the main metabolized energy source is known, and therefore the amount of oxygen required for its catabolism, the rate of O_2 uptake indicates the rate of polychaete metabolism.

2.3.1 Polychaete Collection and Holding

Polychaetes were collected from substrates on the last sampling day from the T2 and T10 trays. The substrates were washed with water originating from deep waters (~100m depth, lab water source), providing similar conditions (temp and salinity) to the polychaetes natural habitat; Previous pilot studies showed that most polychaetes, especially *Ophryotrocha* spp, die rapidly when exposed to surface water (Fang, in press). Polychaetes were then transported back to the lab in coolers, containing deep water (from Bergen), oxygen and ice bricks, keeping the temperature <12 ^{o}C. Polychaetes were held in tanks (50 litres) with constant flow of deep water (Salinity 35 psu, Temp ~11°C) for 5 days. A centre pipe with mesh covered holes allowed the outflow of water without polychaetes escaping. Fish waste and sediments that came from the field were left in the tanks, but no additional food was added in that time. Holding tanks were mostly kept dark, except when preparing for respiration experiments and polychaete handling was kept to a minimum. The *M.fuliginosus* and *Capitella spp* were kept in the same holding tank while *Ophryotrocha* spp were

kept separately to avoid overcrowding. Since *Ophryotrocha* spp is also found on hard bottom, stones were placed in the base of the tank. Small glass beads were placed in the base of the *M.fuliginosus* and *Capitella* spp tank and served as an artificial substrate for these infaunal species.

2.3.2 Experimental Setup

Test runs were performed prior to the actual species comparison experiment in order to familiarize with the system and the polychaetes' oxygen depletion over time (results in appendix). The final set-up for comparison of metabolic rates of the three species (*Ophryotrocha* spp, *Capitella* spp and *M.fuliginosus*) is given below.

Metabolic rates were determined by a closed chamber approach where water was encapsulated and remained unperturbed in 45ml tubes for *M.fuliginosus* and *Capitella* spp and 15ml tubes for *Ophryotrocha* spp. These were placed horizontally to maximize benthic space for the polychaetes. Each chamber included five medium to large individual polychaetes, and small glass beads were included as an artificial substrate/sediment (Fig.8). These polychaetes, especially *Ophryotrocha* spp, are often found living communally and so this was partially replicated in the chambers with five individuals. Each run (one per species, three in total) included nine replicates of five polychaetes and one empty chamber that served as a control (Fig.8).

At the beginning of incubations, chambers were slowly filled with deep water from a small hose to ensure no air bubbles amongst the beads, polychaetes were then added and water refreshed once more before sealing the chamber underwater. During incubations, chambers were placed in a water bath to keep temperature stable (~11°C) and a tarp placed on top to keep it dark and minimize stress responses. A LoggerLite probe was used to measure temperature (°C) of the holding tank/water bath and barometric air pressure (Bp) throughout the experiment. Method details specifically regarding oxygen, nitrogen and CO2 are given below.

At the conclusion of incubations, polychaetes were removed from chambers, rinsed in fresh water to remove salt particles then frozen for later biomass analysis. They were then later dried at 60°C for four days to define Dry Weight (DW) then combusted at 450°C for 6 hours, to define ash weight (AW). Ash Free Dry Weight (AFDW) was subsequently calculated (DW-AW).

Figure.8 Metabolic experimental set up to determine polychaete respiration rates. X10 chambers all with glass beads and x9 with x5 polychaete individuals of one of three polychaete species. Oxygen measured continually with probe and O2 sensor on end then stuck through tight hole in lid.

2.3.3 Metabolic Analysis

2.3.3.1 Oxygen Uptake (O_2)

Oxygen depletion, as percent Dissolved Oxygen (DO %), was measured continuously, every 15 seconds, using a PreSens® OXY-10 mini sensor with fluorometric oxygen spots. These were attached to steel probes and placed through a tight hole in the lid of each chamber. The probe was positioned midway down the chamber, just above the beads. Logging of data began prior to lids being closed, but the incubation start time began 20 minutes after darkness, allowing the polychaetes to first acclimatize. Incubations concluded once DO levels reached ~70% of start value, which was all under 2 hours. This allowed for sufficient oxygen decline to calculate the oxygen uptake rate but not so much that the polychaetes become stressed from hypoxic conditions, possibly altering their metabolic rates.

Analysis; To obtain O_2 in umol mL^{-1}, DO [%] was converted by adjusting for salinity, temperature, Bp, relative humidity and vapor pressure, using coefficients and equations for pressure, salinity and DO factors from Benson and Krause, 1980,1984. Relative humidity was set to 100% and vapor pressure was assumed as the same as water temperature (Rastrick pers com, IMR). The O_2 [umol mL^{-1}] values were then corrected to the actual chamber water volume and experimental incubations (n=9) were adjusted to exclude any natural oxygen decrease, represented by the control chamber.

Polychaete oxygen uptake rates for each species were calculated by selecting a time point beginning at ~20 minutes after the start of each incubation and finishing ~45min later. Linear regression models were then used to find the slope/gradient of O_2 decrease (*m*) in the selected ~45min. The r^2 value was used as the basis for assessing the quality of the selected O_2 gradient, if it was < 0.4 then the selected time was adjusted slightly until it improved.

Mass-specific respiration for polychaete species was calculated using collective Ash Free Dry Weight (AFDW) (g) per chamber and dividing by the gradient of O_2 decrease (*m*) . The AFDW, instead of DW was used as previous studies (Nederlof, in prep) found significant amounts of salt in the dried polychaetes, despite rinsing in fresh water. However, DW mass-specific respiration rates were also calculated to compare with literature (appendix, Table 5).

2.3.3.2 Nitrogen Excretion (NH_4)

Total nitrogen excretion measured as ammonia was found using start and end samples. A water sample at the start of incubation commencement was collected from the same water source (~100m deep water from Austevoll) as used to fill incubation chambers. End samples were collected at the conclusion of each incubation from all chambers. All water samples (20 ml) were filtered through a filter and frozen for later analysis for ammonia concentration. The control chamber had a separate syringe and filter as nitrogen levels were expected to be low, as there were no organisms. Experimental chambers shared a syringe but it was flushed with some of the water from the upcoming sample and filters were replaced as they became clogged.

Nitrogen water samples were analyzed with the phenol blue method of Soloranzo (1969) as described in Small et al (2014). A spectrophotometer (Molecular devices Spectra Max M5) was used to read the absorbency levels of samples and ammonium sulphate was used to make the standard curves. The standards, a series of dilutions with a known concentration of nitrogen (ammonium sulphate in this case), were made to seven dilutions starting with a concentration of 0.0794 and going down to 0 umol ml^{-1} ammonium sulphate. With the absorbency readings from the standards, a linear

regression slope (*m*) was created to translate the respective sample readings into nitrogen concentration. There were two technical replicates of the standards per plate and three technical replicates for each chamber per incubation. The average absorption value from the three technical replicates was then converted to NH4 umol mL^{-1} by subtracting the mean absorbency of the 0 dilution standard and dividing by the slope of the line (*m*). Change in NH4 concentrations over the total incubation time was found by the difference in start and end values. These values were adjusted for water volume in the chamber and any natural change in NH4 values as seen in the control were subtracted. The total run time was used to find the hourly rate of NH4 (umol h^{-1}) excretion for each polychaete species. This was divided by O_2 umol h^{-1}, for the ratio of O_2 production to nitrogen excretion. Due to time restrictions, the lab analysis (excluding calculations) for nitrogen excretion was performed by Henrice Jansen (supervisor) and the data included in this thesis due to its relevance.

2.3.3.3 Carbon Dioxide Production (CO_2)

Carbon dioxide production was determined by pH variation at the start and end of incubations using a Vernier Labquest multimeter. The multimeter was re-calibrated at the start of each incubation with hydrogen chloride buffers of pH 7 and 10 and in accordance with National Bureau of Standards (NBS). Start measurements were taken from the same source of water used in the incubations and an end reading was also taken from here, in case of pH probe variation. End measurements were taken from all chambers immediately after the conclusion of incubations and as quickly as possible (~10min total) to limit time as a variable. Total Alkalinity (TA) was also measured in the holding tanks, with a value of 2330 umol kg^{-1} Sea Water (SW). The pH readings were converted to CO_2 umol kg^{-1} SW using an online program, Co2sys (Pierrot et al, 2006), where TA, pH, temperature and salinity were the known values, while total phosphate and silicate were set to default.

The change in CO2 concentrations during incubations was calculated as the difference between start and end values. Like nitrogen and oxygen, adjustments for the chamber water volume and the natural change in the control were made; total run time was then used to calculate the polychaete hourly CO_2 excretion (umol h^{-1}). This value was when divided by the O_2 umol h^{-1} provided the Respirometry Quotient (RQ) for the polychaete species.

2.4 Statistical Analysis

Statistics were performed using R Studio version 1.0.1. Standard errors were chosen to represent the variation in this study for an indication of the precision of sample means compared to the true mean of the population. This is especially with regards to metabolic rates where a substantial number of factors, just on the individual level can affect the end result. The abundance and biomass field data were tested with a paired t.test. Regarding abundance, difference between substrates (Blue vs Green) and difference between Ophryotrocha dominance and infaunal polychaete abundances were tested. Biomass difference between *Ophryotrocha* spp and *M.fuliginosus* were tested with the paired t.test, *Capitella* was not tested due to low sample number. A correlation test for paired samples (cor.test) was used with the video analysis coverage groups and the Ophryotrocha abundance for nine trays.

The metabolic results; respiration rates, nitrogen and CO_2 production, oxygen/nitrogren ratios and RQ values did not meet the normality assumptions tested with a Shapiro test (p values <0.05). The Barlett homogeneity of variances test was also not met for the majority of the metabolic data. Therefore the non-parametric Kruskal Wallis test (Kruskal and Wallis, 1952) was used to test for significant differences between the three polychaete species in each of the metabolic measurement groups. All incubations were assumed to be independent as each species had its own experiment with each of the 9 experimental chambers separate from the other. In the event of significant, or near significant differences a paired Wilcox post hoc test was used to see which polychaete species were different.

3 Results

3.1 Physical Environment

3.1.1 Current and Conductivity Temperature and Depth

Temperature of bottom water (<80m) during the study period remained at a stable 8-8.3^{o}C and a salinity of 36.2 psu was found for both the 80m and 200m CTD profile at time of sampling. For the duration of the study period (7th Sept-28th Sept) the current between 70-160m depth was quite weak (0.2cm/s) in the Northerly direction. Furthermore, frequent fluctuations between a north and south flowing current were evident (standard deviation 3cm/s). Current velocities at the surface (17-70m depth) were shown to have 1.4cm/s northerly flowing speeds, also with southerly fluctuations (standard deviation 3.4cm/s). The winds however caused upwards of 20cm/s current speeds while the tidal swell was weak (~5cm/s).

3.1.2 Sedimentation

For the duration of the study period there was an average of 4.2 (± 3.1) g m^{-2} d^{-1} of POM settling on the bottom in and immediately around the net pens, compared with 0.6 (± 0.0) g m^{-2} d^{-1} at the reference site (#4)(Fig.9). There was a substantial difference between the POM sedimentation rates measured within the farm with almost 3x more POM in the southern trap (#2) than that of the northern trap (#1), 7.9 (± 2.6) and 2.7 (± 0.1) g m^{-2} d^{-1}, respectively (Fig.9). POM sedimentation just outside the farm (#3) was even lower again with a rate of 2 (± 0.1) g m^{-2} d^{-1}, 3x lower than that of trap #2 which was located ~100m NNW (Fig.2).

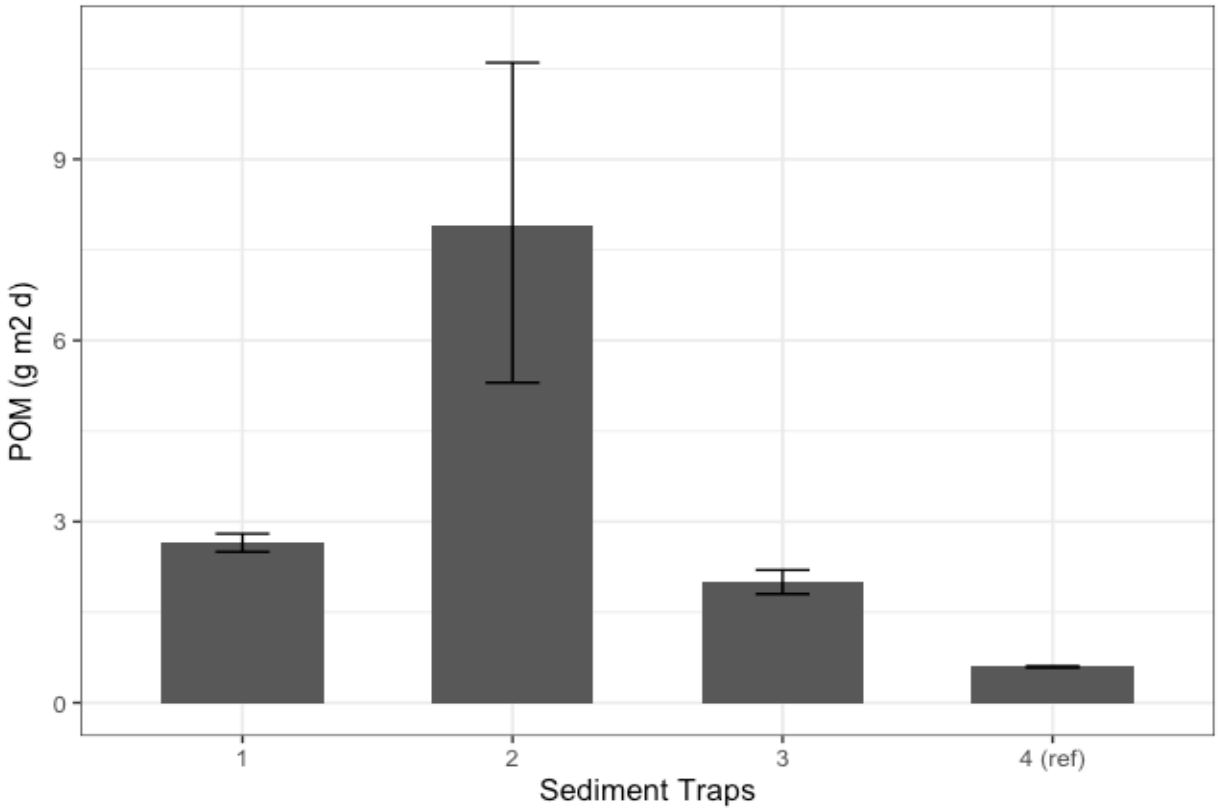

Figure.9. Sedimentation rates (g/m^2/d of Particulate Organic Matter (POM) in September 2017, measured 10m above the bottom, under a Norwegian salmon farm with range (min/max). Trap #1 and #2 are located in the farm towards the north and south, respectively. Trap #3 is ~100m SSE of the farm and #4(ref) is the reference, 1km north of the farm. Bars are the average of each filter from traps with minimum and maximum values marked on each.

3.1.2.1 Sediment Cores Attached to Benthic Trays

Both type of cores (both 20 and 60cm) attached to the trays collected organic material, but also large amounts of polychaetes that became trapped. As the absence of formaline caused large degradation of polychaete biomass, it was impossible to separate deposited material and partly decomposed polychaetes. It was observed during the video surveys, however, that *Ophryotrocha* spp had colonized up, around and into both types of sediment cores on some trays (Appendix, Fig.3), resulting in a higher potential abundance on trays with these structures.

3.2 Polychaete Colonization Patterns

3.2.1.1 Species Diversity

There were three dominant opportunistic polychaete species found on benthic trays; *Ophryotrocha spp* and other *spp, Malacoceros fuliginous*, and *Capitella spp*. A fourth less common species, *Prionospio,* was also found. *M.fuliginosus* was distinguished by its antennae which was longest out of the species seen while *Capitella* spp was red with reduced chaete and head structure. At least 3 different *Ophryotrocha* species were noted during sampling and in the holding tanks for lab studies. It is was observed that the most dominant *Ophryotrocha* species had very visible black P-type jaws, and a red colour along the edges, however, uncertain if visible jaws and colouring a function of age (Fig.10)

Figure.10. Ophryotrocha spp collected from benthic trays under a Norwegian fish farm, September 2017. Red circle is appears to be a different species to the blue rectangle (most dominant) and pictured on the right under 10x magnification.

3.2.1.2 Abundance

The abundance estimates, found an average of 7500 (± 5200) individual polychaetes m^2 on the tray substrates after trays were pulled to the surface. Of these, *Ophryotrocha* spp was significantly dominant (t.test, p=0.01), with an average of 6200 (± 5400) individs m^2, making up ~83% of the total average (7500 ±5200) (Fig.11). Furthermore, it had the highest number of trays (9 of 12) where it was the dominant species (Fig.6). *M.fuliginosus* was the second most abundant polychaete found on the tray substrates, with an average of 970 (± 1370) individuals m^2 and making up 13% of the overall average. *Capitella* spp and *Prionospio* were the least common polychaete found on the trays, with densities of 500 (± 800) and 100 (± 200) individs m^2, making up 7 and 1% of the total average,

respectively (Appendix, Table 2). *Ophryotrocha* spp was found on all trays, *M.fuliginosus* on 11 (of 12), *Capitella* spp on 7 and *Prionospio* on 5 trays (Fig.11). There was substantial variability across the trays with minimum estimates of 30 (T7) and maximum of 16,000 (T9) polychaetes m^2. The highest abundance of polychaetes was at the south end of the farm, T9 and T10, while lowest densities, were at the north end, T3 and T7 (Fig.11). Variability was also in a short distance, with the 3rd and 4th highest tray abundances (T8 and T2) located just south of the lowest abundance estimates (T3 and T7).

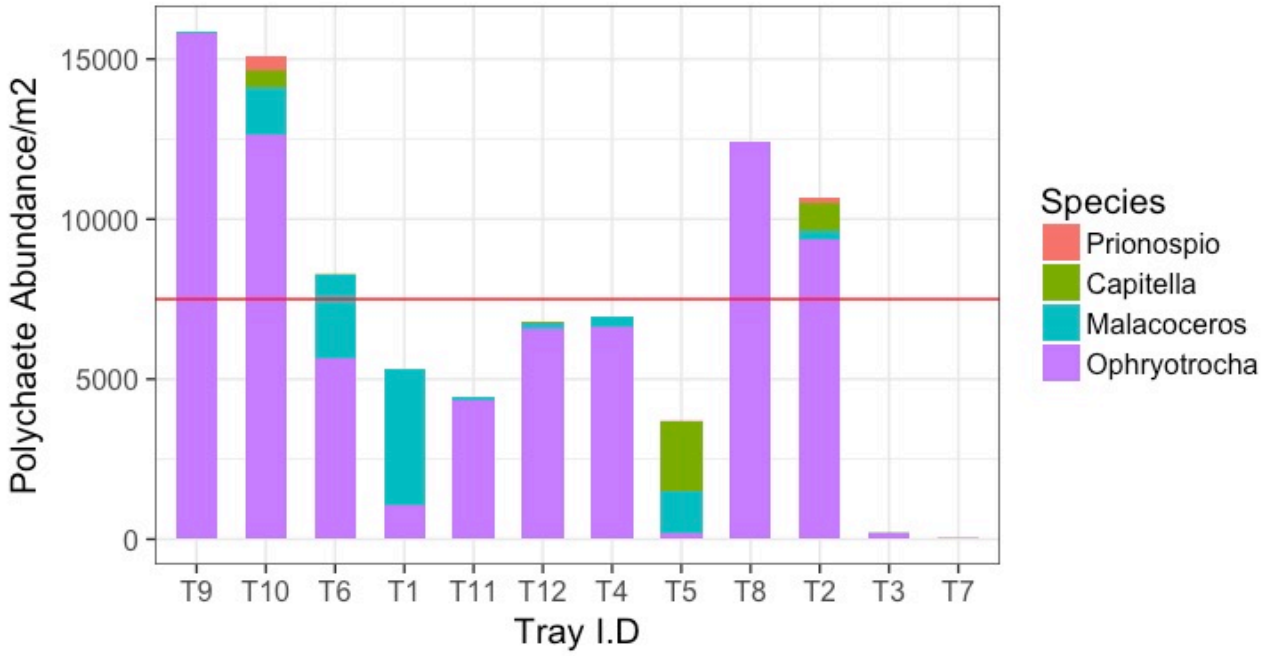

Figure.11. Abundance of the most dominant polychaete species on each of the 12 cultivation trays after retrievement. Trays were deployed for ~3weeks in September 2017 under a Norwegian fish farm. Trays (x axis) are ordered from south (T9) to north (T7), though where two trays had a similar position (T1/T6, T2/T8 and T3/T7) the deeper or most easterly tray is placed first (see also Figure.2 for tray position in farm). The red line presents the mean abundance (m^2) of the 12 trays.

3.2.1.3 Biomass

There was a large difference between the average individual weight (AFDW) of *Ophryotrocha* spp, at 1.8 (±0.5) mg per individual and *M.fuliginosus* at 11.6 (± 5.2, excluding T6) mg (appendix, Table 2). The T6 tray was excluded as an outlier, as the *M.fuliginosus* on that tray were 1.5 times heavier than the second heaviest (T3), weighing 49mg per individual (appendix, Fig 2); *M.fuliginosus* species from T6 were later found to be spawning in the lab (ascending the central ventilation pipe in the process). Due to the lower abundances of *Capitella* spp and *Prionospio*, there was few to no biomass samples taken. However, from the two samples collected from T5, *Capitella* spp individuals (15.6 ± 2.2mg) appeared to be a similar weight to *M.fuliginosus*.

Looking at biomass, *Ophryotrocha* spp was found to still be the dominant polychaete on majority of trays despite its significantly lighter weight (Fig.12). When looking at total biomass and not by number of trays, there was no significant difference (p=0.53) between *Ophryotrocha* spp and *M.fuliginosus*. biomass. This was still the case when T6 individual weights were swapped for the 11.6mg average (p= 0.89). Overall there was an average of 11.1 (±2.8) grams (afdw) m^2 of *Ophryotrocha* spp while *M.fuliginosus,* with the spawning weights was 18.4 (±10.7) and 10.3 (±4.5) g (afdw) m^2.

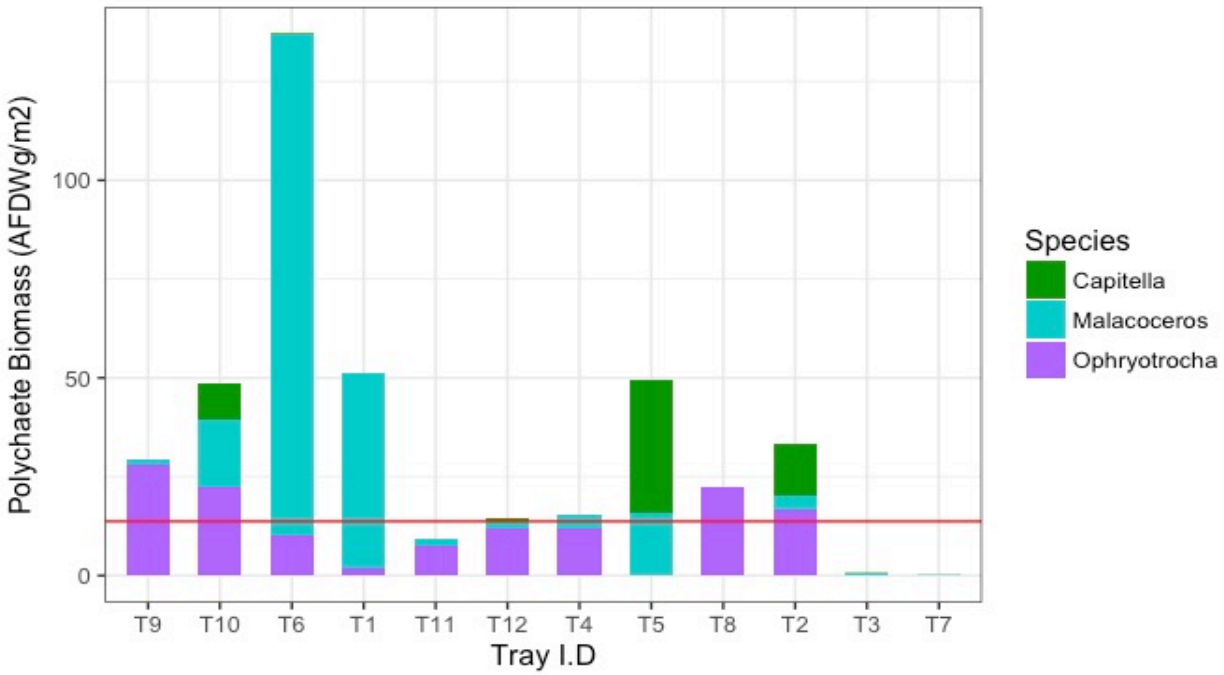

Figure.12. Biomass of the most dominant polychaete species on the 12 cultivation trays deployed for ~3weeks in September 2017 under a Norwegian fish farm. Trays (x axis) are ordered from south (T9) to north(T7), though where two trays had a similar position (T1/T6, T2/T8 and T3/T7) the deeper or most easterly tray is placed first.

Table 4. In situ Waste and Polychaete coverage on trays by video investigations. Data is grouped based on (visual) coverage (Table 3) where 1 (blue)= <10%, 2 (green)= 10-40%, 3 (orange)= 40-70% and 4 (red)= 70-100% substrate coverage. Trays were investigated a few days prior to tray retrievement, except for those indicated with an asterisk (*) which were monitored 10 days prior.

	Tray No.	Waste	Polychaete
South	T9*	3	2
	T10	1	4
	T6	2	4
	T1*	1	2
	T12	4	2
	T4	1	4
	T5	2	1
	T8	1	3
	T2	2	1
North	T3	1	1
	T7	1	1
	Average	1.7	2.3

3.2.1.4 Polychaete and Waste Coverage- In situ

The overall in situ coverage on substrates shows a generally higher amount of material in the south east corner of the farm and less towards the north west. The coverage estimates made by the 9 people were all similar, with only small discrepancies (appendix, Table 1).

The video surveys of substrate coverage, on trays, 1-2 days prior to retrieval showed that most of the substrate was covered by Polychaetes (*Ophryotrocha* spp), especially towards the south of the farm (Table 4). The north end showed generally less substrate coverage overall. Often different types of waste were observed on substrates, ranging from light fluffy material to more solid fish faeces and pellets with bits of old mucus also attached to some trays. The *Ophryotrocha* spp colonies were highest on T10, T6 and T4, (Group 4, 70-100% cover). Of the 11 trays filmed, 4 had higher amounts of Waste than Polychaetes and 2 trays (T3 and T7) had <10% (Group 1) for both coverage types (Table 4). Other observations made during the video investigations were some trays seemed to have higher densities of Ophryotrocha colonies on the trays than the immediate surrounding environment (Fig.13). Furthermore around the south east corner of the farm, near T10 and T6, the water ~5-10metres above the bottom appeared very milky white. Later, the shackle weights on T10 when it was retrieved had a black colouring to them.

Figure 13. Snapshot from video survey of tray and surrounds from Semptember 2017, where trays were placed on the bottom for 4 weeks.

3.2.1.5 Temporal Polychaete and Waste Coverage- In situ

The initial video investigation at the beginning of June indicated that the highest amount of *Ophryotrocha* spp and Waste was found at the north end of the farm, where T8 was later placed, though a high amount of variation was observed. Capitella burrows were also observed covering some soft bottom areas.

In September trays were filmed twice. The first film investigation (14th Sept) found more Waste coverage than *Ophryotrocha* across the 5 trays, with an average grouping of 2.8 and 2, respectively (Table 5). The second film investigation (<3days prior to tray retrieval), found more *Ophryotrocha* than Waste coverage, with average groupings of 3.2 and 1.4 respectively. The minimum time for near complete *Ophryotrocha* coverage (group 4, 70-100% on T10) was <6 days from tray deployment and the maximum was no polychaete colonizations after 24 days deployed (T2). Additionally, T2 had a different development than the other where waste disappeared without an increase in polychaetes. Waste coverage decreased on all trays, except for T10, by the second video survey (Table 5). *Ophryotrocha* meanwhile, only increased on two trays, T4 and T6, but has done so drastically. It was also seen that Ophryotrocha development did not always occur on the tray substrates, T8 (Fig.14) for example, had colonization on tray edges, but waste coverage on substrates still decreased. A further observation made was the difference in shrimp colonies, with few and small shrimp on the first film and relatively large swarms of larger shrimp on the second film; to the point it obstructed all field of view (Fig.14b).

Table 5. *In situ* Waste and *Ophryotrocha* coverage on trays by video investigations at two time intervals (see days deployed for length of trays have been under the farm). Data is grouped based on (visual) coverage where 1 (blue)= <10%, 2 (green)= 10-40%, 3 (orange)= 40-70% and 4 (red)= 70-100% substrate coverage.

	1st Film investigation			2nd Film		
Tray	Days Deployed	Waste	Polych	Days	Waste	Polych
2	13	3	1	24	2	1
4	13	3	1	24	1	4
6	6	4	1	17	2	4
8	13	3	3	24	1	3
10	6	1	4	18	1	4
Average		2.8	2		1.4	3.2

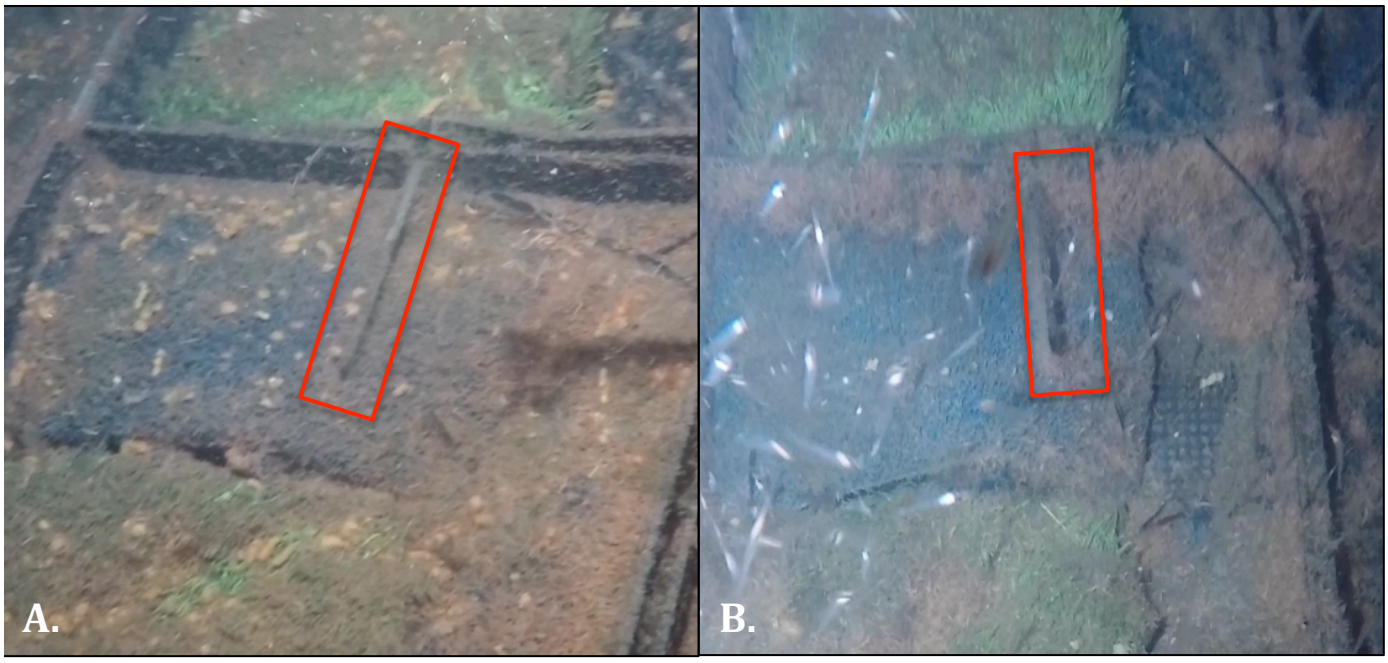

Figure 14. Snapshots from film of T8 tray. The 'mop up' (waste decrease with increase of *Ophryotrocha* spp)effect of *Ophryotrocha* colonies. Image **A**. is from the 1st film taken (14.09.17), two weeks after it was deployed. Waste is identifiable as the small orange pellets and general grey coverage Second image, **B.** is the exact same part of T8, but 10 days later on the 2nd film (25.09.17). *Ophryotrocha* colonies have more distinct borders and gathered on the edges of the substrate. The white blurs in this image are shrimp that were common on most of the second filming. Red rectangle highlights a zip tie, used to attach the substrates, as a reference comparison point.

3.2.3 Benthic Tray Design and Collection

3.2.1.6 Substrates

There was no significant difference between the two substrate types on trays for either abundance (t.test p=0.15) or species diversity (t.test, p=0.2).

There were no significant differences between substrates for *Ophryotrocha* despite 60% of the total abundance being collected from the Green substrate (t.test, p=0.09)(Fig 15). There were 4 more instances when *Ophryotrocha* was found and *Malacoceros* was not, therefore reducing the sample number, reducing the statistical power. Furthermore, 75% of the limited *Prionospio* counts were from the Green substrate, however due to the small sample number (n=6) this difference was not significant (t.test p=0.5)(Fig.15). No differences between substrate types were seen from the video surveys in terms of *Ophryotrocha* coverage.

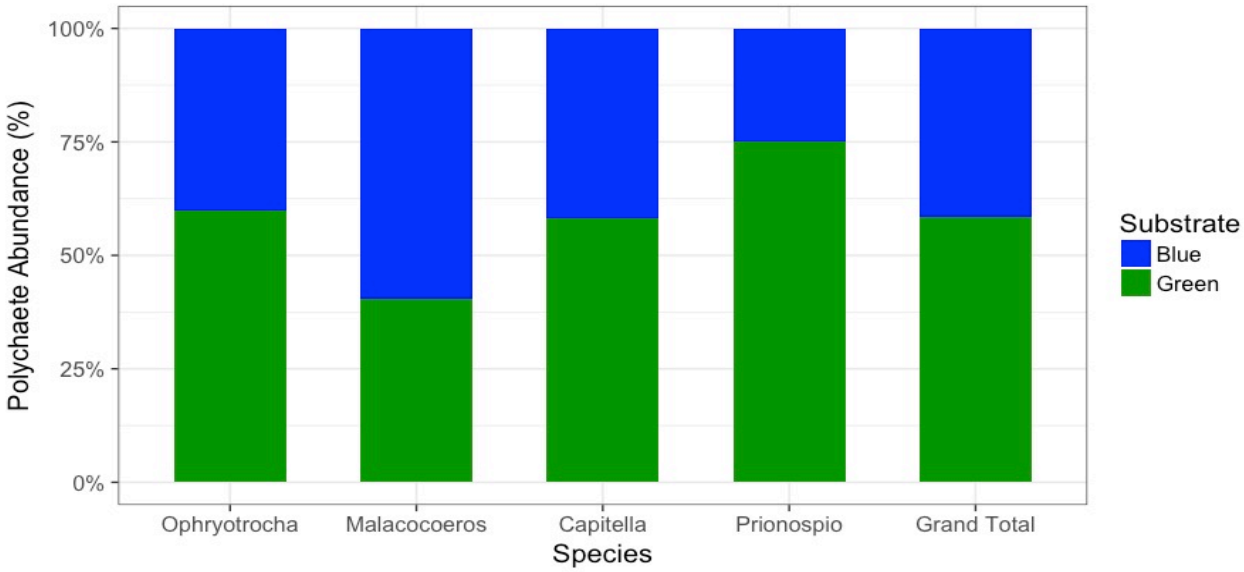

Figure 15. Relative Polychaete abundance (%) from the two different substrate types, Blue and Green, placed under a Norwegian fish farm in September 2017. The x axis is the main polychaete species collected and Grand Total is the abundance sum m^2 of the four species collected from the Blue and Green substrate.

3.2.1.7 Tray Collection

Trays, as a quantification method for polychaete abundance and biomass were flawed in that significant amounts of polychaetes were washed off during retrieval. In order to get an indication of polychaete loss from trays, a comparison between the video survey analysis and *Ophryotrocha* abundance was made. The *Ophryotrocha* coverage groups from the second film investigation (Table 4) were plotted against the *Ophryotrocha* tray abundances (Fig.11). There was a non-significant

correlation between the two variables (r^2=0.4, cor.test, p=0.08). In general the higher grouped trays for *Ophryotrocha* coverage (T6, T10 and T4) fell short of the relative measured densities (Fig.16). It was then the opposite for T2, where low cover was observed (Group 1, <10%) but the fourth highest abundance (9300m^2).

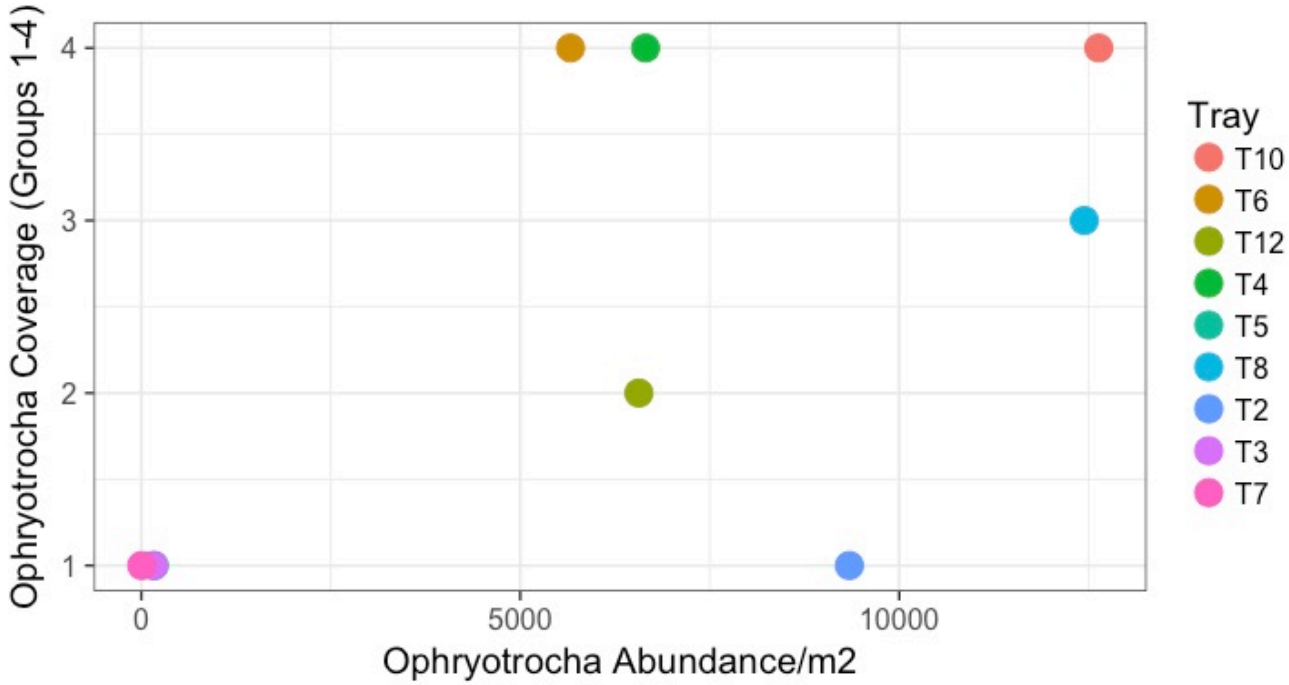

Figure.16. Correlation between polychaete Coverage of Ophryotrocha (categorical) from video analysis and *Ophryotrocha* abundance estimates after trays are brought to the surface. Groupings on y axis are from the Polychaete Cover on trays filmed just prior to sampling (T1 and T9 are excluded here) from Table 4. Film groups refer to a percent range of coverage on the Green substrate (1=<10%, 2=10-40%, 3=40-70% and 4=70-100%, see Table 3). *Ophryotrocha* abundance estimates are from appendix, Table 2.

3.3 Polychaete Metabolic Rates

Incubations were standardized by number of individuals (5 per chamber), the total weights for each polychaete species per incubation: *Ophryotrocha* spp 7.6 (± 0.5), *M.fuliginosus* 123.7 (± 10.4) and *Capitella* spp 86.5 (± 8.2) mg (AFDW).

The mass-specific oxygen consumption rates for the three polychaetes species ranged from a minimum value of 4.5 umol O_2 hr^{-1} $g(afdw)^{-1}$ for *M.fuliginosus* to a maximum of 87.6 umol O_2 hr^{-1} $g(afdw)^{-1}$ for *Capitella*, and a mean of 35 (± 5.5) umol O_2 hr^{-1} $g(afdw)^{-1}$ across species (Fig 17a). Overall, highest average respiration rates were observed for *Ophryotrocha* spp (47.7 ± 9.5 umol h^{-1} $g(afdw)^{-1}$), followed by *Capitella* spp, (36.7 ± 10.3 umol hr^{-1} $g(afdw)^{-1}$), which also had highest

variability (5.4-87.6 umol hr^{-1} $g(afdw)^{-1}$) then *M.fuliginosus*, (20.6 ± 3.9) umol hr^{-1} $g(afdw)^{-1}$). However, no significant difference were seen between the species (p=0.07, Fig.17a)

Higher standard errors were observed within the species groups (*Ophryotrocha* spp ± 9.5 and *Capitella* spp ± 10.3) than between them (± 5.5) (Fig.17a). Furthermore, a steady linear oxygen consumption to anoxic conditions for *Ophryotrocha spp*, *M.fuliginosus* and *Capitella* was observed in test trials with polychaetes still alive after ~6 hours of anoxic conditions (DO <15%).

Ophryotrocha spp (6.6 ±0.8) had significantly higher nitrogen excretion rates than both *Capitella* spp (0.8 ±0.0) and *M.fuliginosus* (1.4 ±0.0) umol hr^{-1} $g(afdw)^{-1}$ (p=0.000006 for both) as well as the largest variation. Furthermore, *M.fuliginosus* also had significantly higher excretion rates in comparison to *Capitella* (p=0.0009).

The same was seen in CO_2 production, where *Ophryotrocha* spp had significantly higher production rates (8.8 ±0.6) and again largest variation compared to *M.fuliginosus* (4.3 ±0.2) and *Capitella* spp (3.9 ±0.2) umol hr^{-1} $g(afdw)^{-1}$ (p=0.00012) (Fig.17b).

The oxygen nitrogen ratios (O/N) ranged between a minimum of 2.6 (*Ophryotrocha*) to a maximum value of 178.4 (*Capitella*) with a mean of 26.4 (±7.9) (Fig.18a). *Ophryotrocha* spp and *M.fuliginosus* showed the lowest O/N ratios, 8.0 (± 1.8) and 15.1 (± 2.3), while *Capitella* spp showed the highest and most variable ratios, 56.1 (± 18.9). The ratios for *Ophryotrocha* spp and *Capitella* spp were significantly different (Wilcox post hoc, p=0.02) and near significance for *M.fuliginosus*and *Ophryotrocha* (P=0.06). No significant difference was found between *Capitella* and *M.fuliginosus* (Wilcox post hoc, p>0.05).

The oxygen to carbon dioxide ratios, or respiratory quotient (RQ) values, for the polychaete species were high, with ~6x more oxygen consumed than CO_2 produced (Fig.18b). RQ values ranged from 0.05 for *Capitella* to 0.98 for *M.fuliginosus* with a mean of 0.26 (± 0.04) (Fig.18b). *M.fuliginosus* had the highest RQ, (0.31 ±0.09), followed by *Ophryotrocha* spp (0.25 ±0.05) then *Capitella* spp, also with the most variance 0.23 (± 0.14). There was, however, no significant difference between species (Kruskal-Wallis, p=0.5).

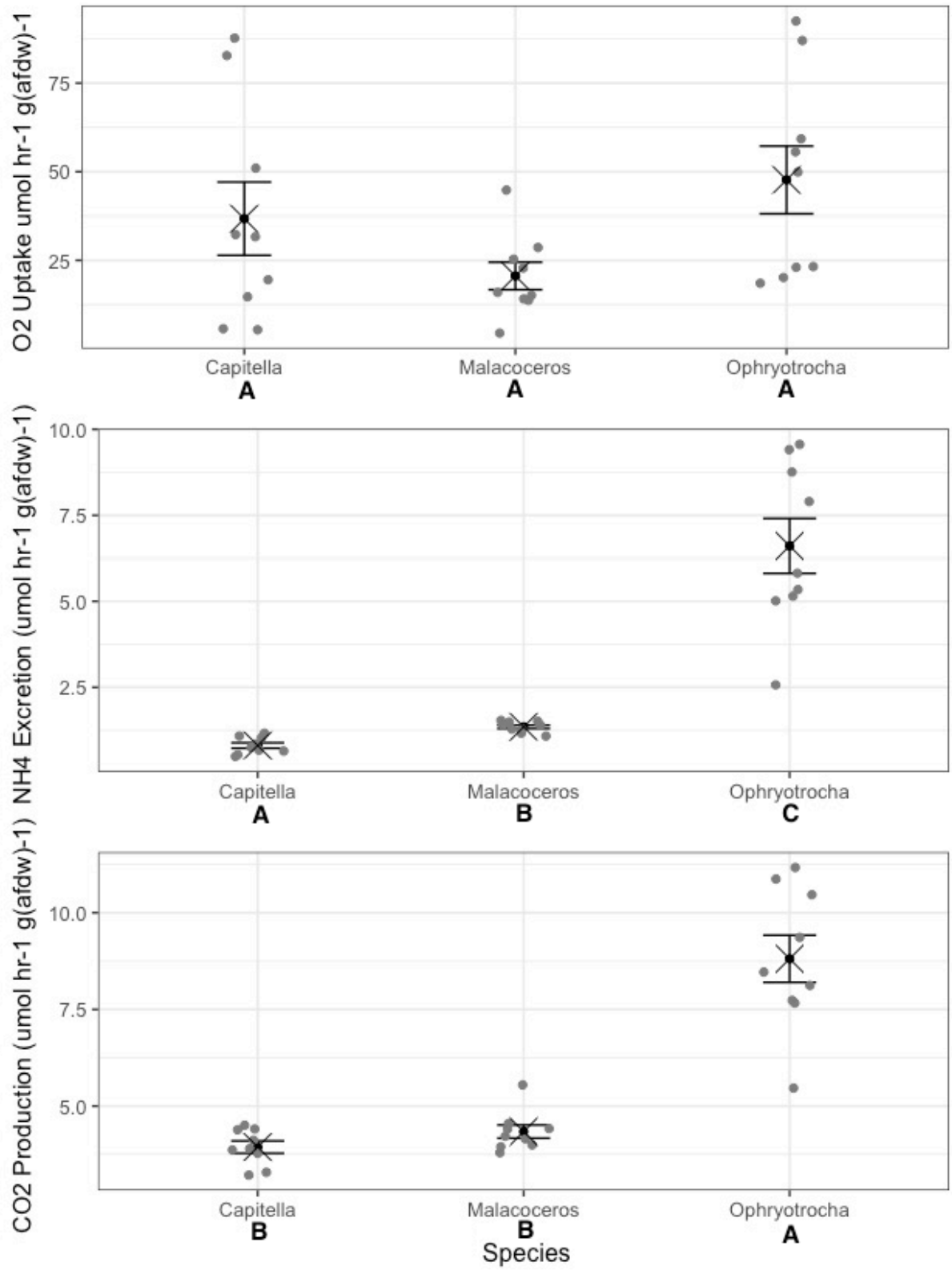

Figure.17. Mass-specific oxygen uptake (**A),** Nitrogen excretion (**B**) and Carbon dioxide production (**C**) for 3 most dominant species of polychaete collected under a fish farm during September 2017. Each chamber (n=9) contains 5 individuals from the same polychaete species (n=21) is represented by a single dot, the cross is the mean of each species and error bars indicate the standard error. Letters on x axis represent the statistical test (post hoc paired Wilcox) outcomes where same letters is no difference and different letters represent significant differences.

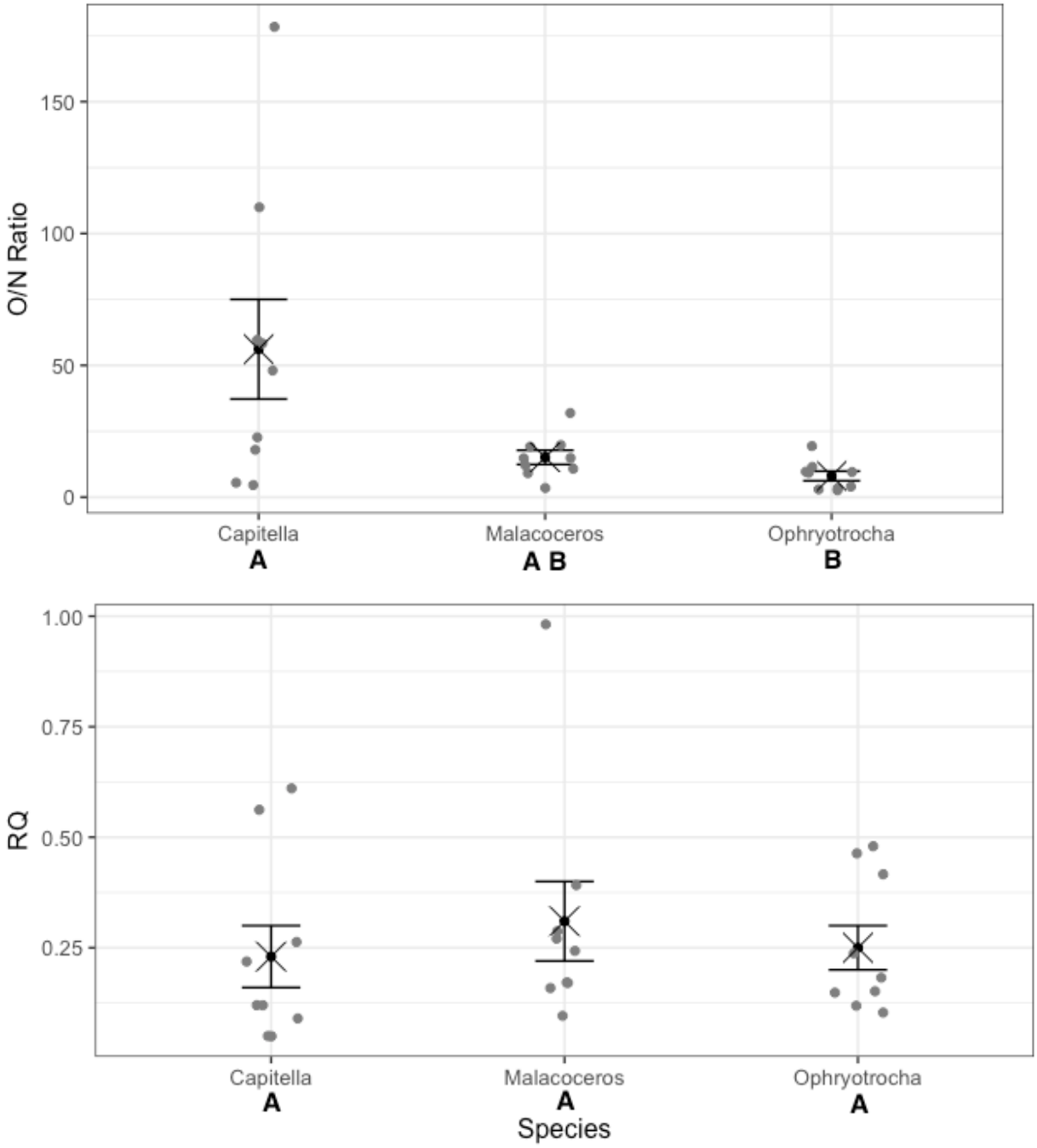

Figure.18. Oxygen Nitrogen ratios **A)** Respiratory Quotient (RQ) **B)** calculated by CO_2 produced/O_2 consumed, for 3 species of polychaete found under a fish farm during September 2017. Each chamber (n=9) contains 5 individuals from the same polychaete species (n=21) is represented by a single dot, the cross is the mean of each species and error bars indicate the standard error. Letters on x axis represent the statistical test (post hoc paired Wilcox) outcomes where same letters is no difference and different letters represent significant differences.

4 Discussion

4.1 Main Findings

Sedimentation was generally higher towards the south of the farm compared to the north, though there is some methodological error with the south trap.

Benthic trays were designed for the cultivation of *Ophryotrocha* spp (hard bottom), however soft bottom polychaetes (*M.fuliginsosus* and *Capitella* spp) were also found in low abundances on the trays. Naturally, *Ophryotrocha* spp was found to be the most abundant species collected from trays. The video surveys, showed large numbers of *Ophryotrocha* spp occupying tray surfaces as well as ropes, shackles and other 3D structures. Additionally, after 1 week there was at least one tray with nearly 100% *Ophryotrocha* coverage, though trays with no *Ophryotrocha* after 4 weeks were also observed, often in areas with little waste. Substrate type on trays did not make a difference for species abundance collected/retained on trays. Video surveys compared with observations after retrieval confirmed that many *Ophryotrocha* spp are washed off, meaning abundances are an underestimate. Both video and abundance highlighted a clear spatial pattern with generally higher amounts of waste and polychaetes in the SSE part of the farm. Metabolic rates for *Ophryotrocha* spp, *Capitella* spp and M.fuliginsosus found no differences in metabolic rate nor nutrient source metabolized. *Ophryotrocha* spp were found to have highest inter individual variation for all metabolic measurements (O_2, nitrogen and CO_2) while Capitella spp had high variation only in respiration rates. Subsequent carbon turnover per gram for each polychaete found that *Ophryotrocha* spp had the highest carbon capacity, followed by *M.fuliginosus* and *Capitella* spp.

4.2 Physical Environment

The current measurements, showed low current velocities at 70-160m, fluctuating between north and south, which matched the sedimentation rates measured at the north and south end of the farm. As the current meter was 800m north of the farm, it is possible that local effects, like the trench running SSE to NNW through the farm could affect the speed as water flows through a narrower area. As *Ophryotrocha* spp is mainly found under fish farms with low current speeds (Eikje, 2013; Valdemarsen et al, 2015 and Hamoutene et al, 2016), it is suggestive similar conditions occur at this site as well. Current speed may be an important factor if considering future sites to use benthic trays to cultivate Ophryotrocha spp.

The sedimentation rates found in this study highlight the spatial variability, though they were likely affectd by number of near by cages as well. The highest area of sedimentation, in the SSE corner of the farm, was also surrounded by more fish pens than that of the northern farm trap. The rates found in this study are generally in line with other Norwegian farms, Kutti et al, (2007a) for example, found variation of 1.6 and 7.5 g m^{-2} day^{-1} of POM (particulate organic matter) within 250m from the farm (range in this study 2.7 - 7.9 g m^{-2} day^{-1}). Some methodological issues with the sediment trap in the SSE corner limit the validity of these rates, specifically only one core was retrieved from the trap and further lab difficulties mean this value could be erroneous. Despite these limitations, the relative trend is likely to be valid, and is in line with the spatial video observations of waste on trays, that is more in the SSE of the form compared to the north.

4.2 Polychaete Colonization Patterns

Species Identification

The identification of *Malacoceros fuliginosus* was made with reasonable confidence, as they are a common and distinct species. There are around 12 different sibling species of *Capitella* spp with *C. capitata* and recently described *C. teleta* being some of the more common (Blake et al, 2009). The larger individuals collected in this study were >10cm long. This means it is unlikely to be *C. teleta*, where the largest collected specimen was 2.4cm (Blake et al, 2009). It is possible the species is *C. capitata*, but as there has been sibling *Capitella* species described in Europe too, it cannot be confirmed (Gamenick et al, 1998 and Blake et al, 2009).

There have been a few different *Ophryotrocha* species found inhabiting areas under fish farms around the world, including *O.cyclops* in Canada, *O.lobifera/Palpiphitime lobifera, O.craigsmithi* in Norway and *O.shieldsi* in Australia (Salvo et al, 2014, Eikje, 2013, Valdemarsen et al, 2015, Wiklund et al, 2009a and Paxton ad Davey, 2010). Based on the red colouring all along the edges, a similar head structure, the P-type jaws and previous known locations, *O.craigsmithi* or *P.lobifera* was deemed the most likely species for the main *Ophryotrocha* spp found. The *Ophryotrocha* genus is known to have diverse feeding strategies (Rouse and Pleijel, 2001) and lab observations saw the different species to inhabit different areas in holding tanks, with some species mainly staying towards the base of the tank. Hence, species identification may be important as different tray colonization patterns and carbon turnover rates could occur.

Colonization Patterns

The dominance of *Ophryotrocha* spp confirms that trays are better suited for hard bottom polychaetes than soft bottom. The presence of *Capitella* spp and *M.fuliginsous* highlights the suitability of substrates for soft bottom polychaetes as well. *Ophryotrocha* spp dominance is likely due to the numerous hard surfaces and structures that extend up into the water column, surfaces that are unavailable for the soft bottom polychaetes. The soft bottom polychaetes were restricted to the essentially two-dimensional space of the substrates instead of the expanse of structures *Ophryotrocha* spp could colonize. As trays lay directly on the seabed, they would restrict the water exchange to the sediment surface and below, where *Capitella* spp and *M.fuliginsosus* reside. These polychaetes, may then only colonize trays as a means to survive from the potentially sudden oxygen depleted environment below. *Ophryotrocha* spp, meanwhile observed both in this study (video surveys) and others (Eikje, 2013, Hansen et al, 2011 and Salvo et al, 2015) dwell on top of sediments, often building rather extensive mucus colonies. The trays essentially increase the available surface area for them to build upon. This is an important consideration for future studies as if the reason for abundances was mere survival and not active preference for trays then the potential of using trays to cultivate polychaetes is reduced. The naturally smaller sizes of *Ophryotrocha* spp (max~2cm) compared to *Capitella* spp or *M.fuliginosus* (max~12cm) further allow more individuals to occupy a unit area (lab observations, as unsure of species i.d). Furthermore, methods of breeding and larval settlement are characteristics that are also shown to affect colonization patterns and success of areas (Levin, 1984).

The presence of *Ophryotrocha* spp*, Capitella* spp and *M.fuliginosus* under the farm was in line with the literature and past monitoring reports (Norwegian Standard NS9410) as all are common opportunistic polychaetes found in association with organically enriched fish farms environments (Pearson and Rosenberg, 1978, Costelloe et al, 1998, Kutti et al, 2007b and Valdemarsen et al, 2015). Overall polychaete abundances reported from other Norwegian fish farms were higher than the average abundance found on trays in this study (7500 individuals m^2). Specifically opportunistic polychaete abundances of >12,000 individuals m^2 were found in Hardanger fjord, south of Bergen at similar depths and current velocities (<5cm/s^{-1}) by Valdemarsen et al (2015). Abundances of *Ophryotrocha* spp on trays compared to natural environments cannot be compared quantitatively as abundances in most of the literature use size of colonies and not percent of coverage (Eikje, 2013 and Salvo et al, 2015). A qualitative comparison, however, shows that more extensive colonies can exist than what was seen on the trays (Eikje, 2013 and Salvo et al, 2015 and Salvo et al, 2018). Valdemarsen et al, (2015) also found that the abundance of *Vigtorniella*, often found in association

with *Ophryotrocha* spp, can be greater than *Capitella* spp under Norwegian fish farms. The abundances on trays for *Capitella* spp and *M.fuliginsosus* were significantly less than what is found in other organically enriched sediments elsewhere. Dauvin et al, (2017) for example found maximum abundances of 21,600 individuals per m2 and 13,000 ind.m2 for *C.capitata* and *M.fuliginosus* in a polluted Algerian harbor while Cardell et al (1999) found >133,000 and 7,000 in a Spanish sewage outlet. In both cases *Capitella* spp was found to be dominant of the two, unlike what was found to colonize trays in this study. This is likely a factor of tray colonization, and not reflective of the actual sediment abundances. Additionally, regarding *Capitella* abundances, there has been extensive taxonomical confusion in the literature between the *C. capitata* and *teleta* species (ref e.gs of confusion, tsutsumi 1990 etc). This is especially relevant with abundance comparison (as well as metabolic rates) as *C.teleta* is much smaller than *C.capitata* so is physically able to have more individuals per unit area. It is not surprising that the polychaete abundances found on trays were lower than other abundances found in literature as polychaetes had 3-4 weeks to actively migrate onto trays. As discussed, there were likely different motivations for tray colonization, which would affect total abundances. Another consideration is that polychaete abundances during a production cycle vary significantly, with fallowing known to drastically reduce opportunistic macrofauna as early as 2 months in (Zhulay et al, 2015). Abundance comparisons could not be made with the other bioremediation attempts as polychaetes were ethier lab based (Kristensen et al, 1995 and Ito et al, 2016), or injected labs cultured pre-known densities under a farm (Chareonpanich et al, 1994 and Kinoshita et al, 2008) where abundances are irrelevant.

The main limitation in determining the number of polychaetes colonizing trays was the large number of polychaetes lost during tray retrieval, especially *Ophryotrocha* spp. Small polychaetes were also lost during the washing process as the sieve mesh size (0.5mm) was too large to collect them. However, a smaller size was tested and shown that the waste and mucus would not sieve through. Meaning both the abundance and biomass estimates for *Ophryotrocha* spp are only for the large sized individuals.

4.2.1 Spatial Variation

From both *in situ* video surveys and abundance estimates it was seen that the south end of the farm generally had more waste and polychaetes, especially *Ophryotrocha*. Benthic variation under Norwegian fish farms is a well-understood concept, monitoring investigations (Norwegian Standard NS9410), for example, take numerous grab samples all through out the farm in order to get a reliable indication of sediment health. This is largely due to the highly varied bottom topography, from cliffs, to trenches to sandy bottoms as well as the depth of sites that often have different layers of current

velocities (ref). The bottom of the trench likely collects OM (organic matter) that has fallen from cliffs higher above, potentially explaining the variation of waste coverage and polychaete abundance observed.

Furthermore, it also shows that the waste up take potential around the farm could also vary greatly with polychaete abundance.

4.2.2 Temporal Variation

The two video surveys of the same trays are thought to be the first *in situ* visual representation of the 'mop up' effect by *Ophryotrocha* spp colonies on fish waste. The 'mop up' effect, is a descriptive term to describe the disappearance of waste on the trays with increasing *Ophryotrocha* colonies. *Ophryotrocha* development was quite rapid in some areas, < 6 days for nearly 100% substrate coverage, while slower rates were found towards the north of the farm. This rapid development is likely due to a high sedimentation rate, as seen with sediment traps or organic fall zones since as there are a number of cliffs where OM can collect then fall. Other opportunistic *Ophryotrocha* spp found under fish farms have been described as generally short lived and reproducing quickly, further contributing to these fast colonization rates (Paxton and Davey, 2010). A third factor, is that of an existing nearby population is also required for such immediate abundance, as described in Keeley et al (2015) and Tsutsumi (1990) with regards to *Capitella* proliferation. This would especially be the case for complete colonization in < 6 days. Determining the contribution of each factor to the results is complicated. There is increasing interest in video surveys for monitoring hard bottom habitats under fish farms in Norway and Canada, and likely Ophryotrocha will be a key indicator species (Salvo et al, 2017, Hamoutene et al, 2015 and Hansen, pers.com). This would help determine the importance and contribution of each factor to the rate of *Ophryotrocha* development.

The small sample number and multiple variables affecting polychaete abundance mean finding an average colonization time is difficult. If conditions are not right, for example, it can be upward of 24 days as seen by the more northern trays. When conditions are right, fast opportunistic polychaete development is a common occurrence (Tstutsumi, 1990, Brooks, 1993 and Keeley et al, 2015). The method used in the temporal analysis attempted to quantify a qualitative response but due to the variation in film quality, was quite subjective. A computer program, if it can distinguish between waste and polychaetes would be a more objective way to estimate cover on substrates. The analysis method was based on estimating the *Ophryotrocha* and Waste coverage percent on just the Green substrate, meaning, *Ophryotrocha* colonies on tray edges were ignored. For the majority of the trays this was ok as the degree in colony development was big enough that differences were seen on the

substrate as well, for example, T8 (Fig.14). Additionally this study is about reducing fish waste in benthic environments, and not solely about growing polychaetes, so waste was an important factor to measure.

Rapid *Ophryotrocha* colony development in Norway has previously been documented in a master thesis (Eikje, 2013), where progression over a 14-month period was monitored using Remote Underwater Video (ROV) surveys. As the areas (trays) and sample size (n=5) were smaller in this study it is difficult to directly compare the rates. Though on two of the trays (T4 and T6) the rates (~3x) of development were similar to that found in Eikje (2013). Furthermore, the video surveys performed under fish farms in Canada have also described *Ophryotrocha* development in association with *Beggiatoa* bacteria as rapid (Salvo et, 2017).

Ophryotrocha colonization on trays was shown to occur with a reduction in waste on trays, which is likely due to the mineralization by the polychaetes. If *Ophryotrocha* colonies can develop under a week in high waste fall areas and therefore decompose or 'mop-up' the waste, this then furthers their potential in the waste recycling process. Though an existing *Ophryotrocha* spp colony would likely be required for the initial colonization.

The other tray, T11, excluded from the video analysis, was seen in a complete vertical orientation off the side of a cliff. As the film was quite limited, with only the trays side being seen, it is quite possible there were more extensive *Ophryotrocha* colonies present. Rapid colonization is not a factor in this case as the film was taken only an hour before sampling. The abundance of *Ophryotrocha* spp (8700 individuals m^2) and *M.fuliginosus* (200 individuals m^2) on T11 highlights the retention abilities of substrates and a vertical axis is not a completely limiting factor for colonization.

4.3 Tray Design and Sampling

The basic tray design used in this study has previously been used in Norway to collect large numbers of *Ophryotrocha* spp for other experiments (Fang et al, in prep, Nederlof, in prep). The results from the Green and Blue substrates suggest, that the blade length and flexibility do not matter for polychaete (specifically *Ophryotrocha* spp) retention during tray retrieval. It was thought the longer blade lengths, which are flexible and collapse down slightly would trap more *Ophryotrocha* spp in comparison to the stiff plastic of the Blue substrate, but this was not the case. The main source of error identified with the substrate comparison was that not all *Capitella* spp and M.fuliginosus were washed out of the Green, as they were intertwined in substrate stitching. The vertical orientation of

the T11 tray and subsequent high *Ophryotrocha* spp abundances, however, do highlight the retention abilities of both substrates while the tray is deployed.

The small sample number (n=8) may have hampered the correlation test between *Ophryotrocha* spp *in situ* substrate coverage and abundance counts from trays. Alternatively, there could be a non-linear relationship, where higher *in situ* cover results in more *Ophryotrocha* spp loss during tray retrieval. The extensive mucus colonies *Ophryotrocha* spp have been observed to make both in this study and others (Eikje, 2013) may mean they stick better together than they do to the substrate. The sub-optimal film quality, however limits this conclusion as of the three trays with most *Ophryotrocha* cover, only one had film where the whole tray was visible.

The very low to almost no polychaete abundances on some trays suggest that they themselves are not sole attraction for polychaete colonisation. The video surveys showed very little to no waste coverage on ethier tray, meaning a high enough rate of food fall is required for polychaetes to colonize trays. The colonization of *Ophryotrocha* onto the 3D structures on some of the trays further hints at ways to maximize their abundance/biomass. It seemed once they had the food, they only required the substrate to then expand to extensive colonies and a 3D tray structure would maximize this. Although it was not specifically studied here, some video observations suggested that abundance of *Ophryotrocha* spp on trays was higher than on the surrounding benthic habitat. This indicates that trays may indeed facilitate *Ophryotrocha* spp abundance, which in turn would further their decomposition potential of fish waste.

3.3 Cultivation Tray Optimization

For this study the trays were set down directly on top of the sediments/bottom. As the trays were in direct contact with polychaete habitats, tray colonization may have occurred faster and to a higher degree than if trays were elevated just off the seabed. This is an important consideration when looking at the long term potential, especially if trays were to be raised and used as a false bottom. This could be done to preserve more of the original benthos, for example or to reduce the overall load to the sediments. Adequate numbers of polychaetes would need to first colonize the trays before they could begin breaking waste down. If limited to no tray colonization would occur then there may be a higher chance of anoxic conditions occurring, depending on the microbial composition on trays and dispersal off trays. Further studies would be needed to investigate the impacts of fish waste build up on elevated trays for an indication of the consequences if polychaetes do not colonize. The substrates used, show that it is possible to also cultivate low abundances, compared to what potentially occurs naturally, of soft sediment polychaetes (*Capitella* and

Malacoceros). Though this colonization would be unlikely if trays were raised off the seabed. After observing *Malacoceros* climbing the central pipe and even emerging from the water, during spawning behaviour, perhaps they would still appear.

With more frequent video surveys and good coverage of all trays, (which was the intention in this study but technical difficulties and weather limited this) a better overall indication of in situ behavior and waste decomposition could be gained.

In terms of optimizing tray design, there was no significant difference between substrates either in the video surveys or the abundances sampled. The video observations of *Ophryotrocha* spp colonizing 3D structures, however, suggest that inclusion of more of these structures could facilitate higher abundance, as long as access to falling POM remained.

4.4 Metabolic Rates

4.4.1 Respiration Rates

The ratios and respiration rates results showed that both metabolic nutrient source and rate are the same for *Ophryotrocha* spp, *Capitella* spp and *M.fuliginosus*. As RQ values are lower than the theoretical 0.6 to 1 range, their validity was questioned and will be discussed below. Furthermore, these findings are only relevant for these particular sized polychaetes (appendix, fig 6) due to the inverse relationship between metabolic rate and body weight within species (Shumway, 1979; Beis et al, 1980). A body weight curve would need to be established for each species in order to know the degree of metabolic change throughout the polychaetes life stages.

Ophryotrocha spp respiration rates were within the range of previous studies (Eikje, 2013 and Nedlerof in prep, appendix, Table 5). *Capitella* spp rates, however, were higher than those found from Nederlof (in prep) though the two likely outlying values in this study would have raised the average. *Capitella* spp respiration rates were also higher than those found in a comparison study with four of the *Capitella capitata* sibling species (Gamenick et al, 1998). Further literature comparisons for *Capitella* spp are difficult due to the number of sibling species of varying size and ecological niche. Literature of respiration rates could not be found for *Malacoceros* genus. Since it is in the same subclass (Sedentaria) as *Capitella* spp and has similar CO_2 output rates, comparisons with *Capitella* literature were used. As *Ophryotrocha* spp and *M.fuliginosus* respirational rates were in line with past

studies (Capitella studies in the case of M.fuliginosus), it is likely they are reasonably accurate. However, *Capitella* spp respiration rates were higher than literature and Nederlof (in prep), suggesting possible erroneous values or non-standard behavior. Potentially abnormal behavior was noted at the end of the *Capitella* spp incubation, where many of the individuals were found curled up in the thread of the lid. As this was not noted in any of the test runs, it may represent abnormal behavior or could have affected the oxygen readings.

Ophryotrocha spp occur in deep and low current benthic areas, therefore, water movement was kept to a minimum during incubations. This was also the case for *Capitella* spp and *M.fuliginosus* in order to keep experiments standardized. *M.fuliginosus* and especially *Capitella* spp were mostly amongst the beads in the chambers during the incubations unlike Ophryotrocha spp who were above. If polychaete movement was insufficient or only amongst the beads in the chamber and not water, then oxygen gradients may have occurred. This would result in non-linear oxygen decline through the incubation, with periods of low oxygen consumption followed by steep drops as the water mixes. Therefore, the variance seen, especially with *Capitella* spp, may be influenced by the degree of water mixing at that time.

4.4.2 Species Difference

One of the more evident differences seen between the polychaete species was the high inter individual variation across all metabolic rates (O_2, nitrogen and CO_2) for *Ophryotrocha* spp. This was shown by small ranges in ratios but comparatively larger variation in the oxygen uptake, nitrogen excretion and carbon dioxide production. The larger ratio standard errors for M.fuliginosus but especially *Capitella* spp, suggest more inter individual variation in metabolic source though respirational measurement errors may also have affected this (as discussed above).

The inter-individual variation in *Ophryotrocha* metabolic rate may be due to its extensive mucus production. A histological analysis on *O.cyclops*, (also found under fish farms) by Murray (2012) found extensive mucoproteins (mucus production) throughout the epithelial tissue of the polychaete. Additionally high metabolic activity in these epidermal areas was also noted and thought to be of physiological significance (Murray, 2012). It may be possible that this then translates up to an overall higher metabolic rate. Mucus production is unlikely to be constant, meaning the varying degrees of mucus production by *Ophryotrocha* spp could result in the varying metabolic rates as seen in the results.

Ophryotrocha spp behavior may also explain the varying rates as different behaviors have been shown to significantly affect metabolic rate (Reinhold, 1999). Numerous interactions, sometimes to

the point of aggression were observed in the *Ophryotrocha* lab holding tank. Though beyond the scope of this thesis, the lab observations may suggest that the degree of different behavior seen may translate up to temporary metabolic rate variations.

The range of O/N ratios seen for Capitella spp, is likely due to very clustered and stable nitrogen excretion rates and high respiration rates which may be due to oxygen gradients or abnormal behavior (as discussed above).

The main species difference found in the metabolic measurements and subsequent ratios was the range with in each species. These findings indicate that there is no statistical difference in rate, though future studies perhaps could benefit from using a higher sample number to minimize the risk of potential type 2 errors.

4.4.3 RQ Values

The CO_2 production was almost 10 times less than the oxygen consumed and the resulting low RQ warrants further consideration. As the main identified reasons were consistently kept factors during the incubations, species differences were unlikely to be affected.

As mentioned the low RQ values suggest a metabolism macronutrient source well away from carbohydrates (RQ=1) and heavier in proteins/lipids, especially ketones (RQ 0.8, 0.7 and 0.6, respectively). The generally low O/N ratios (<60) also suggest a metabolic source away from carbohydrates (Mayzaud and Conover, 1988) and the degree of protein catabolism was seen to be relatively high for *Ophryotrocha* spp and *M.fuliginosus.*

RQ values for marine invertebrates are limited, though Kristensen (1989) found a value of 1.2 for *Nereis virens*, another polychaete found in intertidal areas. The values in this study were all lower than the minimum values found in other studies (0.4 for Nautilis, Boucher-Rodini and Boucher, 1993). As demonstrated in Table 6, the RQ values are all significantly below that, even for other marine invertebrates. The average for these species, 0.75, (0.26 for the polychaetes in this study), has then been used as an assumed RQ in other studies, such as estimating carbon balance in sponges (Koopmans et al, 2010)

Table 6; Mean RQ values from literature for other marine invertebrates with standard errors when available and source.

Genus/Species	**RQ mean (SE)**	**Source**
Ophryotrocha spp (polychaete)	0.25 (±0.05)	This study
Capitella spp (polychaete)	0.23 (±0.14)	This study
Malacoceros fuliginosus (polychaete)	0.31 (±0.09)	This study
Triphyllozoon (bryozoan)	0.75 (±0.03)	Hatcher, 1989
Herdmania momus (ascidian)	0.78 (±0.04)	Hatcher, 1989
Poneroplax albida (chiton)	0.68 (±0.03)	Hatcher, 1989
Haliotis roei (abalone)	0.77 (±0.11)	Hatcher, 1989
Nautilus macromphalus (nautilus)	0.74	Boucher-Rodini and Boucher, 1993
Ophiothrix fragilis (brittle star)	0.69	Migne and Davoult, 1997

An important consideration is that the phyla listed in Table 6 are marine invertebrates not living in deep organically enriched environments where different modes of life and food sources are available. *Ophryotrocha*, for example, are known to partially feed on associated microbes such as *Beggiatoa*, a sulphide-oxidizing bacterium (Salvo et al, 2015 and Decker, 2010). To the best of the authors knowledge, RQs have not been calculated for other organisms found in fish waste environments. It is therefore feasible these values are accurate and they do use an oxygen expensive source of nutrients for metabolic processes. The prevalence of the lipid rich food source around them (fish pellets and faeces) could mean their metabolic fuel may reflect their diet.

Methodological error could also explain values, such as inexperience calibrating pH meters, which in turn may give erroneous readings leading to false CO_2 production rates. Mixing of air into incubation chambers while the pH probe was being agitated may have also altered CO_2 levels to a significant degree. As no additional food was added into holding tanks during the 5 days prior to incubations, it is feasible polychaetes were in a state if starvation and depending on body reserves.

A further explanation is that the polychaetes were all to some degree, storing carbon instead of releasing it as CO_2. This was suggested by Mokrasch et al (1960) where large variations in RQs (0.6-0.84) for hibernating rodents were found. Though the polychaetes aren't likely to hibernate, some may have been in a torpor like state in response to starvation.

The no significant differences seen between polychaetes species in either metabolic source or rate may be reflective of their ecological niche as opportunistic polychaetes in a benthic environment dominated by fish waste. The complexity of physiological energetics makes it difficult to discern reasons. Methodological error in using potentially starved organisms and not having unperturbed water during incubations may also have affected the result. Future studies are recommended to see if the RQ values are true or as a result of potential method errors, specifically ensure that potentially starved animals are not used and explore water mixing methods that do not disturb *Ophryotrocha* spp. Further studies with *Ophryotrocha* spp especially are recommended for investigation into the inter individual variation in metabolic rate seen here. Based on these results, it should be considered that the carbon turnover rates from the CO_2 production from these incubations would likely be an underestimate.

4.5 Waste Recycling Potential

Table 7. Carbon turnover estimates for dominant polychaetes (Ophryotrocha, Capitella and Malacoceros) species collected from the 12 benthic trays placed under a Norwegian fish farm for 4 weeks. Polychaete carbon capacity calculated from RQ values and respiration rates found in this study. Polychaete "Biomass needed" indicates the polychaete biomass in g(afdw) needed to consume 100, 50 and 20% of incoming POC from cages. "Increase required" is how many times the "Current Carbon Consumption" needs to increase to reach 100, 50 and 20% of POC recycling/consumption. POC rates calculated as 36.6% (Wang et al, 2013) of the measured POM sedimentation rates by 3 traps placed within 200m of the farm.

		Ophryotrocha		**Capitella**		**Malacoceros**	
		Calculated	Increase required	Calculated	Increase required	Calculated	Increase required
Current Mean Tray Biomass g (afdw)		11.1		8.1		10.3	
RQs From This Study		0.25		0.23		0.31	
Polychaete Carbon Capacity (mg C/day/g AFDW)	This Study	2.54		1.14		1.25	
	RQ 0.75	10.31		7.94		4.45	
	RQ 0.9	12.37		9.53		5.34	
Current Avg Carbon Consumption (mg C/day/avg biomass (afdw)	This Study	28.19		9.23		12.88	
	RQ 0.75	114.44		64.31		45.84	
	RQ 0.9	137.31		77.19		55.00	
Biomass (g AFDW) Needed for **100%** Mitigation	This Study	601.65	21,34	1340.52	145,17	1222.55	94,96
	RQ 0.75	148.22	5,26	192.47	20,84	343.41	26,67
	RQ 0.9	123.54	4,38	160.36	17,37	286.18	22,23
Biomass (g AFDW) Needed for **50%** Mitigation	This Study	300.83	10,67	670.26	72,59	611.28	47,48
	RQ 0.75	74.11	2,63	96.23	10,42	171.71	13,34
	RQ 0.9	61.77	2,19	80.18	8,68	143.01	11,11
Biomass (g AFDW) Needed for **20%** Mitigation	This Study	120.33	4,27	268.11	29,03	244.51	18,99
	RQ 0.75	29.65	1,05	38.5	4,17	68.68	5,33
	RQ 0.9	24.71	0,88	32.07	3,47	57.24	4,45

Avg

Sediment trap I.D	#1	#2	#3	mg/m2/day
POM g/m2/day	2.65	7.9	1.98	4175.39
Estimated POC input g/m2/day	0.97	2.89	0.72	1528.19
50% POC input g/m2/day	0.49	1.45	0.36	764.1
20% POC input g/m2/day	0.19	0.58	0.14	305.64

Using the respiration rates and CO_2 production (or RQ value) a carbon capacity estimate was made for *Ophryotrocha* spp, *Capitella* spp and *M.fuliginosus* (Table 7). Estimates were also made using an RQ of 0.75 (used in Koopmans et al, 2010) and 0.9 (Cammen, 1985 and Eikje, 2013) for a maximum potential carbon uptake. The average biomass from the tray substrates was then used for an indication of the current amount of carbon being utilized. This was an underestimate due to the degree of polychaetes washed from the trays during retrieval but provides a rough estimate. This was related back to the Particulate Organic Carbon (POC) rate (mg/day/m^2) estimated from the POM sedimentation rates for an indication of polychaete carbon capacity in relation to carbon input. Due to machine malfunctioning, actual POC quantities could not be measured; instead the POC was calculated using the measured carbon content of faeces of 36.6% from Wang et al (2013). Overall *Ophryotrocha* spp had the highest carbon capacity of the polychaete species (2.54 mg C/day/g AFDW this study). *M.fuliginosus* and *Capitella* spp were closer to each other and differed slightly depending on the RQ value used. Naturally, higher RQs yielded a substantially higher carbon estimate for all polychaetes, but these should only be seen as a `What If´ or as a maximum value with the respiration rates measured. None the less, estimates for current carbon consumption for all polychaetes and all RQ values were very small (<8% of the total POC fall).

Using the calculated RQs it was found >600 g (AFDW) of *Ophryotrocha* is needed per m^2 for a theoretical 100% POC consumption (Table 6). This was doubled again for *Capitella* spp and *M.fuliginosus* due to the lower carbon carrying capacities. Theoretical is used as polychaetes do not absorb and use 100% of the carbon they ingest. Furthermore, microbes also make up a significant part of the waste mineralization and would consume a portion (not considered in this study) (Valdemarsen et al, 2012). The 100% POC consumed biomass estimate is also theoretical from a population dynamics standpoint as there is no additional food available once 100% of the input is consumed. This would likely cause the population to collapse or go 'bust'. Opportunistic species especially, generally have a boom and bust population dynamic, as shown with *Capitella* (Chesney and Tenore, 1985 and Ramskov and Forbes, 2008). It was shown with *Capitella* spp huge population

growth occurs in optimal conditions followed by a sharp decline once the capacity of the environment is reached (Chesney and Tenore, 1985). If this decline or bust occurs just prior to peak POM rates then there is a significant imbalance with few polychaetes and high OM loads. The polychaete population must then recover before the OM loads reach levels that overwhelm the aerobic fauna (including bacteria) forcing increased anaerobic mineralization to occur. This ultimately leads into anoxic conditions, where waste mineralization rates slow and macro fauna (i.e polychaetes) have great difficulties re-colonizing as described in Valdemarsen et al (2012). The boom bust population dynamics highlights the importance of long-term studies in assessing the overall potential of polychaete trays under a fish farm.

The polychaete biomass requirements for a 50 or even 20% POC input consumption are more realistic and better for the sustainability of polychaete colonies on the trays. This would require a 11 and 4 fold increase in biomass, respectively for *Ophryotrocha* spp, the closest polychaete to meeting these demands. Considering the degree of wash off from the trays, and the likelihood that the RQs calculated here are an underestimate, it is possible *Ophryotrocha* spp are already decomposing at least 20% of the POC input. An important consideration is that this estimate is for a POM rate 4-5 months into an 18 month production cycle, so maximum OM loading has yet to occur. The amount of POM reaching the sediments increases through a production cycle (Kutti et al 2007a), further requiring more polychaete biomass for waste mitigation. However, due to potential methodological error (sed. trap #2) the POM rate calculated for September may be an overestimate as well.
The carbon consumption estimates were made with the assumption that the polychaetes are only consuming carbon from POM. There is, however, also dissolved organic waste that is expelled from fish farms, commonly utilized by bivalve and seaweed IMTAs. It was suggested in Murray (2012) after noting the microvilli on the epidermal tissue of *O.cyclops,* that the microvilli might be related to uptake of dissolved organic nutrients. Uptake of dissolved nutrients from the water column, especially amino acids, has previously been described in other polychaetes species, such as *Clymenella torquata* and *Nereis diversicolor* (Stephens, 1963 and Gomme, 2001). *Ophryotrocha* and *Malacoceros* were often observed in the lab waving their heads gently in the water column, from their respective mucus and sediment structures. From a stable isotope analysis of likely food sources of *Ophryotrocha*, Salvo et al (2015) found *O.cyclops* were not consuming suspended POM. Perhaps, instead, this unknown behaviour is part of an uptake of dissolved organic nutrients, which in a under a fish farm, there is plentiful amounts. If this were the case, then the carbon capacity estimated in this study would be a further underestimation.

Lastly, a critical factor is that carbon uptake and utilization is not constant through an organisms life time. Metabolic rates and even nutrient sources can vary depending on the growth, reproduction, torpor state of the animal and available food, as seen with Capitella (Gremare et al, 1989).

4.5.1 Further Considerations for Waste Recycling Potential

When looking at the potential for this particular site to invest in cultivating polychaetes in trays, the environmental parameters should also be considered. The current speed at depth at this site was quite slow (<5cm^{-1}), meaning low dispersal of waste and higher sedimentation rates in the close vicinity of the farm. With these conditions, OM decomposition and recycling will more easily be impaired and anoxic conditions more easily reached, as described in Valdemarsen et al (2012). Therefore, the potential gain in even a 20% waste decrease is likely more significant compared to a site with faster current velocities. Other factors that need to be considered when looking at the potential of benthic polychaete cultivation trays is the effort required to install and the upkeep required by the farm. This is not investigated here and further larger scale implementations of the trays would be required for a better indication of the costs and benefits for the farm itself. However, there is still 2% of particulate carbon waste being decomposed producing potentially harvestable polychaete biomass

5 Conclusion

The benthic environment utilized in this study was characterized by bottom depths of 80 to 200m, with low current velocities (<5cm/s) and varied bottom topography with soft, mixed and hard bottom in conjunction with a trench running throughout. These factors likely played a significant part in the polychaete species and abundances found to cultivate trays as well as the sedimentation rates of particulate organic matter (POM), which were fairly normal compared to other Norwegian farms. Calculations for waste recycling on trays were based on species abundance and biomass, tray placement, metabolic rates and assumed nutrient source used for metabolism. *Ophryotrocha* spp

was found to have the best potential for fish waste recycling with its higher abundance and biomass on trays and higher carbon turnover rate.

For future tray designs, the substrate type did not matter but the presence of 3D structures did, with *Ophryotrocha* colonizing up the benthic sediment cores. The polychaete wash off during tray retrieval highlights that collection techniques also need to be developed if an IMTA system is to be achieved in the future.

The minimum carbon turnover rates for *Ophryotrocha* spp was 28.2 mg C/day/ g (afdw), using the average tray biomass and RQs found in this study. The maximum carbon turnover rates assuming an RQ of 0.9 (protein fuelled metabolism) were 137.3 mg C/day/ g (afdw). This equates to 2% and 8%, of the total estimated carbon waste (per m^2) input to the bottom during the study period.

This study has shown that the hard bottom specialist, *Ophryotrocha,* can rapidly colonize benthic trays, as long as there is an organic matter input. The metabolic studies may have been using starved polychaetes, which could have contributed to the low RQ values seen, and future work is required to validate these findings. It was found as a minimum that for a 20% decomposition of the POC coming down the biomass of *Ophryotrocha* spp would have to be 4 times that found in this study.

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Appendix

Table 1: Waste and Polychaete coverage groups on substrates attached to benthic trays determined independently by expert panel (9 people) plus author. Group 1(blue) = <10%, Group 2 (green) =10-40%, Group 3 (orange)= 40-70% and Group 4 (red) =70-100% coverage.

Person		Tray										
		1	2	3	4	5	6	7	8	9	10	12
Author	Group	2	2	1	3	2	4	1	4	3	4	4
1	Group	2	2	1	3	1	4	1	2	3	4	4
2	Group	2	3	1	4	2	4	1	3	3	4	4
3	Group	2	2	1	4	2	4	1	3	2	4	4
4	Group	2	2	1	4	2	4	1	3	3	4	4
5	Group	2	3	1	4	1	4	1	3	3	4	4
6	Group	2	2	1	3	1	4	1	4	3	4	4
7	Group	2	2	1	3	1	4	1	3	3	4	4
8	Group	2	2	1	3	2	4	1	3	3	4	4
9	Group	2	3	1	4	2	4	1	3	2	4	4
Average		2	2,3	1	3,5	1,6	4	1	3,1	2,8	4	4

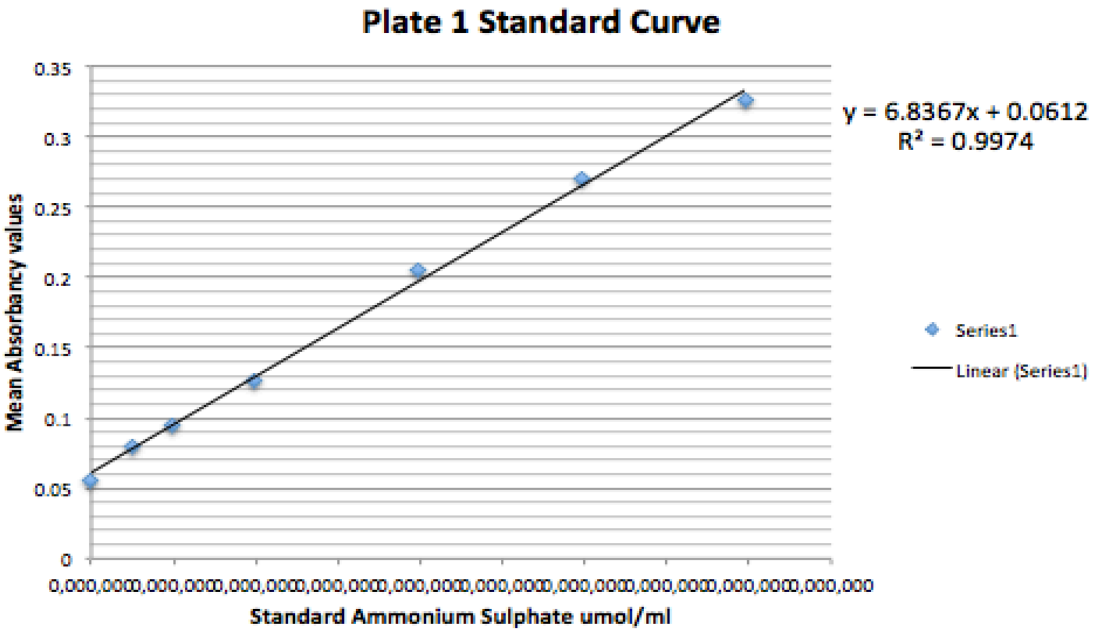

Plate column	Standard umol/ml	Abs r1	Abs r2	Mean
1	0,0397	0,3438	0,3080	0,3259
2	0,0298	0,2643	0,2751	0,2697
3	0,0199	0,2029	0,2062	0,2046
4	0,0099	0,1260	0,1276	0,1268
5	0,0050	0,0972	0,0930	0,0951
6	0,0025	0,0794	0,0814	0,0804
7	0,0000	0,0561	0,0557	0,0559

ID	Abs r1 (row C)	Abs r2	Abs r3	Mean	NH4 conc umol/ml
M1	0,0955	0,0971	0,0913	0,09463	0,00567
M2	0,0875	0,0921	0,0926	0,09073	0,00510
M3	0,0736	0,0698	0,0755	0,07297	0,00250
M4	0,0872	0,0873	0,0827	0,08573	0,00436
M5	0,0873	0,0911	0,0864	0,08827	0,00473
M6	0,075	0,0759	0,0714	0,07410	0,00266
M7	0,0861	0,0819	0,0868	0,08493	0,00425
M8	0,0862	0,0855	0,0855	0,08573	0,00436
M9	0,0807	0,0856	0,0868	0,08437	0,00416
M10	0,0552	0,0552	0,0571	0,05583	-0,00001

ID	Abs r1 (row F)	Abs r2	Abs r3	Mean	NH4 conc umol/ml
N1	0,0694	0,0677	0,0681	0,06840	0,00183
N2	0,0863	0,0729	0,0747	0,07797	0,00323
N3	0,0667	0,0748	0,0725	0,07133	0,00226
N4	0,0773	0,0735	0,0757	0,07550	0,00287
N5	0,0752	0,0750	0,0737	0,07463	0,00274
N6	0,0657	0,0715	0,0685	0,06857	0,00185
N7	0,0715	0,0732	0,0597	0,06813	0,00179
N8	0,0795	0,0793	0,0840	0,08093	0,00366
N9	0,0702	0,0785	0,0718	0,07350	0,00257
N10	0,0553	0,0380	0,0604	0,05123	-0,00068
N start	0,0554	0,0565	0,0550	0,05563	-0,00004

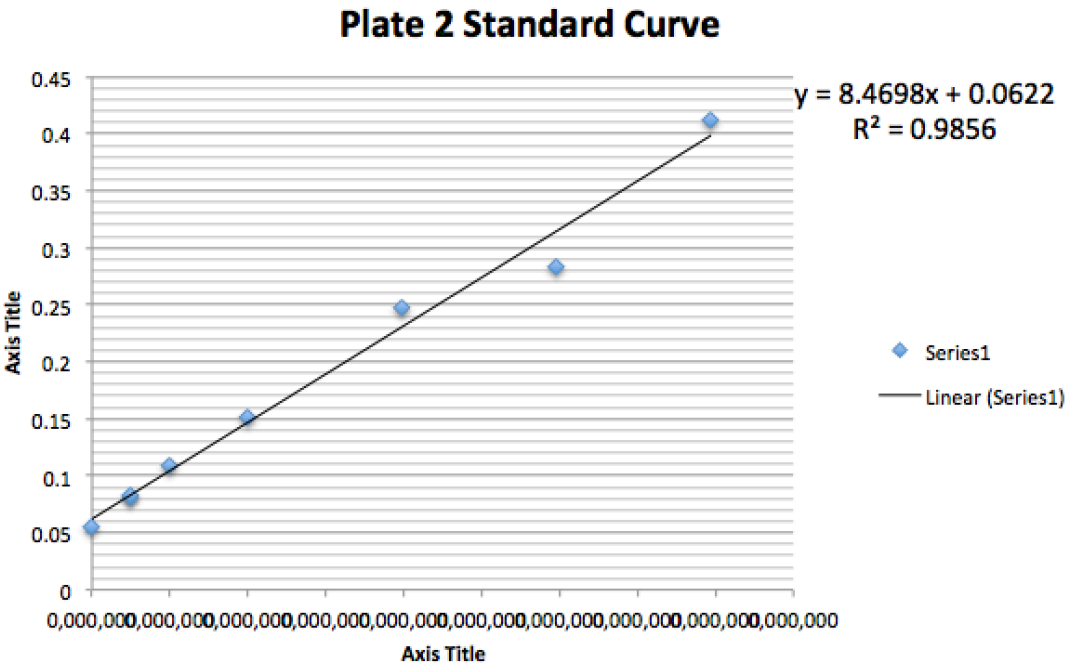

Plate column	andard umol/l	Abs r1	Abs r2	Mean
1	0,0397	0,3662	0,4586	0,4124
2	0,0298	0,2937	0,2719	0,2828
3	0,0199	0,2554	0,2384	0,2469
4	0,0099	0,1615	0,1425	0,1520
5	0,0050	0,1117	0,1070	0,1094
6	0,0025	0,0823	0,0804	0,0814
7	0,0025	0,0821	0,0835	0,0828
8	0,0000	0,0547	0,0560	0,0554

ID	Abs r1	Abs r2	Abs r3	Mean	NH4 conc umol/ml
L1	0,0758	0,0754	0,0776	0,07627	0,00247
L2	0,0811	0,0801	0,0854	0,08220	0,00317
L3	0,0726	0,0766	0,0828	0,07733	0,00260
L4	0,0786	0,0767	0,1135	0,08960	0,00404
L5	0,0767	0,0800	0,0773	0,07800	0,00267
L6	0,0815	0,0775	0,0782	0,07907	0,00280
L7	0,0750	0,0776	0,0791	0,07723	0,00258
L8	0,0795	0,0814	0,0396	0,06683	0,00136
L9	0,0982	0,1025	0,1195	0,10673	0,00607
L10	0,0576	0,0552	0,0568	0,05653	0,00014
Lstart	0,0521	0,0523	0,0513	0,05190	-0,00041

Figure 1. Raw data from nitrogen analysis for dominant polychaete species. Standard curves were made with 7 dilutions from 0.0397 umol ammonium sulphate down to 0 with 2 technical replicates per plate. The 'M' and 'N' are Malacoceros and Capitella incubations, both of which had 3 technical replicates. "Abs r1" refer to the nitrogen level determined from the absorbency readings form the standard dilutions using *y* (the slope of the line).
Plate 2 is the same but for the *Ophryotrocha* spp

Table 2. Abundance and Biomass m^2 and Individual Ash Free Dry Weight AFDW(mg) of polychaete species collected from substrates attached to benthic trays placed under a Norwegian fish farm for 4 weeks. "Biomass m^2" is the average "Individual Polychaete" weight multiplied by abundance.

	Ophryotrocha	Malacoceros	Capitella	Prionospio	Total
Abundance m^2					
Min	5	0	0	0	0
Sum	74818	10647	3666	647	89778
Max	15795	4256	2147	437	15795
Average	6235	968	524	129	7482
Std Deviation	5428	1369	789	185	5239
Percentage of Average	83%	12%	4%	1%	100%
Biomass m^2 AFDW (g)					
Min	0.013	0.0	0.0		0.013
Sum	174.6	419.3	121.4		715.2
Max	33.3	253.2	66.9		353.4
Average	14.5	38.1	17.3		70
Std Deviation	11.9	77,0	24,0		113
Percentage of Average	32%	53%	15%		100%
Individual Polychaete AFDW (mg)					
Min	0.88	5.37	14.02		
Max	2.64	50.12	17.13		
Average	1.78	16.94	15.58		
Std Deviation	0.49	14.32	2.20		
Sample Number	20	14	2		

Table 3. Polychaete biomass (abundance* avg individual weights) on each of the 12 trays deployed under a Norwegian fish for 4 weeks. These are minimum values due to the high amount of polychaetes lost during tray retrival.

Tray No.	*Ophryotrocha*	*Malacoceros*	*Capitella*	Grand Total
T1	1.87023	49.48790		51.35812
T10	22.54443	17.04635	8.956	48.54698
T11	7.74323	1.39534		9.13857
T12	11.71568	1.74417	0.981	14.44114
T2	16.67321	3.48834	13.162	33.32327
T3	0.28553	0.12791	0.187	0.60035
T4	11.87272	3.49997		15.37269
T5	0.30516	15.72078	33.442	49.46761
T6	10.10600	126.61487	0.312	137.03239
T7	0.00929	0.08996	0.069	0.16836
T8	22.20536303			22.20536303
T9	28.1872305	1.034873795		29.2221043
Grand Total	**133.5180807**	**220.2504405**	**57.10842015**	**410.8769413**

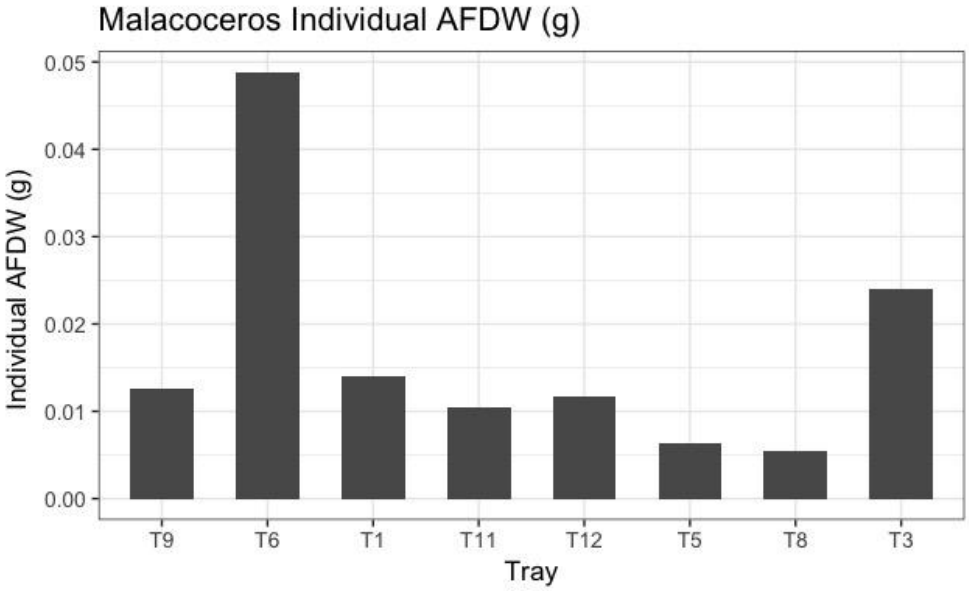

Figure 2. The individual weight (AFDW g) of *Malacoceros* under the Rong farm. Trays are placed in location order from South to North. *Malacoceros* from this tray were later found breeding in the lab.

Figure 3. Snapshots from video surveys of *Ophryotrocha* colonizing the short and long benthic sediment traps and ropes from when trays were deployed under a Norwegian fish farm for a 4 week period in Septemeber, 2017.

Table 4. Metabolic summary for dominant polychaetes species on benthic trays after 4 weeks being under a Norwegian fish farm. Respiration (oxygen uptake), nitrogen excretion and CO2 production averages and standard errors are mass-standardized to total unit Ash Free Dry Weight (AFDW) and Dry Weight (DW). There were 3 incubations run (1 per species) with 9 experimental chambers with 5 individuals each and 1 control chamber.

	Capitella		**Malacoceros**		**Ophryotrocha**	
	Average	SE	Average	SE	Average	SE
Oxygen Uptake (umol/hour/g AFDW)	36.74	10.33	20.58	3.87	47.68	9.54
Oxygen Uptake (umol/hour/g DW)	32.34	9.14	18.36	3.45	40.93	7.71
Nitrogen Excretion (umol/hour/g AFDW)	0.81	0.08	1.35	0.05	6.61	0.80
Nitrogen Excretion (umol/hour/g DW)	0.71	0.07	1.21	0.04	5.70	0.65
Carbon Dioxide Production (umol/hour/g AFDW)	3.94	0.16	4.34	0.17	8.81	0.61
Carbon Dioxide Production (umol/hour/g DW)	3.46	0.13	3.88	0.15	7.67	0.58
Carbon Production from RQ 0.9 (umol/hour/g AFDW)	33.07	9.30	18.52	3.48	42.91	8.59
RQ this Study	0.23	0.07	0.31	0.09	0.25	0.05
O/N Ratio	56.12	18.90	15.11	2.69	8.05	1.83
Carbon Turnover-This Study (mg C/day/g AFDW)	1.14	0.04	1.25	0.05	2.54	0.18
Carbon Turnover-RQ 0.9 (mg C/day/g AFDW)	9.53	2.68	5.34	1.00	12.37	2.48

Table 5. O_2 consumption umol hr^{-1} $g(dw)^{-1}$ for *Ophryotrocha* spp and *Capitella* spp from this study, Nederlof (in prep) and Eikje (2013). Test runs (from equipment familiarization) also included for comparison though method standardized to final runs. "No in chamber" refers to numbers of individual polychaetes in each incubation chamber.

Ophryotrocha	**No in Chamber**	**O_2 umol hr^{-1} g $(DW)^{-1}$**	**Std dev**
This study	5	40.9	23.1
Test run 1	5	18.0	11.4
Test run 2	20	25.4	11.2
Eikje (2013)	1	48.7	4.4
Nederlof	10	23.2	8.5
Nederlof	1	36.1	18.9
Grand Mean		32.1	10.7
Capitella			
N run Nat	5	32.3	27.4
Test run 1	5	15.9	8.3
Test run 2	5	15.0	3.3
Test run 3	5	8.5	4.3
Nederlof	13	10.34	4
Nederlof	1	8.2	4.1
Grand Mean		15.1	9.1

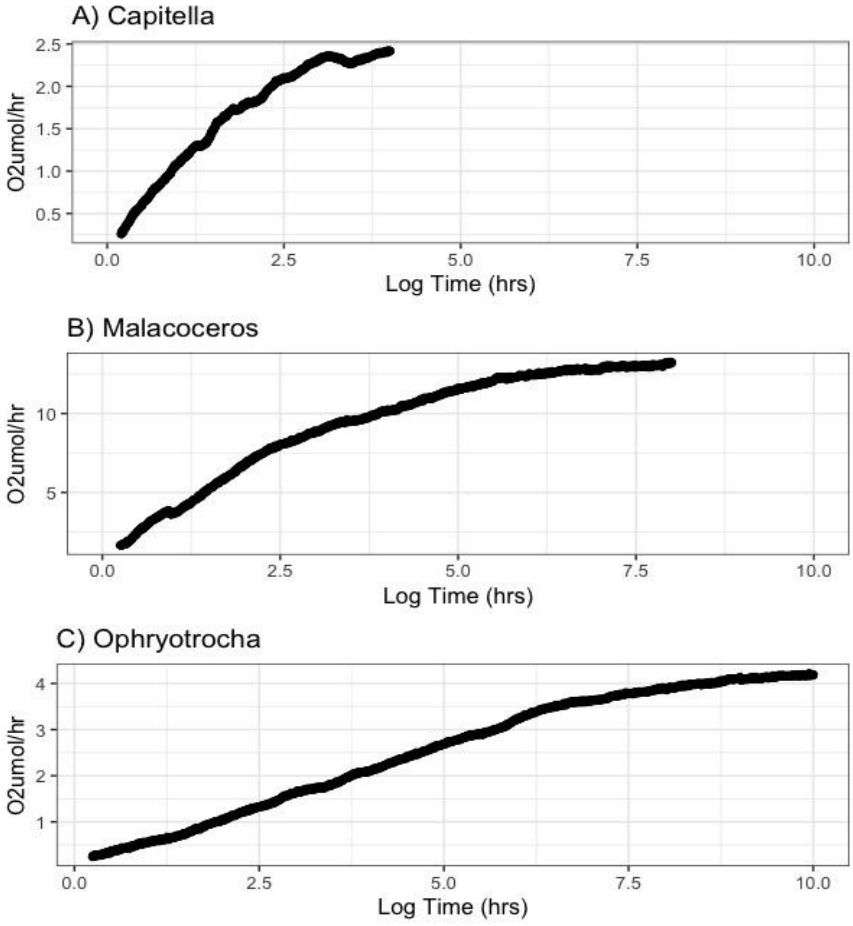

Figure.4. A) *Capitella* O_2umol hr^{-1} consumption to anoxia (at 4 hours) with x5 individuals in each of the 9 incubation chambers **B)** *M.fuliginosus* O_2umol hr^{-1} consumption to anoxia (at 6 hours) with x2 large individuals in each of the 9 incubation chambers **C)** *Ophryotrocha* O_2umol hr^{-1} consumption to anoxia (at 8 hours) with x10 individuals in each of the 9 incubation chambers

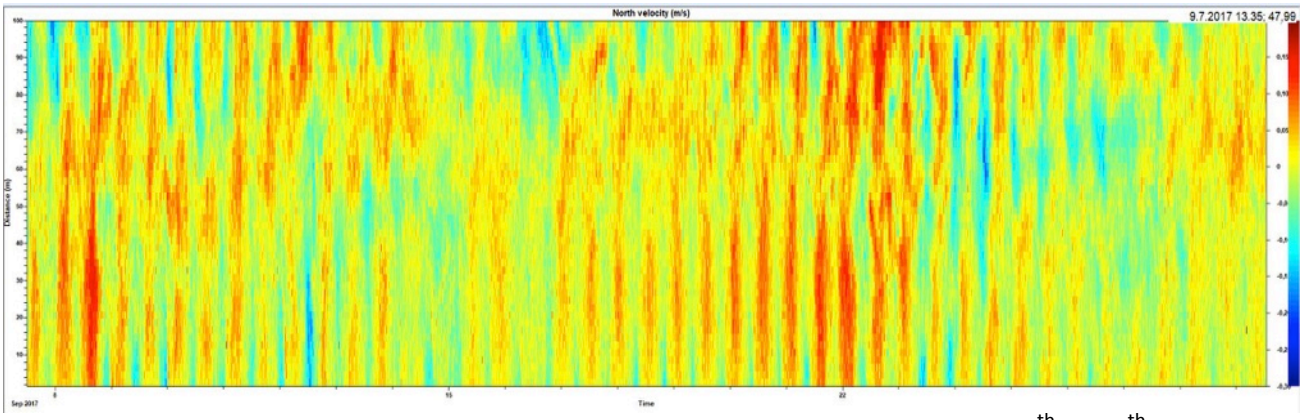

Figure 5. North velocity current measurements for 70 to 160m deep from 8th til 28th of Septmeber. Data from current meter placed 800m north of the Ocean Forest Fish Farm, north west of Bergen during September 2017. Shows fluctuations between north and south direction and at low (<2cm/s) current speeds.

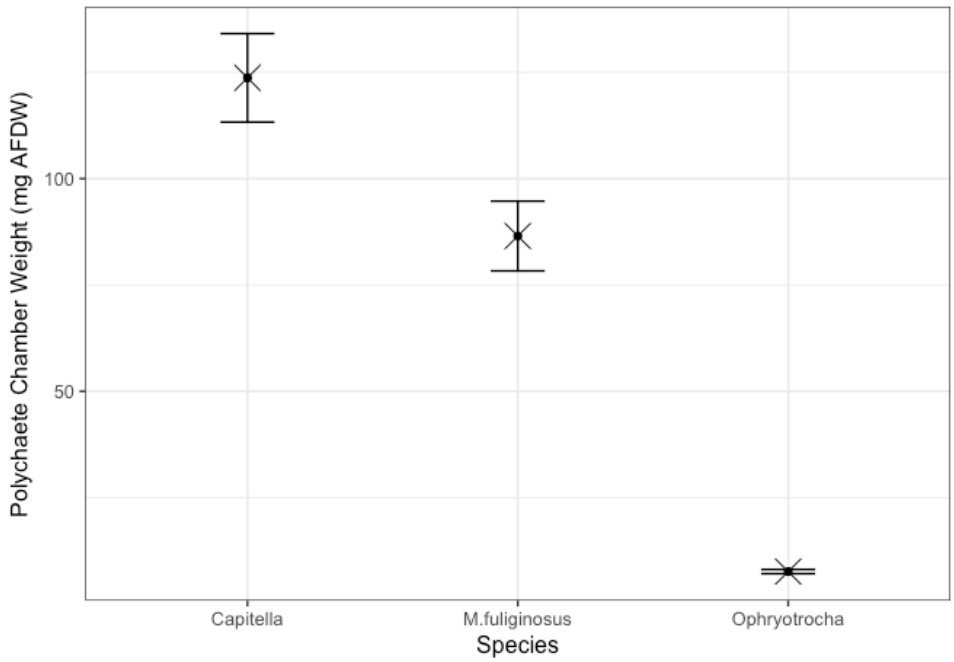

Figure 6. Collective weights of the x5 polychaetes used in metabolic studies.