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Seasonal Variations in Biofouling and Plankton Community Connected to a Large Scale Salmon Farm.

Jayaprabandh Pudota

Marine Coastal Development
Submission date: May 2011
Supervisor: Yngvar Olsen, IBI
Co-supervisor: Johanne mc Arff, SINTEF
Finn Victor Willumsen, ACE/SINTEF

Norwegian University of Science and Technology
Department of Biology

Acknowledgements

This thesis was written at the Department of Biology, NTNU, Trondheim. The experimental work was carried out at ACE aquaculture test facility, Tristeinen, Sør-Trøndelag, Norway from July 2010 to December 2010. This thesis would not have been possible with out my supervisors Professor Yngvar Olsen at NTNU, Johanne Arff at SINTEF and Finn Victor Willumsen at ACE. I am very thankful to all three of my supervisors for their invaluable guidance and support throughout my study.

I am grateful to Dag Altin and Nina Blöcher who has made available their support in many ways throughout my study. I would like to thank Tom EK at ACE, Jana Guenther, Micanti, Maria Bergvik, Tore, Arnesteine, Arne, Salmar ASA employees at the farm who have helped me during my experimental work and laboratory work. I want to thank my fellow students at sea lab for the nice work environment and all help.

Most of all I would like to thank all my family and friends for their support and encouragement. Finally, I would like to thank my girlfriend, Alaka for all her support.

Trondheim, May 2011
Jayaprabandh Pudota.

Summary

Biofouling is one of the main problems in marine finfish aquaculture. Biofouling occludes the nets and incurs major costs to fish farmers in the form of copper containing anti fouling paints used on the net surfaces, cleaning and changing of nets. Copper containing anti fouling paints is the major protective method in use by the fish farmers, but given the toxicity of the copper towards marine invertebrates and its ability to accumulate in the food chain, it may face a ban in marine aquaculture. So, there is a need to develop better anti fouling methods which will be as effective as copper, having less impact on the environment and be more cost effective to use. To design these, better understanding of the process of biofouling is needed. There is little data available about the biofouling in marine aquaculture.

In this regard, I studied biofouling in a marine cage aquaculture farm (ACE/Tristeinen), located in mid-Norwegian coastal waters. In this study, knot less nylon net panels and Micanti net panels were used to compare different aspects of biofouling and to test the effectiveness of the Micanti nets. Zooplankton samples were collected and analyzed with a focus on the larval stages of the fouling organisms, to relate the larval stages of the foulers in the zooplankton sample to the foulers present on the net panels. Sea lice larval stages presence in the zooplankton samples was also detected to study the movement of sea lice larval stages.

Analysis of the net panels and zooplankton samples together showed a trend between the larvae of foulers found in the zooplankton samples and foulers present on the net panels. On net panels hydroids, mussels, algae, amphipods and nudibranchs accounted for the major proportion of the fouling. On nylon net panels hydroids were more compared to mussels and on Micanti net panels mussels were more compared to hydroids. Net occlusion and net fouling wet weight was slightly less on the micanti net panels compared to the nylon net panels. Only a few larval stages of sea lice, *Lepeophtheirus salmonis* were found in the zooplankton samples. In this study, Micanti did not work as expected (as an effective antifouling technology), showing problems with strength, length and density of the fibers flocked on the net. Improving these would help to improve the functionality of the Micanti nets as a better non-toxic antifouling technology.

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1. Introduction

Cage aquaculture is a fish production system used to grow fish in net pens in existing water bodies like lakes, rivers, brackish and marine waters and is a relatively young industry (Beveridge, 2004). Commercial marine cage farming of fish started in Norway in the 1970's (Halwart et al., 2007). Cage aquaculture is practiced in intensive, semi-intensive and extensive systems. Extensive cage farming is practiced based on natural productivity of the water and no external feeding is provided. This is mostly done in fresh waters with high productivity and is mainly in practice in China and Indonesia (Beveridge, 2004). In semi intensive cage farming, fish are fed with plant protein based supplementary feed along with natural food. In intensive cage farming, high densities of fish stocked and fed with high protein artificial feeds.

The global demand for aquatic products resulted in the development and use of the intensive cage farming systems. In intensive cage farming of fish, high value marine fish species are raised. China is the leading country in the production of the cage-farmed fish (29%), followed by Norway (19%) (Halwart et al., 2007). Atlantic salmon is the main cultured marine fish species and makes up to 51% of the total fish cultured in the cages worldwide, and Norway producing 47% of the total Atlantic salmon produced worldwide (Halwart et al., 2007).

Modern cages are made of synthetic nylon net attached to a high-density plastic collar that float on the water surface. Nowadays, marine cage fish farmers are facing many challenges. Escapes of cultured fish, biofouling, impact of cage farming on the environment and parasites like sea lice are some of them. Among these, biofouling is one of the main problems incurring major costs to the cage fish farmers (Hodson et al., 1997).

1.1. Biofouling and history of biofouling

The term ‘fouling’ is employed to differentiate the communities of animals and plants that grow on artificial structures, from those occurring on rocks, stones and other natural objects (WHOI, 1952). The irreversible attachment of organisms to a surface in contact with water for a period of time is known as biofouling (www.crabproject.com). Attached and sessile animals and plants that occur naturally in the shallower waters along the coast form fouling.

Any non-toxic material immersed in water will prone to biofouling (Jones, 2009). Biofouling is ubiquitous in nature, implying great economical costs to industries like shipping, aquaculture, oilrigs, all offshore installations and others. The problem of biofouling varies depending on geographical location, environmental factors, physical and chemical factors of the substrate, species diversity and many others (Braithwaite & Mc Evoy, 2005; de Nys & Guenther, 2009; Greene & Grizzle, 2007; Jones, 2009).

1.1.1. Shipping

In shipping, fouling history dates back to dawn of navigation (Hellio. C., & Yebra, 2009; Jones, 2009). From then onwards till to date fouling is considered as a serious problem in shipping industry. Wooden boats used earlier were prone to attack by the woodborers, mussels and barnacles. To protect from foulers hot pitch, tars, grasses and other materials were used on wooden boats (Jones, 2009; Yebra et al., 2004). During Roman and Greek civilizations, lead sheathing along with copper nails was used to protect the wooden boats (Jones, 2009). Before the 18th Century, lead sheathing was perhaps the most widely used protection against fouling (Yebra et al., 2004).

Decay of wood, limiting length and strength of wooden boats initiated the introduction of iron boats replacing wooden ones in the 19th Century (Jones, 2009; Yebra et al., 2004). Copper sheathing was not practical on iron boats as electrolytic action corroded the hull, which gave rise to need for alternatives and resulted in the antifouling paints introduction into the shipping industry (Hellio, 2010; Jones, 2009).

Introduction of antifouling paints helped the shipping industry to fight better against foulers. Different antifouling paints were used in the shipping industry. Among anti fouling paints used, the organotin compounds tributyltin (TBT) and triphenyltin (TPT) containing paints were the first broad spectrum paints which worked very well against the foulers (Hellio, 2010; Yebra et al., 2004). Their performance even increased with the development of self-polishing polymer paints (Hellio, 2010). However, the use of organotin paints were banned in September 2008 due to their negative impacts on environment and food chains and raised the need for other effective anti fouling solutions.

Since organotin antifouling paints ban, new formulations have been developed containing high levels of copper and herbicides like Iragol 51, Irgarol 1051, diuron, chlorothalonil, dichlorofuanid and zineb (Hellio, 2010; Yebra et al., 2004).These compounds also witnessed negative impact on environment and these will also face a ban in next years to come (de Nys & Guenther, 2009; Hellio, 2010). Therefore, there is a need to develop new anti fouling technologies that will work well against foulers and have less negative environmental effects.

1.1.2. Aquaculture

During earlier stages of marine finfish farming, cotton made nets were used on cages. In Malaysia, cotton fibers soaked in tannins obtained from mangrove trees bark were used to fight against foulers. Later synthetic fibers were introduced into aquaculture. Synthetic fibers are unable to absorb tannins and tannins are no longer used as natural antifoulant (de Nys & Guenther, 2009). Fouling on modern synthetic materials is little known or rarely documented (Braithwaite & Mc Evoy, 2005). Frequent net changing, sun drying and cleaning were the methods used to fight against the biofouling (de Nys & Guenther, 2009). However, frequent changing of nets is a expensive process and also incurs stress on fish (Hodson et al., 1997).

After the introduction of the synthetic fibers introduced into marine aquaculture, effective anti fouling technologies were needed. Learned from shipping industry, tributyltin (TBT) and copper based antifouling paints were introduced into marine aquaculture (Braithwaite & Mc Evoy, 2005). Earlier tributyltin based products were used, but based on its negative effects on the species cultured and the environment, they were banned (Braithwaite et al., 2007; de Nys & Guenther, 2009).

Now a days copper based antifouling paints are in great use in marine finfish aquaculture (Braithwaite & Mc Evoy, 2005, Braithwaite et al., 2007; de Nys & Guenther, 2009). However, considering the toxicity of copper towards marine invertebrates and its capacity to accumulate into food chain, it will likely be banned (de Nys & Guenther, 2009).

1.2. Biofouling in marine finfish aquaculture

Biofouling on the cage netting poses problems by occluding the mesh of the net, thus blocking the water passage through the cage. Blocking the water passage leads to depletion of dissolved oxygen and reduced water quality in the cages that leads to stress in fish (Cronin et al., 1999; de Nys & Guenther, 2009; Guenther et al., 2009).

Fouling adds extra weight to the net and can block the net completely (Fig.3). The extra weight added to nets can be up to 200 fold to the original weight of the net (Braithwaite & Mc Evoy, 2005). Even, within seven days after immersion of nets, up to 37% occlusion of the nets was reported (Braithwaite & Mc Evoy, 2005). Extra weight can disturb the buoyancy of the cages, increase the drag force, deform the cage, thus decreasing cage volume and also add stress on mooring lines (de Nys & Guenther, 2009; Guenther et al., 2009; Swift et al, 2006). Fouling organisms can also host pathogenic organisms posing a disease risk to cultured fish (Braithwaite & Mc Evoy, 2005; Braithwaite et al., 2007; de Nys & Guenther, 2009).

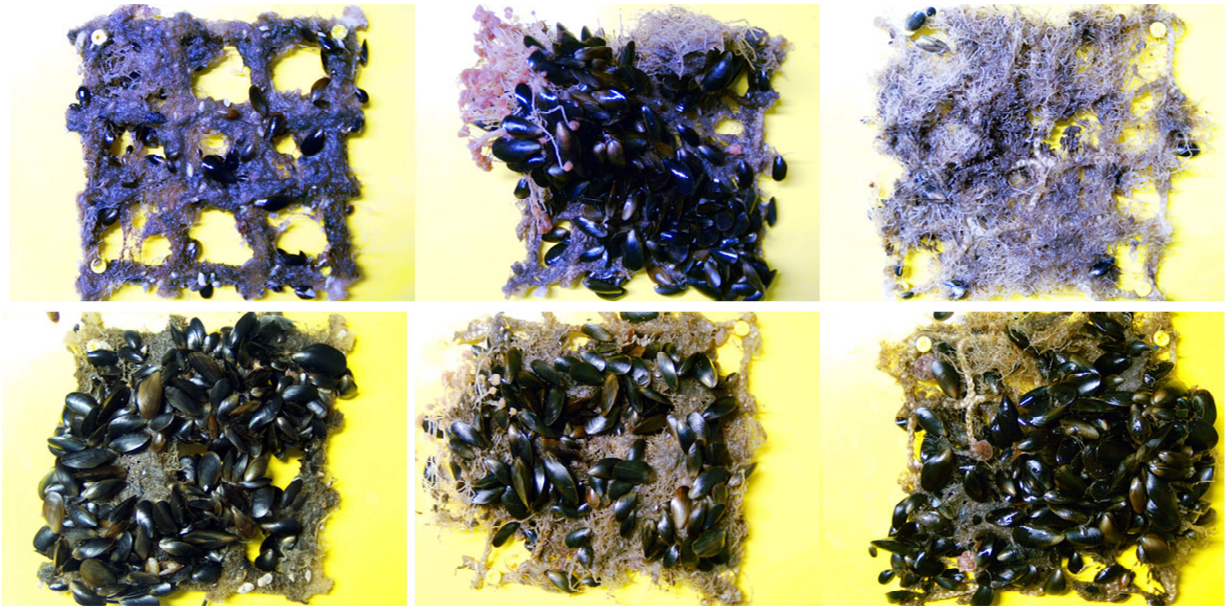

Figure 1: Heavily fouled nets (Photo by Pudota).

Frequent net changing is an expensive process (de Nys & Guenther, 2009; Hodson et al,. 1997). Shore based and underwater cleaning of the nets which requires special expertise, and use of antifouling paints implies a great economical pressure to the farmers (Braithwaite & Mc Evoy, 2005; de Nys & Guenther, 2009; Hodson et al., 1997). According to the CRAB project, biofouling on fish cages and mussel farms costs nearly 5-10% of the industrial value (www.crabproject.com).

1.2.1. Development and factors affecting biofouling

Throughout the global range of the marine environment biofouling development generally follows a consistent pattern that involves an overlapping sequence of a series of discrete, sequential, chemical and biological changes of the substrate (Braithwaite & Mc Evoy, 2005; Corner et al., 2007; www.crabproject.com). Immediately after immersion of the net, net surface will be conditioned by the formation of the conditioning layer (Corner et al., 2007). Microorganisms like bacteria, then colonize the net surface and release extracellular polymeric substances forming a biofilm on the conditioned net surface (Corner et al., 2007) (Fig.2a).

Thereafter macro algae, mussels, hydroids and ascidians fouling develop on the net (Corner et al., 2007; www.crabproject.com) (Fig. 2b). Macro fouling can only occur after the formation of the biofilm on the net surface. Even though fouling development is understand to be a stepwise process, it may not always be true as factors like the abiotic environment, interactions between organisms and the surface characteristics may affect this stepwise process (Braithwaite & Mc Evoy, 2005; Greene & Grizzle, 2007).

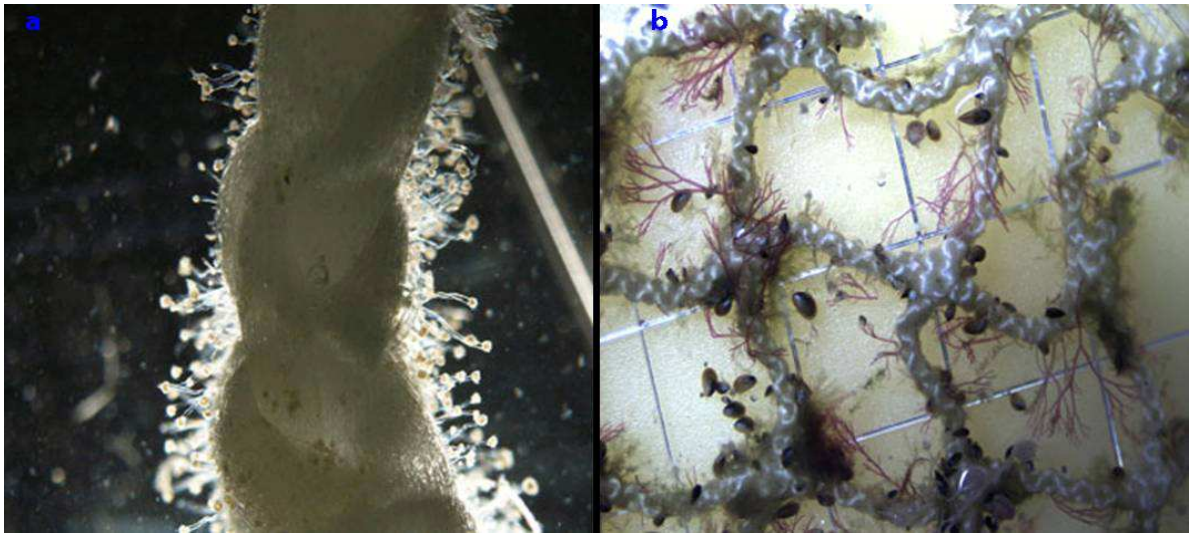

Figure 2: a) Micro fouling and b) Macro fouling on nylon net panels (Photos by Pudota).

Many factors affect the fouling process on cage netting and equipment used in marine fish farming. Development of the fouling on cage structures may change due to the spatial variation and the effective spatial variation can be from 100 km to 1000 km (de Nys & Guenther, 2009). Even within the same sites, development of fouling can change based on local factors like availability of light, water flow and depth (de Nys & Guenther, 2009). Tropical areas with year round light availability and little change in temperatures show year round high pressures of fouling. In temperate waters, biofouling is at peaks in the spring and summer periods (Braithwaite & Mc Evoy, 2005; de Nys & Guenther, 2009; Hellio, 2010).

Marine finfish farming provides a great advantage to foulers as an abundant nutrients supply from cages is present in and around the cages (de Nys & Guenther, 2009; Hodson et al., 1997). The mesh size of the net used plays a role for fouling attached to the cage (Braithwaite & Mc Evoy, 2005; de Nys & Guenther, 2009). Mesh material also affects the fouling amount. The multifilament nylon netting used in cages offers a lot of free surface area to foul and nylon net is also a better surface for fouling compared to metal alloy, galvanized metal nets and flocked nets (Braithwaite and Mc Evoy, 2005; de Nys & Guenther, 2009; Greene and Grizzle, 2007; Hodson et al., 1997; Phillippi et al., 2001). Net color is also supposed to have some impact on the fouling process (Hodson et al., 2000). Diversity of fouling organisms and all these factors involved makes it difficult to control fouling on cages. There are different technologies used to fight against the fouling in the marine aquaculture industry and most of these technologies were learnt from the shipping industry (Braithwaite & Mc Evoy, 2005).

1.3. Antifouling technologies

1.3.1. State of art in antifouling

To combat foulers in marine fish farming, currently different methods are in practice. The major protective method against fouling in aquaculture industry is the use of toxic anti fouling paints (Braithwaite & Mc Evoy, 2005; de Nys & Guenther, 2009; Guenther et al., 2009). Paints are effective and economical to use and copper containing paints are the main antifouling paint used in the marine aquaculture industry (Braithwaite & Mc Evoy, 2005; Braithwaite et al., 2007; de Nys & Guenther, 2009). Copper applied on the net leaches out in the water and this copper is toxic to marine invertebrates (Braithwaite & Mc Evoy, 2005; de Nys & Guenther, 2009). Legislation is permitting the use of these kinds of toxic antifouling methods (Braithwaite & McEvoy, 2005).

Cleaning and changing of the nets are expensive and labor intensive processes that are used in combination with antifouling paints (Guenther et al., 2010). Frequent changing of nets is costly as large number of nets and labor is needed to change the nets (Hodson et al., 1997). This also incurs loss of stock, lower growth rates due to disturbances in feeding regimes and it also cause stress to fish (Hodson et al., 1997). At least 2% of the fish population is affected by the change of nets (www.crabproject.com).
Shore based and underwater cleaning are two types off cleaning processes (Braithwaite & Mc Evoy, 2005; de Nys & Guenther, 2009).Underwater cleaning needs special expertise and effect of this *in situ* cleaning was poorly documented (de Nys & Guenther, 2009). Frequent cleaning of nets also damages the nets (Hodson et al., 1997).

Biological control of the fouling is also in practice. In Norway wrasses are used against fouling and sea lice (Braithwaite and Mc Evoy, 2005; de Nys & Guenther, 2009; Greene and Grizzle, 2007). Sea urchins, hermit crabs, starfish, herbivore grazers (tilapia), mullets, rabbit fish and sea cucumbers can be used against fouling (Braithwaite and Mc Evoy, 2005; de Nys & Guenther et al., 2009; Greene and Grizzle, 2007).

1.3.2. Future antifouling technologies

Assuming a future ban of copper based antifouling paints and the high costs involved in cleaning and changing of nets, new antifouling techniques has to be developed to fight against biofouling in marine finfish aquaculture. The new antifouling strategies are required not to repeat the past TBT experience and should meet the following criteria:

a) Work against the broad spectrum of the fouling taxa

b) Have least possible negative impact on environment and species cultured

c) Be able to withstand on-shore handling and cleaning

d) Be economically viable (de Nys & Guenther, 2009).

Continuous research has to be done to develop new antifouling methods that meet the above criteria.

Micanti, a company working from The Netherlands, has developed a patented non-toxic antifouling net called Thorn-D® (Fig.3). The working principle of the Micanti net is to avoid the organisms to settle instead of killing settled organisms. In Micanti, short spiky Thorn-D fibers are applied on to the net surface with an effective adhesive. Prickliness and swaying action of the fibers helps to form a barrier against the foulers to settle and can thus be used as an antifouling method. Thorn-D® has been developed after continuous trail and errors in places ranging from temperate waters (Norway, Canada) to tropical waters (Turkey) for years. Micanti has revealed some positive results in aquaculture and the use of these net might evolve as an effective antifouling strategy in the future (www.micanti.com).

Figure 3: Micanti Thorn-D® net (Photo from www.Micanti.com).

There are many others methods, which can be used against biofouling like metallic layers (galvanized steel, metal alloys); natural products; non-toxic foul release coatings (silicon PDMS, Flourosilicons); electro chemical and electric methods; vibration; temperature; UV radiation; and pH (www.crabproject.com). So far, these are only theoretical methods and further work has to be done to develop these methods (www.crabproject.com). Among these, the development and commercial production of the natural products is not a practical solution (de Nys & Guenther, 2009). Non-toxic foul release coatings have potential to use in aquaculture as they already proved in shipping industry.

The problem with biofouling is its diversity that no single method suits to all fouling organisms. All these methods have their own drawbacks and there is a need to develop better methods than the existing methods. For this, better understanding of the fouling process is needed.

1.4. The foulers

The organisms that form the biofouling communities are known as foulers. Fouling groups are very diverse and there are organisms from almost every invertebrate phylum (de Nys & Guenther, 2009). The number of fouling species is huge. In 1952, there were 1964 marine fouling organisms recorded and now it is estimated that there are nearly 10,000 identified marine fouling organisms (Jones, 2009). The main fouling groups that form fouling community are macro algae, hydroids, molluscs (bivalves, snails, clams, nudibranch), ascidians bryozoans, polychaets, crustaceans (amphipods, caprellids), echinoderms, sponges (Braithwaite and McEvoy, 2005; WHOI, 1952) (Fig.4). Among all of these groups, the sessile groups poses greater problems compared to the mobile fouling organism groups.

Better knowledge regarding the biology of the foulers and the settlement processes can help in designing the better antifouling protocols. Benthic invertebrates include free-swimming planktonic larval stages in their life history and algae reproduce through spore production. Planktonic larval stages search for a substratum to settle and grow, which later develops as biofouling (Phillippi et al., 2001). Studying these free-swimming planktonic stages present in the water column will give a clear picture of the fouling communities present in that particular area and helps in designing better antifouling methods.

Figure 4: Fouling organisms on net panels in this study. a) Suctorian ciliate, b) Green algae, c) Red algae, d) Hydroid, e) Mussel, f & g) Amphipods, h & i) Ascidian and ascidian larvae, j) Sponges, k) Bryozoan, l) Barnacles, m) Polychaets, n) Nudibranch, o) Starfish, p) Platyhelmenthes. (Photo by Pudota).

1.5. Objectives of my study

The overall objective of this study was to qualitatively and semi quantitatively describe the biofouling and plankton community composition in mid-Norwegian coastal waters through a comparative, monthly study of the biofouling situation on the two types of nets used (untreated nylon and Micanti nets) and through regular sampling of water with a focus on actinula larvae of hydroid *Ectopleura larynx* and other foulers larvae. The study has also included detection of the early life stages of sea lice, *Lepeoptheirus salmonis* as a sub-objective. This study also involves recommendations on the sampling strategies to follow, standardization of sampling in aquaculture farms and considerations on the parallels for robust statistics.

2. Materials and methods

2.1. Study site

The experimental work carried out at ACE aquaculture test facility located at Tristeinen, Sør-Trøndelag, Norway (Fig.5) (Latitude 63.8666667°, Longitude 9.6166667°). ACE is a semi exposed experimental site present in mid-Norwegian coastal waters established for the testing of technology developed in marine sciences. At the time of study, there were six cages present at the site, off which five cages stocked with fish and one was empty. Cages present at the site were 120m-160m in circumference. Salmar ASA was maintaining the operations at ACE when experiment was carried out.

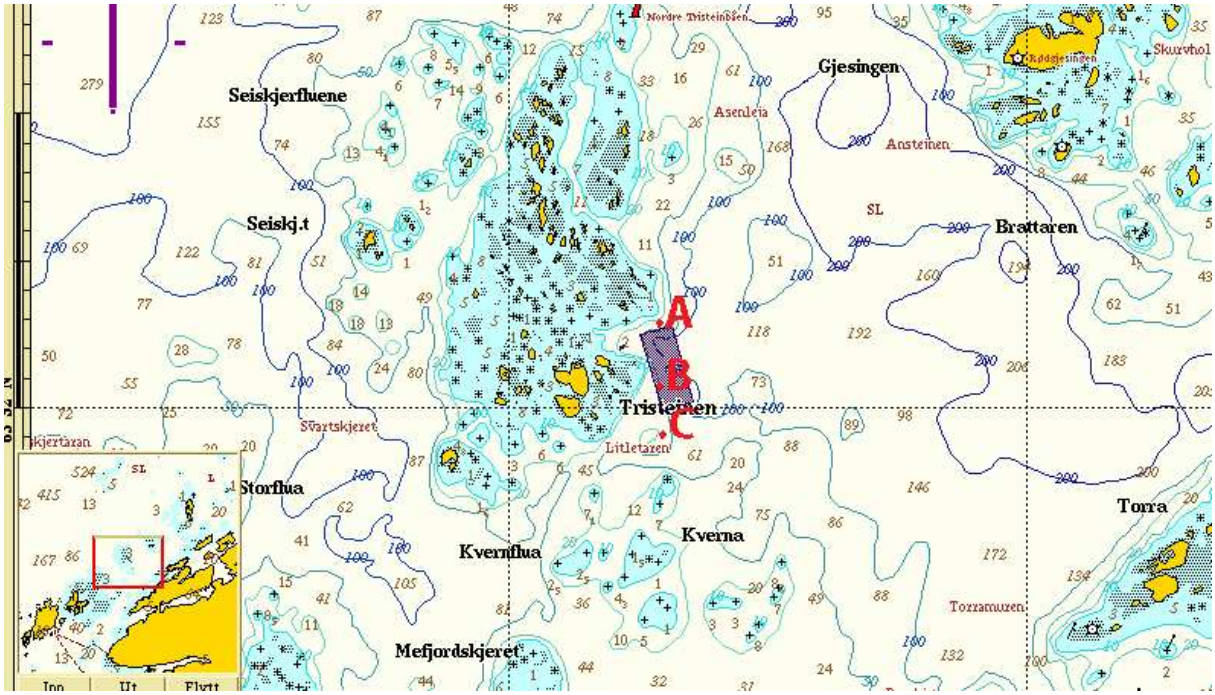

Figure 5: Site map with plankton collection points and frames deploying point. A: Upstream zooplankton sample collection point. B: Frames deployed point and zooplankton sample collection point. C: Down stream zooplankton sample collection point.

2.2. Experimental design

Two frames were prepared with PVC pipes to mount the net panels. Frames were prepared with one-inch black PVC pipe. Each frame was prepared to accommodate 24 net panels, 12 untreated knotless nylon net panels (Fig.6) and 12 Micanti Thorn-D® net panels (Fig.7). Micanti Thorn-D® net is a non-toxic antifouling net produced by Micanti (See 1.3.2 future antifouling technologies, page 8). Net panels used were 10 ×10 cm in dimensions and they were attached to the plastic frame with cable ties (Fig.8). In the frame, heavy gauge nylon rope was fixed internally to tie the weight and to fasten the frame to the side of cage. A five kilograms weight was attached on bottom side of the frame to the rope to keep frames stable in water column. Frames were deployed at five meter depth on the empty cage present in the farm (Fig.5, B point). Frames were deployed on first of July.

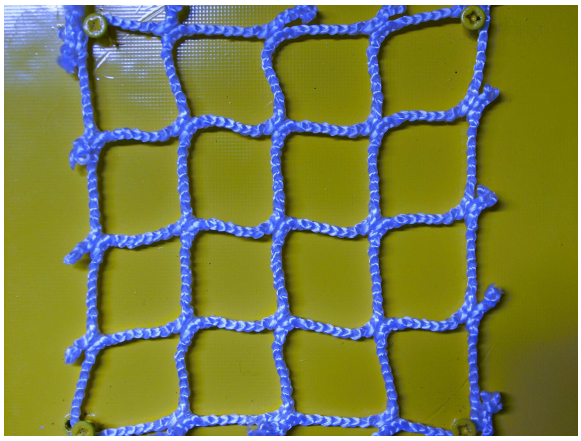

Figure 6: Nylon net panel.

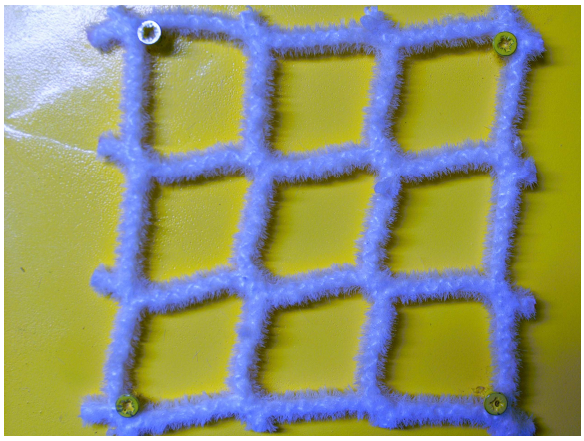

Figure 7: Micanti Thorn-D net panel.

(Photos by Pudota)

Figure 8: PVC frame with nylon and Micanti samples mounted on with cable ties (Photo by Pudota).

2.3. Field methods

2.3.1. Zooplankton sampling

Every month, three vertical zooplankton samples were collected at three stations using 30 cm diameter plankton collecting net made with 100 μm mesh. Sample collections points were, 100 m upstream of the farm, at the frames and 100 m downstream of the farm (Fig.5). Sampling was done from a boat by vertically dropping the plankton net weighted with a two kilograms iron block. Plankton net was vertically dropped to a depth of 25 meters and lifted up to the surface and it was repeated for three times in order to collect a sample.

Due to some wave action there was a slight change in the depth from where the samples were collected. The sampled material was emptied into a 25 ml sampling vials by opening the knob present at the bottom of the plankton net and the vials were marked with sample name, sampling- number, collection point and date of collection after collecting each sample. After 8-10 hours of sampling, formalin was added to plankton samples for preservation.

2.3.2. Net panels sampling

Two nylon and two Micanti net panels from each frame, a total of four nylon and four Micanti net panels from the two frames were sampled every month except for the first sampling that was collected 2 weeks after deployment of the frames. While sampling, each frame was taken out of the water on to the walking platform of cage and net panels mounted on to frame with cable ties (Fig.8) were removed using a cutting player. Then, net panels were placed in the plastic buckets filled with seawater and closed tightly. After 8-10 hours of sampling, formalin was added into the plastic buckets for preservation.

2.4. Laboratory methods

2.4.1. Preservation

37% formaldehyde solution stabilized with about 10% methanol solution was diluted to 20% and used to preserve the sampled net panels with fouling and zooplankton samples. 4 ml of 20% formaldehyde is needed to preserve 100 ml sample volume. Based on this, 1 ml of 20% formaldehyde solution was added to 25 ml of zooplankton sample. As the amount of water in net panel buckets was not constant, formaldehyde was added according to the volume of water and net panels present in the bucket.

2.4.2. Washing process

Exposure to formaldehyde used for the preservation of the samples is toxic, allergenic, and carcinogenic. Therefore, the net panels and the zooplankton samples were washed before analysis, to mitigate the effect of exposure to the formaldehyde while analyzing the samples under microscope. Zooplankton samples collected with 100 μm mesh were washed on a sieve made with 200 μm mesh (Fig.9) for half an hour under continuous seawater flow. Washing the samples on 200 μm mesh which were collected using 100 μm mesh made the sample free from phytoplankton and debris. After washing, sample was concentrated to one point and washed into a beaker from opposite side. Then the washed sample was collected in to a new sampling vial and marked with sample name, sampling number, collection point, and date of collection.

All net panels were washed for 2-3 hours before analysis. An instrument was prepared using a five liters plastic bucket with an outlet on the side fitted with a tap for controlling the out flow. A sieve was prepared using a fifteen centimeters length and ten centimeters diameter PVC pipe by attaching 300 μm mesh on one side. A five centimeters stand was prepared using same type of PVC pipe (Fig.10). Sieve prepared was placed on the stand and kept inside the bucket filled with water. Net panels along with water present in the bucket were poured into the sieve and kept under continuous water flow for 2-3 hours. After washing, net panels were collected into a big Petri dish.

Figure 9: Zooplankton washer.

Figure 10: Net panels washing equipment (Photos by Pudota).

2.5. Microscope and camera unit

Analysis of the zooplankton samples and the net panels done using a stereomicroscope outfitted with a digital camera unit. Stereomicroscope used was Leica made and M205C model. Leica M205C offers up to 20.5:1 zoom and was integrated with an electronic readout to take images of what we see in the sample. Nikon DS-5M-L1 digital sight camera system integrated with a Nikon Digital sight DS-L camera control unit was used to capture the images during the analysis of the samples. To take the images of the net panels a AF Nikkor 14mm 1:28D Nikon made lens connected to above-mentioned system through a F→C adaptor was used.

2.6. Weighing net panels

Net panels were weighed to know the net-fouling wet weight present on the net panels. Both nylon and Micanti net panels weight was measured before immersing into the water and then nets were immersed into buckets filled with seawater. After 24, 48 and 72 hours of immersion, nets were weighed to know the maximum weight gain by the net and if there was any difference in the weight of water absorbed by the net panels. However, there was no difference observed for water absorbed after 24, 48 and 72 hours of immersion. After washing and before analyzing, all net panels were weighed along with the petri dish to determine the wet weight of fouled net. While washing the net panels, loosely attached fouling was detached from the net and this fall off was collected into an other petri dish and weighed. Net-fouling wet weight was calculated by subtracting the weight of the net panels after immersing in to seawater from the total weight of the net with fouling (gross net weight + fall of weight). Throughout the study, the same weighing machine was used to weigh all net panels to minimize the errors in calculated weight.

2.7. Analysis of samples

2.7.1. Zooplankton samples analysis

Samples were analyzed after washing, using a Leica M205C stereomicroscope outfitted with a digital camera unit (See chapter 2.5) for capturing images of the organisms observed in the sample for further study. The analysis of plankton samples was focused on actinulae of hydroids, sea lice and larvae of the main fouling groups. Qualitative analysis of the sample was done depicting the different organism groups present in the samples. Details concerning the sample were noted down after analyzing each sample. The samples were preserved using formaldehyde after analysis. All zooplankton samples were analyzed twice.

2.7.2. Net samples analysis

After weighing and before analysis, an image of the complete net panel was taken with a digital camera unit (See chapter 2.5) to keep as an evidence of the net since all the fouling present on the net panel was scraped off from the net during the analysis. The analysis of the net panels was done with a focus on all organisms present on the net panel and in the fall off. All net panels were analyzed qualitatively and semi quantitatively. The qualitative analysis of the net panels was done depicting the biodiversity present on the net panels and semi quantitative analysis gave the relative proportion of the different organism groups present on the net panels. Fouling scraped off while analyzing the net panels and different groups were stored in separate containers. Approximate percentage of different groups that formed most of the fouling on the net panels was estimated by the amount of the different groups stored in separate containers and through the visual observations of the net panels. Sub samples prepared were preserved with formaldehyde for a second analysis. All subsamples were also analyzed qualitatively. After analysis, all samples were preserved with formaldehyde.

2.8. Data analysis and processing

The qualitative data of the Zooplankton samples and net panels was obtained based on the presence or absence of each group in each sample. Presence of a group in a sample is noted down as one and absence of a group is noted with zero and these data was entered into the Microsoft excel sheets. Qualitative data graphs were generated combining the data sheets with Sigma plot software. Semi-quantitative data of each group on each net panel was obtained through the visual observations of net panels and through the amount of the different groups stored in separate containers. For the ease of data processing, an average value of the four nets for each net type was drawn and standard deviation was calculated and entered into excel data sheets. From the excel data sheet values, graphs were generated for the total percentage occluded and the composition of the different fouling groups on net panels. Weights data of the net panel was also processed like the semi quantitative data. All of the tables were prepared using Microsoft excel, 2003 version and from the tables graphs were made by Sigma plot 10.0 (Systad Software Inc, USA).

3. Results

3.1. Zooplankton samples

Analysis of the zooplankton samples depicted the heterogeneity in the zooplankton community present at the test site. There were eight phylums represented in the samples consisting of 14 functional organism groups. Among these 14 groups, most of which are meroplankton stages, actinulae of hydroids, medusa, mussels, snails and bryozoans were important considering their biofouling activity and abundance in the samples. Amphipods, polychaets, echinoderms, larvaceans and decapods were the minor groups. Samples hold many copepod stages and few sea lice. Fig.11, presents larvae found in the plankton samples.

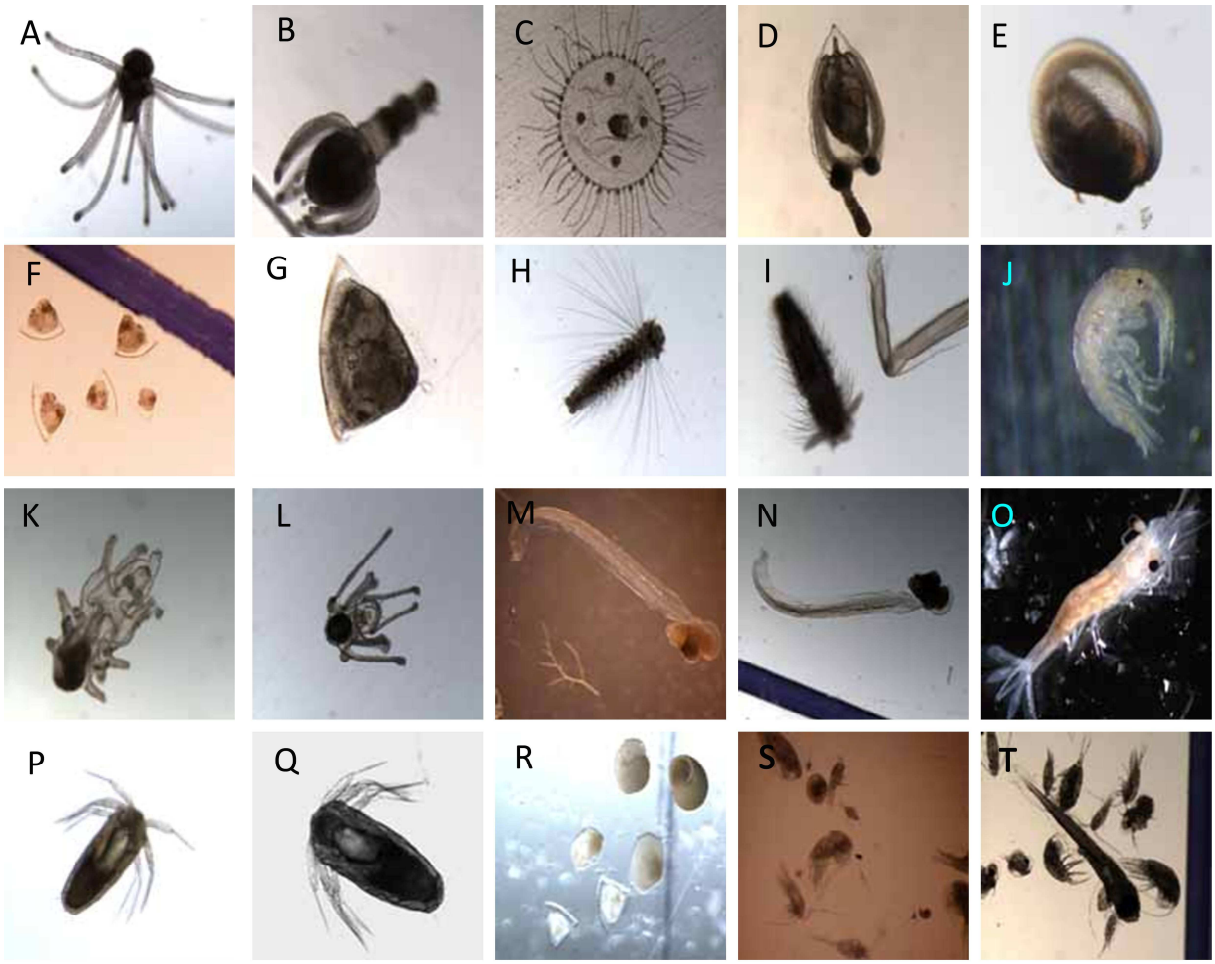

Figure 11: Different organisms found in the zooplankton samples.

A&B: Actinula, C&D: Medusa, E: Mussel larva, F&G: Bryozoan larvae, H&I: Polychaet larvae, J: Amphipod, K: Starfish larva, L: Brittle star larvae, M&N: Larvacean larvae,

O: Decapod, P&Q: Sea lice larvae, R: Snail & bryozoan larvae, S&T: Copepods (Photo by Pudota).

Actinula of hydroids present in the samples were hard to identify to species level (Fig.11A&B). They might be actinula of *Ectopleura larynx*, since it is the most abundant fouling hydroid species in mid-Norwegian coastal waters. Samples from September to December samplings had actinulae stages (Fig.12A). All of the samples in October, two of three samples in September and November and one of three samples in December contained actinulae stages. Actinula stages were abundant in the samples and were of different size.

Obelia sp (Fig.11C), *Ectopleura sp*, *Hybocodon sp* (Fig.11D), hydromedusa and few other unidentified medusas were present in the samples. Except in December, at least one sample in all months had medusa stages (Fig.12B). All of three samples in August, October and the second sampling in July hold medusa stages. Two of three samples in the first sampling in July and in one sample in September and November had medusa forms. *Obelia sp* was abundant in the samples in July, while *Ectopleura sp* medusas were not that many observed in the samples.

Mussel and snail larvae were very small and it was difficult to identify those (Fig.11E&R). Two of three samples in the first sampling in July and only one sample in August and October, had mussel larvae (Fig.12C). Snails were present in all samplings and samples except for the first and last samplings i.e. July and December, where they were present only in two samples (Fig13K). Snail larvae were abundant in the samples.

Larvae of bryozoan, *Membranipora membranacea* were found in all samples except in August and November (Fig.12D). Only one sample in August had bryozoan larvae, while they were absent in November. The polychaet larvae present in the samples were difficult to identify to species level (Fig.11H&I). Two of three samples in July and October and only one sample in August, November and December contained polychaet larvae (Fig.12E).

Very few amphipods (Fig.11J) were present in the samples. One sample in August and two samples in November contained amphipods (Fig.12F). Among the Echinoderms, starfish and brittle star larval stages (Fig.11K&L) were present in July, September, October and December samplings (Fig.12G). All of the samples in first four samplings (July-September) had larvacean, *Oikopluera sp* larval stages and they were abundant (Fig.11M&N & Fig13.H).

Except in November, at least one sample had decapod larval stages in rest of the samplings (Fig.11O & Fig.12I). Very few (2-3) planktonic free-swimming nauplius II stage of sea lice, *Lepeophtheirus salmonis* (Fig.11P&Q) were present in October and December. In October, all three samples and in December only two samples showed nauplius II stages of sea lice (Fig.12J). All samples in all samplings contained many copepods (Fig.11S&T and Fig.12L).

Apart from groups presented in Fig.11 and Fig.12, a few cladocerans (*Evadne sp, Podon sp*), phoronids and three barnacle larvae were present in the samples. There were very few unidentified organisms present in zooplankton samples.

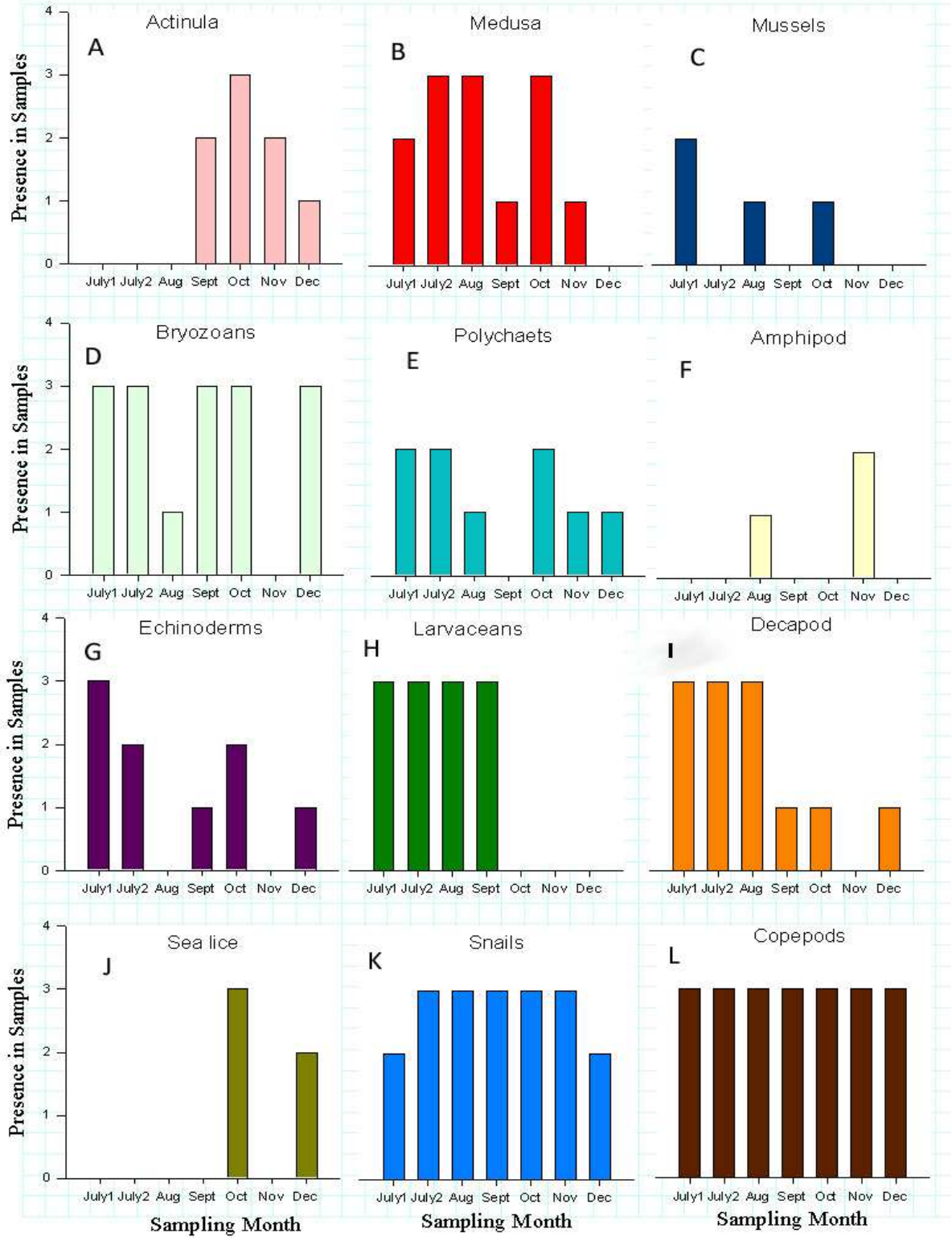

Figure 12: Presence of different organism groups in the zooplankton samples collected monthly. Data presented in the graphs is based on presence or absence of different organism groups in the samples. 1= present in one sample, 2= present in two samples, 3= present in all three samples.

3.2. Net panels

Analysis of the net panels showed the presence of different biofouling groups at the test site. There were 12 phylums represented on the net panels consisting of 18 functional organism groups. Red and green algae, blue mussels, hydroids, amphipods (gammarid and caprellid) and nudibranchs were the main fouling groups, which made up most of the fouling. Bryozoans, polychaets and echinoderms were the minor fouling groups on the net panels, and accounted for a minor part of the fouling. Sponges, suctorian ciliates and ascidians were present on very few net panels. There were also nematodes, platyhelmenthes and barnacle larval stages present on the net panels. Fig.13 presents all these 15 groups found in the samples.

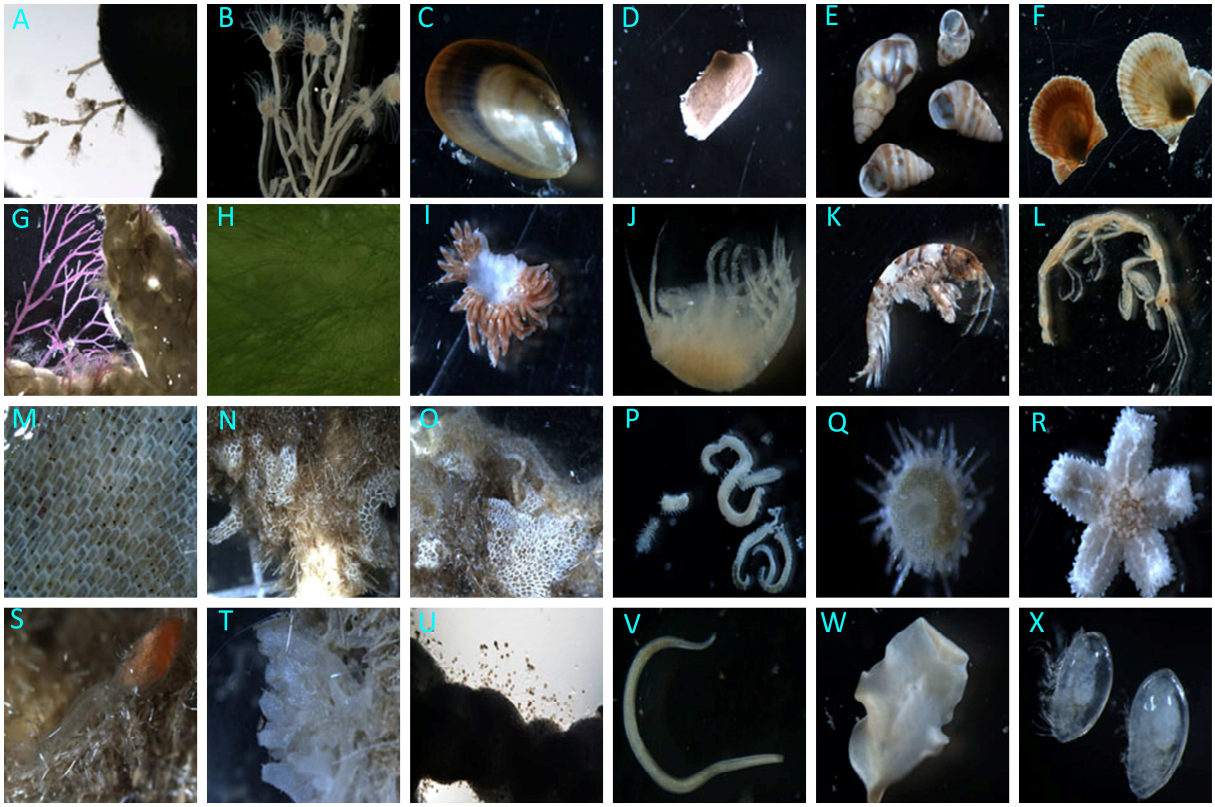

Figure 13: Different fouling organisms present on the net panels.
A&B: Hydroids, C: Mussel, D: Clam, E: Snails, F: Scallops, G: Red algae, H: Green algae, I: Nudibranch, J-L: Amphipods, M-O: Bryozoans, P: Polychaets, Q&R: Echinoderms, S: Ascidian, T: Sponges, U: Suctorian Ciliates, V: Nematode, W: Platyhelmenthes, X: Barnacle larvae. (Photo by Pudota).

The Hydroid, *Obelia sp* (Fig.13A) was present on all nylon net panels and on one Micanti net panel in July and it was microscopic. All nylon and Micanti net panels from August to December had hydroids (Fig.14A and Fig.15A) and the dominant species was *Ectopleura larynx* (Fig.13B). Most of the net panels had all growth stages of the hydroids (from budding stage to fully matured stage).

Blue mussel (*Mytilus edulis*, Fig.13C), clam (*Hiatella sp*, Fig.13D), snails (Fig.13E) and scallops (Fig.13F) were present on net panels. All net panels both nylon and Micanti throughout the study months contained blue mussels (Fig.14B and Fig.15B). Net panels had larvae of blue mussel in July. These larval stages grow with the time progressing and succession of growth was observed on the net panels. Small amounts of clams, snails and scallops were observed on the net panels and they were of same size throughout the study.

Red and green algae were present on the net panels (Fig.13G&H) and the red algae were identified as *Ceramium sp*. All of the nylon and micanti net panels from August to December and one micanti net panel in July had red algae (Fig. 14C & Fig. 15C). All nylon and micanti net panels from August to November had green algae (Fig. 14D & Fig. 15D). Three of four nylon net panels in December and three of four Micanti net panels from July had green algae.

Nudibranchs were present on the net panels and the dominant species was *Coryphella sp* (Fig.13I). All nylon and Micanti net panels from September to December had nudibranchs (Fig. 14E & Fig. 15E). Two of four nylon net panels in July and August and one of four Micanti net panels in August had nudibranchs (Fig.14E & Fig.15E). Amphipods were one of the main fouling groups on net panels (Fig.13J-14L). All nylon net panels in all months and Micanti net panels from August to November contained amphipods (Fig.14F & Fig.15F). Only three of four micanti net panels in July and December had amphipods.

Net panels had wide mats of bryozoan, *Membranipora membranacea* (Fig.13M-O) and they first appeared in August. Among nylon net panels, bryozoans found on all net panels in October and on three of four net panels in September. In November and December, two of four panels had bryozoans and in August only one panel contained bryozoans (Fig.14G). Among Micanti net panels, bryozoans were present on all panels in September and October. In December, three of four panels and in August and November, two of four panels contained bryozoans (Fig.15G).

There were six types of polychaets present on the net panels (Fig.13P). All nylon and Micanti net panels in September and October contained polychaets (Fig.14H) (Fig.15H). Three of four nylon net panels in November and December, two of four nylon net panels in August and one of four nylon net panels in July contained polychaets. All four Micanti net panels in November, three of four Micanti net panels in August and December contained polychaets.

Among echinoderms, starfish and sea urchins were present on the net panels (Fig.13Q&R). Among nylon net panels, echinoderms were present in September, October and December. In October all nylon net panels, in September two nylon net panels and in December one nylon net panel contained echinoderms (Fig.14I). Among the Micanti net panels, three of four net panels in August, two of four net panels in October and December and one of four net panels in September and November had echinoderms (Fig.15I).

Apart from the above main fouling groups, which have been represented in the graphs, very few (3-5) ascidians (Fig.13S) were present among all of the net panels and platyhelmenthes (Fig.13W) were present on six net panels. Sponges (Fig.13T) were present only on nylon net panels in December and suctorian ciliates (Fig.13U) were present on all net panels in July. Nematodes (Fig.13V) present on all net panels from August to December and barnacle larvae (Fig.13X) were present on all net panels from September to December. All these were observed in a very small amount and they did not contribute much to the fouling.

Nylon net panels:

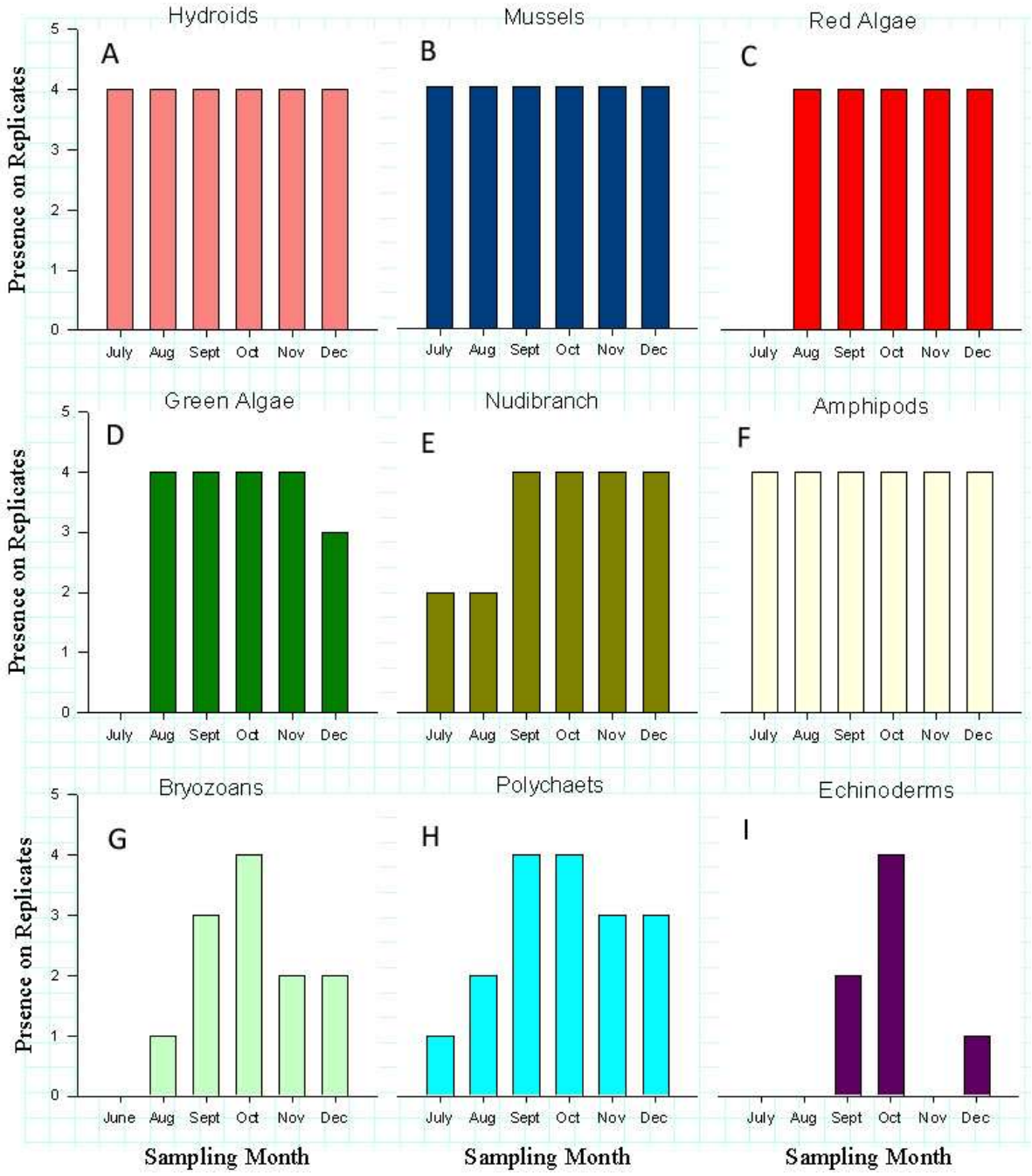

Figure 14: Different fouling organism groups present on nylon net panels sampled every month. Data presented in the graphs is based on presence or absence of different organism groups on the net panels. 1= present on one net panel, 2= present on two panels, 3= present on three net panels and 4= present on all four net panels.

Micanti net panels:

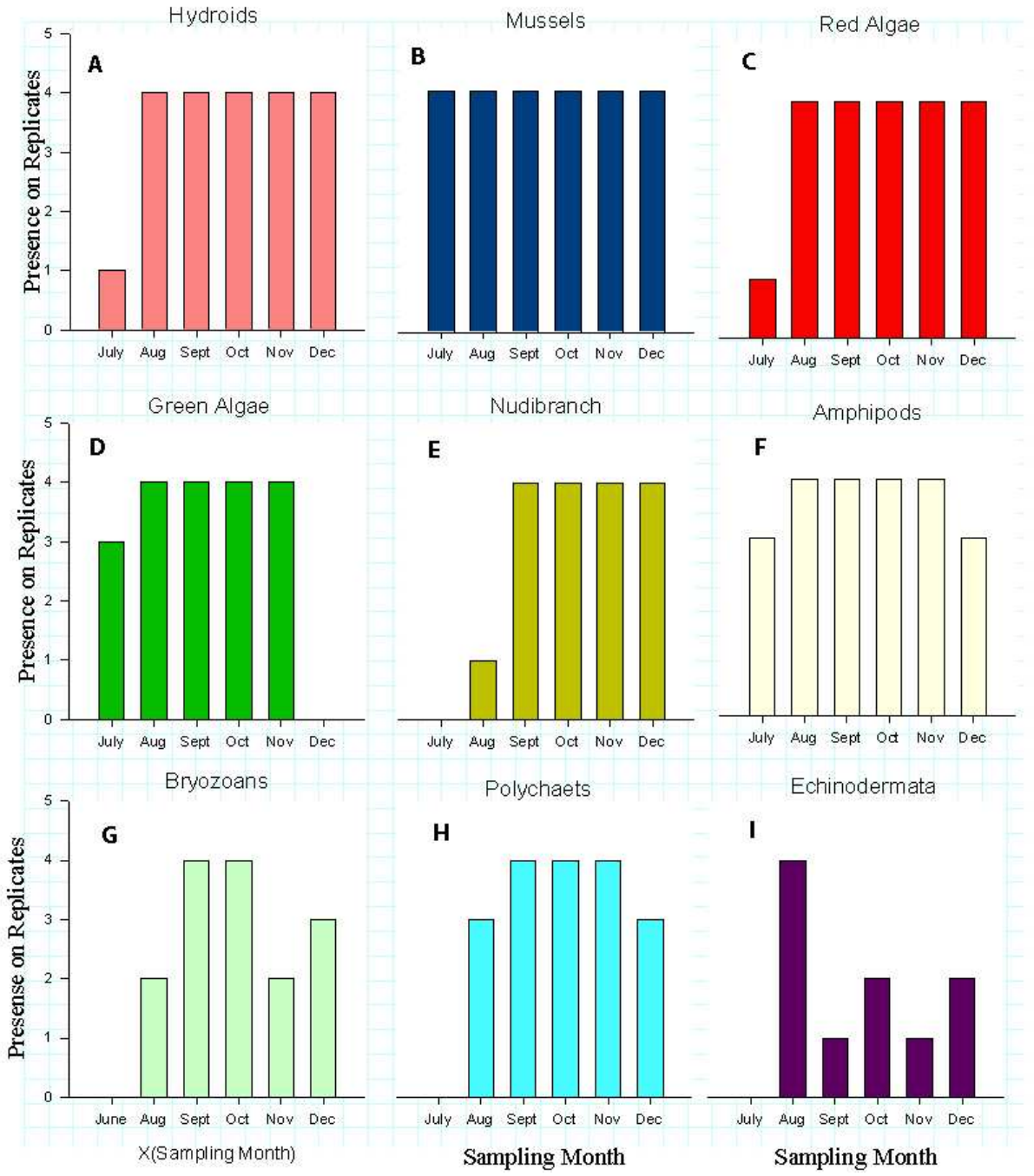

Figure 15: Different fouling organism groups present on Micanti net panels sampled every month. Data presented in the graphs is based on presence or absence of different organism groups on the net panels. 1= present on one net panel, 2= present on two panels, 3= present on three net panels and 4= present on all four net panels.

3.3. Fouling composition

3.3.1. Nylon net panels

Fig.16 presents the composition of fouling organisms on nylon net panels. Hydroids were one of the dominant fouling groups, forming 15-40% of total fouling on nylon net panels except in July (Fig.16). In July, hydroids formed no visual fouling that could be observed with the naked eye. Molluscs were present on all net panels in all months but on net panels in July, they formed no visual fouling, as only microscopic larvae were present on the net panels. Molluscs were one of the dominant fouling groups from August to November forming 5-60% of the total fouling (Fig.16). In November, molluscs strongly dominated the net panels forming about half of the total fouling but they constitute only 10% of fouling on panels in December

Algae were present in all months except in July and were the dominant group in August, forming 35% of the total fouling. From September to December algae formed 5- 20% of the fouling on net panels (Fig.16). Amphipods formed 5-10% of the total fouling on net panels from September to November but in December, they formed the major part (40%) of the fouling on net panels (Fig.16). Other organisms, mainly nudibranchs, bryozoans and polychaets formed 5-20%of the total fouling on all net panels except in July (Fig.16).

3.3.2. Micanti net panels

Fig. 17 presents the composition of fouling organisms on Micanti net panels. Hydroids were present on all net panels from August to December forming 5-20% of the total fouling (Fig.17).Molluscs were the dominant group on all frames during all months and they formed up to 20-70% of the total fouling on net panels during all months (Fig.17).

Algae were present in all months except in July. From August to December, algae formed 5-30% of the fouling and were one of the dominant fouling groups on net panels in September (Fig.17). Amphipods were one of the dominant groups in December and were absent in July. On net panels, they formed 5-20% of total fouling (Fig.17). Other organisms, mainly nudibranchs, bryozoans and polychaets formed 10-20% of the total fouling on all net panels except in July (Fig.17)

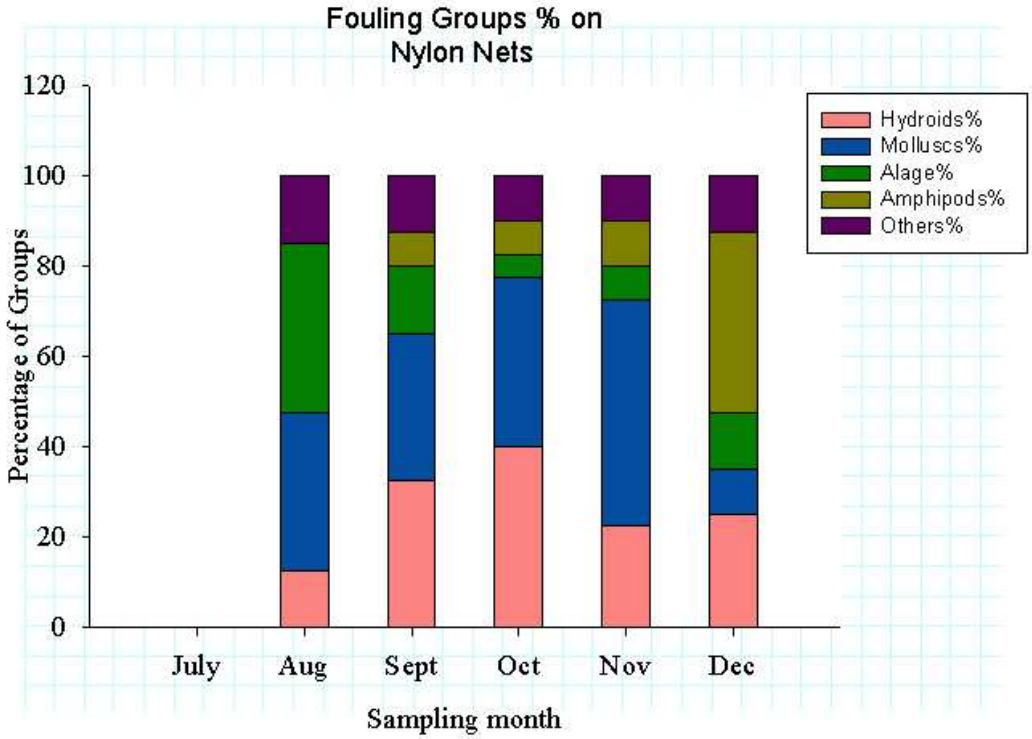

Figure 16: Relative proportion of different fouling organism groups on nylon nets.

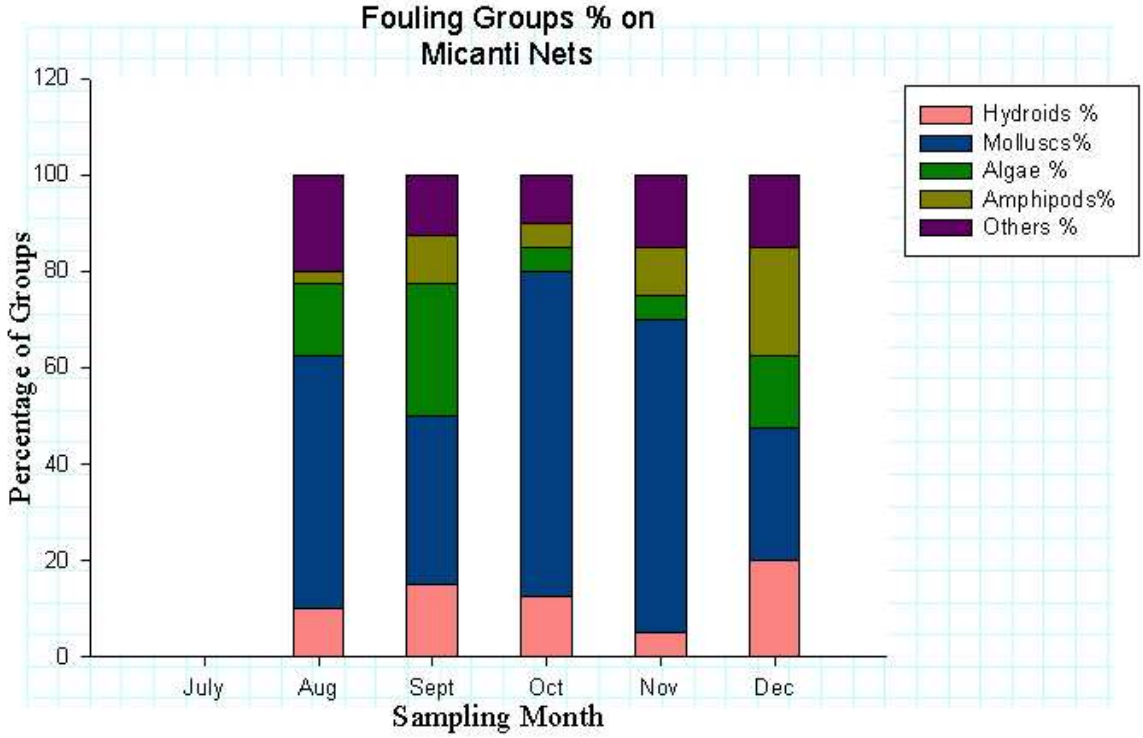

Figure 17: Relative proportion of different fouling organism groups on Micanti nets.

3.4. Occlusion of net panels

Fig.18 and Fig.19 represents the development of fouling on nylon and Micanti net panels through out the study (July to December).

Nylon net panels

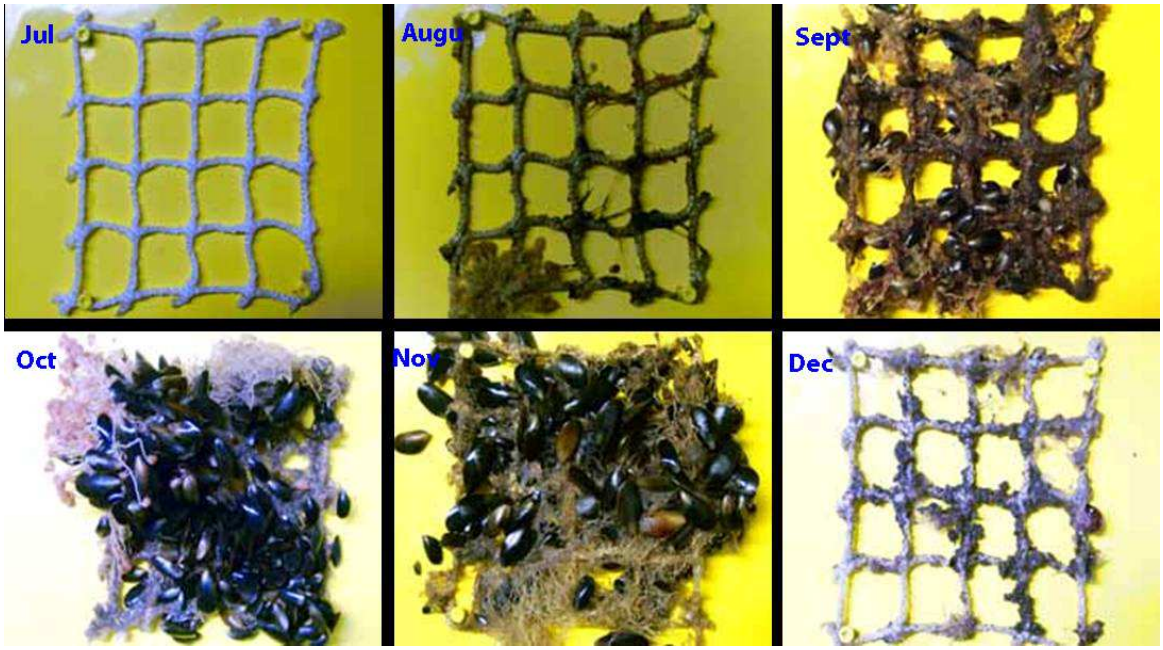

Figure 18: Development of fouling on nylon net panels from July to December.

Micanti net panels

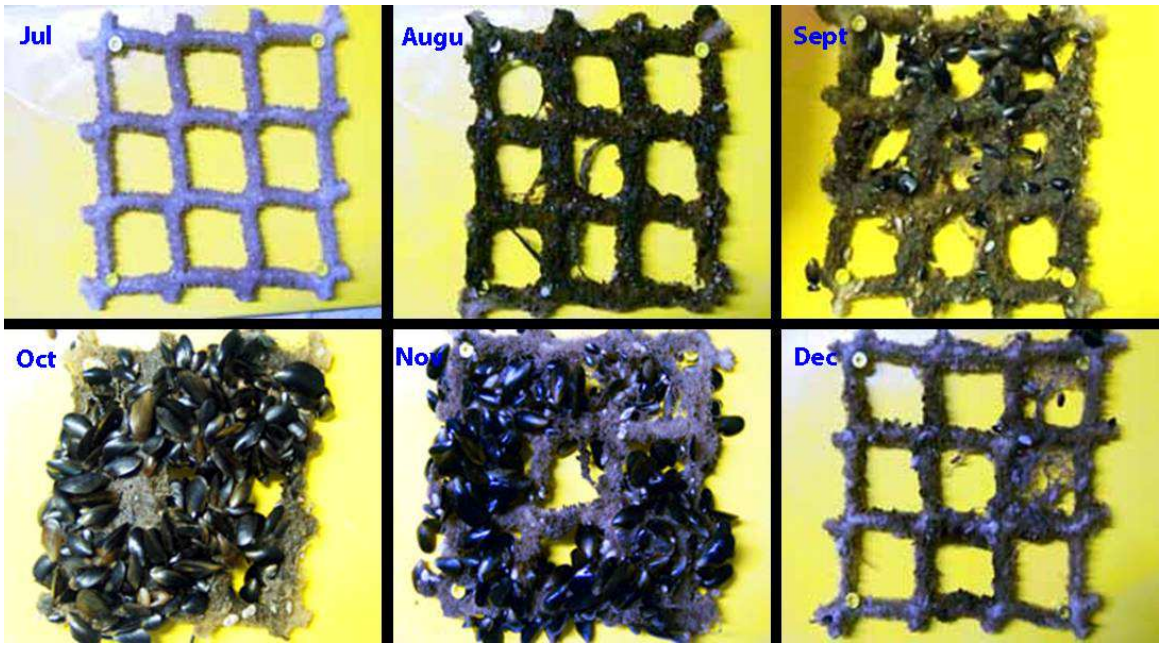

Figure 19: Development of fouling on Micanti net panels from July to December.

Suctorian ciliates, hydroids and blue mussel larvae settled on the net panels in July. Very few (1-3) amphipods, snails and nudibranchs were also settled on the net. In August, the size of mussel larvae and hydroids increased and algae appeared on the net panels. Some other fouling organisms were also developed on the net panels. The foulers occluded the net thread and net mesh openings were not blocked. In September, foulers started to occlude the net mesh opening and in October, foulers occluded the entire net mesh opening. Due to some unknown reasons, fouling on the net panels started to fall off by November and in December, foulers were completely fell off from the net panels leaving the entire net mesh opening almost free of fouling. Successional development of fouling on Micanti net panel was also the same as like on nylon net panels.

On both nylon and Micanti net panels, the maximum fouling was after 117 days of immersion (in October) (Fig.20). On Micanti nets, more detritus and sediments were trapped between the fibers. In August, Micanti nets were more fouled compared to nylon nets but in all later months nylon net panels fouled more compared to Micanti net panels (Fig.20). By November, fouling fall off started on net panels and resulted in decrease in the percentage of occlusion. By the last sampling in December, the percentage occlusion on Micanti net panels dropped to below 30% and on nylon net panels it was below 40% (Fig.20). Hydroids were more on the nylon net panels compared to Micanti net panels. Mussels were more on Micanti net panels compared to nylon net panels. In all other main fouling groups, no such trends were observed in the selectivity of the net panels.

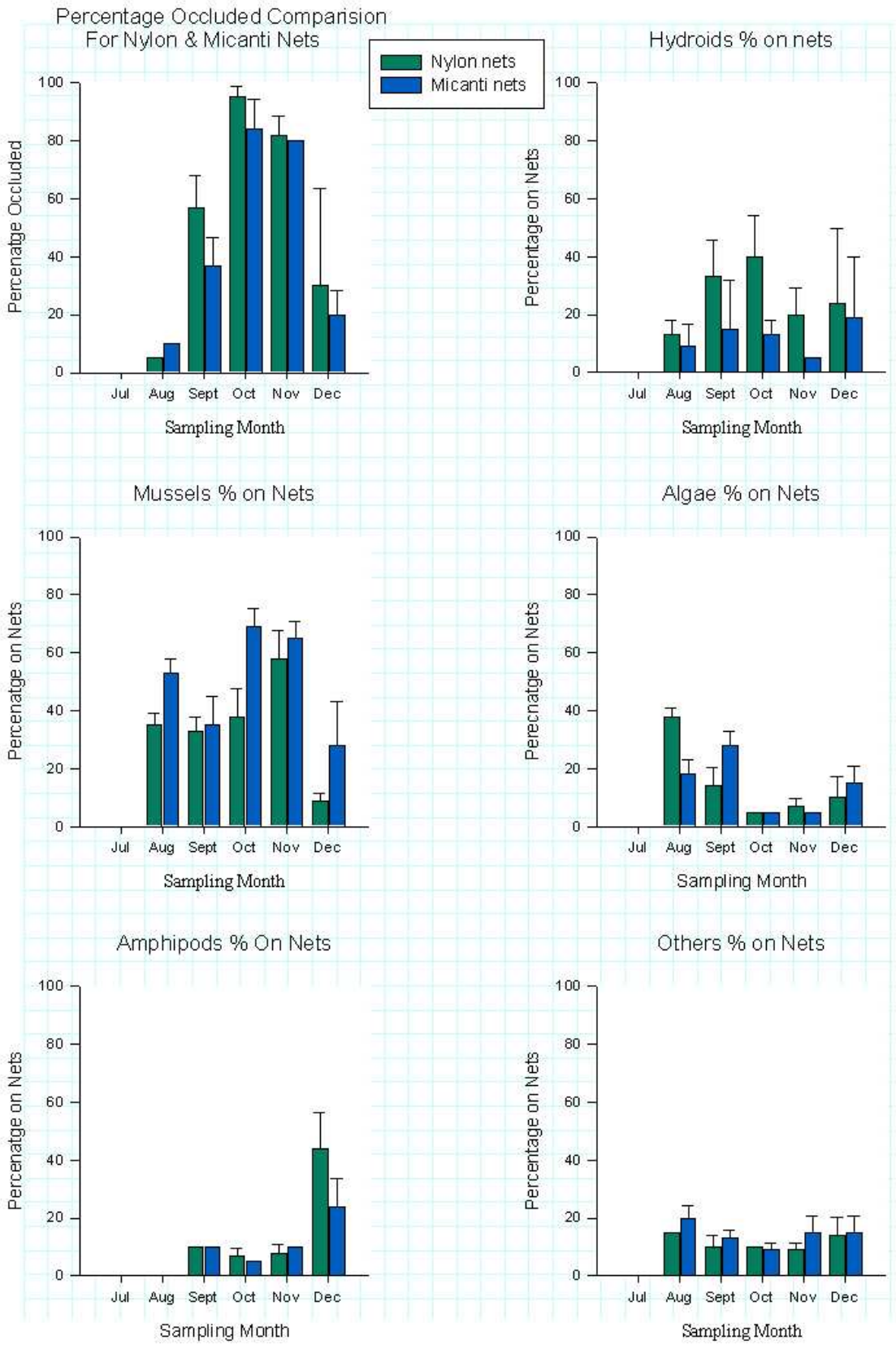

Figure 20: Comparing the percentage occluded and different groups relative proportions on the nylon and Micanti net panels (n=4).

3.5. Fouling wet weight on nets

The Micanti-net panels had more net fouling wet weight compared to that of nylon-net panels in July and August. In July, Micanti nets had considerable fouling weight along with trapped detritus and sediments. Nylon nets had no considerable fouling weight in July. In all remaining months, Micanti nets showed slightly less fouling weight compared to nylon nets and had considerable deference in October (Fig.21).The maximum average net fouling wet weight observed was 21.2 kg / m² for nylon net panels and 16.1 kg / m² for Micanti net panels in October.

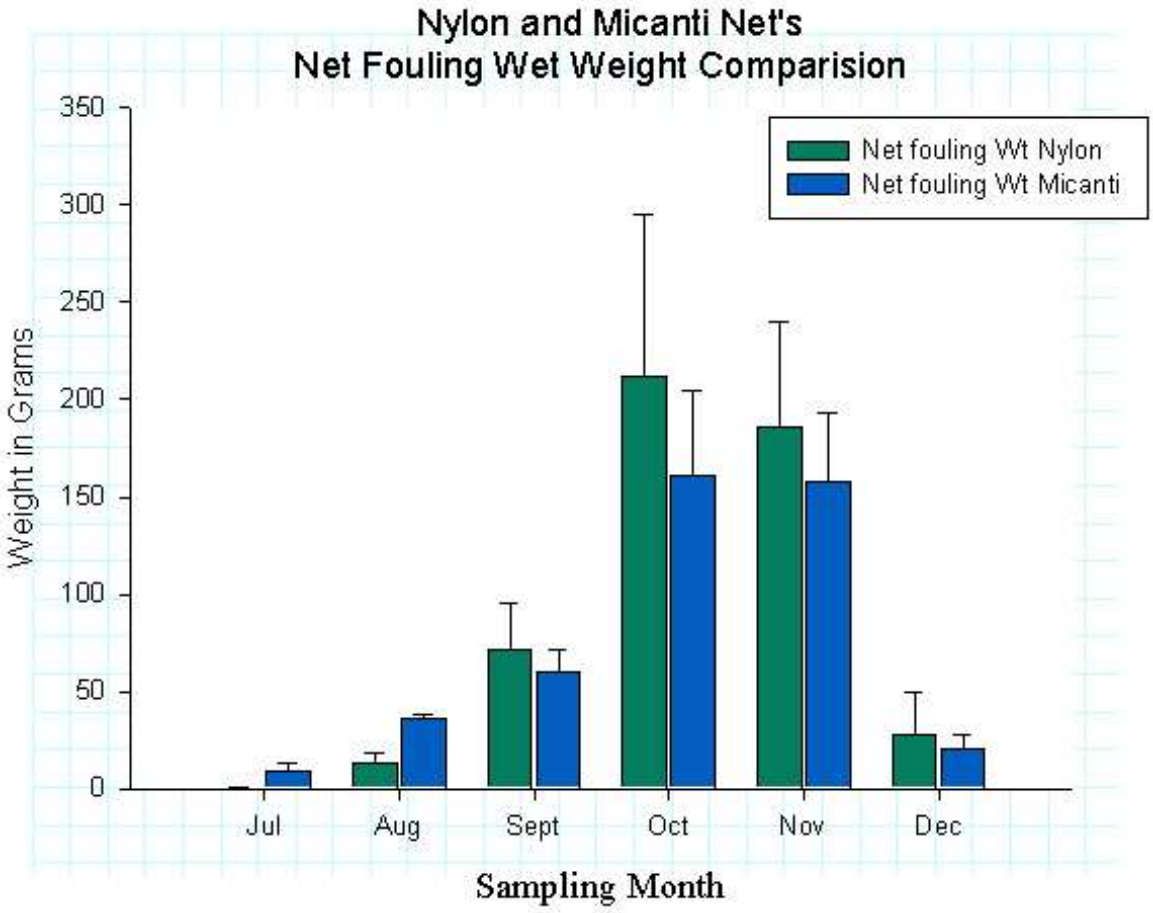

Figure 21: Net fouling wet weight on nylon and Micanti net panels. (n=4)

4. Discussion

Biofouling as a big cost-incurring problem in marine fish farming needs to be studied in detail to develop better antifouling methods that are environment friendly and cost-effective unlike the present antifouling methods. Biofouling varies, following change in substrate, spatial variation and local conditions (Braithwaite and Mc Evoy, 2005; de Nys and Guenther, 2009).

In this regard, I studied biofouling on untreated nylon and Micanti nets in mid-Norwegian coastal waters, to identify the detailed biofouling profile. In this experiment, I used nylon and Micanti net panels (short fibers flocked on a net surface) to compare and analyze the main biofouling groups, biofouling composition, biofouling weights and factors affecting the biofouling process. Zooplankton samples were also collected and studied to find larvae of fouling groups and to be able to identify any possible relation with the fouling on net samples.

Water velocities and nutrients availability are different inside the cages with fish cultured, cages without fish and in the water column outside the cages (Madin et al., 2010). The position of the frames with net panels can also affect the fouling (de Nys and Guenther, 2009). In this experiment, frames were deployed on an empty cage without net installed and present at the downstream point of the farm. So, fouling on the net panels might be different from the fouling on the actual cage netting. Deploying the frames at different points (preferably on the cages with fish, on cages without fish and outside the cage in water column) would have been better to get a more clear picture of the biofouling. To compare different aspects of biofouling in this study, no much references are available. This study might help to get a better idea about biofouling in mid-Norwegian coastal waters.

4.1. Biofoulers larvae and net fouling

Benthic invertebrate organisms release planktonic larvae and algae release spores that are carried with the currents to a new substrate and settle for further recruitment (Phillippi et al., 2001). Most of the fouling organisms reproduce through production of free-living planktonic larval stages that will settle on the substrate and grow. Settlement of fouling organisms on the substrate depends on the abundance of the larvae of fouling organisms and spores of algae in the water column and the properties of the substrate (Larsson, 1997). Analysis of the zooplankton samples and the net panels in this study was in contract with above statement as there was a relation found between the presence of the larval stages in the zooplankton samples and fouling organisms found on the net panels.

In the zooplankton samples there were no algal spores observed as it is not possible to sample algal spores with zooplankton sampling net. In the zooplankton samples no larval stages of amphipods were observed, as they do not have larval stage included in their life cycle (Wade et al., 2004). Copepods and larvaceans are holoplanktonic larval stages found in the zooplankton samples and they did not account for any of the fouling. Meroplanktonic larval stages found in the samples accounted for the most of the fouling on the net panels.

As the net panels used in this experiment were heavily fouled by the time actinula stages appeared in the zooplankton samples (September), I was not able to identify them as hydroids on the net panels used in this study. However, they appeared on fresh net panels deployed close to the net panels deployed in this study (Nina Blöcher, personal communication). This might explain the effect of space limitation and succession of hydroid fouling on the development of fouling on the net panels. Medusa identified in the zooplankton samples were found as hydroids on net panels. *Obelia sp* medusa observed in July zooplankton samples found as hydroids on the net panels in July (Fig.13A).

Plankton samples collected when the net panels were deployed at the farm had mussel larvae and the same mussel larvae were observed on the net panels sampled two weeks later. Snails were present in both plankton samples and net panel throughout the study. Plankton samples throughout all samplings had larvae of bryozoan, *Membraniopora membranacia* and the same *Membraniopora membranacia* was observed as wide-mats on the net panels (Fig.13M-O).

Polychaets observed in the plankton samples were also observed on the net panels. In addition, there were more polychaet types on the net panels. Planula larvae of starfish observed in the plankton samples were found as starfish on the net panels. All these trends observed between the zooplankton samples and net panels, confirms the relation between larvae present in the water column and foulers observed on the net panels.

Madin et al., 2010 explained that based on the food availability there is a difference between fouling on net panels deployed on cages with fish, without fish and nets deployed outside the cages in the water column. They also explained that fouling would be less on the nets exposed to high water flow velocities. According to Madin et al., 2010, due to the high water velocities, it would be difficult for fouler's larvae to settle on the substrate and high water currents flush off the food particles that decreases the contact of larvae with food particles, thus decreasing the availability of the food. Net panels used in this study were deployed on an empty cage without net installed and that cage was present at the downstream point of the farm. Following Madin et al., 2010 explanation fouling on net panels used in this study was different from the fouling on the cage netting with fish.

In this study, only three zooplankton samples were taken in each sampling and all net panels were deployed at same point. Increasing the frequency of sampling and studying more number of zooplankton samples and more number of net panels that were sampled from different points of the farm (cage with fish, cage without fish, out side the cage and outside the farm) might give more clear picture of the biofouling organisms profile in that area.

Since a small zooplankton sampling net (30 cm diameter) was used in this study, sampling of the free-swimming planktonic nauplius I, II and copepod stages of sea lice *Lepeophtheirus salmonis* was difficult (Schram, 2004). However, plankton samples in October and December had nauplius II stages of sea lice, which was associated with the sea lice infection of the farmed fish cultured in the fish farm. Due to restricted sampling regime (three samples collected in each sampling), low number of sea lice found in each sample (2-3) and difficulty in sampling sea lice larval stages, it is not easy to tell about the movement of the sea lice larval stages in and around cage farms as described by Schram (2004).

4.2. Biofouling on nets

Guenther et al., (2010) have described the main fouling organism groups present in the mid-Norwegian coastal waters. In their article, they identified filamentous algae, hydroids (*Ectopleura larynx*), mussels (*Mytilus edulis*), solitary ascidians, caprellid amphipods and nudibranchs (*Aeolidia papillosa*) as the main fouling groups on the nets. Except ascidians, all of these groups were found in this study while the main nudibranch species found in this study was *Coryphella sp*.

Algae were believed to be the first macro foulers on the fresh surfaces deployed in the water (Corner et al., 2007; Greene & Grizzle, 2007; Scheer., 1945), but in the present study algae came after the hydroids and mussel settled on the net. Algae were found on all net panels sampled in August (after 45 days of deploying) (Fig.14 and Fig.15).

In this study, hydroids, in particular *Ectopleura larynx* was one of the dominant fouling groups on all of the net panels sampled along with the blue mussels (*Mytilus edulis*). Mussels present on the net panels secreted many bysus threads and they formed a mesh on the nets. This mesh provided space and support for the other fouling organisms (nudibranchs, polychaets and echinoderms).

Guenther et al., (2010) stated that caprellid amphipods formed a negligible part of the fouling community compared to hydroids and mussel fouling. However, in this study, gammarid amphipods and caprellid amphipods made significant part of the fouling on the net panels. There were many tube-building amphipods present on the net panels and they formed many brown tubes (their shelters) on the net. Together with the bysus threads secreted by mussels, brown tubes formed shelter for other minor foulers (nudibranchs, polychaets and echinoderms) to settle on the net panels and accounted for a significant portion of the total fouling.

Nudibranchs were common on the net panels and they accounted for a considerable amount of the total fouling. With time, they grew in size and the main species *Coryphella sp* dominated the net samples. Among other fouling groups bryozoans, polychaets and echinoderms were present in considerable number and amount. However, these formed relatively a lesser amount of fouling compared to the main fouling groups (hydroids and blue mussels).

Ascidians are one of the space competitors on the net surfaces (Greene & Grizzle, 2007). There are solitary ascidians and colonial ascidians that colonize the net surfaces and other aquaculture equipments. Except few (3-5) tadpole larval stages, no fully grown ascidians were observed on the net panels. In the study by Guenther et al., (2010) in mid-Norwegian coastal waters, less numbers of ascidians recorded which is supporting ascidian results in this study. Many barnacle larvae observed on the net panels but they formed no fouling.

4.3. Comparing the occlusion of nylon and Micanti panels

Hydroids were the main fouling group on the nylon net panels followed by mussels, the second dominant group on nylon net panels. (Fig.16). Algae were in significant proportions in early months. Proportion of algae on the net panels decreased with the increase of amphipods on the net panels, which are known to feed on algae (Madin et al., 2009). The fouling fall off in December removed most of the main fouling groups (hydroids, blue mussels and algae) from the net panels, leaving the amphipods as the main fouling group. Following nudibranchs presence on net panels, all hydroids lost their typical pink heads. Predation of nudibranchs on the hydroids might have caused this (Greene & Grizzle, 2007).

Larsson, (1997) and Phillippi et al., (2001) concluded that compared to normal surfaces used in aquaculture (like untreated knotless nylon nets) flocked surfaces would have more mussel and less green algal fouling. In this study, Micanti nets with flocked fibers had a lot more blue mussels compared to the untreated nylon nets (Fig 18). However, no impact of flocking against green algae fouling was observed

Braithwaite et al., (2007) stated that in their study uncoated nets started to foul after 50 days after immersion. In this study, biofouling was visually evident on both nylon and Micanti nets after 45 days of immersion but only net thread fouling leaving the mesh open. Following 117 days after deploying, fouling completely occluded the nylon net panels while Micanti net panels were slightly less occluded compared to nylon net panels. Surprisingly by November, fouling started to fall of and by December, net panels were without much fouling (Fig 19). Possible reasons that were responsible for the fouling fall off might be environmental changes, birds that are known to feed on the mussels, strength of attachment of the fouling to the net panel and space limitation.

Fouling fall off started between October and November and there were no abrupt changes observed in temperature and wave velocities in this period. Unfortunately, there was no data available for the period between November and December when most of the fouling fall off happened. As the net panels were small, there was no much space available for the growing fouling organisms to attach firmly to the net panels. So, foulers grew by attaching to the mussels that have been already firmly established on the net panels. The attachment of mussels might not be strong enough to bare the heavy weight of fouling (Fig.21, net wet weights of fouling in October on net panels), and this might have resulted in the fouling fall off. However, there is no known reason that explain the fall-off of fouling organisms.

Micanti in their presentation claimed that their nets will have more fouling in the earlier months and with the time as the foulers grow, they will fall off from the nets and Larsson, (1997) have stated the same. However, the results of this study are contradictory to these. Successional development of fouling on the Micanti nets was almost the same like as on the nylon net panels. Only a small effect of fibers flocking observed on the Micanti nets against fouling. The possible reasons for this less effectiveness of Micanti nets against fouling might be density, length, thickness and strength of the fibers. Larsson, 1997 found that, density of the fibers is very crucial to have the antifouling action by the flocked surfaces. Density of flocked fibers on the Micanti net panels was low and there was a lot of free space available between the fibers that could be sufficient for the fouling organism's larvae to settle on and develop. In this study, many mussel larvae were observed on the Micanti net panels within the spaces between the flocked fibers. To avoid the fouling organism's larvae to settle, the space between the flocked fibers should be less than the size of the fouling organism's larvae.

Length and thickness of the fibers should be considered. Short fibers and long fibers work different against fouling. Thin fibers show more resistance against fouling than the thicker fibers and different lengths control different foulers. So, having at least two different length thin fibers might be effective (Larsson, 1997; Phillippi et al., 2001). Strength of the fibers attachment is also important which was poor in case of the Micanti net and this explains the poor performance of Micanti as better antifouling method.

4.4. Fouling weight

The maximum average net fouling wet weight observed among the net panels was more than 33 kg/m² on nylon net panels in October. Braithwaite et al., (2007) recorded 4.5 kg/m² biofouling wet weight average on untreated nylon nets following 10months of immersion. There were 2.2 kg/m², 4.5 kg/m², 7.8 kg/m² biofouling wet weights recorded (Braithwaite and Mc Evoy, 2005; Hodson et al ., 2000). Compared to all these, weights recorded on the untreated nets in this study are far more high and weights increased maximum to 33 fold.

Phillippi et al., (2001) explained that the fiber flocked PVC surfaces would get less fouling compared to unflocked PVC surfaces. Micanti nets had slightly less fouling wet weight compared to nylon nets. Micanti nets had more mussel fouling compared to nylon nets and mussels weigh more than hydroids, even then net fouling wet weight on Micanti net panels was less than on nylon net panels. Changing the density, length and strength of fibers on the Micanti nets might work well in fighting against the fouling (Phillippi et al., 2001). Micanti producers recently changed the length of fibers and increased the strength of fibers and they might perform well now. However, more research and development is needed to prove the Micanti net as a good solution against fouling.

5. Conclusion

Through out the study, there was a clear relation observed in the presence of fouling organisms in the zooplankton samples and fouling organism found on the net panels. Therefore, studying plankton samples and net panels together can give a better idea on the biofouling profile in a particular area. On net panels hydroids, mussels, algae, amphipods and nudibranchs accounted for the major proportion of the fouling. Throughout the study (from July to December), on nylon net panels hydroids were more compared to mussels and on Micanti net panels mussels were more compared to hydroids. There was no trend observed in the remaining fouling groups present in zooplankton samples and on the net panels. Nets occlusion and net fouling wet weight was slightly less on the micanti net panels compared to nylon net panels.

For a detailed picture of the fouling organisms, the number of samples, the zooplankton sampling points and the net panels deploying points should be considered. Micanti net panels used in this study might evolve as a good solution against fouling replacing copper antifouling paints, if the density, strength and length of the fibers flocked on the net surface are improved. To find out any detail regarding the sea lice movement between the farms, sampling device, number of samples and frequency of sampling should be considered.

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7. Appendix

Appendix.1 Presence of different organism groups in zooplankton samples.

	Actinula	Medusa	Mussels	Bryozoans	Polychaets	Amphipod	Echinoderms	Larvaeceans	Decapod	Snails	Copepods	Cladoceran	Phoronids	Others
J1A	0	1	1	1	1	0	1	1	1	1	1	1	1	Star fish larvae1present
J1F	0	1	1	1	1	0	1	1	1	1	1	1	0	
J1B	0	0	0	1	0	0	1	1	1	0	1	1	1	Star fish 1 present
J2A	0	1	0	1	1	0	1	1	1	1	1	1	1	
J2F	0	1	0	1	0	0	0	1	1	1	1	1	0	
J2B	0	1	0	1	1	0	1	1	1	1	1	1	1	Star fish ,phoronid
AA	0	1	1	1	1	1	0	1	1	1	1	1	0	
AF	0	1	0	0	0	0	0	1	1	1	1	1	1	nudibranch,sea lice,unknown
AB	0	1	0	0	0	0	0	1	1	1	1	1	0	
SA	0	0	0	1	0	0	0	1	1	1	1	1	0	
SF	1	0	0	1	0	0	1	1	0	1	1	0	0	
SB	1	1	0	1	0	0	0	1	0	1	1	0	0	
OA	1	1	1	1	1	0	0	0	0	1	1	1	0	3 sea lice larval stags present
OF	1	1	0	1	1	0	1	0	1	1	1	0	0	3 sea lice larval stags present
OB	1	1	0	1	0	0	1	0	0	1	1	0	0	Sea lice larval stages present
NA	1	0	0	0	0	1	0	0	0	1	1	0	0	poor sample with debris
NF	1	1	0	0	1	0	0	0	0	1	1	0	0	poor sample with debris
NB	0	0	0	0	0	1	0	0	0	1	1	0	0	poor sample with debris
DA	0	0	0	1	0	0	1	0	0	1	1	1	0	
DF	1	0	0	1	1	0	0	0	1	0	1	0	0	sea lice larvae present
DB	0	0	0	1	0	0	0	0	0	1	1	1	0	nudibranch,sea lice,

A=100m Above farm	J=July	1-Prsent
F=At Frames	A=August	0-Absent
B=100m Below farm	S=September	
	O=October	
	N=November	
	D=December	

Appendix.2 Different fouling organism groups present on nylon net panels sampled every month.

	Hydroids	Mussel	Red Alga	Green Alg	Nudibran	Amphipoc	Bryozoa	Polychae	Echinode	Barnacle	Sponges	Suctoria	Platyhelm	Nematods	Star fish	Snails	Hiatella
JNA1	1	1	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0
JNA2	1	1	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0
JNN1	1	1	0	0	1	1	0	0	0	0	0	1	0	0	0	1	0
JNN2	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0
ANA1	1	1	1	1	0	1	1	1	0	0	0	0	0	1	0	1	0
ANA2	1	1	1	1	1	1	0	0	0	0	0	0	0	1	0	1	0
ANN1	1	1	1	1	1	1	0	0	0	1	0	0	0	1	0	1	0
ANN2	1	1	1	1	0	1	0	1	0	0	0	0	0	1	0	1	0
SNA1	1	1	1	1	1	1	1	1	0	1	0	0	0	1	0	1	0
SNA2	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	1	0
SNN1	1	1	1	1	1	1	1	1	0	1	0	0	0	1	0	1	0
SNN2	1	1	1	1	1	1	0	1	1	1	0	0	0	1	1	1	1
ONA1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	1	1
ONA2	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1
ONN1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1
ONN2	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	1	1
NNA1	1	1	1	1	1	1	0	0	0	1	0	0	0	1	0	1	1
NNA2	1	1	1	1	1	1	1	1	0	1	0	0	1	1	0	1	1
NNN1	1	1	1	1	1	1	0	1	0	1	0	0	0	1	0	1	1
NNN2	1	1	1	1	1	1	1	1	0	1	0	0	0	1	0	1	1
DNA1	1	1	1	1	1	1	1	0	0	1	0	0	1	1	0	1	1
DNA2	1	1	1	0	1	1	1	1	1	1	0	0	1	1	0	1	1
DNN1	1	1	1	1	1	1	0	1	0	1	0	0	0	1	0	1	1
DNN2	1	1	1	1	1	1	0	1	0	1	0	0	1	1	0	1	1
		J-July		N-Nylon		1-Present											
		A-August				0-Absent											
		S-September		A-Amma													
		O-October		N-Naanna													
		N-November															
		D-December															

Appendix.3 Different fouling organism groups present on Micanti net panels sampled every month

	Hydroids	Mussels	Red Algae	Green Alg	Nudibranc	Amphipoc	Bryozoar	Polychae	Echinoderm	Barnacles	Sponges	Suctorian Ci	Platyhelmen	Nematoc	Star fish	Snails	Hiatella	
JMA1	1	1	1	1	0	1	0	0	0	0	0	1	0	0	0	1	0	
JMA2	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	
JMN1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	
JMN2	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	
AMA1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1	1	0	
AMA2	1	1	1	1	0	1	0	1	1	0	0	0	0	1	0	1	0	1 Ascidian P
AMN1	1	1	1	1	0	1	1	1	1	0	0	0	0	1	1	1	1	
AMN2	1	1	1	1	0	1	1	1	1	0	0	0	0	1	1	1	1	
SMA1	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	
SMA2	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	
SMN1	1	1	1	1	1	1	1	1	0	1	0	0	0	1	0	1	1	
SMN2	1	1	1	1	1	1	1	1	0	1	0	0	0	1	1	1	1	
OMA1	1	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	
OMA2	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	1 Ascidian P
OMN1	1	1	1	1	1	1	1	1	0	1	0	0	0	1	0	1	1	
OMN2	1	1	1	1	1	1	1	1	0	1	0	0	0	1	0	1	1	
NMA1	1	1	1	1	1	1	1	1	0	1	0	0	1	1	0	1	1	1 Ascidian P
NMA2	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	1	1	
NMN1	1	1	1	1	1	1	0	1	0	1	0	0	0	1	0	1	1	
NMN2	1	1	1	1	1	1	0	1	0	1	0	0	0	1	0	1	1	
DMA1	1	1	1	0	1	1	1	0	0	1	1	0	0	1	0	1	1	
DMA2	1	1	1	0	1	1	1	1	0	1	1	0	0	1	0	1	1	
DMN1	1	1	1	0	1	1	0	1	1	1	1	0	0	1	0	1	1	
DMN2	1	1	1	0	1	0	1	1	1	1	1	0	0	1	0	1	1	
		J-July		M-Micanti		1-Present												
		A-August				0-Absent												
		S-September		A-Amma														
		O-October		N-Naanna														
		N-November																
		D-December																

Appendix.4 Relative percentage of different fouling organism groups on nylon nets

Sam. No	% Occluded	Hydroids %	Molluscs %	Algae %	Amphipods %	Others %				
JNA1	0	0	0	0	0	0				
JNA2	0	0	0	0	0	0				
JNN1	0	0	0	0	0	0				
JNN2	0	0	0	0	0	0				
ANA1	5	15	30	40	0	15				
ANA2	5	5	40	40	0	15				
ANN1	5	15	35	35	0	15				
ANN2	5	15	35	35	0	15				
SNA1	45	20	40	15	10	15		J-July		N-Nylon
SNA2	50	30	30	15	10	10		A-August		A-Amma
SNN1	70	50	30	5	10	5		S-September		N-Naanna
SNN2	60	30	30	20	10	10		O-October		
ONA1	90	20	50	5	10	10		N-November		
ONA2	95	40	40	5	5	10		D-December		
ONN1	99	50	30	5	5	10				
ONN2	95	50	30	5	5	10				
NNA1	80	30	50	5	5	10				
NNA2	75	15	60	5	10	10				
NNN1	90	25	50	5	10	10				
NNN2	80	10	70	10	5	5				
DNA1	10	0	10	20	50	20				
DNA2	80	60	5	5	25	5				
DNN1	10	15	10	10	50	15				
DNN2	20	20	10	5	50	15				

Appendix.5 Relative percentage of different fouling organism groups on Micanti nets

Sam.No	% Occluded	Hydroids %	Molluscs %	Algae %	Amphipods %	Others %				
JMA1	0	0	0	0	0	0				
JMA2	0	0	0	0	0	0				
JMN1	0	0	0	0	0	0				
JMN2	0	0	0	0	0	0				
AMA1	10	20	50	10	0	20				
AMA2	10	5	60	20	0	15				
AMN1	10	5	50	20	0	20		J-July		M-Micanti
AMN2	10	5	50	20	0	25		A-August		A-Amma
SMA1	30	5	40	30	10	15		S-September		N-Naanna
SMA2	35	10	40	30	10	10		O-October		
SMN1	30	5	40	30	10	15		N-November		
SMN2	50	40	20	20	10	10		D-December		
OMA1	95	20	60	5	5	10				
OMA2	75	10	70	5	5	10				
OMN1	90	10	75	5	5	5				
OMN2	75	10	70	5	5	10				
NMA1	80	5	60	5	10	20				
NMA2	80	5	60	5	10	20				
NMN1	80	5	70	5	10	10				
NMN2	80	5	70	5	10	10				
DMA1	30	50	20	10	10	10				
DMA2	20	10	20	20	30	20				
DMN1	10	5	50	10	25	10				
DMN2	20	10	20	20	30	20				

Appendix.6 Nylon and Micanti nets, net fouling wet weight data

Sam no	Gross Wt	Petridish Wt	Net, Fouled-net Wt	Gross fal of wt	Petridish Wt	Net fall of Wt	Original net wet Wt	Net fouling Wt		
JNA	11,81	0	11,81	0	0	0	11,66	0,15		
JNN	11,87	0	11,87	0	0	0	11,66	0,21		
JMA	64,64	27,75	36,89	0	0	0	24,59	12,30		
JMN	57,02	27,75	29,27	0	0	0	24,59	4,68		
ANA	46,28	27,75	18,53	13,67	7,35	6,32	11,66	13,19		
ANN	44,95	27,75	17,20	13,57	7,35	6,22	11,66	11,76		J-July
AMA	82,46	27,75	54,71	13,06	7,35	5,71	24,59	35,83		A-August
AMN	80,45	27,75	52,70	15,3	7,35	7,95	24,59	36,06		S-Septemb
SNA	90,46	27,75	62,71	26,13	7,35	18,78	11,66	69,83		O-October
SNN	95,55	27,75	67,80	24,58	7,35	17,23	11,66	73,37		N-Novembe
SMA	96,62	27,75	68,87	26,44	7,35	19,09	24,59	63,37		D-Decembe
SMN	96,46	27,75	68,71	19,59	7,35	12,24	24,59	56,36		
ONA	167,45	27,75	139,70	61,46	17,55	43,91	11,66	171,95		N-Nylon
ONN	256,66	27,75	228,91	62,46	27,75	34,71	11,66	251,96		M-Micanti
OMA	167,28	27,75	139,53	47,71	27,75	19,96	24,59	134,90		
OMN	188,41	27,75	160,66	77,24	27,75	49,49	24,59	185,56		A-Amma
NNA	151,86	27,75	124,11	64,49	27,75	36,74	11,66	149,19		N-Naanna
NNN	187,82	27,75	160,07	101,12	27,75	73,37	11,66	221,78		
NMA	149,97	27,75	122,22	70,18	27,75	42,43	24,59	140,06		
NMN	176,35	27,75	148,60	78,84	27,75	51,09	24,59	175,10		
DNA	75,38	27,75	47,63	0	0	0	11,66	35,97		
DNN	54,36	27,75	26,61	0	0	0	11,66	14,95		
DMA	73,98	27,75	46,23	0	0	0	24,59	21,64		
DMN	70,8	27,75	43,05	0	0	0	24,59	18,46		