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Development of biofouling on salmon cage nets and the effects of anti-fouling treatments on the survival of the hydroid (*Ectopleura larynx*) (Ellis & Solander, 1786)

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Marine Coastal Development

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Abstract

Biofouling has become a serious problem for farmers in the Norwegian finfish aquaculture industry in recent years; with the hydroid *Ectopleura larynx* dominating the fouling community in southwestern and mid-Norwegian waters. Most farmers in Norway use a combination of strategies to deal with fouling: the use of copper coated nets combined with washing, the use of copper coated nets combined with drying, and the use of uncoated nets combined with frequent washing. Concerns have been raised about the use of copper coatings on nets due to possible environmental threats. A better understanding of fouling patterns with depth and time; as well as the effectiveness of environmentally friendly treatments is needed. The aims of the study were to identify the temporal and depth variability of biofouling on salmon cage nets from a farm in mid-Norway. Additionally, the effect of drying on hydroid survival and the effects of environmentally friendly anti-fouling treatments on the survival of *E. larynx* were investigated. The first experiment was to look at the development of biofouling on salmon cage nets at 3 different depths (1, 5 and 10 m) over a six month period (June-November). The second experiment involved the drying of hydroid colonies at 6 different drying times to determine the shortest time needed to ensure complete mortality of hydroids. The third experiment was to determine the effects of washing and drying on the recovery of hydroid colonies, using 5 different treatments; after which hydroids were allowed to recover for a two week period and analysed. The results showed that the major fouling groups were algae, molluscs, hydroids, crustaceans and bryozoans, with hydroids becoming abundant from August onwards. Hydroids began to completely colonise the nets from 10 m in September and then completely colonized the nets at all three depths in October and November. 48 hours of air drying caused a complete mortality of hydroids. Dead hydroids shed their hydranths and cut or damaged hydroids were capable of regeneration. Nets which had hydroids removed, damaged or cut by the washing process had the highest percentage growth increase than the other treatments after a two week recovery period. This study demonstrated that fouling communities differ with depth and time but are driven by some ecological interactions, and that, a combination of washing and then killing of hydroids with hot water; or washing and then drying can help farmers deal effectively with fouling. Further research into the feasibility of these on an industrial scale is recommended.

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1. Introduction

1.1. Background

Fouling refers to the process by which unwanted material accumulates at an interface; with the fouling material made up of living or non-living compounds (Dürr & Thomason, 2010). Fouling has been reported from various substrates and industries including shipping, oil and gas platforms, aquaculture, on other living organisms, in water bodies, heating installations, power stations, water pipes, in medicine among others (Dobretsov; Jass, Surman &Walker, 2005; Henderson, 2010; Cowie, 2010; Romani, 2010).

Fouling by living organisms is termed as biofouling (Lovegrove, 1979). Other non-biological forms of fouling include the settlement of suspended solids on surfaces, chemical reaction fouling, and the precipitation of certain solids out of solution onto pipes and other surfaces (www.wlv.com). Biofouling is the most detrimental form of fouling; as it is almost always a problem where it occurs due to the fact that it negatively affects surfaces; the materials they are made from and even destroys these surfaces; and also because invasive fouling species are difficult to manage (Durr & Thomason; 2010; Piola, Dunmore & Forrest, 2010).

1.2. Biofouling in the marine environment

Biofouling in the marine environment is a succession of organisms; which progresses from a bacterial biofilm, diatoms, filamenous green algae, red and brown algae, sessile animals, errant benthic and epibenthic organisms; to commensals, parasites and pathogens. (Dennington, n.d.; GISP, 2008). Records of biofouling in the marine environment date as far back as the 5th century B.C; and was mostly in connection to shipping vessels; although it is known that marine fouling must have been known for many years before a written language was ever devised (WHOI, 1952). Among the earliest references to fouling organisms are of the sharksucker, *Remora sp.*, which is found firmly attached to sharks and other large marine organisms. These fish were reported in ancient Roman times attached to ship hulls and slowed ships speeds greatly; which might often be a great cost in battle (Leao, 2002; McClane, 1998, WHO1, 1952). Other species which have been reported since ancient times include barnacles, shipworms, woodborers and bivalves (GSIP, 2008).

Over 5000 different species of fouling organisms worldwide have been reported from various literature (Abdul Azis et al., 2003). Biofouling is much more extensive in tropical waters than it is in temperate waters; but seasonal changes in fouling communities are not as pronounced as seen in temperate waters (WHOI, 1952; Poloczanska & Butler, 2010). On a global scale, the most common fouling organisms are ascidians, bryozoans, barnacles and mussels, with ascidians reported from the northern hemisphere and barnacles having an almost universal distribution (Canning-Clode & Wahl, 2010). Fouling macroalgae also tend to have a vast distribution with the genera *Ulva, Ceramium* and *Polysiphonia* found at several sites worldwide (Canning-Clode & Wahl, 2010).

Fouling on offshore oil and gas platforms is dominated by space-holding taxa, typically made up of macroalgae, molluscs, bryozoans, coral, sponges and hydroids among others (Page, Dugan & Piltz, 2010). Fouling communities of offshore platforms, piers and marinas have been found to differ greatly from those on nearby rocky outcrops or shores (Telizza & Fiamali, 2011; Greene & Grizzle, 2007; Glasby, 1999).

In industry, biofilms form on high pressure filtration membranes in desalination plants; disrupting the flow of water (Henderson, 2010). Fouling by macroalgae, hydroids and bivalves (mussels) may block intake pipes, filters, and even affect the motion of turbines and wheels, leading to flow disruptions, breakdown of machine parts, erosion damage and a risk of infection by pathogens (Henderson, 2010; Stanczak, 2004; Dennignton, n.d.). Biofouling is also a big problem reported from mariculture installations with the most documented impact for sea based aquaculture (de Nys & Guenther, 2009; Braithwaite & McEvoy; 2005).

Over the years; biofouling has also increased the rate of species invasions (Lewis & Coutts, 2010). Fouling organisms which hitch rides on the hulls of ships and in ballast water are introduced inadvertently through deliberate or unknown means and have taken over from native species in areas where there are no natural predators and where environmental conditions are favourable (Lewis & Coutts, 2010; Coutts, 1999; Stanczak, 2004; GISP, 2008). Among the most well documented species invasions is the case of the zebra mussel (*Dreissena polymorpha*) which is a freshwater species that has been transported via ship hulls and ballast water through oceans, canals and seaways into inland water bodies where they have outcompeted native bivalve species, clogged intake pipes, and harbour potential

pathogens due to bioaccumulation from their filter feeding (GISP, 2008; Bruner, Fisher & Landrum, 1994). *Mytilus edulis* and *M. galloprovincialis*; which are temperate bivalve species, have also been recently introduced into non-native areas through increased maritime activity (Lewis & Coutts; 2010).

Although biofouling seems to have several detrimental effects, farmers have been able to take advantage of fouling by using this phenomenon to seed mussel and scallop lines in shellfish aquaculture (Bardach, Ryther & McLarney, 1972; Mallet & Carver, 1991; Aypa, 1990; Cyr et al., 2007).

1.3. Biofouling in mariculture

Aquaculture is becoming an important industry because of the decline in global fisheries; with global aquaculture production reaching 59.4 million tonnes per year, with 7.68 % of the global value contributed by the Western European Region (Dürr & Watson, 2010; Cook et al., 2008). The mariculture industry has reported significant impacts of fouling on aquaculture installations, and the problem is expected to become more severe with time due to the increased growth in the aquaculture industry (Braithwaite & McEvoy, 2005; Carl, 2008; Dürr & Watson, 2010). The high level of waste generated from aquaculture systems through losses of fish food and faeces increases nutrient levels which becomes a suitable food material for non-selective filter feeders; among them ascidians, polychaetes, barnacles and bivalves (Braithwaite & McEvoy, 2005).

Of all aquaculture systems, the production of diadromous and marine finfish species is dominated by the use of sophisticated cage aquaculture systems, with marine salmonid culture being dominated by Norway, Chile, the United Kingdom and Canada (de Nys & Guenther, 2009). Fouling can decrease the product value of farmed species by up to 90%, and about 5-10% of the industry value is used for dealing with biofouling and its related problems on a yearly basis (Dürr & Watson, 2010, Lane & Willemsen, 2004).

Multi-filament netting is an ideal substrate for fouling since it has many crevices which can entrap and protect settling organisms (Hodson, Lewis & Burke, 1997). Fouling in mariculture is dominated by mussels (*Mytilus edulis*); ascidians (*Ciona intestinalis*), barnacles (*Balanus spp*. and hydroids (*Tubularia/Ectolpleura* genus). Other mobile species like amphipods, sea urchins and sea stars are also included since they can be found as a part of fouling

communities. In Western Europe, most fouling consists of six groups, including algae, hydroids, serpulids, mussels, barnacles and ascidians, with the algae dominated by *Enteromorpha (Ulva*) s*p*. and *Ectocarpus sp*. Macroalgae, however, do not pose as much of a problem as the other fouling species do because they occur in lower numbers, are restricted to the upper part of the cages and do not possess the weight and size of other fouling organisms (Braithwaite & McEvoy, 2005, de Nys & Gunether, 2009, Dürr &Watson, 2010).

1.3.1. Effects of biofouling in mariculture

The principal effect of biofouling on cages is the restriction of water flow through the occlusion of nets, with an increase in the effect as the fouling communities are forming a complex three dimensional community (de Nys & Guenther, 2009).The tendency of fish farmers to increase the size of net pens and maintain the net pens in the sea for an entire production cycle causes the effect of restricted water exchange to become more severe (Sunde et al., 2003).

When the flow of water though cage netting is restricted through fouling; dissolved oxygen levels in the cage drop dramatically, leading to stress on the fish, reduced feed uptake, and even mortality in some cases. This is a problem in temperate countries in summer, when the high temperatures and increased oxygen demand by organisms drop dissolved oxygen levels even further (de Nys & Guenther, 2009; Dürr & Watson, 2010, Braithwaite & McEvoy; 2005).

Heavily fouled nets can also increase drag on nets three fold, increase the drag coefficient by up to 900% which affects cage structure and behaviour in rough seas and high current conditions. The increased stress on the netting could weaken the netting and also compromise the integrity of cages (Dürr & Watson, 2010, Greene & Grizzle, 2007).

Fouling on cage nets may also serve as a reservoir for pathogens or parasites; which could lead to a greater susceptibility of the already stressed fish, causing disease. Viral pathogens of fish could also be bioaccumulated in fouling shellfish; and the high stocking densities in fish farms could also facilitate the rapid transmission of disease from attached fouling communities to the fish in the cages (de Nys & Guenther, 2009, Dürr &Watson, 2010; Swift et al., 2006).

1.3.2. Anti-fouling strategies in mariculture

Most of the antifouling strategies in use for aquaculture have been limited to cleaning, natural predators and the use of some antifouling coatings; with multiple strategies being used simultaneously (Dürr & Watson, 2010). Antifouling strategies adapted methods from the shipping industry through the use of antifouling paints and coatings (Abdul Azis et al., 2003, WHOI, 1952, Braithwaite & McEvoy, 2005).

Shore–based and *in-situ* net cleaners involve the use of automated cleaners which use a strong jet of water to dislodge fouling organisms. However, the in-situ cleaning of cages is complicated by the three-dimensional nature of the netting; and the fact that frequent cleaning selects for fast growing fouling communities (Hodson et al., 1997).The frequent changing of nets for cleaning is cost and labour intensive, and the drying of nets to compost and kill off fouling also reduces the efficiency of fish farm operations (Dürr & Watson, 2010; deNys & Guenther, 2009, Braithwaite & McEvoy; 2005).

The use of biocides and anti-fouling coatings from natural sources has also been documented. Lai et al. (1993) reported the use of tannins from plant sources as a source of biocides to control fouling on absorbent cotton nets (Baithwaite & McEvoy, 2005). However, the most common means of combating fouling has been through the use of antifouling coatings which are mostly made from toxic materials. Tributylin coatings, which are highly toxic to the environment have led to problems related to imposex in dog whelk species, accumulation in salmon and human tissue among others, were used as an antifouling coating in aquaculture nets until they were banned over environmental concerns (Davies & McKie, 1987, Dürr & Watson, 2010, de Nys & Guenther, 2009). Antifouling coatings in use today involve the coating of cage netting with copper based paints. Copper based antifouling netting has proven to be effective against biofouling; and it has been shown that these coatings do repel most fouling species (Dürr & Watson, 2010; de Nys & Guenther, 2009). Concerns have however also been raised over the possible leaching of copper from the coatings into the marine environment and the potential problems it could cause to marine organisms (Willemsen, 2005; de Nys & Gunether, 2009).

Based on these concerns, studies into the possible use of practical, cost effective and environmentally friendly methods of dealing with biofouling have been carried out or considered. Trials involving the use of hot water and steam (Blakemore & Forrest, 2007;

Guenther, Fitridge & Misimi, 2011); acetic acid, hypochlorite and hydrated lime Piola, Dunmore & Forrest (2010) as antifouling strategies were have shown to be effective against most fouling organisms.

1.4. Biofouling in Norwegian aquaculture

Norwegian aquaculture is highly sophisticated and geared mostly towards salmonid production; with cages reaching to up to 20 m in depth and circumferences from 90 to 160m (Carl, 2008, Guenther, Misimi & Sunde, 2011).

The hydroid *Ectopleura larynx* (syn. *Tubularia larynx*) has become the most common and dominant fouling species on aquaculture nets in southwest and central Norway within the last 20 years, dominating the community between July and November; with farmers having serious problems with hydroid fouling from July to October (Carl, 2008; Guenther et al., 2010). Other fouling organisms in the fouling community in these waters are filamentous algae, molluscs (*Mytilus edulis*), solitary ascidians, caprellid amphipods and nudibranchs (Guenther et al., 2010; Carl, 2008; Pudota, 2011).

Filamentous algae are found at the upper depths; while hydroids and caprellid amphipods are found at all depths from September until December (Guenther et al., 2010). A survey carried out by Carl (2008) revealed that farmers had different opinions about hydroid colonization of nets, with some farmers reporting that hydroids colonized the nets from the surface downwards, with others reporting that fouling colonization started from the bottom upwards. Most of the farmers however reported a shift in fouling composition as hydroids became the dominant fouling species.

Most Norwegian fish farmers clean their nets at least once a month during the summer, and employ a combination of different cleaning methods to curb fouling (Carl, 2008). Copper makes up the active ingredient used in marine antifouling coatings in Norway (Olasfen & Sandberg, 2006). However, two of the most common fouling species (*E. larynx* and caprellid amphipods) in Norwegian waters have been found to be tolerant to copper exposure (Guenther et al., 2010). The three main strategies used to get rid of fouling by farmers include: copper- based coatings on nets combined with washing, copper-based coatings on nets combined with frequent drying of nets onsite; and the use of uncoated nets combined with frequent cleaning (Olafsen, 2006). Most farmers tend to use the copper-based coating

combined with washing strategy, since the copper-based coatings become less effective with time and do not deter hydroid settlement (Carl, 2008; Olafsen, 2006, Guenther et al., 2010).

The complete removal of *E.larynx* from fouled nets is virtually impossible due to the complex mode of attachment to the filaments of salmon cage netting (Carl, 2008, Hodson et al., 1997). Hydroids which have been cut during the washing process are reported to grow back even faster than before; and the washing process creates loose filaments which could actually facilitate better hydroid attachment; and the reproductive fragments released into the water column could immediately recolonise net surfaces (Carl, Guenther & Sunde, 2011; Guenther et al., 2010; Hodson et al., 1997).

1.5. The hydroid *Ectopleura larynx*

E. larynx (Ellis & Solander, 1786) is a marine hydroid which belongs to the family Tubulariidae (Hydroida, Cnidaria, Hydrozoa); and is common along the boreal coast, and can be found in shallow waters up to 35m from the surface (Schuchert, 2011). It is made up of the hydrorhiza which is formed by the horizontal, branched stolons which are affixed to the substrate, the hydrocaulus which is the erect stem; and the hydranth which is the feeding polyp. "Polyp" is also used to refer collectively to the different feeding and reproductive polyps on the hydroids (Carl, 2008; Gili & Hughes, 1995). The polyps of a hydroid are connected to other polyps by a continuous periscarc which facilitates the transport of materials from the hydranths to other parts of the colony (Gili & Hughes, 1995). Hydroid morphology is greatly affected by factors such as pollutants, temperature, and salinity. Low concentrations of metal ions may actually increase the growth rate of hydroids. Hydroids will also be more numerous in areas where water movements are sufficient but not fast enough to cause damage. *Ectopleura* are favoured in relatively exposed areas (Gili & Hughes, 1995). Studies by Nellis & Bourget (1996) showed that the maximum hydroid (*E. larynx*) biomass occurred at 12 m; where the minimum average current speed of the year of the experiment was also recorded.

The diet of hydroids is varied; but dominated by zooplankton which can in turn be found in dense numbers around fish farms due to the increased nutrient input. Some prey items include nauplii, copepodids, foraminiferans and larvae of larger invertebrates; and even fish may be consumed (Gili & Hughes, 2005).

Many caprellids often use hydroids as a shield to hide from predators, and also attach themselves to hydroids to take advantage of faster currents for suspension feeding. Hydroids themselves are in turn heavily preyed upon by nudibranchs, gastropods, pycnogonids, and polychaetes among others (Gili & Hughes, 2005, Salvini-Plawen, 1972, Boero, 1984).

1.6. Aims of study

There are conflicting reports on the development of the fouling community on salmon cages in Norwegian waters, and there is a need to develop environmentally friendly solutions to curb biofouling. It would be necessary to test the efficacy of the methods that are already in use by farmers and find ways of reducing the amount of labour and time needed for the removal of biofouling. This study therefore seeks to examine fouling development on salmon cage netting and analyze the effects of various treatments on the survival of *Ectopleura larynx*. The specific aims of this study are to:

1. Identify the temporal and depth variability of biofouling on salmon cage nets from a farm in mid- Norway.
2. Determine the effect of air drying on the survival of hydroids
3. Determine the effects of environmentally friendly anti-fouling treatments on hydroid dominated communities.

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2. Materials and methods

2.1. Study site

The field experiments were carried out on the SalMar ASA fish farm site situated at Korseneset, Halsa (63°08.565N, 08°13.496'E). Halsa is situated on the western coast of central Norway, in the Møre og Romsdal region. The fish farm is approximately 147 kilometers south-west of Trondheim. SalMar is one of the world's largest and efficient producers of farmed salmon (www.salmar.no). They harvested a total of 78,500 tonnes of Atlantic salmon in 2010; out of which 47,200 tonnes were farmed in central Norway (SalMar yearly report, http://hugin.info/138695/R/1510833/446008.pdf). The sampling site is one of several in central Norway which engage in the farming aspect of the company's operations. A total of six cages were in use at the site. The cages were 150 m circumference and the nets were copper-coated nylon (50 mm stretched mesh). The cages had sinker tubes at 15 m depth, after which the nets taper off into a cone at a total depth of 30 m.

The cages were anchored in the littoral area of the fjord and very close to the rocky shore. 6 cages were in use at the site. One cage was empty. Two of the cages were deployed in March 2010 and the fish were slaughtered in the summer; three had been in the water since May 2010 and the fish were slaughtered in October 2011. The net frames for the experiment were therefore deployed on the side of one of the cages to be slaughtered in late October.

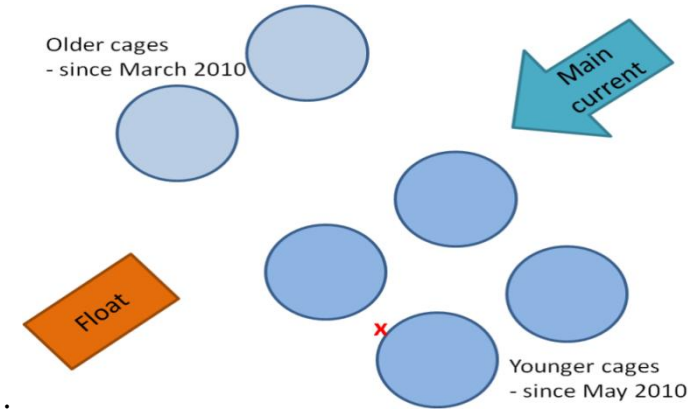

Figure 1: Cage position at the study site. The red cross shows the site of placement of the net panels.

2.2. Field methods

2.2.1. Monthly development of biofouling

Three experimental frames were built and connected to each other at 1, 5 and 10 m with nylon rope. Each frame was made of 35mm inside diameter PVC pipe, and was designed to accommodate 4 net replicates (white, knotless uncoated nylon netting). The nets were had a stretched mesh size of 50 mm. All the net pieces had 7x7 mesh openings, and were 0.4 m² in area. The entire set-up was anchored with a 5 kg iron weight. The other end of the rope was secured to the floats of the cage. The PVC frames were deployed outside the cages to allow for easy sampling of the net panels.

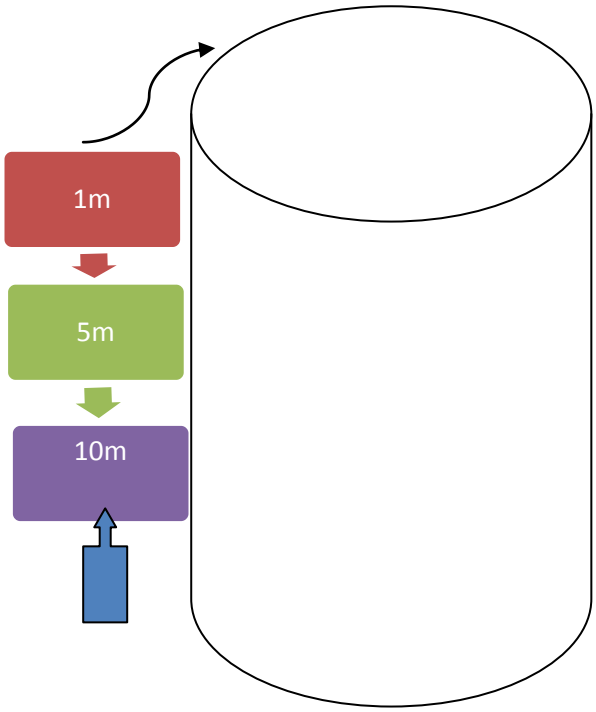

Figure 2: Frame placement beside cage. Cylinder represents the cage; and the coloured boxes represent the three panels.

Figure 3: Net panel with net pieces.

The first placement of nets was carried out on the 24th of May, 2011. The initial panels were harvested a month later in June, and new nets fixed for sampling in July. This was repeated monthly until November when the field work ended.

The collected nets were grouped according to depth and placed in marked 10 L plastic buckets filled with about two-thirds seawater from the site and transported to the laboratory for weighing, preservation and analysis.

2.2.2. Effect of drying on hydroid survival

To determine the effect of different drying regimes on hydroids, collector panels for the drying experiments were deployed on the 2nd of August, 2011. These panels consisted of 3 separate experimental panels bearing 12 nets each. The dimensions were 50 mm stretched mesh size, with 7x7 mesh openings. These were randomly placed at 10 m depth on different parts of another cage for hydroid collection in September.

Mature hydroid colonies were plucked off the collector nets on the 20th of September, 2011. These colonies were rinsed in seawater to release trapped matter and debris, and then placed

into 3 plastic 10 L buckets filled to about two-thirds of their volume and transported to the laboratory.

2.2.3. Effects of anti-fouling treatments on the survival of *E. larynx*

A rectangular PVC panel with 20 net pieces was placed beside the cage at 5 m in late August for field experiments. White, uncoated knotless nylon nets (26 mm stretched mesh size) with 9x9 mesh openings were used. Each net panel was 0.0225 m^2 in area. This panel was harvested in early October for treatments.

Four treatments with 5 replicates each were employed in the study. These treatments involved 'new nets' (five nets with hydroids were removed and sent to the laboratory for community analysis and replaced with the new nets), 'alive' (live hydroids), 'alive and washed', 'dead' (immersed in hot water), and 'dead and washed'. The nets were photographed and weighed on the farms' feeding barge.

Hydroids for the 'dead' treatment were killed on-site by immersing the hydroids in hot water at 60°C for 5 seconds. This was done for the two treatments involving 'dead hydroids'. This has been proven by Guenther et al (2011) to be highly effective at causing mortality to adult hydroids. Where after immersion, the temperature had dropped a few degrees; the hydroids were kept for a few more seconds to ensure all the hydroids were dead. Dead hydroids turned a dull shade of brown.

The 'washed' treatment involved flushing the nets with a high pressure jet of water to stimulate the automated net cleaning washing process employed by the fish farmers. Only one side of the net panels was washed in this study. The nets were weighed again after the treatments; and randomly fixed back to the frame to be placed in the fjord for a recovery period of two weeks.

At the end of the recovery period, all the nets were photographed, and weighed. Each net was then cut up into 6x6 openings (0.01 m^2) and then fixed with a seawater solution of 10% strength formalin buffered with borax (di-sodium tetraborate decahydrate by Merck Pharmaceuticals) for laboratory analysis.

Figure 4: Panel before treatment.

Figure 5: Panel after recovery period

2.3. Laboratory methods

2.3.1. Monthly development of biofouling

Each net replicate was blotted and wet weights measured. A Mettler Toledo AG204 Delta Range® electronic balance was used and weights were rounded off to two decimal places. After each net was weighed, 3x3 mesh openings (0.01 m^2) were cut out and then weighed. These were used later for biomass analysis and species composition. After weighing, each 3x3 piece attached to a frame and then placed into a rectangular 19L plastic bucket which had been filled with seawater. A frame with an aperture for a camera lens was positioned above the bucket. The purpose of the frame was to give a constant distance from the net panel to the lens for consistency in the photographs. A Canon PowerShot S80 camera housed in a waterproof casing was used to take photographs of each net.

Each net was then placed into a plastic bottle containing about 200ml of seawater. Formalin (37% stabilized with 10% methanol, Merck Pharmaceuticals) was added to make up about 10% buffered seawater concentration and borax was added to serve as a buffer. This brought the concentration of formalin itself to about 3.7% buffered strength.

2.3.1.1. Microscopic analysis:

Preserved net samples were thoroughly washed in a fume hood (air flow 0.5 m/s) under running water to remove as much formalin as possible. This is necessary since formalin and borax are both highly toxic; borax affecting fertility (container label) and formalin being carcinogenic, allergenic and highly toxic upon direct exposure even to its' fumes (www.osha.gov , 2006).

The washing was done with the aid of an improvised continuous flow system which consisted of a cylindrical sieve with 300 micron mesh. The sieve was 12 cm high and 16 cm in diameter. This was placed on a stand which was in turn placed in a plastic container which was fitted with an outlet pipe. Each preserved net sample was placed in the sieve and washed with freshwater from a tap for about an hour.

After a net was washed, it was transferred into a Petri dish and the sieve back-washed to remove any remaining fouling organisms from the cylinder.

This was then observed under a Leica MZ7S stereomicroscope which was fitted with a Nikon DS-5M L1 digital camera system. Species were identified to the lowest possible taxon, and a presence /absence inventory recorded. All fouling was removed from the nets and sorted into fouling groups. Species which could not be identified immediately were photographed and preserved for later identification.

The five broad groups used for quantitative biomass analysis were: hydroids, molluscs, algae, crustaceans and bryozoans. This method was chosen because all the organisms observed in the study fell into these broad groups.

The sorted organisms were placed in labeled, pre weighted glass vials; and then dried in an oven at of 60 °C for 72 hours. The vials were then placed in a desiccator for two hours; and the dry weight of the groups recorded as biomass. The dry weights were recorded to four decimal places using a Mettler AE260 Delta Range® electronic scale. This was done for all the months and for all depths.

2.3.2. Effect of drying on hydroid survival

Hydroids collected from the experimental panels were acclimatized overnight in plastic containers which had a continuous flow of filtered seawater to maintain freshness and give aeration. The water was kept at a constant temperature of 10 °C to mimic the temperature of the seawater from the fish farm. Room temperature was kept at 12 °C, and humidity was kept at 93%.

The next day, 60 live hydroid colonies with at least 60 live hydranths each were selected and then each colony then transferred randomly into sixty 2 L glass beakers which had a continuous flow of filtered seawater. The hydroids were deemed to be alive when they were a bright pink in colour, were observed to be moving their tentacles in seawater, and /or reacted to touch (Ushakov et al., 1977) under a stereomicroscope (see chapter 2.3.1.1). The glass beakers were fed with seawater from Pasteur pipettes. The pipettes were connected to plastic tubing, which were in turn also connected to four inlet pipes. 4 large plastic buckets fitted with outlet pipes housed 15 beakers each. Seawater from a mains supply was provided via the four pipes. There were 10 replicates for each of the 6 treatments.

An improvised drying rack was made with wire gauze, and 50 individual colonies were placed on them to air dry. The other 10 were randomly distributed in the beakers and were the control group. The drying times were 0, 2, 6, 12, 24 and 48 hours respectively. 10 hydroids were removed from the rack at the end of each drying period and randomly placed in the glass beakers. The position of each replicate was marked and noted. Especially for the 12, 24 and 48 hours drying treatments; the hydroids sometimes stuck fast to the gauze.

To prevent a loss of hydranths which could compromise the outcome of the experiment, these colonies were cut out with the wire gauze still attached and placed in their beakers. The hydroids came free off the wire gauze after a few hours; after which the gauze was taken out with no damage to the hydroids.

The hydroids for each treatment were allowed to recover for 48 hours, after which they were transferred to Petri dishes filled with seawater and then observed under the microscope (see chapter 2.3.1.1). 50 individuals (hydranths) were counted for each colony and the percentage survival of each replicate per treatment recorded.

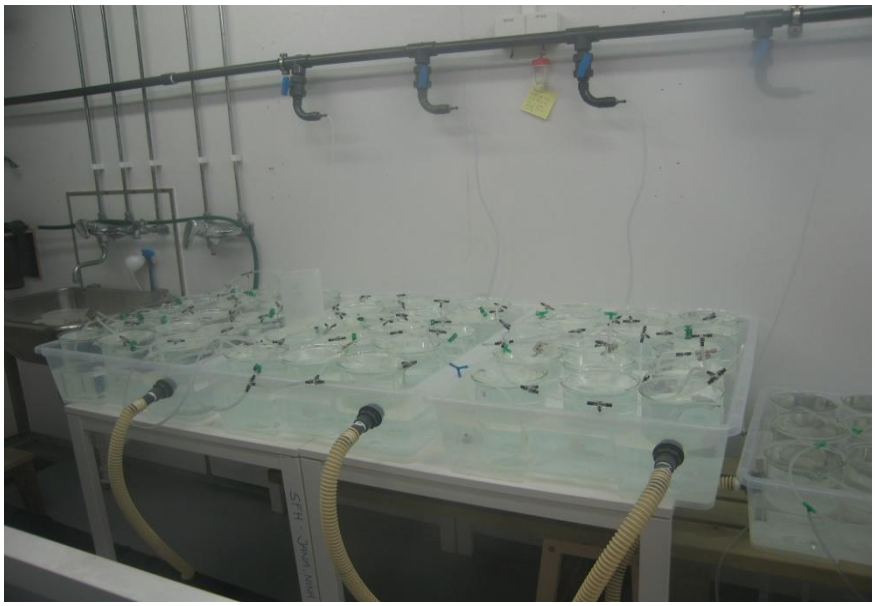

Fig. 6: Laboratory set- up for drying experiments

2.3.3. Effects of anti-fouling treatments on the survival of *E. larynx*

The net pieces for this experiment had already been fixed in formalin in the field**,** and were washed and sorted under the same conditions and microscope as outlined in chapter 2.3.1.1.

2.4. Statistical analysis

Statistical analysis for three experiments was performed using IBM SPSS Statistics version 19 (SPSS Inc, 2010). This software was used for all descriptive statistics. Two-factor ANOVA (analysis of variance) was employed to test for the any interactions between depth and month in fouling development; followed by Tukey's HSD multiple comparison test ($\alpha = 0.05$).

The results of the drying experiments were tested using one-way ANOVA to compare means and test for significant differences between drying times. In the case of the fate of dead hydroids experiments, repeated measures analysis, followed by Tukey's HSD multiple comparison test ($\alpha = 0.05$) was carried out. Where the assumptions of tests of normality and homogeneity (Levene's test) were not met; a square root transformation was carried out to normalize the data. Since ANOVA is assumed to be robust when such assumptions are not met and sample sizes were equal in all the experiments; the results of the ANOVA was reported (Underwood, 1997; Quinn & Keough, 2002).

MDS (Multidimensional scaling) plots generated using PRIMER (Clarke & Gorley, 2006). MDS is a useful tool for exploring similarities in species composition over the sampling period due to its flexibility (Clarke & Warwick, 1994).

All data were presented as mean ± SE (standard errors), and error bars on graphs showed 95% confidence intervals.

3. Results

3.1. Biofouling on nets

3.1.1. Monthly development of biofouling

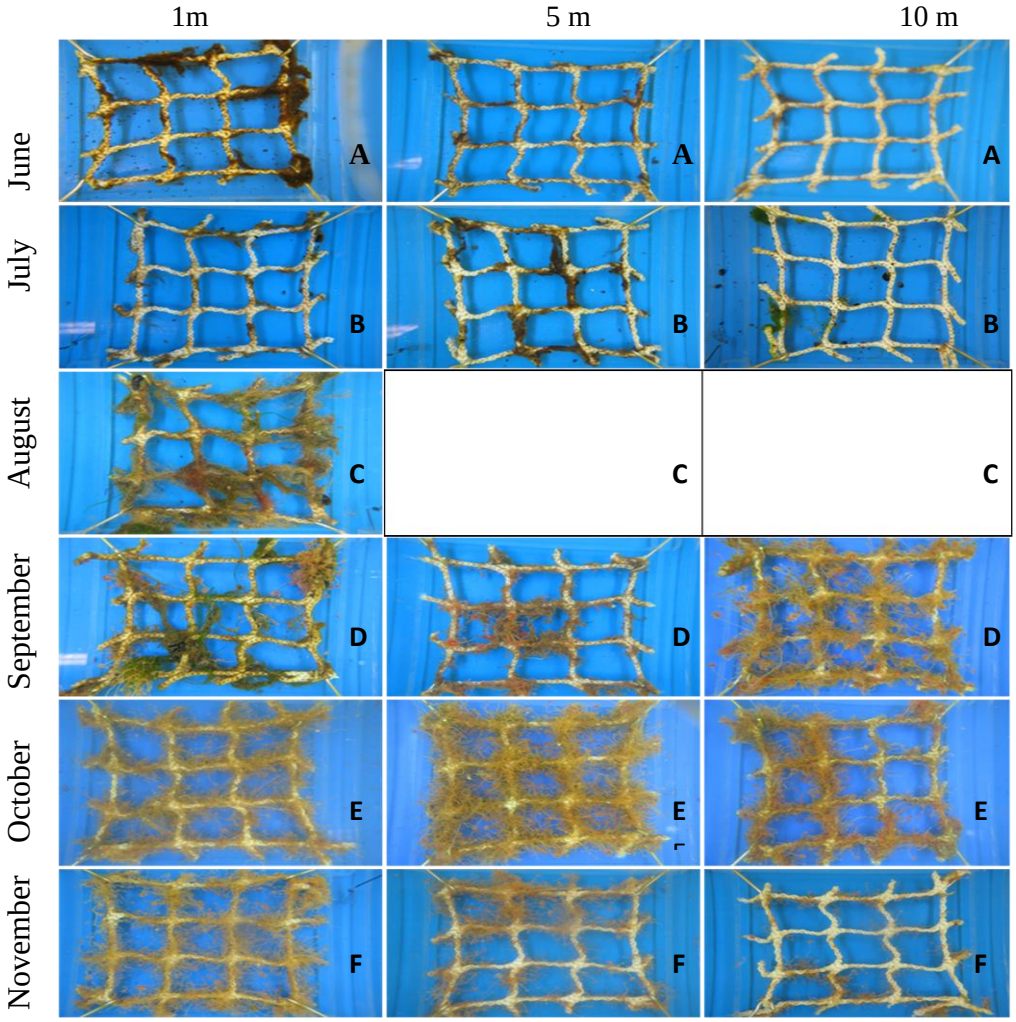

Figure 7: Pictorial representation of the monthly development of biofouling on cut-out nets with depth and time. The first column from left shows 1 m, middle column, 5 m and third column , 10 m. Rows show months from June through to November: June (A), July (B), August (C), September (D), October (E) and November (F). Note the blanks for August at 5 and 10 m, when no data was available.

Biofouling was observed on all the monthly net panels at all depths (Fig. 7), except for the 1 and 5 m depths in August; when the no data was available. It was however varied, with differences in community composition and biomass.

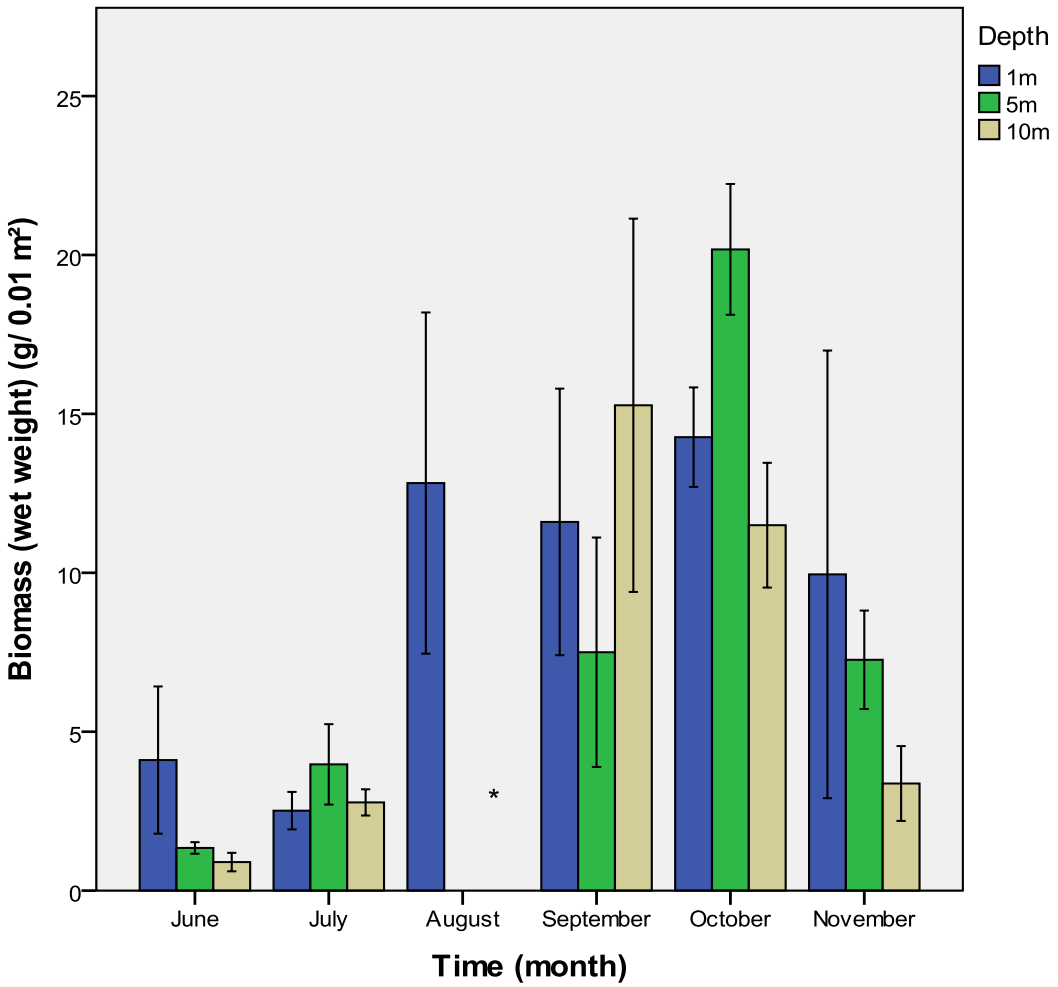

Figure 8: Mean biomass (wet weights) from the cut out net pieces at the three different sampling depths with time. There was no data available for 1 and 5 m in August (*). Error bars express 95% confidence interval.

The highest biomass in wet weights of fouling during the study occurred in the month of October at 5 m (Figures 7 & 8). The lowest biomass was recorded in the month of June at 10 m. Fouling at 1 m fluctuated with time. At 5 m, fouling increased steadily from June until October. Fouling at 10 m also increased monthly from June until September, after which it

began to decrease. Fouling was at higher at 5 m in July and in October than at 1 m and 10 m for the same months.

At 1 m, the highest wet weight recorded was for the month of October (mean ± SE, 14.27 ± 0.49 g), and the lowest was in July, 2.51 ± 0.19 g. At 5 m, the highest weight was recorded in October, 20.2 ± 0.65g), and the lowest in June, 1.34 ± 0.06 g. At 10m, the highest wet weight was recorded in September, 15.27 g ± 1.85; and the lowest was 0.90 ± 0.09 g for the month of June.

Table 1: ANOVA tests of between-subject effects of biomass (wet weights) on cut net panels at different depths (1, 5 and 10 m) over the six sampling months. These results are based on the square root transformation of the data.

Tests of Between-Subjects Effects

Dependent Variable: square root

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	68.284[a]	15	4.552	43.963	.000
Intercept	428.373	1	428.373	4136.985	.000
Depth	1.858	2	.929	8.972	.000
Month	55.259	5	11.052	106.733	.000
Depth * Month	9.610	8	1.201	11.601	.000
Error	4.970	48	.104		
Total	517.300	64			
Corrected Total	73.254	63			

a. R Squared = .932 (Adjusted R Squared = .911)

Although there was a significant effect of time ($p < 0.05$) and depth ($p< 0.05$) on biomass, there was also a significant interaction between depth and month; ($F= 11.60$, $p < 0.05$; Table 1). This interaction was driven by the fact that the wet weight of fouling in the months of June and July were lower than in the succeeding months (August-November). Also, fouling was generally concentrated at 1 and 5 m, and was higher than at 10m. After August however, fouling at the lower depths increased (at 5 and 10 m) in the months of September and October (see Figure 8).

3.1.2. Fouling diversity

Table 2: Taxa and the major species observed on net panels during monthly study

Taxa	**Key species**
Algae	*Chaetomorpha sp., Enteromorpha (Ulva) sp., Ectocarpus sp., Polysiphonia sp.*
Mollusca	*Mytilus edulis, Littorina sp., Hiatella arctica, Flabellina sp.*
Cnidaria (Hydrozoa)	*Ectopleura larynx, Obelia sp.*
Crustacea	*Caprella sp., Corophium sp.*
Bryozoa	*Membranipora membranacea, Electra pilosa*

A total of 28 organisms were identified from the laboratory experiments. These organisms were placed into 5 broad groups: algae, hydroids, molluscs, crustaceans and bryozoans (see Table 2).

Algae and molluscs dominated in June, July and August and were followed by hydroids from August onwards. The dominant hydroid in the samples over the duration of the 6 month sampling period was *E. larynx*. However; mollusc diversity changed from *M. edulis* and littorinids (June- August), to nudibranchs in November.

Crustaceans and bryozoans occurred on net panels in lesser amounts. No molluscs were recorded in October at all three depths. Solitary ascidians, mussels, numerous encrusting bryozoans and calcareous tube- building polychaetes were also seen in high numbers attached onto the main PVC frames as the months progressed. From July onwards, mussels and bryozoans were prevalent on the nylon rope used to connect the frames and also on the main PVC pipes (Figure 9).

Figure 9: Newly settled mussel spat (right arrow) and encrusting bryozoans (downwards arrow) attached to PVC pipe and nylon rope at 1m.

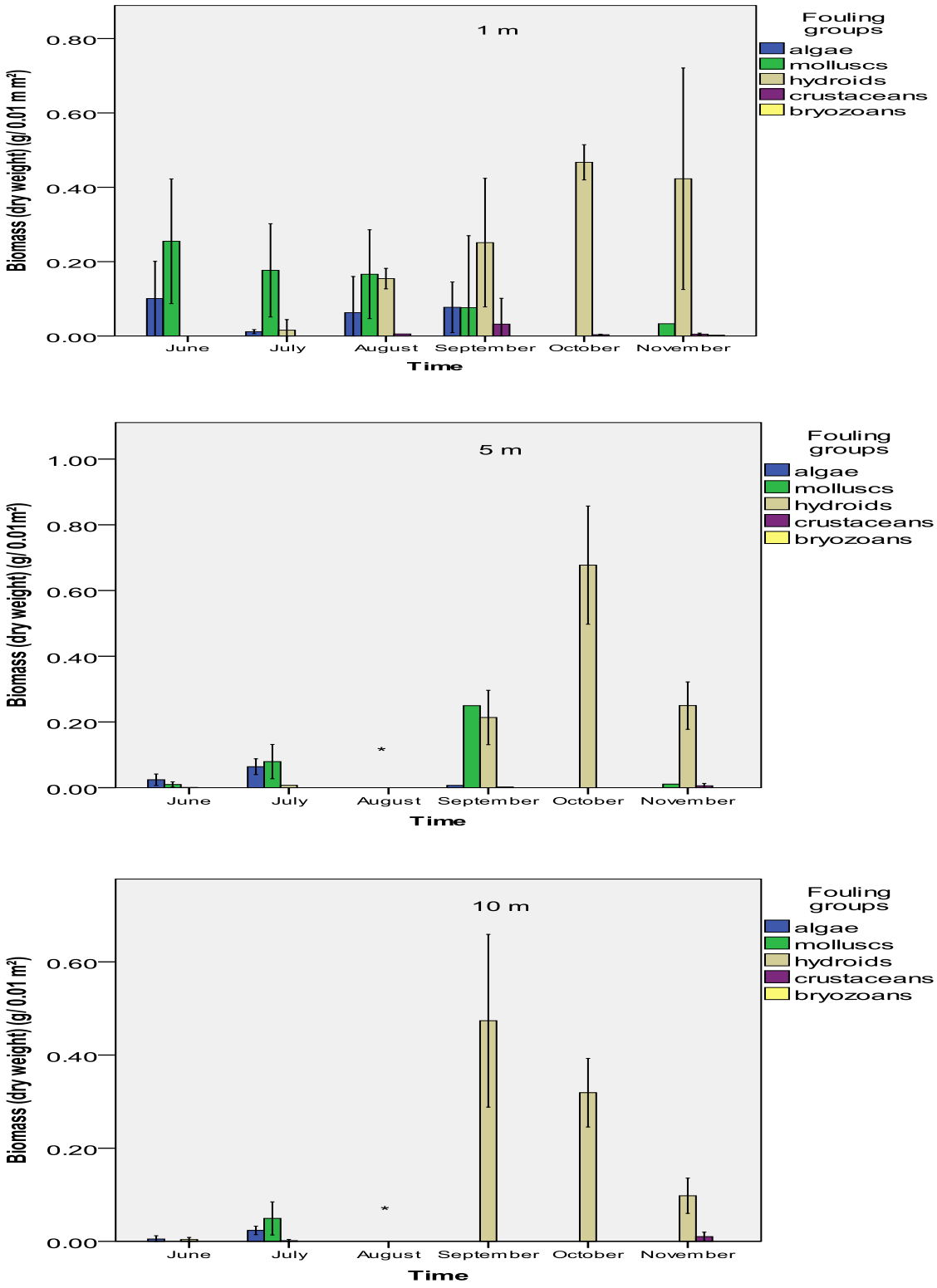

Figure 10: Mean fouling group distribution with depth (1, 5 and 10 m) over the six sampling months. Note the different y-axes at all three depths (*- no data was available). Error bars express 95% confidence interval.

At 1 m, fouling mainly consisted of algae and molluscs from June until August. The molluscs consisted mostly of newly settled mussel spat and littorinid snails. The mussels had attached themselves via byssus. There was a steady decline of molluscs at 1 m from June until the end of the field experiments in November; especially at 1 m. From August onwards, hydroids (E. larynx) and crustcaceans (amhipods) began to occur in high numbers (see Figure 10). Nudibranchs were the main molluscs recorded in November. Hydroids (*E. larynx* and *Obelia sp*.) began to dominate samples in September. The highest hydroid biomass at 1 m was recorded in October.

At 5 m, fouling also consisted mainly of algae and molluscs in June and July. There was no data obtained for August at this depth, but the highest number of molluscs recorded at this depth occurred in September. The highest hydroid biomass was in October. This was also the highest biomass recorded in the study (see Table 3 below). Crustaceans were recorded in high numbers in November.

At 10 m, the numbers of algae and molluscs for June and July were very lower than at 1 and 5 m (see Figure 10). There was no data for August. The highest hydroid biomass at this depth was in September. Crustaceans (amphipods) were recorded in November at this depth. Bryozoa occurred in negligible amounts in November.

Table 3: Biomass (dry weights) of taxa (where present) showing where the highest and lowest counts recorded, where present.

Taxa	**Highest biomass (dry weight) per cut net (g/0.01 m² ± SE) where present**	**Lowest biomass (dry weight) (g / 0.01 m² ± SE) where present**
Algae	0.1006 g ± .0315 (June, 1 m)	0.0050 g ± .0022 (June, 10 m)
Mollusca	0.2548 g ± .0527 (June, 1 m)	0.0097 g ± .0006 (June, 5 m)
Cnidaria (Hydrozoa)	0.6773g ± .0260 (October, 5 m)	0.0007 g ± .0000 (June, 5 m)
Crustacea	0.0120g ± .0030 (November, 10 m)	0.0021 g ± .0000
Bryozoa	0.0023 g ± .0000 (November, 1 m)	

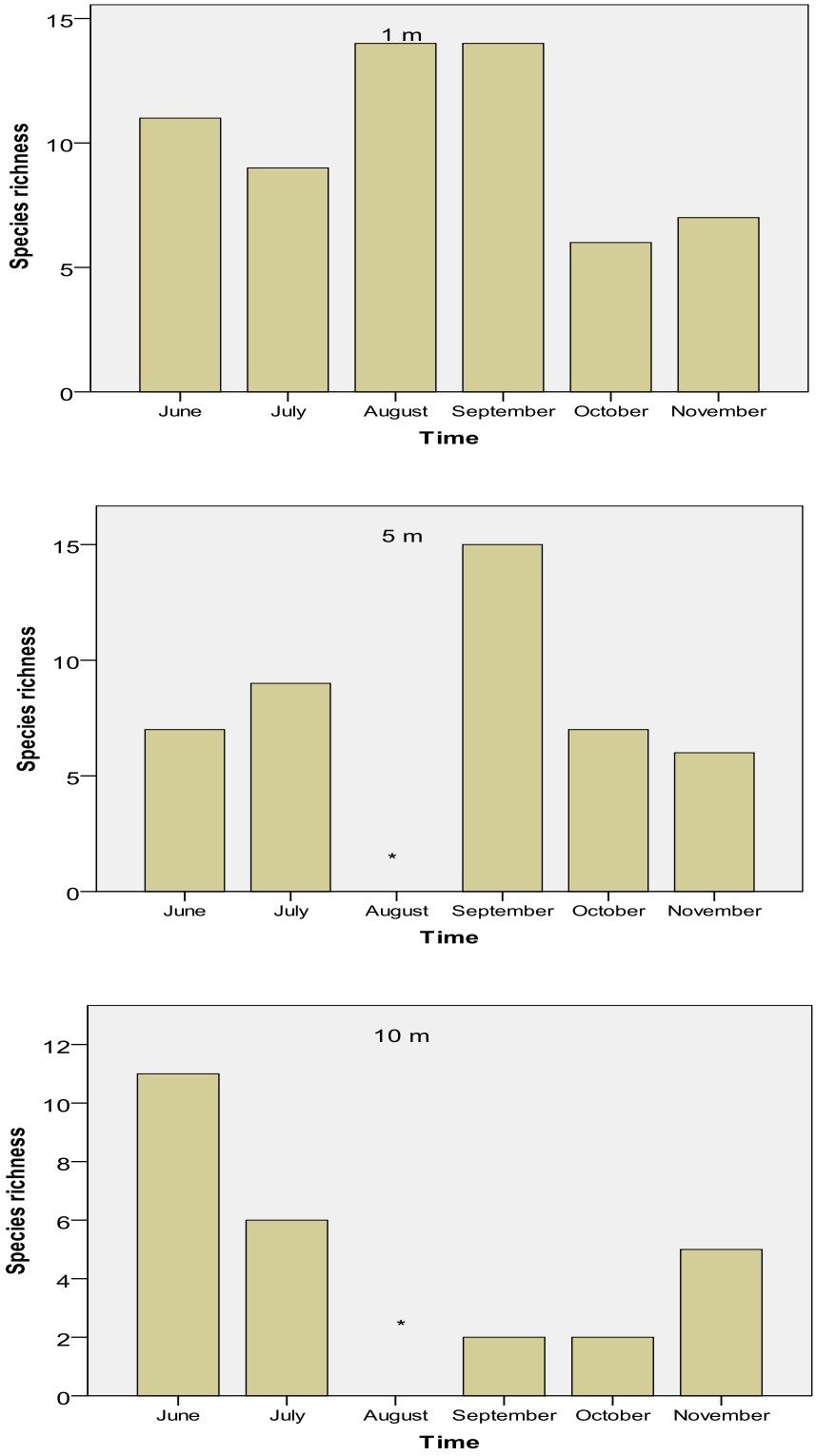

Figure 11: Species richness of fouling with depth (1, 5 and 10 m) over the six sampling months.. Note the different scale of the y- axis at 10m. No data was available for August at 5 and 10 m (*).

Species richness varied with depth and time (Figure 11). The highest species richness occurred in September at 5 m. Although the highest biomass at 10 m was in September, species richness was very low (2, see Figure 11). The same was also seen in October at 5 m, had a very low species richness (2); although the highest wet weight of fouling for entire experiment was from this depth.

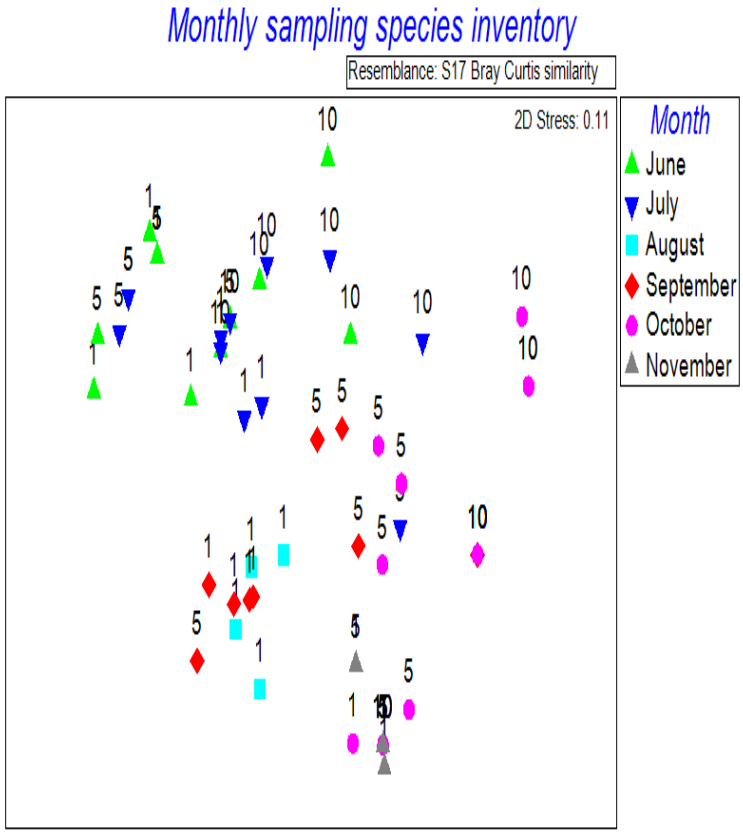

Figure 12: MDS of species abundance (using presence/absence data) compared from the three sampling depths (1, 5 and 10) over the six sampling months (stress= 0.11).

The results of the MDS plot for species diversity showed that species from November at all depths are similar to those from October at 1m (see Figure 12). Species recorded from August and September at 1m were also similar. There was also a similarity between June samples at 1 m and June samples at 10 m. Species at all depths for June and July also showed more similarity than samples for August and September at 1 and 5 m.

3.2. Effect of drying on hydroid survival

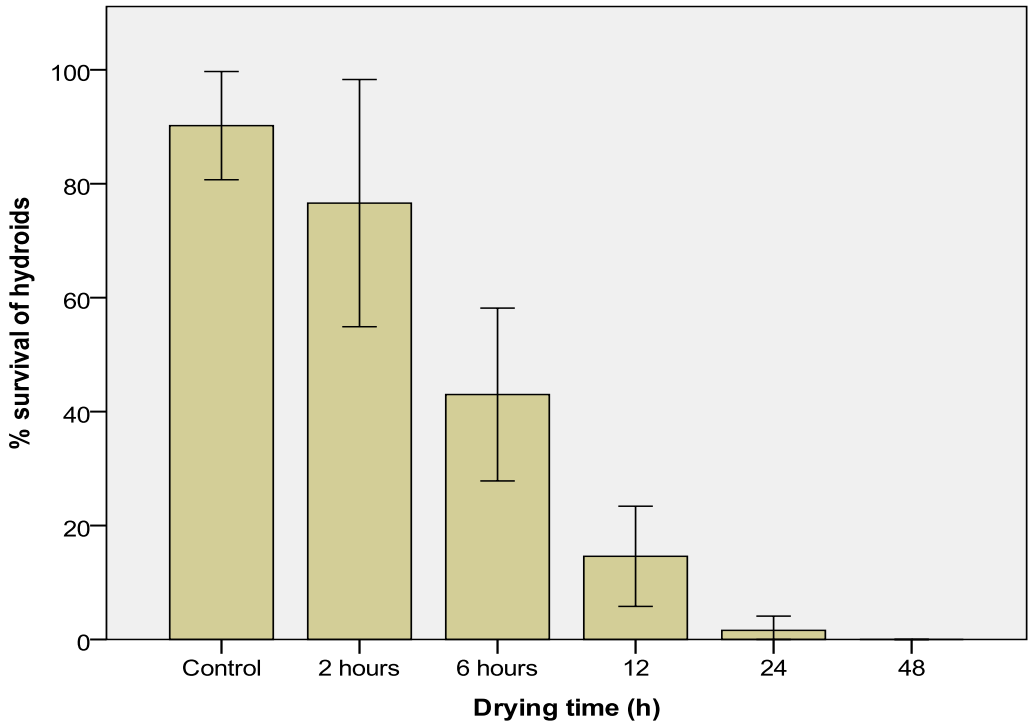

Figure 13: Percentage survival of *Ectopleura larynx* after different drying times. Error bars express 95% confidence interval.

Hydroid survival showed a steady decline with increased drying time. The control group showed the highest mean survival rate of 90.2 ± 4.20%, the 2 hour group; of 76.6 ± 9.60%, and the 6 hour group, 43.0 ± 6.71%. The 12 hour group had a survival rate of 14.6 ± 3.90 %, whereas 24 hours of drying gave a low survival rate of 1.6 ± 1.11%. 48 hours of drying led to a complete mortality of all the hydroid colonies (see Figure 13).

Live *E. larynx* colonies had their tentacles extended and moving, and were bright pink in colour. However, the longer the drying time, more the number of drying hours, the more pale and feeble the hydranths looked under the microscope, with a complete loss of hydranths, especially in the 24 and 48 hour treatments. Hydroids which showed a loss of hydranths or mortality had no moving tentacles, and showed a pale brown in colour.

There was a significant effect of drying time on the percentage survival of hydroids, $F_{(5, 54)} = 53.03$, $p < .05$, $\omega = .90$. It was also observed that, there was a "shielding effect" of sorts, where the uppermost hydranths died, but the new polyps at the base of the colony were able to survive. This was seen for drying treatments lasting for 6, 12 and 24 hours.

3.3. Effects of anti-fouling treatments on the survival of *E. larynx*

3.3.1. General trends

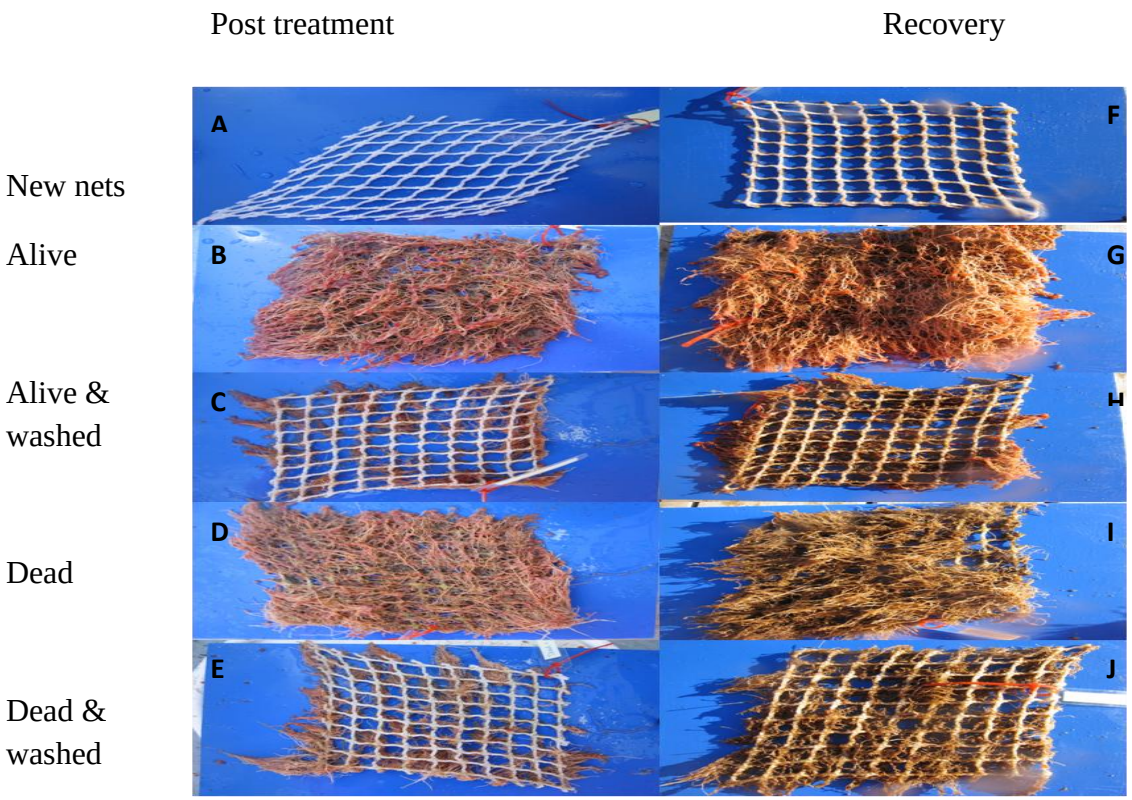

Figure 14: A series of photographs showing fouling on net pieces post treatment, and after a two week recovery period. The column on the left shows post treatment, the right column shows recovery. Treatments are in rows; new nets (A, F), alive (B, G), alive and washed (C, H), dead (D, I) and dead and washed (E, J). Note the brownish colour of dead hydroids after recovery in I (dead) and J (dead and washed).

Hydroids from the 'alive' treatments were pink in colour (Figure 14: B, G, C & H); but hydroids from the 'dead' treatments turned brownish in colour after both the treatment and recovery periods (Figure 14: D, 1, E & J). Although only one side of the nets involving 'washed' were treated, it is obvious (Figure 14: D & E) that washing with high pressure net cleaners is effective at dislodging fouling communities found on fish cage netting.

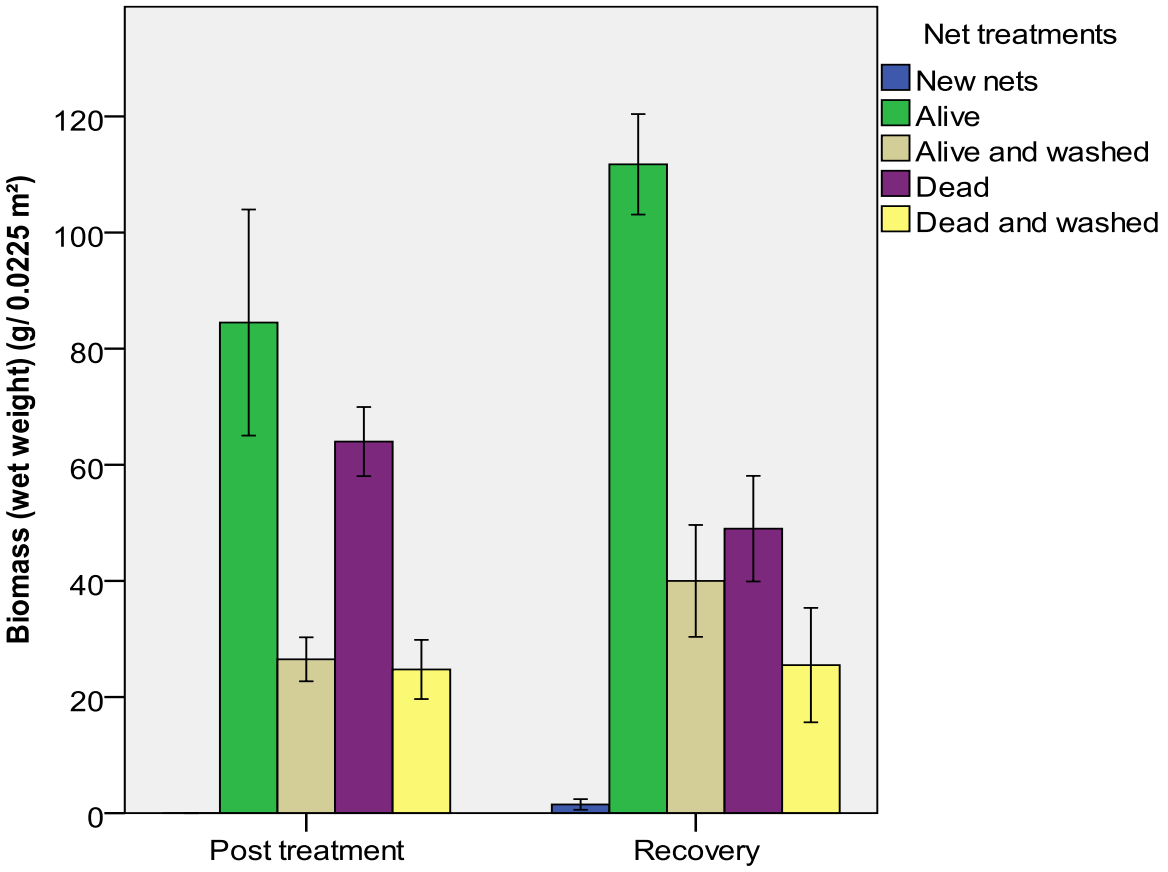

Figure 15: Mean wet weights of fouling on net panels post treatment and after recovery. Error bars express 95% confidence interval. New nets post treatment does not appear on the graph because the new nets had no fouling post treatment.

Net wet weights varied after the treatments were first administered (Figure 15). Nets which were exchanged (new nets) had no fouling. All the treatments except the control (alive) exhibited a reduction in wet weights after the treatments were administered. Thus; the control group weighed (mean fouling wet weight (g) ± SE) 84 g ± 6.112 after treatment. This weight was representative of the initial fouling wet weights of all the nets in the study before the treatments were applied. The 'alive and washed' treatment weighed 26.5 g ± 1.19, and the 'dead' treatment weighed 64 g ± 1.871. The 'dead and washed' treatment weighed 24.75 g ± 1.601.

After the two week recovery period; the mean wet weight of fouling on the new nets was 1.50 g ± 0.29. The control treatment ('alive') had increased in wet weight by 32.3 % to weigh 111.8 ± 2.720 g. The 'alive and washed' nets had the highest percentage increase of 51%,

weighing 40.0 ± 3.03 g. However; the ‘dead’ nets actually decreased in fouling wet weight by 23.4 % to weigh 49.00 ± 2.856 g at the end of the recovery period. The ‘dead and washed’ treatment increased very little in wet weight (3.03 %), to weigh 25.50 ± 3.091 g after recovery.

Hence, at the end of the recovery period, new nets had the lowest increase in fouling, the ‘dead’ treatment actually decreased in fouling; while ‘dead and washed’ increased only slightly in weight. The ‘alive and washed’ treatment recorded the highest percentage increase in fouling; followed by the ‘alive’ treatment (see Figure 15).

Table 4: Repeated measures analysis of hydroid regrowth two weeks after the use of 5 different treatments (new nets, alive, alive and washed, dead, and dead and washed).

Source	Time	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Linear	313.600	1	313.600	22.427	.000
Time * Treatment	Linear	1991.650	4	497.912	35.608	.000
Error(Time)	Linear	209.750	15	13.983		

There was a significant interaction term between ‘time’ and ‘treatment’ (repeated measures analysis, $F = 35.608$, $p < 0.05$, Table 4). This interaction was mainly driven by the fact that, after treatment, new nets had no fouling while the other nets had a considerable amount of fouling on them (see Figure 15). This persisted into the recovery period, where the new nets had the lowest biomass (1.50 g ± 0.29) of fouling among the five treatment groups.

3.3.2. Biomass (dry weight) analysis

The dominant fouling organisms were hydroids; in turn made up almost entirely of *E. larynx.* A total of 9 species were identified during the recovery phase of this experiment. Three mollusc species (Pectinidae, *Mytilus edulis,* and *Flabellina sp*); two hydroids (*Ectopleura larynx, Obelia sp*); two amphipod groups (*Caprella sp, Corophium* sp), one nereid polychaete and the bryozoan *Membranipora membranacea* were the species identified.

Prior to the start of the experiments; all the net panels were completely occluded with hydroids (mostly *Ectopleura larynx*). As a result; debris and sediment were trapped in the dense growth. These were however gently released by gentle agitation of the nets in seawater before transport to the laboratory.

The species identified were then placed into four fouling groups. The pre-treatment nets used as a reference group however recorded five fouling groups. The groups recorded for the treatments after recovery were hydroids, molluscs, crustaceans and polychaetes.

Table 5: Fouling group distribution after a two-week recovery period

Treatment	**Fouling groups**
Alive	Hydroids, molluscs, crustaceans, polychaetes
Alive and washed	Hydroids, crustaceans
Dead	Hydroids
Dead and washed	Hydroids, crustaceans
New nets	Hydroids

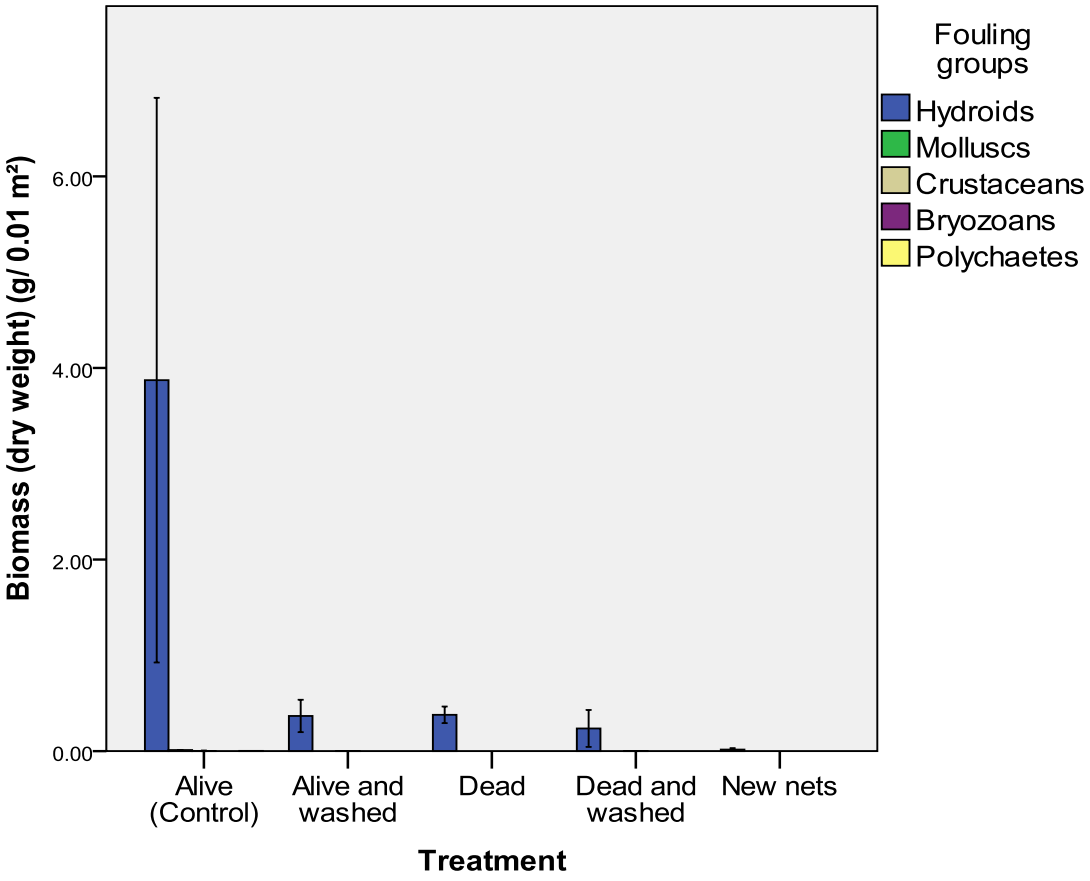

Figure 16: Clustered bar graphs showing mean biomass (dry weight) of the fouling groups found after recovery. Note the dominance of hydroids in all the samples and how the other fouling groups are not visible due to extremely low values. Error bars express 95% confidence interval.

In all the 5 treatments analyzed after recovery, hydroids were the most dominant fouling group and contributed to over 95% of the total biomass in each treatment (see Figures 16 & 17). The 'alive' treatment had the highest biomass of hydroids (mean dry weight (g) ± SE, 3.87 g ± 0.93). The 'dead' treatment recorded the next highest value for hydroids of 0.38 g ± 0.03, 'alive and washed' recorded a similar biomass of 0.37 g ± 0.05, 'dead and washed', 0.24 g ± 0.04; and new nets recorded the lowest value of 0.02 g ± 0.00. The values recorded for the other fouling groups were very minimal. The pre-treatment (reference group) had a hydroid biomass of 1.42 g ± 0.13.

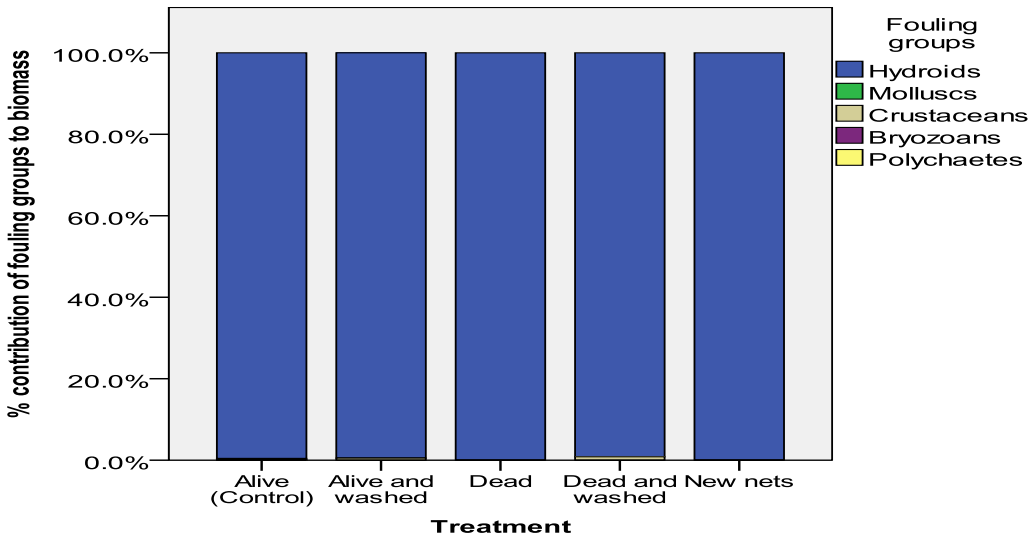

Figure 17: Relative contribution of fouling groups to biomass in the various treatments. Note the dominance of hydroids in all the treatments.

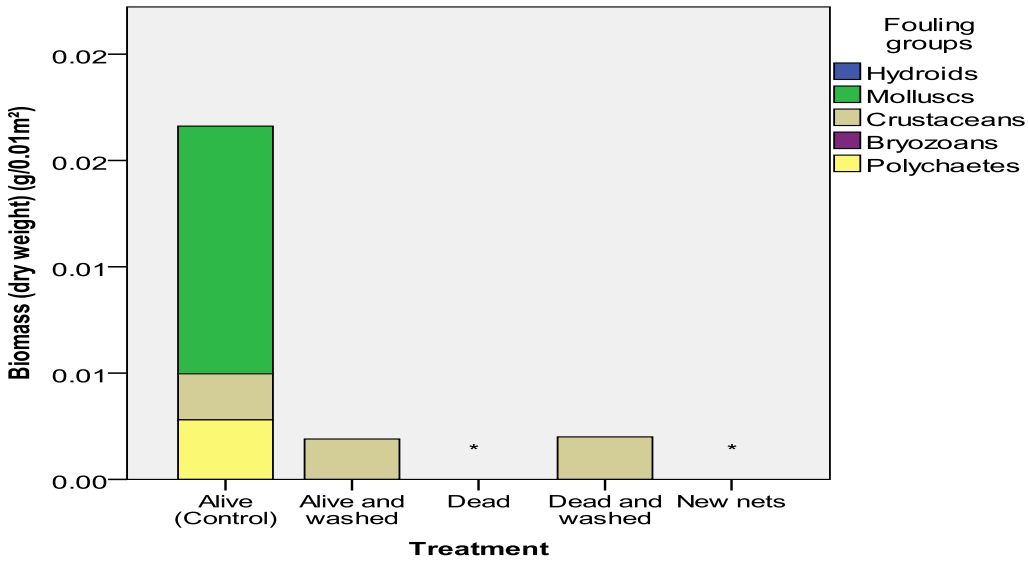

Figure 18: Stacked bar graphs showing the other fouling groups when hydroids are excluded. Note the difference in scale between this and in Fig. 8. *-Note the absence of any other fouling groups in the dead and new nets treatments. Values for polychaetes in the 'alive' treatment are so low they do not show.

The fouling community of 'alive' nets differed from the other treatments (Figure 18). While the 'alive' treatment had molluscs, crustaceans and polychaetes, the 'alive and washed' and 'dead and washed' treatments had crustaceans, but the dead nets and new nets had no other fouling groups except for hydroids.

4. Discussion

4.1. Overview

Biofouling is a significant problem for all aquaculture industries and has its most well documented impacts in sea based aquaculture (de Nys & Guenther, 2009). Olafsen (2006) also outlined the damaging effect of biofouling on the Norwegian fish farming industry. The multifilament nature of the nylon netting used in fish culture also provides a suitable substrate which harbours settling organisms (Hodson et al., 1997).

Cronin, Cheshire, Clarke & Melville (1999) also stated that, the artificial substrate of cage netting combined with the high waste input allows for the proliferation of an abundant fouling community. de Nys & Guenther (2009) reported that *E. larynx* is the dominant fouling organism in Norwegian aquaculture; and that fouling communities on salmon cage netting decrease flow rate and create low oxygen levels in cages; which has deleterious effects on farming activities. Fouling communities also alter cage behaviour in rough seas and under high current conditions (Greene & Grizzle, 2007; Swift et al., 2006).

The ecology of hydroids on fish farms still has room to be explored, (Carl, 2008); and several studies have been carried out on aquaculture farms with respect to fouling distribution, development and control (Greene & Grizzle, 2007; Braithwaite, Carrascosa & McEvoy, 2007; Braithwaite & McEvoy; 2005; de Nys & Guenther, 2009). This study confirms that biofouling is a major problem on farms in South-West and Mid- Norway from July to October (Carl, 2008; Guenther et al. 2009); and contributes to a greater understanding of fouling development on aquaculture nets, and the potential benefits of using environmentally friendly methods to curb the growth of hydroids on nets.

4.2. Biofouling on nets

4.2.1. Monthly development of biofouling

Fouling occurred on all the net panels throughout the study; with fouling showing variation with depth and time. In June; fouling was highest at 1 m depth in terms of wet weight. There was a steady increase in wet weights at 5 m from June until values peaked in October and then reduced in November. Wet weights of fouling also increased steadily at 10 m from June until they peaked in September, and then gradually reduced in October and November (see

Figure 7). The significant interaction between depth and time in the data could have possibly been influenced by a combination of abiotic and biotic factors.

Seasonality in the environment is thought to have an influence on the composition and structure of species (Jenkins & Martins, 2010). In the month of November, when winter had set in; there was a pronounced decrease in the cover of fouling on nets. The drop in fouling in November could be due to a response to lower temperatures, mortality, increased predation, and food availability among others. This study is consistent with reports by Guenther, Carl and Sunde (2009); that farmers have a major problem with fouling; especially with hydroids from July to November.

The high fouling biomass (wet weight) in the study recorded in October at 5 m (20.2 g) translates to 2.02 kg/ m^2. Braithwaite et al. (2007) reported fouling of 4.9 kg/ m^2 at 3 m on uncoated nets on a salmon farm in Shetland; and Cronin et al. (1999) also reported 2.2 kg/ m^2. Other researchers have reported higher numbers of fouling: Rothwell and Nash (1977) reported 13 kg/ m^2 on nets that had been immersed for one month. Pudota (2011) also reported 21.2 kg/ m^2 on cage nets from mid-Norway. It is possible that differences in geographic local ecological factors have a part to play in the differences in wet weight of fouling reported in literature, since these values are drawn from different parts of the globe.

Fouling assemblages on nets have been shown to be vary significantly with depth (Svane, Cheshing & Barnett; 2006); which was also seen in this study. This is consistent with literature from Nellis and Bourget (1996); who found a significant effect of depth on recruitment of hydroids in Canada; and Guenther et al. (2010) who also reported a significant difference in net aperture occlusion of fouling with depth from Norway.

The differences between fouling at the various depths could be due to various factors which affect vertical zonation of fouling organisms. Cowie (2010) outlined the fact that light intensity, recruitment from larval stages, current velocity, nutrient input; and variations in oxygen conditions, among others plays a very important role in determining community composition with depth.

4.2.2. Fouling diversity

The 28 organisms identified in this study is consistent that found in literature for fouling species on aquaculture nets. Braithwaite et al. (2007) reported 36 fouling species from untreated netting which had been immersed for 10 months; Cheah and Chua (1979) reported 34 species from nets that had been immersed for two months.

Most of the fouling organisms from this study are also associated with rocky, benthic communities. The siting of the fish farm within a sheltered, rocky bottom fjord environment possibly provides an easy recruitment of larval species from the surrounding water onto the nets. Pudota (2011) found a clear relationship between species occurring in zooplankton samples and foulers on net panels from a fish farm in mid- Norway. It is therefore possible that the species from this study were recruited on the net panels from the immediate environment.

The five broad taxa into which the fouling organisms from this study were grouped are typical of fouling communities reported in literature: algae, molluscs, hydroids, crustaceans, bryozoans and ascidians are the typical fouling taxa seen on cage netting (de Nys & Guenther, 2009; Braithwaite & McEvoy, 2005). In this study; ascidians were not observed on the net panels, but occurred in high numbers on the PVC panels used to house the nets between September and November. This could be due to the fact that nylon netting is not a rigid enough surface for settlement when compared with solid PVC pipes, which is more preferred.

Algae were seen mostly at 1 and 5 m between June and September at 1 and 5 m; which is indicative of the preference of algae for well lit environments and upper portions of cage netting (de Nys & Guenther, 2009). The dominant algae from this study were *Chaetomorpha sp., Enteromorpha (Ulva) sp., Ectocarpus sp.* and *Polysiphonia sp*. This is consistent with reports of some of the major fouling algae reported in aquaculture (Cronin et al., 1999; de Nys and Guenther, 2009; Braithwaite and McEvoy 2005).

Mytilus edulis, Littorina sp, and Hiatella arctica are among the most prolific fouling molluscs (Greene & Grizzle, 2007; Braithwaithe & Mc Evoy, 2005; Dürr & Watson, 2010). The monthly decline in mollusc numbers at 1m could be explained by a shift that occurred in

the community diversity. From June until September, algae were present in all the samples (especially at 1 and 5 m). *M. edulis* was found on the nylon rope as the months progressed, and littorinid snails occurred in large numbers on the net panels. Algae serve as a food source for herbivorous littorinid snails Salvini-Plawen (1972); and the shift in mollusc diversity could be explained by the fact that as the cold months progressed; the decline in algae also led to a decline in littorinid numbers since their prey item (algae) were no longer found on the nets.

As algae biomass changed with the onset of colder and darker months; hydroids bloomed and nudibranchs replaced the other mollusc species. The high numbers of the nudibranch (*Flabellina sp*) in September and November could be explained by the fact that they were feeding on the hydroid community which had bloomed from September to November at all three depths. Greene and Grizzle (2007); and Pudota (2011) reported the occurrence of nudibranchs feeding on *Tubularia (Ectopleura) larynx*; serving as a form of biological control. Crickenberger and Sotka (2009) also reported a recruitment of nudibranchs which was timed to mirror the period of peak development of hydroids. Several of the nets from which nudibranchs were recorded in this study had their hydranths missing, and when net panels were harvested, the nudibranchs were seen to have been grazing on fouling hydroids.

Hydroids are the most abundant fouling species connected with Norwegian aquaculture, and they are most prolific from July to November (Guenther, Misimi & Sunde, 2010). Carl (2008) also reported a peak in hydroid growth in mid- Norway from July to October. The results of this study are consistent with these reports since hydroids began to feature prominently in the samples from the month of July, albeit at 1 m; and tapered off in November. Gili and Hughes (1995) ascribed 20% of fouling community biomass to be made up of hydroids. However, in the month of September, hydroids formed the bulk of biofouling at all three depths and were the only fouling group found at 10m. From then on, hydroid numbers increased and dominated the fouling community by more than 90% in September at 10 m and for all depths in October and November.

Norwegian fish farmers have varied opinions of hydroid colonisation with depth (Carl, 2008). Some farmers report hydroid colonization starts from the bottom up while others think it is from the surface towards the bottom. While upper portions of nets tend to be fouled with algae, lower depths are fouled with hydroids, bivalves and amphipods (Cronin et al., 1999).

A few juvenile scallops were seen attached to some hydroids, which is also consistent with reports by Harvey, Bourget and Mirron (1993) that juvenile Iceland scallop larvae exhibit a preference for filamentous substrates, especially hydroid communities. Olafsen (2006) reported that hydroids seemed to settle at lower depths; and Guenther et al. (2010) stressed the need for further research into this.

Complete colonization and dominance of the net panels by hydroids (in turn made up almost exclusively of *E. larynx*) was first seen in September at 10 m. At this depth, wet weights of hydroids higher than 40 g/ 0.01m² were first recorded. The net panels at all three depths for the months of October and November were dominated by hydroids. Hydroids tend to be found at lower depths in well lit areas due to competition with algae (non-epiphytic hydroids) for attachment to the substrate and also since some larval hydroids become negatively phototactic after settlement (Gili & Hughes, 1995). This study therefore confirms that hydroid colonisation starts from the bottom of the cages and moves upwards towards the upper portions. Had there been data for August at 1 and 10 m, it is believed that a better understanding of the fouling pattern could have been established.

Crustaceans (amphipods) were made of up caprellid and tube- building amphipods. *Caprella sp*. were firmly attached to the stalks of E. larynx and very well camouflaged. This observation was also reported by Swift et al. (2006); that the majority of fouling growth at a depth of 15 was made up of caprellid amphipods attached to hydroids. Corophid amphipods were also conspicuous and had built brownish tunes which were attached to the nylon netting. Caprellid and corophiid amphipods are usually found living in close association with hydroids; where they feed on trapped organic matter, invertebrates and scavenge on decaying organic matter (Lalli & Parsons, 1997; Gili & Hughes, 1995). The input of nutrients and organic matter from the salmon cages could also be of benefit to the amphipods; since the hydroid colonies tend to trap sediment and organic matter which the amphipods can then feed on.

The two bryozoans in this study (*Membranipora membranacea and Electra pilosa)* are encrusting filter feeders, who could also be taking advantage of the possible increase food source available in the fouling associated community and also from the increased nutrient emission from the salmon cages.

Although only one pycnogonid (*Nymphon gracile*) was seen during the study; the numerous nudibranchs recorded from the study on the hydroid colonies confirm that the hydroids are prey for the above mentioned species.

The high species richness of fouling organisms found on the nets at 1 m depth can be explained by the fact that all the major taxa were recorded at this depth over time. From June-September, fouling was made up of different species of algae, molluscs, hydroids and crustaceans. In October and November, species richness reduced and was made up of hydroids, crustaceans (amphipods) and molluscs (scallops and nudibranchs). At 5 m; species richness was low in June and July (7 & 9) and then peaked in September, after which numbers dropped in October and November. Although the highest fouling was recorded at 5 m in the month of October, we can clearly see that species richness is not correlated to biomass. This was clearly seen at 10 m, when the lowest species richness was 2 for the months of September and October; when biomass was in fact very high. This is explained by the fact that fouling at this stage was made up entirely of *E. larynx* colonies. Also; the monthly replacement of net panels could have caused disturbance by preventing the sucession of a fouling community. The monthly exchange of nets could have possibly reduced the species richness, since disturbance leads to a decline in species richness (Guenther et al., 2010; Valdivia et al., 2008).

The results of the MDS plots (Figure 12) can be explained by the species richness data. The similarity of species at all depths in November can be explained by the fact that in the cold sampling months (October- November), fouling was low (7, 6 and 5 at 1, 5, and 10 m) was made up entirely of hydroids, amphipods and molluscs (nudibranchs and scallops). Samples for the month of October were not similar to the other groups because fouling was made up almost exclusively of *E. larynx*.

The similarity between species in August and September could be due to the fact that species richness was high (14 in both cases); and was made up of similar species from the various fouling groups. Sample for June and July were also very similar, and this can be explained by the fact that these two months were very similar in species composition, being made up largely of various filamentous algae, molluscs, a few hydroids and other invertebrates (see Appendix 2).

The results of this study indicate that species diversity varies with time and depth, and also that species richness is not indicative of biomass; since a very high biomass could be made of very few species and vice-versa.

4.3. Effect of drying on hydroid survival

Intertidal hydroids tend to occupy areas such as overhangs, under boulders; or contract their hydranths to escape desiccation for a few hours (Gili & Hughes, 2005). The hydroids in the present study experienced low mortalities after 0 and 2 hours of drying time; and just about half survived after 6 hours of exposure. It is possible these results mimic what happens in the natural environment during the daily exposure to air at low tide. One of the cost-effective methods for dealing with biofouling is the air drying of nets. Fouled netting is left to compost for 1-2 weeks, and then cleaned with high pressure water hoses or automated net cleaners (Cronin et al., 2009). The Norwegian finfish industry also uses copper-based coating on nets in combination with drying on site to curb fouling; while other farmers also use the double net system, where the upper portion of nets is lifted out of the water column to air dry while the other half in left in the water column (CRABa; Olafsen, 2005).

The results of this study show that a period of 48 hours is enough to ensure complete mortality of hydroids, provided there is a complete exposure of the hydroids to air. Therefore, the 1-2 week composting time, which is followed by washing can be shortened by exposing the net completely to air for two days before washing; which can increase efficiency. Complete exposure to air is important to also ensure the mortality of emergent polyps which could possibly be shielded in densely fouled communities by the outermost hydranths. This phenomenon was seen in the case of colonies which had been dried for 6 and 12 hours: the outermost hydranths died, while emergent polyps at the base of the colonies survived. Hydroids have devised ingenious ways of firm attachment to the nylon filaments; and even when nets are washed, the remaining stolons intertwined with the filaments are capable of fast regeneration (Carl, 2008; Hodson, Lewis & Burke, 2007). Shielded emergent polyps are therefore not adversely affected by the washing process, since cut or damaged hydroids have the ability to grow back faster; and washing loosens the nylon filament which facilitates better hydroid attachment (Carl, Guenther & Sunde, 2011).

It is recommended that farmers first wash the nets to completely expose stolons or filaments; and then proceed to dry the cleaned nets instead of the other way round as has been standard practice. Drying of the washed nets will then ensure the complete mortality of any emergents and/or stolons. On the re-immersion of these nets, the need for frequent washing and drying will be eliminated since the dead filaments/ stolons are not capable of regeneration.

4.4. Effects of anti-fouling treatments on the survival of *E. larynx*

4.4.1. General trends

As part of the methods to fight fouling on cage nets; a variety of methods exist. The commonest means of dealing with the biofouling problem involve cleaning in-situ or on land using either mechanical or hand cleaning; the use of electricity, antifouling coatings (copper, fouling release coatings, metallic layers) among others (CRABb). Fouling strategies are not mutually exclusive from each other; and farmers often use a combination of methods to deal with fouling (Dürr & Watson, 2010). In the Norwegian finfish industry; farmers use three different strategies: - copper-based coating combined with drying, copper-based coating combined with washing and sometimes drying; and the use of uncoated nets combined with frequent cleaning. Concerns have been raised about the potential ecological effects of antifouling coatings on the marine ecosystem via leakage and bioaccumulation (Braithwaite et al., 2007; Hall & Anderson, 1999; Dürr & Watson, 2010). The washing of nets also comes with attendant problems, since cut or damaged hydroids still have their living stolons intertwined in the nylon filaments; this leads to an even faster regrowth of hydroids after the washing process (Carl, 2008; Hodson et al., 2007; Guenther et al., 2010).

A growing need had arisen for the potential use of practical, cost-effective and environmentally- friendly control tools to manage marine fouling pest species (Piola et al., 2010). Among some of these methods are the use of heat, hydrated lime, natural biocides from plant sources and hypochlorite among others (Graham, Moncrieff, Benson & Stock, 1975; Lai, Kessler & Khoo, 1993; Piola et al., 2010; Guenther et al., 2011). The present study sought to quantify the effect of washing and a combination of killing with washing on the regrowth of E. larynx. The results of the field experiments showed that short term immersions of hydroids in 60 ° of heated seawater led to a total mortality of hydroids, as had been found out by Guenther et al. (2011). After the two week recovery period, the hydroids which had been killed had shed their hydranths which led to an actual decrease in biomass; although

their dead stolons remained on the treated panels. This observation was also made by Guenther et al. (2011). While the 'alive' group experienced growth, the highest percentage growth occurred in the 'alive and washed' treatment. This finding concurs with findings by Guenther et al. (2010); that cut *E. larynx* regenerates its polyps rapidly after they have been cut off; and that cut or damaged hydroids have the tendency to grow back more rapidly than before. The dead and washed nets had very little increase in fouling; which could be explained by the fact that dead hydroids may not be capable of regeneration. Although the new nets had the lowest increase in fouling after two weeks, they could have been fouled completely had the recovery time been much longer, since results from the monthly sampling show that hydroids can colonise nets within one month. Although only one side of the nets were washed in this study; the results show that killing of hydroids and then washing them could be looked at by farmers as a cost-effective and efficient means of dealing with biofouling. The results from the drying experiments suggest that it will more successful to first wash the nets and then immerse them in hot water to ensure the total mortality of hydroids.

4.4.2. Biomass (dry weight) analysis

The results of the biomass analysis are similar to the trends in species richness discussed in section 4.2.2. The presence of molluscs and polychaetes on the 'alive' treatments can be explained by the fact that nudibranchs and some polychaetes prey on hydroids, and that some scallop species display a preference for settling on hydroid filaments (see 4.2.2). The presence of amphipods on the 'dead and washed' and 'alive and washed' nets could be due to the fact that only one side of the nets were washed. Hence, the few remaining stolons and empty spaces provided a suitable substrate for attachment, or may have provided the amphipods with a food source since they are known to scavenge on organic matter and detritus probably left behind by the washing process (Gili & Hughes, 1995; Summers, Delong & Thorp, 1997). The absence of any fouling groups except hydroids on the new nets and 'dead' treatments could be explained by the fact that the new nets had just newly emerging hydroids. The lack of other groups on the nets could be because the hydroids; though dead were still attached to the net in dense numbers. It is possible that had there been a longer recovery period, the risk of the dead hydroids providing a suitable substrate for settlement by other fouling species would have been higher (Guenther et al., 2011).

5. Conclusion and further work

Biofouling has a very serious effect on the efficiency of aquaculture operations. This study shows that there is a temporal and depth variation in the development of biofouling. There was a significant interaction and of depth with time which could be driven by differences in fouling community composition at different depths with time; and these differences are in turn influenced by abiotic and biotic factors. The major fouling species were algae, molluscs, hydroids, crustaceans and bryozoans. Fouling in the early months of June and July was dominated by molluscs and hydroids, and replaced by hydroids and amphipods in the later months. The major fouling organism was the hydroid *Ectopleura larynx,* and several caprellid and corophiid amphipods were found living on the hydroid colonies. The study also confirms reports by some Norwegian farmers that complete colonisation of nets by hydroids starts from lower depths and then progresses to the surface. The lack of hydroids for 5 and 10 m in August would have given a much clearer picture of fouling development.

48 hours of air drying leads to a complete mortality of hydroids. However; dense fouling can enable the survival of younger, emerging hydroids located at the base of the colony; and these are capable of regeneration upon re-immersion. To ensure a total mortality of hydroids; nets will have to be evenly exposed to air on both sides. It is recommended that a combination of washing to expose intertwined stolons and emergent hydroids; followed by drying for 48 hours will reduce the total time used in drying of nets by 1 week or more. This will also lead to efficiency in farm operations; and reduce the chances of the rapid recolonisation of nets by hydroids.

The study also confirms findings that cut or damaged E. larynx colonies can regenerate rapidly. The killing of hydroids with hot water, followed by washing them will be safe, non-toxic method of eliminating the hydroid problem since it leads to an actual reduction of fouling and less regrowth of hydroids. Alternatively; farmers could first wash the nets, and then immerse them in hot water to cause total mortality to any remaining hydroid fragments intertwined in the net filaments.

Research into new models of automated net cleaners which can use hot water for cleaning nets on land should be investigated further. The feasibility of implementing these ideas on an industrial scale (heating costs, electricity, huge water vats etc.) are required to know their efficacy and cost-saving potential to the farmer.

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Appendix

Appendix 1

Table A.1: List of wet weights (g/0.01 m²) and standard error of mean of fouling on cut out nets from monthly sampling.

Sampling month	Biomass (wet weights) (g/ 0.01m²)								
	Sampling depth								
	1m			5m			10m		
	Mean	Standard Error of Mean	Standard Deviation	Mean	Standard Error of Mean	Standard Deviation	Mean	Standard Error of Mean	Standard Deviation
June	4.11	.73	1.46	1.34	.06	.11	.90	.09	.18
July	2.51	.19	.37	3.97	.40	.80	2.78	.13	.26
August	12.82	1.69	3.37	.	.	.	.	.	.
September	11.60	1.32	2.64	7.50	1.13	2.27	15.27	1.85	3.69
October	14.27	.49	.98	20.18	.65	1.29	11.50	.62	1.23
November	9.95	2.21	4.43	7.26	.49	.97	3.37	.37	.74

Appendix 2

Table A.2: Mean biomass of fouling groups (dry weights) in g/0.01 m² and standard error of mean at 1 m depth from cut out net pieces.

Fouling groups	Month											
	June		**July**		**August**		**September**		**October**		**November**	
	Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)	
	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean
algae	.1006	.0315	.0117	.0017	.0627	.0306	.0772	.0215	.	.	.	.
molluscs	.2548	.0527	.1766	.0394	.1661	.0375	.0762	.0608	.	.	.0330	.
hydroids	.	.	.0157	.0089	.1544	.0087	.2511	.0623	.4672	.0149	.4229	.0936
crustaceans	.	.	.	.	.0055	.	.0320	.0162	.0036	.0005	.0050	.0007
bryozoans	.	.	.	.	.	.	.	.	.	.	.0023	.

Table A. 3: Mean biomass of fouling groups (dry weights) in g/0.01 m² and standard error of mean at 5 m depth from cut out net pieces.

Fouling groups	Month											
	June		July		August		September		October		November	
	Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)	
	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean
algae	.0242	.0055	.0638	.0075	.	.	.0070	.	.	.	.	.
molluscs	.0097	.0006	.0793	.0164	.	.	.2496	.	.	.	.0108	.
hydroids	.0007	.	.0072	.	.	.	.2136	.0260	.6773	.0565	.2499	.0227
crustaceans	.	.	.	.	.	.	.0021	.	.	.	.0057	.0023
bryozoans	.	.	.	.	.	.	.	.	.	.	.	.

Table A. 4: Mean biomass of fouling groups (dry weights) in g/0.01 m² and standard error of mean at 10 m depth from cut out net pieces.

Fouling groups	Month											
	June		July		August		September		October		November	
	Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)		Dry weight of fouling species (g)	
	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean
algae	.0050	.0022	.0235	.0029	.	.	.	.	.	.	.	.
molluscs	.	.	.0493	.0111	.	.	.	.	.	.	.	.
hydroids	.0037	.0015	.0015	.0006	.	.	.4737	.0583	.3192	.0231	.0979	.0119
crustaceans	.	.	.	.	.	.	.	.	.	.	.0102	.0030
bryozoans	.	.	.	.	.	.	.	.	.	.	.	.

Appendix 3

Table A.5: Species richness with depth (1, 5 and 10 m) over time.

Time	Depth		
	1	**5**	**10**
	Species richness	Species richness	Species richness
June	11	7	11
July	9	9	6
August	14	.	.
September	14	15	2
October	6	7	2
November	7	6	5

Appendix 4

Table A.6: Species identified during the study and their taxonomic groupings. (AL= algae, MO= mollusc, HY= hydroid, CRU= crustacean, BRY= bryozoan, ARTH= arthropod, DIA= diatom, ECH= echinoderm, NEM= nematode, PLA= platyhelminthes, ANN= annelid. Only filamentous algae, molluscs, hydroids, crustaceans and bryozoans were used for data analysis. The other groups were either too minute to be measured or were incomplete specimens.

Species	**Grouping**
Ascophyllum sp.	AL
Polyshiphonia sp.	AL
Chaetomorpha sp.	AL
Porphyra sp.	AL
Chordaria flagelliformis	AL
Enteromorpha (Ulva) sp.	AL
Ectocarpus sp.	AL
Halidrys siliquosa	AL
Sphacelaria sp.	AL
Mytilus edulis	MO
Hiatella arctica	MO
Flabellina sp.	MO
Pectinidae	MO
Littorina sp.	MO
Nucella sp.	MO
Ectopleura larynx	HY
Obelia sp.	HY
Campanularia sp.	HY
Caprella sp.	CRU
Jassa sp.	CRU
Corophuim sp.	CRU
Nymphon gracile	ARTH
Electra pilosa	BRY
Membranipora membranacea	BRY
Licmophora sp.	DIA
Nematode	NEM
Flatworm	PLA
Astropecten sp.	ECH

Appendix 5

Table A.7: Mean percentage survival of hydranths per colony from drying experiments (0, 2, 6, 12, 24, & 48 h). 60 hydranths were counted per colony (replicate), and there were 10 replicates per drying time.

Drying time (h)	Percentage survival of hydranths		
	Mean	Standard Error of Mean	Total N
Control	90	4	10
2	77	10	10
6	43	7	10
12	15	4	10
24	2	1	10
48	0	0	10

Table A.8: Results of one-way ANOVA from drying experiments

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	75556.533	5	15111.307	53.026	.000
Within Groups	15388.800	54	284.978		
Total	90945.333	59			

Appendix 6

Table A.9: Wet weights (g/0.0225 m²) of fouling from nets used for repeated measures analysis. Table shows means and standard errors of nets post treatment.

Treatments	Post treatment	
	Mean	Standard Error of Mean
New nets	0	0
Alive	85	6
Alive and washed	27	1
Dead	64	2
Dead and washed	25	2

Table A.10: Wet weights (g /0.025 m²) of fouling from nets used for repeated measures analysis. Table shows means and standard errors of nets after a two week recovery period.

Treatments	Recovery	
	Mean	Standard Error of Mean
New nets	2	0
Alive	112	3
Alive and washed	40	3
Dead	49	3
Dead and washed	26	3

Appendix 7

Table A.12: Mean biomass (dry weight) (g/0.01 m²) of fouling groups of various treatment groups after a two week recovery period.

Fouling groups	Mean biomass (dry weight) (g/0.01 m²)											
	Treatment used											
	Reference group		Alive (Control)		Alive and washed		Dead		Dead and washed		New nets	
	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean	Mean	Standard Error of Mean
Hydroids	1.4233	.1304	3.8728	.9261	.3672	.0531	.3792	.0272	.2367	.0448	.0165	.0047
Molluscs	.0251	.0116	.0116	.0000	.	.	.	.	.	.	.	.
Crustaceans	.0014	.0006	.0022	.0010	.0019	.	.	.	.0020	.	.	.
Bryozoans	.0079	.	.	.	.	.	.	.	.	.	.	.
Polychaetes	.	.	.0028	.	.	.	.	.	.	.	.	.