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# Biofouling in the Norwegian Salmon Farming Industry

Thesis for the degree of Philosophiae Doctor

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Norwegian University of Science and Technology
Faculty of Natural Sciences and Technology
Department of Biology

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## Abstract

Biofouling, the unwanted growth of organisms on submerged artificial surfaces, is ubiquitous in the marine environment and a particular problem in the salmon farming industry. In Norway, one of the most common and problematic fouling species is the hydroid *Ectopleura larynx*. Together with other biofouling organisms such as blue mussels and algae, it may reduce the water flow through the cage nets, increase the weight of equipment and the disease risk, and reduce the performance of cleaner fish. Therefore, biofouling not only affects farm management practices, but may also impact fish health.

This thesis aims to increase the overall understanding of the development, the impacts and the prevention and management of biofouling on salmon farms. Further aims were to extend the knowledge on the hydroid *E. larynx* in order to improve current farm management practices, provide information for future aquaculture risk assessments and contribute to the development of novel antifouling technologies.

A 1-year field study was conducted at a commercial salmon farm to provide background knowledge about how natural and farm operational factors influence the biomass, species richness and community composition of biofouling on cage nets. The effects of immersion period, sampling time, mesh size and variability between three individual cages were investigated. The biofouling community on the cage nets consisted of up to 90 species and multi-species groups. Among the four tested factors, immersion period and sampling time had the strongest influence on the community, resulting in clear successional and seasonal patterns in biomass, species richness and community composition, while mesh size and variability between cages had only limited influence. By controlling the timing of when nets are introduced into the water and the cleaning schedule, farm management has a large influence on the seasonality and succession within the fouling community.

The potential food sources of hydroids and caprellids living on fish cages were analysed in order to identify a possible link between fish farm wastes and high biofouling abundances. The stable isotope analysis of plankton, particulate organic matter, caprellids (which may be preyed on by hydroids), fish feed and fish faeces showed that hydroids mainly feed on zooplankton and that they are unlikely to include fish feed or fish faeces into their diet. This data was supported by estimations of a maximum hydroid biomass of 6.7 t wet weight on a cage with a daily food intake of 0.2 t C which could be met by the local zooplankton biomass. Consequently, the extensive growth of hydroids on cage nets may be largely independent of fish farm wastes. However, the selective feeding on specific prey species could negatively affect the zooplankton community, a fact that should be taken into account in future aquaculture

risk assessments. In contrast, the caprellid data suggested that this species may utilize fish feed and therefore could benefit from living at a fish farm.

In a survey of salmon farmers operating along the Norwegian coast, the temporal and spatial prevalence of the hydroid *E. larynx* was analysed. In addition, the presence or absence of other problematic fouling species was investigated. *E. larynx* was reported from salmon farms between the southern tip of Norway to regions north of Tromsø, with a high presence in South-West and Central Norway. On some farms hydroids are found all year round while further north hydroids are not found on all farms or occur only during the main fouling season. Besides hydroids, blue mussels, kelps, caprellid amphipods, small algae and diatoms, and occasionally sea squirts were identified as problematic fouling species.

Hydroid biofouling can affect the oxygen levels in a cage through the reduction of the water exchange across the net. In order to clarify if, in addition, the oxygen consumption of the hydroid population can affect the oxygen budget of a cage, the oxygen consumption of hydroids was measured. At ambient water temperatures of 12, 14 and 16°C, *E. larynx* had an oxygen consumption rate of 0.54, 1.22 and 1.09 ml $O_2$ $g^{-1}$DW $h^{-1}$, respectively. These values are similar to the respiration rate of Atlantic salmon. However, because the biomass of the cultivated salmon by far exceeded the biofouling biomass on the cage, the oxygen consumption of the hydroid population equated only to 1.1% of the oxygen consumption of salmon. Therefore, the oxygen consumption of the hydroids is unlikely to reduce the oxygen budget of the cage below critical levels that would endanger the health of the cultivated salmon.

Finally, to find a non-toxic alternative to copper-based antifouling coatings, the effects of the physical surface properties wettability and microtopography on the settlement of the hydroid *E. larynx* were analysed. The settlement preferences of hydroid larvae for materials with wettabilities ranging from hydrophobic to hydrophilic were tested. Although settlement differed between materials, no trend regarding the tested wettabilities could be found and none of the tested materials were able to reduce average settlement below 50%. In a second experiment, surfaces with microtextures between 40–600 µm were analysed, but there was no systematic effect of microtopography on the settlement of *E. larynx*. Similarly, there were no preferences for any of the examined microtopographies in a 12-day field test at a commercial salmon farm. In conclusion, neither surface wettability nor microtopographies are effective at deterring the settlement of the hydroid *E. larynx*. The high plasticity of the aboral pole and the hydrorhiza of the hydroids may explain settlement even under unfavourable conditions and suggests that antifouling methods based on the tested physical properties may not provide a solution to the hydroid fouling problem.

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I NB and JG planned the experiment. NB performed the experiment, analysed the data and wrote the manuscript with help from JG and YO.

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III NB, JG and RJB planned the experiment. NB performed the experiment with help from RJB. NB analysed the data and wrote the manuscript with help from JG and RJB.

IV NB, JG and RdN planned the experiments. AJP was responsible for the manufacturing of the tested surfaces. NB performed the experiments with help from JG. NB and JG analysed the data. NB wrote the manuscript with help from JG, RdN and AJP.

# 1. Introduction

## 1.1. Biofouling in the marine environment

Marine biofouling is the unwanted accumulation of living organisms on submerged artificial surfaces, and since settlement space is a limiting resource in the marine environment, all surfaces will eventually become fouled (Wahl 1989). The development of biofouling can be successional; first, organic and inorganic molecules become absorbed to the surface and form a conditional film (molecular fouling). Onto this film, bacteria will settle, thereby establishing a biofilm. This is followed by settlement of protozoans and diatoms (micro-fouling) and finally by macroalgae spores and invertebrate larvae (macro-fouling). However, biofouling does not always follow a successional pattern. Alternatively, after the formation of the conditioning film, the fouling stages can occur concurrently in a probabilistic fashion, where organisms accumulate according to their prevalence in the environment (reviewed in Wahl 1989; Maki & Mitchell 2002).

The settlement of marine organisms is not only determined by the availability of space, but is influenced by the interaction of a wide range of physical, chemical and biological factors (settlement cues), which may affect the survival, growth and/or reproduction of fouling organisms. Known settlement cues are for example hydrodynamics, temperature, salinity, depth, light, surface texture, colour and chemistry, biofilm composition, food availability, resource competitors, availability of propagules and presence of predators or conspecifics (reviewed in Maki & Mitchell 2002; Ettinger-Epstein et al. 2008; Prendergast 2010). These factors may vary temporally and spatially, resulting in high natural variations in the composition of biofouling communities.

With few exceptions, such as mussel mariculture or algae cultivation for biofuel production, biofouling is generally considered a nuisance. For centuries it has been known and treated mainly in the shipping industry, but it is also a common problem in other industries such as aquaculture, power plants or the oil and gas industry, affecting net cages, cooling water systems, platform structures and pipeline constructions (Dürr & Thomason 2009).

## 1.2. Biofouling in salmon aquaculture

### *1.2.1. Norwegian salmon aquaculture*

Norway is the world's largest supplier of farmed salmon (FAO Fisheries and Aquaculture Department 2012), with a production of more than 1 million tonnes of Atlantic salmon (*Salmo salar*). In 2011, approximately 1000 farms were in operation along the Norwegian coast. (Norwegian Directorate of Fisheries 2012). Norwegian salmon farms are typically constructed after two different construction types:

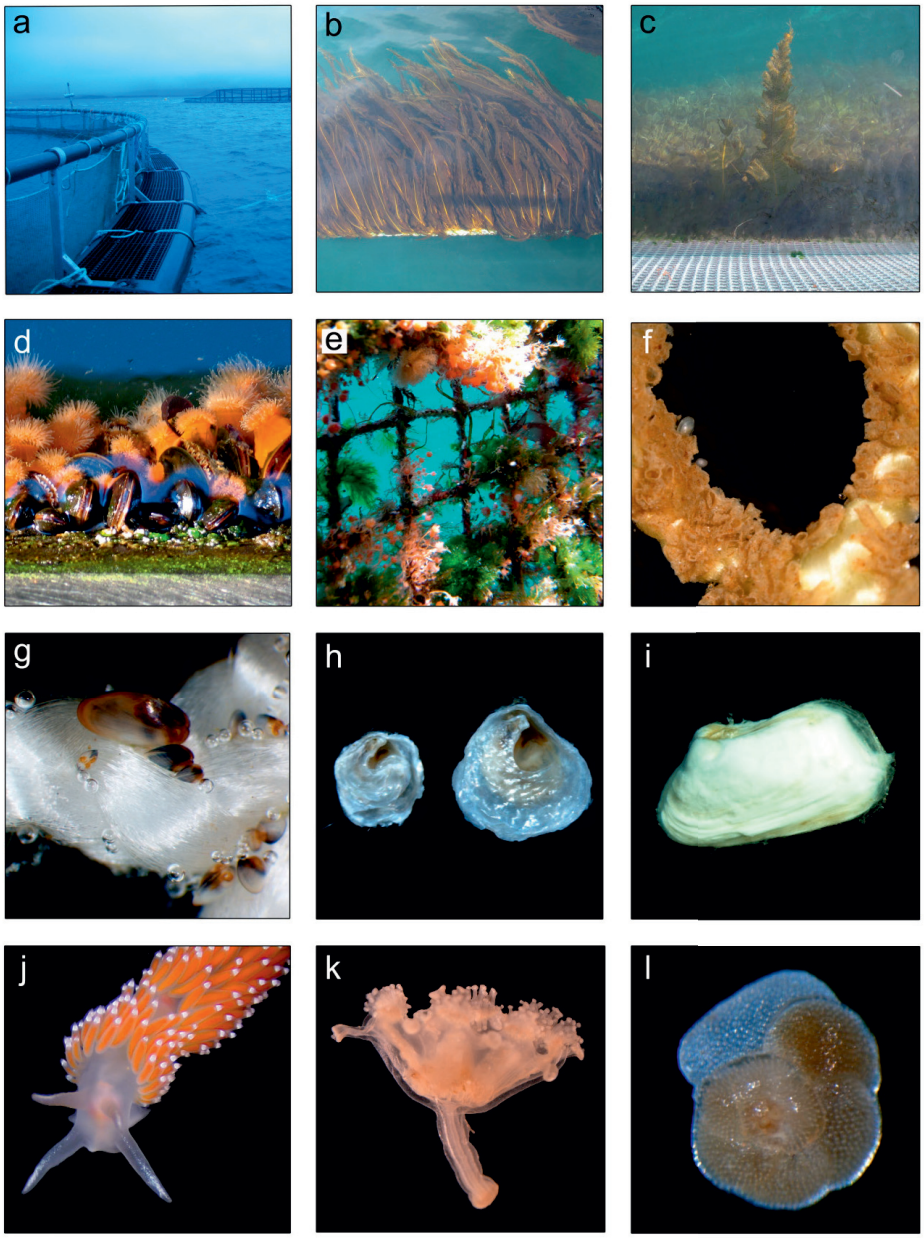


**Fig. 1.1:** The diversity of biofouling on salmon cages: (a) a salmon cage; (b) algae fouling on a rope and (c) on a cage net; (d) a fouling community on a plastic cage ring and (e) on a net; burrows of the fouling amphipod *Jassa falcata* on a net; the common fouling bivalves (g) *Mytilus edulis* (on a net thread), (h) *Heteranomia squamula* and (i) *Hiatella arctica*; (j) the gastropod *Flabellina verrucosa* that preys on hydroids; (k) the stauromedusa *Haliclystus salpinx*, and (l) a foraminifera.

(1) rectangular cages with a cage side-length between 20-40 m and a depth of up to 35 m, clustered close together around a steel platform (up to 28 cages per site), or (2) circular plastic cages individually moored to a grid in single or double rows with greater distances between the cages. Circular cages may reach a diameter of 50 m, corresponding to a circumference of 157 m, and a depth of 45 m. Up to 15 cages may be collated at one site (Oppedal et al. 2011a). The most frequently used nets are knotless multifilament nylon nets, but other materials such as plastic or steel are also employed. After being raised in land-based freshwater systems, the growth of juvenile salmon to a slaughter weight of approximately 5 kg takes 18 months on average (Personal comm. with farm personnel). During this time a cage may contain up to 200 000 individual fish (Norwegian Ministry of Fisheries and Coastal Affairs 2011).

With their combination of large net surfaces, support structures, mooring lines and permanently anchored feed barges, salmon culture facilities offer a considerable amount of space for biofouling organisms (Fig. 1.1). At the same time, the use of toxic antifouling agents is restricted compared to other industries where no food for human consumption is produced. This leads to a challenging situation where biofouling is ubiquitous while there are only few antifouling solutions that are safe for both human and fish health, and also environmentally friendly.

### *1.2.2. Common fouling species*

The most common fouling species found on salmon aquaculture nets in Norwegian waters and other temperate regions are the hydroid *Ectopleura larynx* (syn. *Tubularia larynx*) Ellis and Solander 1986 (Fig. 1.2 and 1.3), the blue mussel *Mytilus edulis* (Fig. 1.1), the ascidian *Ciona intestinalis* and algae of the genus *Ulva* and *Ectocarpus* (Braithwaite & McEvoy 2005; Olafsen 2006; Greene & Grizzle 2007; de Nys & Guenther 2009; Guenther et al. 2010). In addition, caprellid amphipods, a polyphyletic group containing both native and invasive species, can be found in high quantities on cage nets in Norway and other countries (Olafsen 2006; Greene & Grizzle 2007; Rensel & Forster 2007; Woods 2009; Cook et al. 2010).

With the exception of these main species, little is known about the composition of biofouling communities developing on cage nets at Norwegian salmon farms. In addition to the natural variation that leads to temporal as well as spatial variation within and between farms, the production schedules of the farming facilities also have a strong influence on the biofouling development. By defining the time of introduction of nets into the water as well as the length of their immersion period or frequency of disturbance by *in situ* net cleaning, farming operations affect the seasonality and succession of the biofouling communities. Currently, there is no data available on the magnitude of effects of these factors on the biofouling development in Norwegian salmon culture.

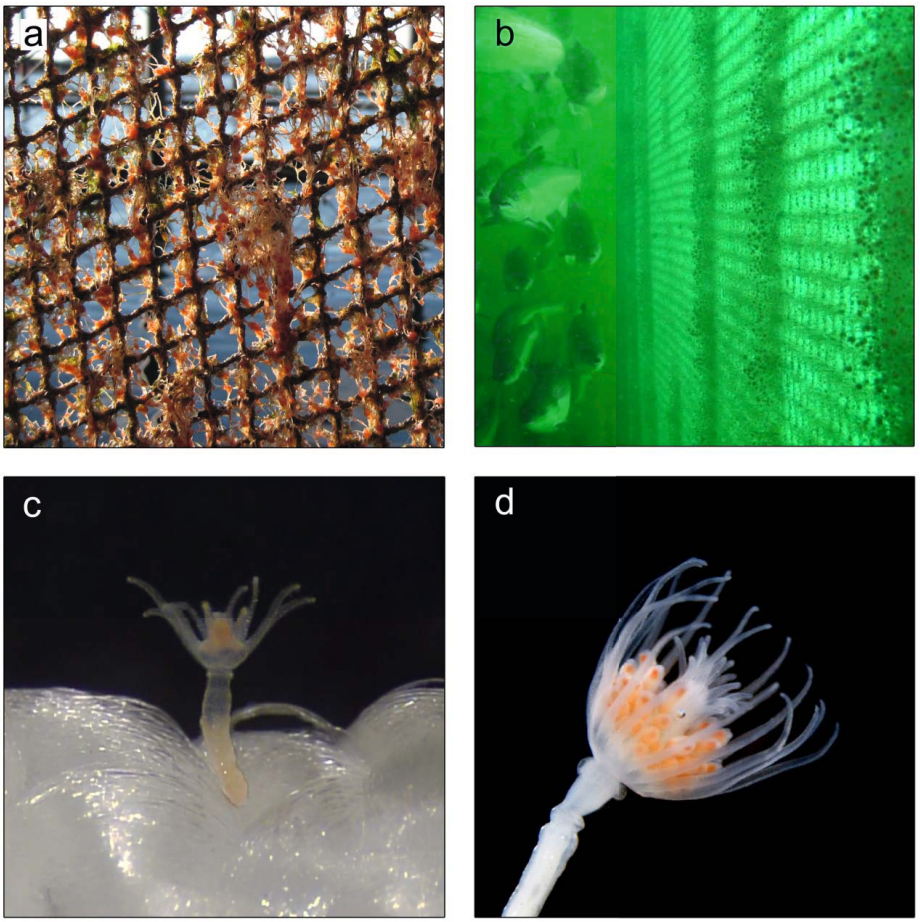


**Fig. 1.2:** The hydroid *Ectopleura larynx* (a) on a net; (b) hydroids remaining after *in situ* cleaning (photo by J. Guenther); (c) young polyp on a net and (d) an adult polyp.

### *1.2.3. The hydroid* Ectopleura larynx

One of the most common and problematic species in Norwegian salmon farming is the hydroid *E. larynx*, or ringed tubularia (Fig. 1.2 and 1.3; Olafsen 2006; Guenther et al. 2010). This colonial species occurs throughout the North Atlantic and in colder regions of the Pacific Ocean (Calder 2012). It can be found on cage nets of Atlantic salmon farms along the Norwegian coast with the highest abundances being reached in South-West and Central Norway (Olafsen 2006; Guenther et al. 2010). Outside of Norway, *E. larynx* is known to cause problems on finfish farms in Ireland (Baxter et al. 2012) and Scotland (Cook et al. 2006), while related species are responsible for cage biofouling in the USA (*Ectopleura crocea*, Chambers et al. 2012; *Ectopleura marina*,

Rensel & Forster 2007; *Tubularia* sp., Greene & Grizzle 2007) and are considered severe pests in both finfish and shellfish aquaculture in Australia and Japan (*E. crocea*, Yamashita et al. 2003; Fitridge & Keough 2013). In Norway, the highest abundances of *E. larynx* are reached between August and November, but this species can occur all year round. The reason for the high variation in the temporal and spatial distribution is not fully understood.

*E. larynx* has no medusa stage and the polyps have separate sexes (Schuchert 2010). The body of the polyp, that can reach a height of 17 cm (Schuchert 2010), consists of a net of hydrorhiza (stolons) from which several hydrocauli arise, each carrying a single hydranth (Fig. 1.3). The species grows without branching, but through the settlement of actinula larvae onto existing hydrocauli, subsequent branching does occur (Schuchert 2010; Nawrocki & Cartwright 2012). The stolons and hydrocauli, including secondary branches, are mostly covered by a chitinous perisarc and are connected through a common coelenteron (Hawes 1955; Schuchert 2010; Nawrocki & Cartwright 2012). The hydranth is athecate with a hypostome that is surrounded by an oral and aboral whorl of nematocycst-bearing tentacles. These tentacles, whose numbers vary between 14-28 and 16-29 for the oral and aboral whorl, respectively (Schuchert 2010), are used to catch prey (Gili & Hughes 1995; Gili et al. 1996).

Female gonophore development is completed approximately 24 days after settlement and is followed by the release of actinula larvae over two weeks. Afterwards, polyps may be autotomized and subsequently regenerated (Pyefinch & Downing 1949). After release, the negatively buoyant, star-shaped actinula larvae sink through the water column while elongating the initially spherical body that already bears an oral and aboral ring of tentacles (Pyefinch & Downing 1949). Upon reaching a surface, the larvae may temporarily attach with their aboral tentacles by discharging nematocysts from the tips and may crawl over the surface. Final attachment is made with the aboral pole and is followed immediately by the development of hydrorhiza that provide additional fixtures (Pyefinch & Downing 1949; Moate 1985). Because the actinula larvae are not able to swim, they mostly settle in the immediate vicinity of the adult colony (Pyefinch & Downing 1949).

*E. larynx* commonly feeds on zooplankton, but may occasionally also rely on epibenthic prey. The diet mainly consists of crustaceans of various life stages, especially copepods and cladocerans, with a maximum prey size of 3 mm (Gili & Hughes 1995; Gili et al. 1996). Although the feed intake of *E. larynx* is related to the zooplankton abundance in the surrounding water, the hydroids are able to enrich their diet through selective feeding of preferred prey items. Their diel mass-specific ingestion rate can be as high as 90% of the body mass (Gili & Hughes 1995; Gili et al. 1996). Whether hydroids are able to rely on fish farm wastes, such as the mussels *M. edulis* and *Perna viridis* (Gao et al. 2006; Handå et al. 2012), has not been determined yet. Their ability to consume particulate organic matter (POM; Gili et al. 1996) may enable them to ingest fish feed or fish faeces of small size and thereby profit from fish farm derived nutrients.

In addition to the hypothesised supplementation through fish farm wastes, other factors exists that may facilitate high hydroid abundances on cage nets. One of these factors is the high plasticity of the hydrorhiza. By wrapping the hydrorhiza around the threads of the net, through loose filaments of the multifilament netting and by incorporating nylon filaments into their perisarc, *E. larynx* is able to secure a strong hold on the cage nets (Carl et al. 2011). Furthermore, the species has a high potential for regeneration, since it can regrow and recover after disturbances such as *in situ* cleaning of the nets within approximately 5 days (Guenther et al. 2010). Although hydroids are deterred from settling when copper concentrations exceed 10 µg $cm^{-2}$ $d^{-1}$ (Barnes 1948), they show increased attachment with elevated copper levels in the water (Pyefinch & Downing 1949). These factors make *E. larynx* a well-adapted species to colonise cage nets and allow it to exploit this resourceful settlement place, where constant water movement that is common on aquaculture sites, supplies them with food and oxygen and distributes their gametes and larvae.

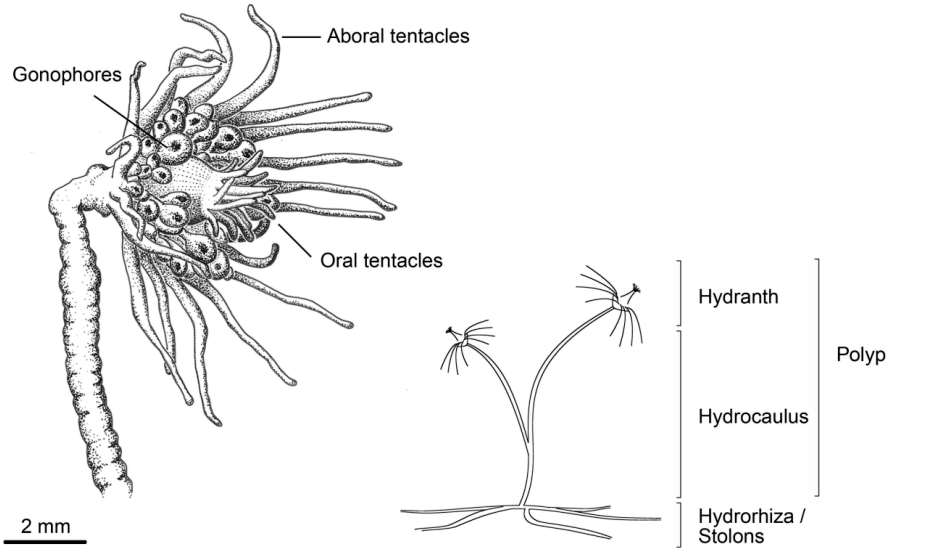


**Fig. 1.3**: Habitus of the hydroid *Ectopleura larynx* (right, schematic) with a detailed description of an adult hydranth (left).

### *1.2.4. Negative effects of biofouling*

In aquaculture, four main problems arise from biofouling on cage nets and structures:

(1) Reduced water flow across the net due to mesh blockage by fouling (Madin et al. 2009; Madin et al. 2010). This decreases the efficiency of waste removal from the cage and reduces the amount of oxygen available to the cultivated fish, both of which may negatively affect fish health (Kennedy et al. 1977; Wildish et al. 1993; Oppedal et al. 2011a). A study in an Australian tuna farm showed that the oxygen budget of the cage can be affected by the oxygen consumption of fouling species on the cage nets (Cronin et al. 1999). However, it is not known if this also applies to biofouling organisms such as hydroids in Norwegian salmon farming. In addition to impeding waste removal and oxygen supply, the inhibition of the water flow increases the drag (Swift et al. 2006) and subsequently the deformation of the cage (Lader et al. 2008). This can lead to crowding of the fish beyond desired densities which negatively impacts fish health (Oppedal et al. 2011b).

(2) Increased weight of the equipment due to the weight of accumulated fouling organisms. Combined with the elevated drag and deformation, this will enhance the load on the moorings and impact the stability of the cage, increasing structural fatigue and making handling of the net more difficult, and eventually increasing the possibility of fish escapes (Jensen et al. 2010).

(3) Increased risk of diseases due to the elevated presence of parasite pathogens associated with the fouling species (e.g. Andersen et al. 1993; Cribb et al. 2011). There may also be a direct health risk associated with fouling species, for example through gill injuries by nematocycts of biofouling cnidarians (Baxter et al. 2012).

(4) Decreased performance of cleaner wrasse. These fish are kept together with the cultivated salmon as a natural treatment against salmon lice, which they are supposed to pick from the salmon. However, if biofouling is available as an alternative food source, the cleaner wrasse prefer to feed on fouling organisms and their delousing performance drops, raising the number of lice-infested fish (Kvenseth 1996). Consequently, in order to ensure optimal growth and fish welfare, the monitoring and control of biofouling is an essential part of salmon husbandry.

## 1.3. Antifouling strategies in salmon aquaculture

### *1.3.1. Current antifouling strategies and their limitations*

The control of biofouling has been estimated to account for 5 to 10% of the annual production costs in finfish and shellfish aquaculture (Lane & Willemsen 2004), depending on the severity of the problem and the applied antifouling strategy. The currently employed treatments in Norway are copper based coatings on nets, drying of nets, *in situ* cleaning and the exchange of nets for larger mesh sizes to minimize the exposed surface area of nets. Among these, copper based coatings on nets are most

widely used (Olafsen 2006 and pers. comm. with farm personnel). Unfortunately, due to progressive wear and leaching of copper, the coatings are only efficient for up to 6 months (Braithwaite & McEvoy 2005; Braithwaite et al. 2007), necessitating additional *in situ* cleaning of the nets with high-pressure water jets (Olafsen 2006). While cleaning removes most of the fouling from the nets, some organisms or their regenerative body parts may remain and facilitate fast re-colonisation of the now available free space (Guenther et al. 2010). In addition, the stress of cleaning promotes the release of larvae of fouling organisms such as the hydroid *E. larynx* (Carl et al. 2011), which can settle immediately onto the newly cleaned net. As a consequence, some farms adopt regular, fortnightly cleaning schedules independent of the state of biofouling accumulation on the nets, while others clean on demand after inspection of the net condition, resulting in intervals of approximately 8 weeks during winter and up to once a week during the main fouling season in late summer.

Besides the limited efficiency of copper coatings due to leaching, wear (Braithwaite & McEvoy 2005; Braithwaite et al. 2007) and resistance of several species such as *E. larynx*, caprellids and the fouling alga *Ectocarpus siliculosus* (Barnes 1948; Pyefinch & Downing 1949; Hall 1980; Perrett et al. 2006), copper coatings may pose an environmental hazard (Brooks & Mahnken 2003; Burridge et al. 2010; Guardiola et al. 2012). Although no elevated copper levels were found in cultivated salmon or biofouling organisms such as the mussel *M. edulis* and the brown seaweed *Ascophyllum nodosum* (Solberg et al. 2002), copper levels in sediments and seawater surface layers surrounding farms can exceed recommended threshold levels even after more than a year of fallowing (Burridge et al. 2010; Loucks et al. 2012). Furthermore, several non-target species may be affected by copper leaching into the environment, with the most sensitive being algae, molluscs, crustaceans and phytoplankton (Burridge et al. 2010). The mechanisms of impact are as diverse as the affected organisms and include reduced swimming speed, molt delay, decreased embryonic development, reduced germination, reduced growth, changes in enzymatic activity and damaged gill filaments (reviewed in de Nys & Guenther 2009; Burridge et al. 2010). Consequently, governmental regulations aim to minimise the release of copper into the environment, and emphasise the need for alternative antifouling technologies. Although several alternative antifouling compounds exist, most of them are derived directly from the shipping industry instead of being specifically tailored for the use in aquaculture; and due to the lack of extensive long-term studies carried out *in situ*, their environmental impact at chronic exposures is currently difficult to assess (de Nys & Guenther 2009; Guardiola et al. 2012).

### *1.3.2. Novel antifouling technologies*

In order to find alternatives to the copper-based antifoulants, the development of non-toxic physical or biological antifouling technologies is encouraged by governments and

consumers. In the shipping industry, the development of surface materials that prevent settlement based on surface characteristics such as wettability and microtopography has made promising advances. Lowering the wettability of a surface by reducing its surface energy to 20–30 mN $m^{-1}$, reduces the attachment of many fouling organisms and facilitates their removal, a technique that is applied successfully in foul-release coatings made from e.g. silicone (Baier 1972; Callow & Fletcher 1994; Genzer & Efimenko 2006; Townsin & Anderson 2009). Alternatively, surfaces can be designed following the principles of the 'attachment point theory'. According to this theory, the settlement of active settling organisms is reduced on surfaces with microtopographies smaller than the width of the settler, restricting the contact between the body of the organism and the material surface (Scardino et al. 2006; Scardino et al. 2008). Following this principle, two microtextured surfaces were developed that reduced the settlement of the ubiquitous fouling algae *Ulva linza* by 85% (Carman et al. 2006) or the settlement of the barnacle *Balanus amphitrite*, another nuisance species in the shipping industry, by 97% (Schumacher et al. 2007).

In the aquaculture industry, non-toxic silicone coatings have been tested, but have not been applied yet on a commercial scale due to the lack of durability (Hodson et al. 2000). In addition, there have been tests of a latex coating combined with low concentrations of a chemical (isothiazolinones) which was more effective than uncoated nets yet only with a small margin (Svane et al. 2006). Furthermore, nets with bristly micro-fibers (Thorn-D® by Micanti) are available, but in Norway these are not utilised on a commercial scale due to the lack of proof of superiority over copper coated nets under local conditions. Despite the promising examples from the shipping industry, no solutions based on microtexture exist for the aquaculture industry.

Another challenge in the context of the development of novel and non-biocidal antifouling technologies to minimise and ultimately avoid the application of copper coatings is that especially non-toxic antifouling technologies are often species-specific, therefore requiring a combination of technologies for a comprehensive solution (Magin et al. 2010; Ralston & Swain 2011; Scardino & de Nys 2011). However, for such tailored approaches the community structuring processes as well as the species involved have to be known. Consequently, there is not only a need for more alternative antifouling technologies, but also for more knowledge on the organisms present on the cages.

Finally, biological control methods on fish farm cages rely on grazing by sea cucumbers (Ahlgren 1998) or certain fish species (e.g. wrasse; Kvenseth 1996). However, these methods are not commercially employed either, because associated animals are difficult to keep on a net in environments where high velocities are common, and because the organisms may be selective in their action against biofouling and therefore not sufficient.

### 1.4. Aims of the thesis

Biofouling research can be divided into three main areas: biofouling development, biofouling impacts and biofouling prevention and management (Fig. 1.4). By approaching knowledge gaps identified in the Introduction in all three areas, this thesis aims to increase the overall understanding of biofouling on salmon farms. Furthermore, by focusing part of the study on the hydroid *E. larynx*, it aims to extend the knowledge on one of the most problematic fouling species in the Norwegian salmon farming industry; knowledge which can improve current farm management practices, provide information for future aquaculture risk assessments and contribute to the development of novel antifouling technologies. To achieve this, the following research questions were posed:

1. How do immersion period, sampling time, mesh size and variability between cages influence the development of biofouling on cage nets? (***Paper I***)
2. Does the hydroid *E. larynx* benefit from fish farm wastes by incorporating them into its diet? (***Paper II***)
3. Is the oxygen consumption of the hydroid *E. larynx* a threat to the cultivated salmon? (***Paper III***)
4. Do novel antifouling technologies based on surface wettability and microtopography deter the settlement of *E. larynx*? (***Paper IV***)

To answer these research questions, two additional questions had to be addressed:

- What is the distribution of the hydroid *E. larynx* on fish farms along the Norwegian coast? (***Paper III***)
- How much hydroid biomass can grow on a fish cage? (***Paper II***)

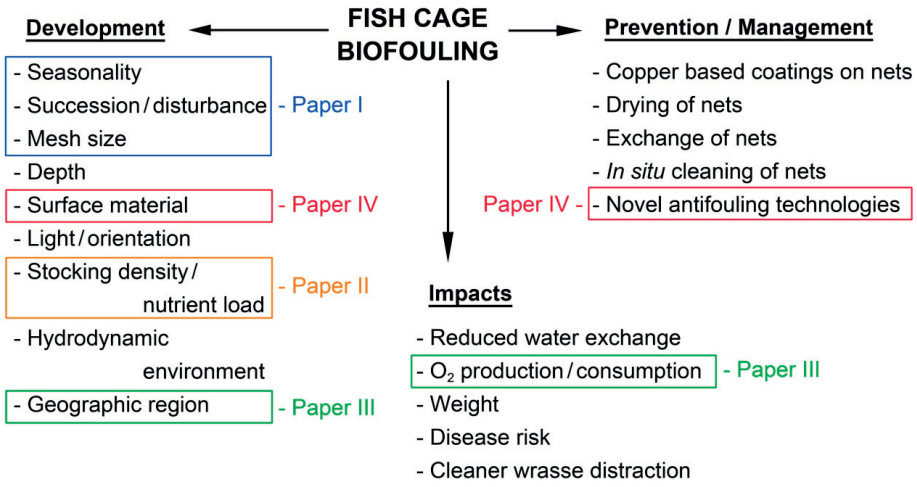


**Fig. 1.4:** Overview of factors that influence biofouling development, the main impacts of biofouling, and common prevention and management strategies. Coloured boxes indicate the research areas investigated in the individual papers of this thesis.

## 2. Results

To answer the research questions posed in the previous chapter (Section 1.4), four main experiments were conducted that resulted in four papers investigating aspects of the development, the impacts and the prevention and management of biofouling on cage nets (Fig. 1.4).

### 2.1. Influence of immersion period, sampling time, mesh size and variability between cages on the development of biofouling on cage nets

(This description is based on Paper I: Variability of biofouling communities on fish cage nets: A 1-year field study at a Norwegian salmon farm; see Chapter 5)

Biofouling is a problem that is prevalent throughout the year in the Norwegian salmon industry, yet little is known about the natural and farm operational factors that influence the abundance and community composition. To answer Research Question 1, this experiment investigated the importance of immersion period, sampling time, mesh size and variability between cages regarding the development of biofouling on net cages. In a 1−year field study at a commercial salmon farm in Central Norway, the effects of these four factors on the biomass, species richness and community composition of biofouling on net panels were measured by comparing 10×10 cm net panels with two different mesh sizes (13 and 25 mm half-mesh), immersed for periods of 1, 3, 6 and 12 months, and replicated over three individual cages.

A total number of 83 species and 7 multi-species categories were identified on 432 net panels. Biomass accumulation ranged from 0.5 to 83.1 g per panel and was strongly influenced by immersion period and sampling time while at the same time no significant differences could be found between the two mesh sizes or the three individual cages. The highest species richness added over all panels of one immersion period was found on 3-month panels (72 species), followed by 6-month (67 species), 1-month (65 species) and 12-month (51 species) panels. Total species richness per sampling time ranged from 1 to 52 species. Both immersion period and sampling time significantly influenced the species richness on 1-, 3- and 6-month panels. Variability between cages affected species richness on 1- and 3-month panels, while differences between the two mesh sizes were only found on some 1-month panels.

The five most frequent sessile macrofoulers were the amphipod *Jassa falcata*, the blue mussel *Mytilus edulis*, the hydroid *Ectopleura larynx* and the algae *Polysiphonia stricta* and *Saccharina latissima*. *M. edulis* and *E. larynx* contributed most to the biomass and, together with the algae, they showed a distinct successional pattern. While the dominance of *M. edulis* increased with length of immersion period, the proportion of *E. larynx* decreased. The community composition on 1-, 3-, and 6-month panels differed significantly among sampling times and varied between the two mesh

sizes and the three cages. After 12 months of immersion, the biofouling community had reached a climax state where neither mesh size nor variability between cages had an effect.

Immersion period and sampling time were the two factors with the strongest effect on biomass. Immersion period reflects the influence of succession, as shown in the declining proportion of the hydroid biomass on the panels while the dominance of *M. edulis* increased with increasing length of immersion period. Hydroids are one of the first colonisers of newly available substrate and are often inferior in competition with other macrofouling organisms (Boero 1984; Migotto et al. 2001), while blue mussels are known for their strong performance as competitors in communities of later successional stages (Dean & Hurd 1980; Greene & Grizzle 2007). Similar successional patterns were found by Cook et al. (2006) for the hydroid *Obelia longissima* on a Scottish salmon farm.

Sampling time reflects the influence of season on the rate and composition of biofouling. Especially the 1- and 3-month panels showed a pronounced seasonal pattern with a biomass peak during summer, a pattern that is typical for temperate marine waters (Raymont 1980; Zheng 1989; CRAB 2007) and was also observed by Greene and Grizzle (2007) on net panels at an aquaculture facility.

No difference in biomass between the two mesh sizes could be found although the small meshed nets offered 30% more surface area for settlement than the large meshed nets. A study by Tseng and Yuen (1978) concurs with these findings, however, several other studies show that small meshed nets accumulate more fouling in a short time while large meshed nets may have a greater carrying capacity over longer time (Milne 1975; Mak 1982; Cheah & Chua 1983).

While there were no differences in biomass, species richness and community composition varied between individual cages. These variations may be caused by several factors, such as the hydrodynamic micro-environment that influences for example the water flow (Madin et al. 2009; 2010), the number of larvae arriving and attaching to a substrate (Todd 1998) or the physical living conditions (Leichter & Witman 1997). However, since none of these variables was directly measured at the individual cages, it is impossible to discern the sources of variation, which may, in addition, be masked by natural small-scale variation (Hodson et al. 1995).

Another factor that was expected to influence the development of biofouling on the net panels was fish stocking density and feed input, because the nutrient input of feed and faeces of stocked fish may enhance the amount of biofouling on cage nets (Madin et al. 2010). Although the three investigated cages differed in stocking density and feed input, the differences between the cages were either not high enough to induce a measurable difference in biofouling, or other factors had a much stronger effect on the developing biofouling on the individual cages.

The results of this first comprehensive analysis of the species present on Norwegian salmon cages highlights the susceptibility of Norwegian salmon farms to a

diverse range of fouling species and provides valuable information for farm operators, implemented biofouling monitoring projects, and for the design of novel antifouling technologies.

### 2.2. Investigation of the link between fish farm nutrients and biofouling organisms

(This description is based on Paper II: Using stable isotopes to identify the link between fish farm nutrients and associated biofouling organisms; see Chapter 5)

Several species living in the vicinity of aquaculture sites are known to include fish farm wastes into their diet (Gao et al. 2006; Handå et al. 2012). This may have a facilitating effect on the abundance of the species within the influence range of a fish farm. Hydroids and caprellids are common fouling species that can reach high abundances on cage nets. This experiment analysed if the incorporation of fish farm derived nutrients by hydroids may explain these high abundances. Caprellids were investigated in addition. The potential food sources which are plankton, particulate organic matter (POM; collected upstream and at the cage), caprellids (they may be preyed on by hydroids), fish feed and fish faeces were analysed using stable carbon ($\delta^{13}C$) and stable nitrogen ($\delta^{15}N$) isotopes after acid treatment of the samples to account for the potential influence of inorganic carbon in the samples (Jacob et al. 2005; Serrano et al. 2008). In addition, a reference population of hydroids was sampled.

*E. larynx* collected from a salmon farm had $\delta^{13}C$ and $\delta^{15}N$ values of $-22.1 \pm 0.3$‰ and $9.9 \pm 0.1$‰, respectively, and was significantly enriched in $\delta^{13}C$ and $\delta^{15}N$ by 1‰ and 0.6‰, respectively, compared to the reference hydroids. A likely reason for this is the closer proximity to land of the reference hydroids where they are under stronger influence of terrestrial carbon food sources with a more depleted $\delta^{13}C$ signature compared to marine food sources (Riera & Richard 1997).

The $\delta^{13}C$ and $\delta^{15}N$ values for plankton were both depleted relative to *E. larynx* from the salmon farm by on average 0.6‰ and 2.2‰, respectively, suggesting that zooplankton most likely was the primary dietary source for the hydroids. All other potential food sources were highly depleted in both $\delta^{13}C$ (1.6–4.0‰) and $\delta^{15}N$ (3.7–5.4‰) relative to *E. larynx* from the farm and appear not to contribute to the hydroids diet. These results are supported by a study of Rensel and Forster (2007) who found no indication that the diet of the planktivorous cnidarian *Metridium senile*, growing at a reference location, would differ from organisms sampled from a fish farm, concluding that they, similar to *E. larynx*, do not utilize farm wastes.

The analysis of caprellid nutrition based on the $\delta^{13}C$ value of $-23.7 \pm 0.1$‰, identified upstream POM and fish feed, which were depleted by 0.6‰ and 0.9‰, respectively, as the most likely food sources. All other potential food sources were either too depleted (cage POM and fish faeces) or enriched (plankton) compared to the

caprellids. Due to the high variability in the $\delta^{15}N$ data, the samples were re-evaluated based on the results of the non-acidified samples ($\delta^{15}N = 8.1 \pm 0.0‰$). According to this data, fish feed as well as upstream and cage POM (1.9, 2.9 and 3.1‰ enrichment, respectively) were within the predicted range of enrichment employed in this study. These results indicate that the caprellids living on the fish cages incorporated fish farm wastes into their diet. Stable isotope studies by Rensel and Forster (2007) and fatty acid analyses by Cook et al. (2010) also show that caprellids, in contrast to *E. larynx*, may enrich their diets with fish farm wastes.

As the stable isotope analyses showed that hydroids mainly feed on zooplankton, it was further assessed whether the food demand of a hydroid colony growing on a salmon cage could be met by the zooplankton that was available from surrounding waters. The biomass and respective nutritional demand of a hydroid colony was estimated and compared with the zooplankton biomass transported through the cage. The biomass accumulation of hydroids on a cage was estimated to be up to 6.7 t wet weight with a respective food requirement of approximately 0.2 t C $d^{-1}$ (based on data from Guenther et al. 2010). Calculations suggest that this can be supplied by the local plankton community where approximately 0.5 t C $d^{-1}$ zooplankton are available in the water masses passing the cage (Y. Olsen, unpublished data). This supports the results from the stable isotope analysis that *E. larynx* growth is independent from fish farm derived nutrients. However, it also suggests that hydroids, through their ability to selectively feed on specific prey species (Gili et al. 1996), may be able to diminish specific species and affect the zooplankton community composition.

### 2.3. Prevalence and oxygen consumption of hydroids

(This description is based on Paper III: Prevalence and oxygen consumption of the fouling hydroid *Ectopleura larynx*: implications for salmon aquaculture; see Chapter 5)

In order to assess the spatial and temporal variability of hydroids on farms along the Norwegian coast, a survey was conducted with farm managers, including an investigation of the presence or absence of other problematic fouling species. The results from the survey show that the hydroid *E. larynx* can be found on salmon farms between the southern tip of Norway to regions north of Tromsø, with a high presence in South-West and Central Norway. On some farms in these regions, hydroids are found all year round with a strong presence between July/August and November, when cleaning is more frequent. Further north, hydroids are not found on all farms, and biofouling on cage nets is generally considered less severe resulting in less frequent cleaning. Besides hydroids, five other taxa were identified as problematic fouling species by the farm managers. These were blue mussels (*Mytilus edulis*), kelps, caprellid amphipods, small algae and diatoms, and occasionally sea squirts. The results from the survey show that the hydroid *E. larynx* is a problematic fouling species on

salmon cages along the entire Norwegian coast and that hydroid fouling may be more common in regions further north than previously thought.

Biofouling that occludes the nets of the fish cages leads to reduced water exchange in the cage (Madin et al. 2009; Madin et al. 2010). In addition, the fouling species on the cage nets may affect the oxygen levels in the cage through their own respiration, which may lead to a further reduction of the oxygen budget in the cage and increase the negative impact upon fish health (Wildish et al. 1993; Cronin et al. 1999; Oppedal et al. 2011a). In this experiment, the oxygen consumption of *E. larynx* was measured at three water temperatures (12, 14 and 16°C), reflecting ambient conditions during the main fouling season. Measurements were conducted for approximately 100 minutes using non-invasive oxygen-sensitive spot technology.

The oxygen consumption of hydroids measured at 12°C ($0.54 \pm 0.03$ ml $O_2$ $g^{-1}$DW $h^{-1}$) was significantly lower than the oxygen consumption at 14 and 16°C. The oxygen consumption measured at 14°C ($1.22 \pm 0.04$ ml $O_2$ $g^{-1}$DW $h^{-1}$) did not differ significantly from the consumption measured at 16°C ($1.09 \pm 0.04$ ml $O_2$ $g^{-1}$DW $h^{-1}$).

To estimate whether the presence of hydroids on cage nets could affect the oxygen budget of a cage and distinctly lower the amount of oxygen available to the cultivated salmon, the oxygen consumption of *E. larynx* and Atlantic salmon was compared. For the simulation of a worst case scenario, the calculation was based on the highest measured oxygen consumption of the hydroids. Furthermore, a fully stocked and heavily fouled fish cage was assumed, containing 200 000 fish at 2.5 kg each, equalling a total fish biomass of 500 t with an oxygen consumption of 4 mg $O_2$ $kg^{-1}$WW $min^{-1}$ (Folkedal 2010), and carrying a hydroid biomass of 6.7 t (Bloecher et al. in review). Under these assumptions, the salmon would consume 2000 g $O_2$ $min^{-1}$ while the hydroid population on the cage net would consume 23 g $O_2$ $min^{-1}$, equating to 1.1% of the oxygen consumption of the salmon. Cronin et al. (1999) argue that an oxygen consumption of 3% by biofouling organisms would not significantly affect the oxygen budget of a cage, but may be sufficient to decrease the oxygen concentration below critical levels in an already stressed environment. However, even under stressed conditions, a 1.1% reduction of the oxygen budget of a cage due to the oxygen consumption of hydroids is unlikely to lower oxygen levels beyond critical values. Instead, the main threat of hydroid biofouling for the salmon is still the occlusion of the net that reduces the water exchange.

## 2.4. Influence of surface wettability and microtopography on the settlement of *Ectopleura larynx*

(This description is based on Paper IV: The fouling hydroid *Ectopleura larynx*: a lack of effect of next generation antifouling technologies; see Chapter 5)

Copper based coatings on nets are the most commonly used antifouling treatment in the salmon aquaculture industry, but novel antifouling technologies are needed due to the limited effectiveness of the coatings and environmental concerns regarding the leaching of copper into the water. Materials where the physical surface properties, such as surface wettability and microtopography, are specifically tailored to reduce or prevent the settlement of biofouling organisms provide a potential solution (Magin et al. 2010; Callow & Callow 2011; Ralston & Swain 2011; Scardino & de Nys 2011). In order to identify the effects of surface wettability and microtopography and their potential as novel antifouling technologies against the settlement of *E. larynx*, both laboratory- and field-based settlement assays were conducted.

In the first experiment, the settlement preferences of hydroid larvae for 12 materials with wettabilities ranging from hydrophobic (54° water contact angle) to hydrophilic (112°) were tested in a no-choice bioassay. Although settlement differed significantly between materials, with the highest average settlement on polytetrafluoroethylene (PTFE, 95%) and the lowest on untreated polyurethane (PU, 53%), no trend regarding the tested wettabilities could be found. Furthermore, both the most preferred and the least preferred materials had similar water contact angles (untreated PU: 96°, PTFE: 103°), and none of the tested materials were able to reduce average settlement below 50%. This is in accordance with previous studies where settlement of hydroid larvae on glass rods with various wettabilities did not differ, while concurrently settled barnacle and bryozoan larvae showed clear preferences (Roberts et al. 1991; Holm et al. 1997).

In the second set of no-choice assays, the effects of selected microtopographies on the settlement of *E. larynx* were tested. Prior to the assays the tentacle tips and the aboral pole, which play a crucial role during settlement, were measured in newly released, elongated and just settled larvae. The average diameter of the aboral pole was 143, 139 and 194 µm, respectively, and the tentacle tips had average length/width dimensions of 65×55 µm (for both newly released and elongated larvae) and 83×57 µm (settled larvae). According to the attachment point theory (Scardino et al. 2006; Scardino et al. 2008), settlement was expected to be reduced on microtopographies below the size of the aboral pole (i.e. 200 µm). Therefore, microtopographies between 100 and 600 µm were selected for testing on high-density polyethylene (HDPE), a material commonly used in aquaculture, while microtopographies between 40 and 400 were tested on polydimethylsiloxane (PDMS), which is similar to many foul-release coatings.

No systematic effect of microtopography on the settlement of *E. larynx* could be found. Similarly, there were no preferences for any of the examined microtopographies between 40 and 400 µm in a 12-day field test using PDMS surfaces at a commercial salmon farm. These results contrast with Nellis & Bourget (1996) who found reduced settlement on sand-coated panels with 250 and 500 µm heterogeneity levels. However, their explanation was based on related measurements of the aboral pole of approximately 1000 µm, which strongly deviates from this study as well as measurements by Pyefinch and Downing (1949).

The hydroids in this study preferred to settle in the flat-bottomed channels on PDMS microtopographies between 80 and 300 µm compared to the equally wide ridges. This suggests that the hydroids can actively choose a settlement site, a behaviour that has been documented before in *E. larynx* during choice assays regarding colour preferences (Guenther et al. 2009) and also in *Ectopleura crocea* during settlement on complex surfaces (Lemire & Bourget 1996).

A reason for the lack of deterrence of wettability and microtopography may be the high plasticity of both the aboral pole, fitting into channels as narrow as 80 µm, and the hydrorhiza, whose immediate growth into even the smallest channels can give additional support to the hydroids. In contrast to organisms such as the bryozoan *Bugula neretina*, which are attached by a single attachment point, resulting in a strong preferences for a specific wettability and microtopography (Roberts et al. 1991; Rittschof 2001), hydroids quickly develop multiple attachment points through their expanding stolonal system. This may explain their settlement even under unfavourable conditions (Ralston & Swain 2011).

### 2.5. Summary of main results

The present study has increased our understanding of the development, impacts and management of biofouling on aquaculture facilities, and has established background information for farm management practices. By focussing on one of the main fouling species, the hydroid *E. larynx*, questions regarding growth facilitation by nutrient wastes released from salmon farms and the impact of biofouling on the cage oxygen budget could be answered. In addition, the potential of novel antifouling strategies based on physical surface characteristics was investigated.

**The main results of the presented work are:**

1. The biofouling community on a salmon farm in Central Norway consisted of up to 90 species and multi-species groups. Of the four tested factors, immersion period and sampling time had the strongest influence on the biofouling community, while mesh size and variability between cages had only limited influence.

2. Based on a survey of fish farmers, hydroids were found to occur on most salmon farms along the Norwegian coast, and at some farms even throughout the year. However, they are more abundant and are consequently a bigger problem in South-West and Central Norway. In addition to hydroids, there are five other groups of problematic species; mussels, caprellids, small and large algae and sea squirts.

3. Estimations suggest that a single cage can be fouled by up to 6.7 t wet weight of hydroids, which will require a daily food intake of 0.2 t C.

4. Nutrients derived from salmon farm wastes did not contribute to the diet of *E. larynx*. Instead, the hydroids mainly fed on zooplankton, which was supplied in sufficient amounts by the local environment. Consequently, the extensive growth of hydroids on cage nets may be largely independent of fish farm wastes. Caprellids, however, may incorporate fish farm wastes into their diet.

5. The oxygen consumption of *E. larynx* at water temperatures of 12, 14 and 16°C was 0.54, 1.22 and 1.09 ml $O_2$ $g^{-1}$DW $h^{-1}$, respectively, which is similar to the respiration rate of Atlantic salmon. However, because the biomass of the cultivated salmon by far exceeded the biofouling biomass on the cage, the oxygen consumption of the hydroid population equalled only 1.1% of the salmon's oxygen consumption. This minimal reduction of the oxygen budget of a cage is unlikely to be a threat to the health of the salmon.

6. Although settlement of *E. larynx* actinula larvae differed between materials with various wettabilities, no trend regarding the tested wettabilities could be found, and none of the materials was able to reduce the settlement below 50%. Furthermore, the tested microtopographies had no effect on the settlement of the hydroid larvae, neither in laboratory nor field tests, thereby excluding the tested materials and microtopographies as potential future antifouling strategies against hydroid settlement.

# 3. Synthesis and future research

In this chapter the results of this thesis are discussed and evaluated regarding their implications for the understanding of biofouling development and its consequences, as well as for biofouling management and prevention at Norwegian salmon farms. Where appropriate, areas of interest and important knowledge gaps are highlighted as possible directions for future research.

## 3.1. Biofouling development

Biofouling development depends on a variety of factors (Fig. 1.4). The data presented in this thesis suggests that among the four factors tested, seasonality (simulated by the sampling time) and succession or disturbance (simulated by the length of the immersion period) had the strongest effects on biofouling biomass, species richness and community composition (Paper I). Especially on the 1-month panels, biomass peaked strongly during the summer months, the main fouling season, as reported by farmers and reflected in the more frequent net cleaning common during summer. Seasonality and succession are closely connected, and successional patterns differed strongly during the year due to the variations in species and numbers of propagules available in the water (Paper I). By controlling the timing of when nets are introduced into the water and the cleaning schedule, farm management has a large influence on seasonality and succession within the fouling community, by favouring for example early colonists with short generation times (e.g. hydroids; Sousa 1979; Guenther et al. 2010).

The third factor tested, mesh size, had an effect on community composition, but not on biomass. One of the possible reasons is natural small scale variation, which could also explain the variation in community composition measured between panels of different cages (Paper I). For farm management this implies that even though the cages of a farm were treated equally regarding biofouling prevention and management, variations in community composition may still occur.

Fish farm wastes can have a facilitating effect on the growth of biofouling (Cook et al. 2006; Madin et al. 2010). In the presented 1-year field study where the biofouling on three cages was analysed, the differences in fish stocking density and respective feed input where too small or masked by other factors to lead to measurable differences in the biofouling community between the individual cages (Paper I). However, this does not exclude the possibility of a general influence of fish farm wastes on biofouling development. The data in Paper II showed that caprellids were likely to include fish farm wastes in their diet. This may have a facilitating effect on population growth, and may explain high abundances of caprellids on fish farm cage nets described in other studies (Ashton 2006; Rensel & Forster 2007). However, this does not apply to all biofouling organisms. The hydroid *E. larynx* did not include fish farm derived nutrients

into its diet, but relied mainly on zooplankton (Paper II). Therefore, the large biomass of hydroids was not further enhanced by released waste products from the farm, but can be attributed to the favourable living conditions at the farm (Paper II). For hydroids, these include sufficient food supply through zooplankton, available settlement space on the nets and a population of potential partners for reproduction (Gili & Hughes 1995; Prendergast 2010), all of which is available at a farm. Unfortunately, the fact that hydroids are mostly independent from nutrient wastes released from the farms removes the possibility to manage population growth through controlling the feed input. For other species, such as caprellids, the reduction of available feed particles through the development of e.g. feed with lower solubility and fragmentation, could be a possibility to reduce population growth.

The independence of the hydroids from fish farm wastes may also lead to some environmental concerns. With zooplankton being the principal basis of nutrition, the hydroid biomass on a cage, which can amount to as much as 6.7 t wet weight (Paper II), may influence the zooplankton community in the area. Although there is a sufficient amount of zooplankton available to sustain the hydroid population, the fact that *E. larynx* is able to feed selectively on preferred prey organisms may lead to the depletion of selected species from the local species pool (Gili et al. 1996; Maar et al. 2008). As this may reduce the biomass and alter the composition of the zooplankton community of a region, and especially that of copepod species (Gili et al. 1996), future aquaculture risk assessments should include considerations regarding the selective depletion of zooplankton by the hydroid population associated with a fish farm.

The Norwegian salmon farming industry intends to become one of the world's leading seafood producers to meet the growing demand for seafood that comes with the increase of population numbers, buying power and awareness of health benefits of a diet that contains fish. These plans include an increase of today's annual salmon production of approximately 1 million tonnes to 5 million tonnes by 2050 (Olafsen et al. 2012). To reach such ambitious goals, the exploration of new sites suitable for cage culture is inevitable and will most likely result in a spread of facilities up north and further offshore (Olafsen et al. 2012). However, the establishment of fish cages in more exposed locations and offshore areas still poses many challenges. Regarding the regional differences shown in the hydroid distribution survey, such as the latitudinal cline in prevalence throughout the year (Paper III), analogous differences in biofouling are expected between coastal and offshore sites. However, it is difficult to simulate an offshore site because the presence of farmed fish and the respective nutrient input can play an important role for the development of biofouling (Madin et al. 2010). In order to predict some of the challenges a further offshore site may present, conclusions drawn from comparisons between farm sites at exposed, coastal locations and sheltered fjords could provide some basic knowledge that is currently lacking.

### 3.2. Biofouling impacts

The impacts of biofouling are numerous and diverse, and include decreased water exchange, increased weight, elevated disease risk and decreased performance of the cleaner wrasse (Fig. 1.4). In addition, biofouling on cage nets may represent a net production or consumption of oxygen, depending on the proportion of algae, which affects the oxygen budget of the cage. If the oxygen consumption outweighs the primary production, this may critically reduce the amount of oxygen available to the cultivated fish (Wildish et al. 1993; Cronin et al. 1999). The specific rate of oxygen consumption of the hydroid *E. larynx*, one of the main fouling species in Norwegian aquaculture, is comparable to the consumption of salmon. However, as the biomass of the hydroids on the cage, which can amount to up to 6.7 t, is exceeded markedly by the biomass of the cultivated salmon, the hydroids consumption equates to only 1.1% of that of the cultivated salmon (Paper III). Consequently, the oxygen consumption of the *E. larynx* population is unlikely to be an additional threat to the salmon's health and does not need to be included in models where oxygen flows and budgets in and around cages are calculated.

In the present study, biofouling development was assessed based on biomass, species richness and community composition (Paper I). These are reliable indicators for the measurement of biofouling impacts such as increased weight or risks of diseases associated with specific fouling species. However, some impacts, such as reduction of water flow and increased drag, are caused by mesh blockage (Swift et al. 2006; Lader et al. 2008), which can be better assessed through measurements of percentage net aperture occlusion (PNO; Braithwaite et al. 2007; Guenther et al. 2010). PNO measurements were planned for this study, but the high variation in fouling cover and an inappropriate choice of a background colour made the analysis of the data impossible. While the biomass of hydroids correlated well with PNO up to PNO values of ~85% (L. Gansel, unpublished data), correlations for other species are not available yet. Especially multi-species communities require direct PNO measurements as species with soft bodies (e.g. hydroids, algae) are likely to differ in their influence on the water flow and drag compared to hard bodied species (e.g. mussels, Swift et al. 2006). Therefore, PNO measurements are suggested for future studies to make predictions of water flow and drag more precise and to improve real-time monitoring of biofouling for fish farmers.

### 3.3. Biofouling prevention and management

The various surface materials tested in this thesis with regard to development of potential novel antifouling technologies (Paper IV) were ineffective at preventing the settlement of the hydroid *E. larynx* in both laboratory and field tests. Neither surface

wettability nor microtopography reduced the settlement below 50% in the bioassays. Most likely the high plasticity of the attachment organs that maximised the contact area between the organism and the surface, and the flexible growth form enabled the hydroids to overcome unfavourable settlement conditions. Although only a limited number of materials with various wettabilities and microtopographies were tested, the data suggested that antifouling methods based on these physical properties may not provide a solution to the hydroid fouling problem (Paper IV).

Paper III showed differences in hydroid prevalence on farms along the Norwegian coast. While on some farms hydroids grow all year round, many farmers have reported that they are present during the main fouling season between August and November, but absent throughout the rest of the year. Where hydroids are absent for some time, the population has to re-establish itself every year. There are two possible explanations for the re-colonisation of such seasonally impacted farms: (1) Larvae arrive from the surrounding 'wild' population and colonise the cage net; or (2) some hydroids remain at the farm and retreat into a resting stage when local environmental conditions are unfavourable. When the conditions improve again the hydroids re-emerge (Bavestrello et al. 2006) and rapidly re-colonise the nets. If the re-colonisation of farms occurs from wild populations, the possibilities for prevention will be very limited. However, if the main number of recruits re-colonises nets from resting stages, adjusted cleaning strategies that focus on the complete removal of these resting stages and the elimination of potential reservoirs on surfaces other than the net, such as the feed barge or mooring lines, could help delay the onset of the hydroid fouling period.

A third source of viable colonisers in areas with a high density of salmon farms could be the large quantity of colony fragments and actinula larvae that are released into the water as wastes generated during the widely employed method of *in situ* cleaning of cage nets (Carl et al. 2011). Although laboratory experiments indicate that the spreading of actinula larvae of *E. larynx* is limited because of their negative buoyancy (Pyefinch & Downing 1949), reports of floating adult colonies of other hydroids such as *Clytia hemisphaerica* (Clare et al. 1971) suggest that free-swimming colonies could distribute and release larvae over greater distances. Consequently, depending on their ability to drift with water currents, the colony fragments and actinula larvae could lead to the establishment of a population on farms downstream. Therefore, the understanding of the mode of colonisation of individual farms and the ability to estimate the distribution range of larvae and adult colonies may help reduce hydroid colonisation of salmon farms.

In addition to the potential infestation of other farms located downstream, the actinula larvae released during *in situ* cleaning may directly settle on the cleaned net. Furthermore, *in situ* cleaning potentially supports the development of hydroid monocultures through the often incomplete removal of biofouling, which allows a rapid recovery and subsequent re-colonisation of the cleaned cage net (Guenther et al. 2010).

By constructing washing equipment that completely removes biofouling from the nets or kills the remaining organisms, for example by combining cleaning with the use of hot water or acetic acid (Guenther et al. 2011), the intervals between cleaning events could most likely be prolonged. An associated capturing unit that collects the cleaning waste could prevent the release of potential settlers and pathogens associated with biofouling and furthermore reduce the nutrient load of the environment (Brooks et al. 2002).

Finally, the collection of cleaning waste may lead to a change in perception of biofouling in aquaculture, from generally being considered as a problem to acknowledging its potential as a resource. Depending on production costs that are difficult to estimate based on the current state of knowledge, the collected cleaning waste could be used for the production of biofuels, as fertilizer, or for the extraction of raw materials such as omega−3 fatty acids, which are valuable additives to fish feed as well as human food (Rubio-Rodríguez et al. 2010). In comparison to Integrated Multi-Trophic Aquaculture (Chopin et al. 2001; Troell et al. 2009; Handå et al. 2012) where selected species are cultivated on special structures in the vicinity of fish cages in order to reduce the environmental consequences of nutrient emissions and offer additional application for biofuel production (kelp species) or human consumption (mussel culture), the harvest of biofouling biomass would not need additional substrates or care. Under optimal conditions, these two concepts could be combined, maximising the profit of the farm while minimizing the ecological impacts.

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## 5. Paper I – IV

## Paper I

**Variability of biofouling communities on fish cage nets:**
**A 1-year field study at a Norwegian salmon farm**

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# Variability of biofouling communities on fish cage nets: A 1-year field study at a Norwegian salmon farm

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ABSTRACT

The accumulation of biofouling on cage nets is a major problem and cost factor in finfish aquaculture worldwide. Norway is one of the main producers of Atlantic salmon, but biofouling on salmon farms has not been studied systematically. In a 1-year field study at a commercial salmon farm in Central Norway the effects of immersion period (1, 3, 6 and 12 months), sampling time, mesh size (13 and 25 mm half-mesh) and variability between three individual cages on the biomass, species richness and community composition of biofouling on net panels were investigated. Biomass accumulation ranged from 0.5 to 83.1 g per panel. A total number of 90 species and multi-species categories were identified with total species richness ranging from 1 to 52 species per sampling time. The five most frequent sessile macrofoulers were the amphipod *Jassa falcata*, the blue mussel *Mytilus edulis*, the hydroid *Ectopleura larynx* and the algae *Polysiphonia stricta* and *Saccharina latissima*. *M. edulis* and *E. larynx*, along with the mussel *Hiatella arctica*, contributed most to the biomass. Immersion period and sampling time had a strong effect on the biomass accumulation, the species richness and the community composition. The variability between cages and, to a lesser extent, the differences in mesh size only influenced the community composition for 1-, 3- and 6-month samples. After 12 months of immersion, the biofouling community had reached a climax state where neither mesh size nor variability between cages had a significant effect. The results of this study may contribute to the optimisation of current antifouling treatments of aquaculture nets and provide background knowledge for farm operation and management with regard to monitoring and cleaning procedures.



## 1. Introduction

Of the 1.14 million tonnes of fish cultured in Norway in 2011, 93% were Atlantic salmon (*Salmo salar*; Norwegian Directorate of Fisheries, 2012), making Norway the world's largest supplier of farmed salmon (FAO Fisheries and Aquaculture Department, 2012). Juvenile salmon are raised in land-based freshwater systems before being transferred to the sea, where the grow-out phase until a slaughter weight of approximately 5 kg takes 18 months on average. Small meshed nets (~13 mm half-mesh) are employed at the beginning of the grow-out and later replaced with large meshed nets (~25 mm half-mesh), as the salmon increase in size, in order to allow higher water flow through the net and minimise the surface available for biofouling. The cage structures may be exchanged for ones with larger diameters, along with the nets, when the fish are sorted according to their size and redistributed among the cages. Due to considerable variation in the farming processes, cage nets can be exposed to biofouling pressure for periods of up to one year or longer.

Biofouling is a major problem and expense factor in finfish aquaculture worldwide. The development of biofouling communities on fish nets can reduce water flow through the net and affect oxygen supply, waste removal and the susceptibility of farmed fish to diseases. The occlusion and increased weight of the net can also negatively impact cage structure and stability (reviewed in Braithwaite and McEvoy, 2005; de Nys and Guenther, 2009; Dürr and Watson, 2010; Fitridge et al., 2012). The biofouling communities that develop on aquaculture installations are dominated by mussels, ascidians, hydroids and – depending on light availability – algae (reviewed in Braithwaite and McEvoy, 2005; de Nys and Guenther, 2009; Dürr and Watson, 2010; Fitridge et al., 2012). In Norwegian waters and other temperate regions, the most common species are the blue mussel *Mytilus edulis*, the ascidian *Ciona intestinalis*, the hydroid *Ectopleura larynx* (syn. *Tubularia larynx*) and algae of the genus *Ulva* and *Ectocarpus*. In addition, caprellid amphipods can reach critical abundances (Braithwaite and McEvoy, 2005; de Nys and Guenther, 2009; Guenther et al., 2010; Olafsen, 2006). The development of the communities may be influenced by a wide range of factors, including seasonality, succession, depth, physical and chemical water properties, hydrodynamic conditions and substrate orientation and material. (Braithwaite and McEvoy, 2005; de Nys and Guenther, 2009; Dürr and Watson, 2010; Fitridge et al., 2012). In a more applied context, the

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production schedule of the farming facility determines the seasonality as well as the succession through the introduction of nets into the water and their immersion period or disturbance by washing.

The main strategy in Norwegian aquaculture to reduce cage net biofouling is the use of copper-based antifouling coatings on nets. However, these coatings prevent biofouling for only up to 6 months (Braithwaite and McEvoy, 2005; Braithwaite et al., 2007) due to progressive wear and leaching of the copper, and are therefore combined with regular *in situ* washing of the nets (Olafsen, 2006). Washing removes much of the biofouling (Guenther et al., 2010), but because the antifouling agent is not renewed, it also creates more suitable settlement surfaces. Washing of nets is generally conducted every eight (winter) to 2 weeks (summer), but can occur as often as weekly during periods of high biofouling pressure.

Although biofouling on nets is a cost- and labour-intensive part of fish farming operations, it has not been investigated systematically at farms situated in Norwegian coastal waters. So far, knowledge is limited to the dominant species groups and their occurrence at certain times of the year (Guenther et al., 2010; Kvenseth, 1996; Olafsen, 2006). There is a profound lack of knowledge on the more detailed community composition of cage biofouling communities and its seasonal variability. With almost 1000 salmon farms operating in Norwegian waters in 2011 (Norwegian Directorate of Fisheries, 2012), fish cages have become one of the main artificial habitats along the Norwegian coast with its remote and pristine environments. Effective management of farming practices and natural resources require a better understanding of this increasingly important non-natural habitat.

A better understanding of the development of biofouling on fish cage nets is also important for several other reasons. First, the accuracy and utility of biofouling monitoring depends on the placement of sensors or the choice of sampling locations that express the biofouling development on a cage in a representative and diagnostically conclusive way. Second, understanding the composition and dynamics of fish farm biofouling is important for the design of novel antifouling technologies, as biofouling organisms vary greatly in their reaction and sensitivity towards different antifouling technologies (reviewed in *e.g.* Magin et al., 2010; Ralston and Swain, 2011; Scardino and de Nys, 2011). Lastly, understanding the factors that influence biofouling abundance and composition on individual fish farms can be used to optimise the design of larger-scale biological surveys of aquaculture operations.

The aim of this study was to characterise biofouling development on cage nets at a Norwegian salmon farm throughout one year and to determine the effects of immersion period, sampling time, mesh size of nets and the variability between cages on biofouling abundance, species richness and community composition. A range of studies have examined these factors in isolation or in pairs (*e.g.* Braithwaite et al., 2007; Greene and Grizzle, 2007; Madin et al., 2009, 2010), but their complex interactions are currently not understood.

## 2. Material and methods

### 2.1. Study site

The field experiment was conducted from December 2009 to December 2010 at a commercial salmon farm and research facility at Tristein, Central Norway, the Aquaculture Engineering (ACE) facility (63°86.91′N, 9°62.06′E, Fig. 1). The water depth at the site varies between 35 and 115 m, and the mean current flow at 5 m depth was between 0 and 0.15 m $s^{-1}$, coming from a main direction of 210° (Frank, 2012). The operation of the farm is representative of salmon farms throughout the region. In October 2009, the farm was stocked with 1.04 million Atlantic salmon divided over five cages (120 m in circumference, 12 m to the sinker tube, 16 to 18 m in total depth). The cages were equipped with 13 mm half-mesh copper-coated (Netwax Gold; Netkem AS) knotless multifilament nylon nets (Mørenot AS) and lined up in one row at 45° to the main water current (Fig. 1). From November 2009 to April 2010, cages were illuminated by two lamps per cage with 1000 W each at 5 m depth to control maturation of the fish. During June 2010, the salmon were split and redistributed over six cages (157 m in circumference, 15 m to the sinker tube, 22 to 25 m in total depth, with 25 mm half-mesh copper-coated nylon nets). Water temperature, fish biomass and daily feed input were continuously monitored (Fig. 2).

### 2.2. Experimental setup

Between December 2009 and December 2010, the effects of immersion period (or succession), sampling time (or season), mesh size, and variability between individual cages on biomass, species richness and composition of biofouling communities on net panels at the farm were examined. Biofouling development during four immersion periods (1, 3, 6, and 12 months) and on two mesh sizes (small meshed *vs.* large meshed) was analysed for three cages of the farm ($n = 4$) (Fig. 2). Two PVC frames with 16 uncoated white net panels each ($10 \times 10$ cm, Egersund Net) were suspended at a depth of 5 m on the southwest side of each cage (Fig. 1). One frame had small meshed nets ($6 \times 6$ apertures, 13 mm half-mesh) similar in mesh size to the nets used in the first phase of the salmon production cycle, while the other frame had large meshed nets ($3 \times 3$ apertures, 25 mm half-mesh) similar in mesh size to the nets used in the second phase after splitting and redistributing the fish.

### 2.3. Sampling and sample analysis

Nets corresponding to 1-, 3- or 6-month immersion periods were replaced with new ones as appropriate (Fig. 2). The samples were labelled and transported to land in buckets with seawater, each comprising the four replicates taken off one individual frame. Net panels were slightly shaken to remove free-swimming organisms before they were individually stored in PVC bottles with seawater and transported to the laboratory. There, net panels were carefully blotted dry, their total wet weights to the nearest 0.01 g measured, preserved in seawater with formalin (4%) buffered with borax, and stored in the dark at 6 °C until further processing. The total wet weight of fouling was calculated by subtracting the mean wet weight of 12 clean net panels of each mesh size from the total wet weight of the fouled net panel. Although the small meshed net had 30% more surface area than the large meshed net (net solidity analysis based on photographic assessment by pixel-count relationship between projected net material area and the total area of the panel (Standard Norge, 2009)), the total wet weight and species richness were not standardised because the aim of this study was to identify eventual differences between the two commonly used mesh sizes.

Before identification under a dissecting microscope, the net panels were placed into a 200 μm sieve and rinsed with running freshwater to remove the formalin. All organisms on each panel were identified to the lowest taxonomic level possible and their presence/absence recorded. In most cases, these were monophyletic categories; however, some species were grouped into multi-species categories if the distinction into several species was ambiguous (*e.g. Nematoda* spp., diatoms). Planktonic species were excluded from the analysis. Mobile animals living attached to the net but theoretically able to move short distances between nets (*e.g.* platyhelminthes, mobile anthozoans, swimming polychaetes, caprellids, echinoderms, and gastropods) were included in the analysis of total wet weight and species richness since they are essential parts of the net fauna. However, to allow a comparison to already published studies, the community composition analysis was based on only sedentary species. Species richness (number of species present) was calculated for every panel. In addition, if the collated biomass of a species on a panel was sufficient, it was dried at 80 °C until constant weight, and the collective dry weight was taken. An arbitrarily chosen basic weight of $1 \times 10^{-6}$ g was assumed for the collective dry weight of every species present on a panel, and the actual measured

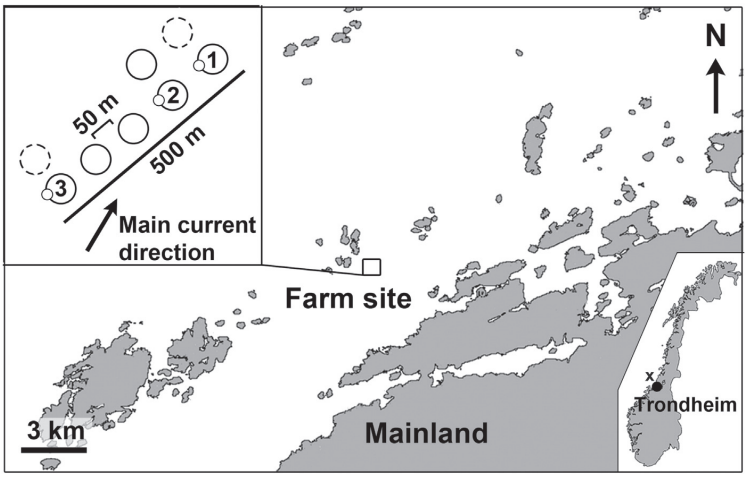


**Fig. 1.** Location of the ACE facility Tristein off the coast of Central Norway (indicated by an x) and an overview of the cage arrangement (after the splitting of fish in June 2010). Numbers correspond to cages used in the experiment with a sphere indicating the sample position. Cages depicted with dashed lines did not hold any fish.

dry weight of the assorted species was added to this value. Thus, species with few small individuals, where dry weight could not be measured, could still be included in the community analysis. Furthermore, the same community analysis was conducted based only on the presence/absence of species.

No biofouling measurements were taken in July because between June and July all PVC frames had been transferred to an empty salmon cage without a net to avoid disturbance of the fish-sorting and -splitting process taking place at the experimental fish cages. The samples were returned to their designated cages in mid-July.

### 2.4. Statistical analysis

The data were analysed separately for the individual immersion periods because of the large differences in the number of samples per period. The majority of the data was not normally distributed and had no

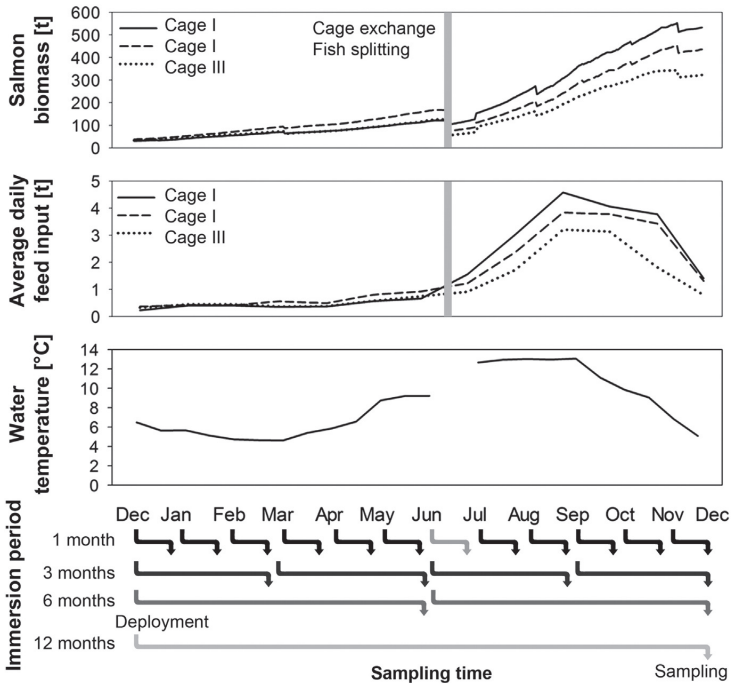


**Fig. 2.** Salmon biomass development per cage, average daily feed input per cage, water temperature (averaged over 2 weeks) and schematic overview of the sampling process. Vertical grey bars indicate the time when the cages and nets were exchanged and the fish was split. The July-samples were excluded from the analyses (see Section 2.3).

homogeneous variances due to strong seasonal differences in accumulated fouling which transformations of the data could not amend. Therefore, the effects of the fixed factors sampling time and mesh size and the random factor variability between cages on total biomass (wet weight), species richness and community composition based on biomass (dry weight) and presence/absence data were analysed with a permutational multivariate analysis of variance (PERMANOVA). PERMANOVA does not rely on assumptions of a specific data distribution (Anderson et al., 2008). Analyses were performed using the PERMANOVA+ routine in PRIMER v6.0 (Plymouth Routines In Multivariate Ecological Research, UK). The analysis of the univariate data for total biomass and species richness was based on Euclidean distance with 9999 unrestricted permutations of raw data, while the analysis of the multivariate community composition data was based on Bray–Curtis similarity with 9999 permutations of residuals under a reduced model. For all analyses, a significance level of 5% was used. However, where the number of unique permutations was ≤100, the Monte-Carlo asymptotic $p_{MC}$-value was consulted (Anderson et al., 2008). Pairwise comparisons were used to examine differences where main or interaction terms associated with immersion period and/or mesh size were significant. Since the factor variability between cages is a random factor, no pairwise tests were conducted.

For the interpretation of Figs. 3 to 6, please note that x-axis labels for 1-month panels represent the time when the net panels were collected following immersion. X-axis labels for the 3-month panels correspond to the season of immersion (winter represents December to March, spring represents March to June, summer represents June to September, and autumn represents September to December). X-axis labels for 6-month panels depict the first half (December to June) and the second half (June to December) of the year. Results are reported as mean per 0.01 $m^{-2}$ (equalling one net panel) ± 1 standard error (SE).

## 3. Results

In total, 83 species and 7 multi-species categories were identified on 432 net panels, which included ciliates, diatoms, foraminiferans, porifera, bryozoans, cnidarians, platyhelminthes, nematodes, nemertheans, annelids, molluscs, echinoderms, crustaceans, ascidians, as well as green, red, and brown algae (for details see Table 2 in the appendix). These 90 species and multi-species categories belonged to 17 different phyla and included 14 mobile species. Although only 40% of all the sessile species were filter-feeders, they made up 97% of the total dry weight biomass.

### 3.1. Total biomass

The abundance (total wet weight) of biofouling on 1- and 3-month panels varied between sampling times ($F_{(10,198)} = 112.61$, $p < 0.001$ and $F_{(3,72)} = 75.04$, $p < 0.001$, respectively; Table 1, see appendix for further results of the statistical analysis), showing a strong seasonal trend with values ranging from 0.5 ± 0.0 g in February to 63.9 ± 8.4 g in September for 1-month panels, and from 2.5 ± 0.1 g in winter to 83.1 ± 9.5 g in summer for 3-month panels (Fig. 3). In contrast, on 6-month panels, biomass did not differ significantly between the first (14.7 ± 1.1 g) and second (10.2 ± 0.8 g) half of the year ($F_{(1,36)} = 12.95$, $p_{MC} = 0.071$, Table 1; Fig. 3). The 12-month panels accumulated an average biomass of 27.9 ± 6.1 g. It should be noted that some of the accumulated biomass (mainly blue mussels) fell off these panels during early autumn before the sampling. There were no significant differences in total biomass between the two mesh sizes or the three cages for any of the tested immersion periods (Table 1; Fig. 3).

### 3.2. Species richness

The five most frequent macrofouling organisms were the amphipod *Jassa falcata*, the blue mussel *Mytilus edulis*, the hydroid *Ectopleura larynx*, the red alga *Polysiphonia stricta* and the snail *Hydrobia* sp. When excluding mobile species, the latter was replaced by the sugar kelp *Saccharina latissima*. The most frequent species, *J. falcata*, did not obtain high biomass values, but it was among the earliest macrofouling settlers, attaching its tubes to the net while the community still consisted mostly of small algae sporelings and protists. The highest species richness added over all panels of one immersion period was found on 3-month panels (72 species), followed by 6-month (67 species), 1-month (65 species) and 12-month (51 species) panels (Fig. 4, Table 2 in the appendix).

All immersion periods showed clear seasonal differences in species richness. On 1-month panels, total species richness peaked in September (51 species), while average species richness showed a less pronounced seasonality than biomass, ranging from 1.0 ± 0.0 species in January to 19.0 ± 0.6 species in September. Conversely, on 3-month panels, the highest total species richness was found in spring (52 species), followed by a steady decline. The 6-month panels showed a higher total species richness after the first half of the year than the second half (51 and 36 species, respectively; Table 1; Fig. 4). In addition to the seasonal variation, species richness on 1-month panels varied between the two mesh sizes and the three cages ('Time × Mesh size × Cage': $F_{(20,198)} = 1.66$, $p = 0.045$, while on 3-month panels significant differences were found between cages ('Time × Cage': $F_{(6,72)} = 2.92$, $p = 0.0139$), but not between mesh sizes. The 6- and 12-month panels showed no significant influence of either mesh size or cage (Table 1; Fig. 4).

### 3.3. Community composition

The community biomass (dry weight) composition of sedentary species on the 1-, 3- and 6-month panels differed significantly among seasons and varied between the two mesh sizes and the three cages ('Time × Mesh size × Cage' for 1-, 3- and 6-month panels, respectively: $F_{(20,198)} = 1.63$, $p < 0.001$; $F_{(6,72)} = 1.53$, $p = 0.022$; $F_{(2,36)} = 2.18$, $p = 0.014$; Table 1). However, the analysis of presence/absence data showed differences only among sampling times and individual cages (1-month: 'Time × Cage' $F_{(20,198)} = 2.15$, $p < 0.001$; 3-months: 'Time': $F_{(3,72)} = 124.94$, $p < 0.001$; 'Cage': $F_{(2,72)} = 3.35$, $p < 0.001$; Table 1) and not between the two mesh sizes. Therefore, the variability between

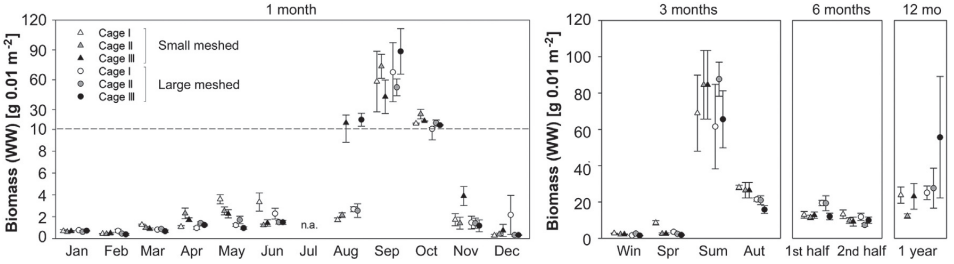


**Fig. 3.** Average (±1SE, n = 4) fouling biomass (wet weight) accumulated on small and large meshed net panels deployed at 3 individual cages for immersion periods of 1, 3, 6 and 12 months. Please note the change of scale of the y-axis in the 1 month graph.

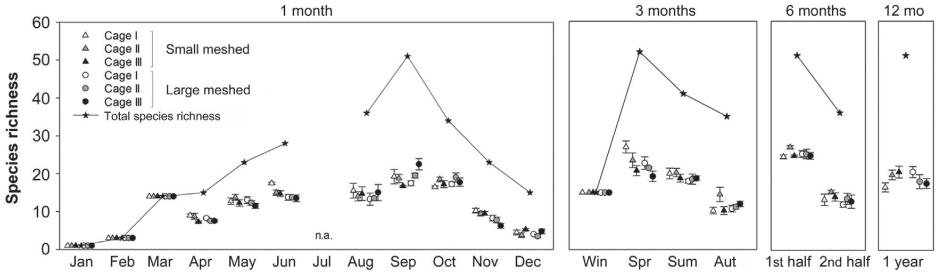


**Fig. 4.** Average (±1SE, n = 4) species richness on small- and large-meshed net panels and total species richness per sampling time deployed at 3 individual cages for immersion periods of 1, 3, 6 and 12 months.

mesh sizes was due to differences in biomass of individual species. The 12-month panels showed a similar community composition with similar biomass distributions on all panels (Table 1).

The five species that contributed most to the biomass were *M. edulis*, *E. larynx*, the mussel *Hiatella arctica*, the ascidian *Ciona intestinalis*, and *S. latissima*. When adding up the dry weight of all algal species, they had the third-highest biomass. The brown film found on the cage nets during the winter months preceding the algal growth was identified as a mixture of diatoms, ciliates and small algae sporelings.

Concentrating on the three main contributors (*E. larynx*, *M. edulis* and a mixture of algae), a strong influence of immersion period and season was found, translating into a successional pattern (Figs. 5 and 6). *M. edulis* dominated 1- and 3-month panels sampled during summer. With lengthening immersion period, the mussels made up an increasing percentage of the community in samples taken during the autumn months or the second half of the year. While the *M. edulis* dominance increased with length of immersion period, the proportion of *E. larynx* decreased (Fig. 5). On 1-month panels, the hydroids peaked after *M. edulis* and *H. arctica* had declined (Fig. 6). Hydroids accounted for 60 and 76% of the dry weight biomass in October and November, respectively, but represented only 1% of the biomass on the 12-month panels (Fig. 5). Furthermore, algae could be found throughout the year and reached a maximum in August (Fig. 6). However, they dominated the community biomass of 1-, 3- and 6-month panels only in the spring months, whereas there was no substantial contribution of algae to the dry weight biomass of 12-month panels (Fig. 5).

## 4. Discussion

A total of 90 species and multi-species categories were identified during recruitment experiments on a salmon farm in Central Norway, clearly highlighting the susceptibility of fish farms to a diverse range of marine biofouling organisms. Immersion period and sampling time were the two factors with the strongest influence on biomass, species richness and community composition, while mesh size and variability between cages had less strong effects.

### 4.1. Immersion period

Immersion period, which reflects the influence of succession, strongly affected the biofouling communities. While the community composition on 1-month panels consisted of successful settlers of the pool of available propagules, the longer immersed panels showed communities in progressing states of succession. Hydroids are one of the first colonisers of newly available substrate and are often inferior in competition to other macrofouling organisms (Boero, 1984; Migotto et al., 2001), while blue mussels are known for their strong performance as competitors in communities of later successional stages (Dean and Hurd, 1980; Greene and Grizzle, 2007). This was reflected in the declining proportion of hydroid biomass and the increasing dominance of *M. edulis* on the panels with increasing length of immersion period. Similar successional patterns were found by Cook et al. (2006) for the hydroid *Obelia longissima* on a Scottish salmon farm.

In contrast to the 1-, 3- and 6-month panels, the succession on the 12-month panels may have reached a climax state (Greene and Grizzle, 2007; Jenkins and Martins, 2010), where the communities on the panels did not show any influence of mesh size or variability between cages. However, in an aquaculture environment, the biofouling on cage nets rarely has time to grow to a climax community, because many fish farmers regularly wash their cage nets *in situ*. Regular cleaning intervals are often strictly scheduled and independent of the level of biofouling on the cage nets. These cleaning intervals are employed not only to allow sufficient water flow through the nets, but also to ensure a good performance of the cleaner wrasse. These small fish are kept in the cage with the salmon and are used as a natural remedy to reduce the presence of salmon lice, which they pick off the cultured fish (Kvenseth, 1996). However, given the opportunity, the

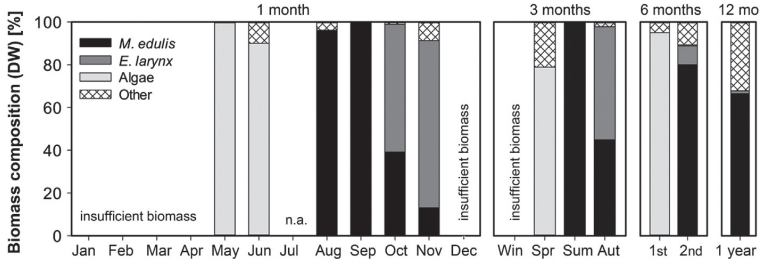


**Fig. 5.** Biomass composition of the 2 main sedentary species and algae according to dry weight measurements. Data was pooled over 2 mesh sizes and 3 cages (n = 24). Immersion periods (1, 3, 6, 12 months) are graphed separately. There was no substantial biomass to take dry weight samples during the winter (Jan–Mar) and April samples.

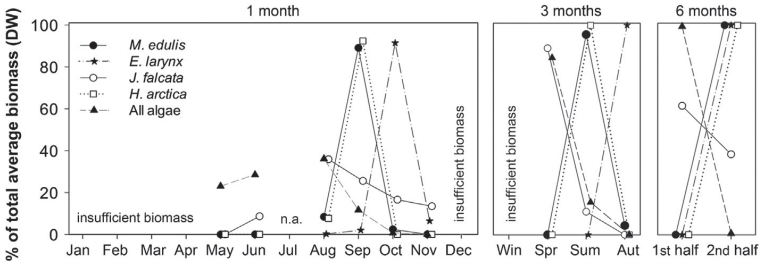


**Fig. 6.** Biomass (dry weight) development of the 5 most influential species and all algae for 3 different immersion periods. Data was pooled over 2 mesh sizes and 3 cages (n = 24) and y-axis values are standardised as % of the total average biomass of all panels collected with the same length of immersion. Immersion periods (1, 3, 6, 12 months) are graphed separately.

wrasse preferentially feed on biofouling organisms on the cage nets instead of on the salmon lice, and as a result, their de-lousing performance drops (Kvenseth, 1996). Furthermore, regular washing of the cage nets may hold the biofouling communities in an early successional stage (Guenther et al., 2010; Sousa, 1979) which allows the settlement, and often dominance, of first colonisers such as the hydroid *E. larynx*. For monitoring purposes, it is therefore advisable to immerse net panels for periods which reflect the washing intervals of the cage nets and take samples of biofouling communities in comparative successional phases.

**Table 1**
Results (permutational *P*) of PERMANOVA for main effects and pairwise comparisons (shown as superscripts) for the influence of sampling time ('Time'), mesh size and variability between cages ('Cage'), including respective interactions, on biomass, species richness and community composition based on dry weight or presence/absence of species. Data were analysed separately for each immersion period. Bold entries indicate relevant significant results for the individual biological variables. For details see Table I in the appendix.

| Immersion period & factor | Biomass | Species richness | Composition (dry weight) | Composition (pres/abs) |
|---|---|---|---|---|
| **1 month** | | | | |
| Time | <**0.001** | <0.001 | <0.001 | <0.001 |
| Mesh size | 0.872[a] | 0.276[a] | 0.849[a] | 0.041[a] |
| Cage | 0.655 | 0.633 | <0.001 | <0.001 |
| Time × mesh size | 0.881 | 0.074 | 0.901 | 0.077 |
| Time × cage | 0.992 | 0.188 | <0.001 | <**0.001** |
| Mesh size × cage | 0.284 | 0.053 | 0.044 | 0.708 |
| Time × mesh size × cage | 0.318 | **0.045** | <**0.001** | 0.662 |
| **3 months** | | | | |
| Time | <**0.001** | <0.001 | <0.001 | <0.001 |
| Mesh size | 0.856[a] | 0.193[a] | 0.259[a] | 0.198[a] |
| Cage | 0.439 | 0.065 | <0.001 | <**0.001** |
| Time × mesh size | 0.655 | 0.244 | 0.146 | 0.466 |
| Time × cage | 0.564 | **0.013** | <0.001 | 0.344 |
| Mesh size × cage | 0.186 | 0.171 | 0.087 | 0.650 |
| Time × mesh size × cage | 0.329 | 0.487 | **0.022** | 0.815 |
| **6 months** | | | | |
| Time | 0.071[a] | <**0.001**[a,b] | <0.001[a] | <**0.001**[a] |
| Mesh size | 0.176[a] | 0.073[a] | 0.578[a] | 0.880[a] |
| Cage | 0.076 | 0.056 | 0.580 | **0.007** |
| Time × mesh size | 0.277 | 0.382 | 0.483 | 0.319 |
| Time × cage | 0.355 | 0.752 | 0.591 | 0.302 |
| Mesh size × cage | 0.607 | 0.796 | 0.010 | 0.262 |
| Time × mesh size × cage | 0.124 | 0.771 | **0.014** | 0.536 |
| **12 months** | | | | |
| Mesh size | 0.214[a] | 0.867[a] | 0.694[a] | 0.528[a] |
| Cage | 0.411 | 0.871 | 0.205 | 0.070 |
| Mesh size × cage | 0.586 | 0.074 | 0.226 | 0.378 |

[a] $p_{MC}$ values instead of permutational p-values.
[b] First half > second half.

### 4.2. Sampling time

Sampling time, which reflects the influence of season on the rate and composition of biofouling, had a strong structuring effect on the communities. Especially the 1- and 3-month panels showed a pronounced seasonal pattern with a biomass peak during summer. This pattern is typical for temperate marine waters with strong seasonal variation in temperature and propagule abundance in the water (CRAB, 2007; Raymont, 1980; Zheng, 1989). Similar seasonal changes in community biomass with a peak in summer on 1- and 3-month panels were reported by Greene and Grizzle (2007). However, in their comparison of net panels of equal mesh size, submerged at 15 m depth at the coast of Maine for similar immersion periods, the maximum of the average biomass was almost four-fold higher (approximately 300 g) than in the present study (83 g), while the maximum of the average species richness was lower (<12 species) compared to that found in the present study (<25 species).

### 4.3. Mesh size

Although the small meshed nets offered 30% more surface area for settlement than the large meshed nets, there was no difference in biomass between the two mesh sizes. Similarly, Tseng and Yuen (1978) did not measure significant differences in fouling accumulation on nets with 19, 20, 38 and 50 mm mesh size submerged at four different locations for 2 months. These findings are in contrast with other studies suggesting that small meshed nets accumulate more fouling in a short time while large meshed nets may have a greater carrying capacity over longer time (Cheah and Chua, 1983; Mak, 1982; Milne, 1975). In the present study, species richness on 1-month panels as well as community composition based on biomass data on 1- to 6-month panels showed some variation with mesh size, yet only in interaction with both sampling time and cage. This may reflect the natural small-scale variation between panels (Hodson et al., 1995), or differences in the hydrodynamic micro-environment discussed below.

One reason for the exchange of nets during the farming process is that net solidity decreases with increasing mesh size, resulting in lower drag forces (reviewed in Klebert et al., 2013). The main factor when determining the impact of biofouling on the water exchange capabilities of nets is the percentage net-aperture occlusion (PNO; Braithwaite et al., 2007; Guenther et al., 2010). However, because PNO values were not investigated in the present study, no conclusions could be drawn from the lack of differences in biofouling between the two mesh sizes regarding their eventual impact on the water flow. Therefore, a comparison of biofouling accumulation on nets with different mesh sizes including PNO measurements, conducted at several farms, will increase the knowledge about the impact of mesh size.

### 4.4. Variability between cages

While there were no differences in biomass, species richness and community composition varied between individual cages. Only few studies have compared biofouling between cages. For example, Svane et al. (2006) found differences in two out of six test months between cages (coupled with depth) regarding biofouling cover when comparing anti-fouling treated cages with untreated cages with three replicate cages each. Bwathondi and Ngoile (1982) found differences in both abundance and community composition of various mussel species on two cages during a period of 103 days. In contrast, no differences were found by Hincapie-Cardenas (2007) when analysing the abundance and community composition of fouling on nets sampled every other month from two cages during a 10-month period, although the cages were stocked with different fish species and varied with regard to feed input. However, because samples were not replicated in the latter two studies, the results cannot be assessed regarding their significance and general validity.

Several factors are known to influence biofouling communities and may cause variations within small spatial scales. One important factor is the hydrodynamic micro-environment. Reduced water flow in cages may result in increased biofouling growth by sessile organisms (Madin et al., 2009, 2010). Although the water velocity in the present study was not measured at each individual cage, but at a central point at the farm site, it is highly likely that the flow regime at the individual cages showed some degree of variation, due to shadowing or water deflection from adjacent cages (Madin et al., 2010) and the specific bottom hydrography at the site. Such hydrographical variations may result in differences in the number of larvae that attach to a substrate (Todd, 1998) or in differences in physical living conditions, which finally may result in variations in biofouling (Leichter and Witman, 1997). In addition, the water flow differs in the short-term with season and weather conditions, which may also explain the variability in community composition found on individual cages.

Furthermore, fish stocking density and feed input were also factors that were expected to have an impact on the development of biofouling on the net panels, given that the nutrient input of feed and faeces of stocked fish may enhance the amount of biofouling on cage nets (Madin et al., 2010). The three investigated cages differed in stocking density and feed input (Cage 2 > Cage 1 and Cage 3 before the splitting of the fish, Cage 1 > Cage 2 > Cage 3 after the splitting; Fig. 2). However, the differences in species richness and community composition did not follow the same pattern and there were no differences in biomass at all. This suggests, that the differences between the cages were either not high enough to induce a measurable difference in biofouling, especially regarding the biomass, or that other factors had much stronger effects on the developing biofouling on the individual cages.

Finally, as the biofouling on cage nets can show a high small-scale variation within replicates from one sampling site (Hodson et al., 1995), it may be that the measured variability between cages is due to the natural variation in the spatial distribution of the biofouling cover, for example caused by post-settlement interactions. Consequently, future studies may profit from higher replicate numbers to more adequately represent the large mesh surface of the cage. Furthermore, spatial replication in relation to the depth and orientation of the cage may provide further insights regarding the drivers of small-scale variation in biofouling. To discern the natural variation from the influence of other factors, such as the hydrodynamic micro-environment or the influence of nutrients, more physical variables, such as water flow velocities on a smaller scale directly at the cage should be measured to be able to relate them to the observed differences.

### 4.5. Implications for aquaculture

This first comprehensive analysis of the species present on Norwegian fish farm cages can provide valuable information for farm operators, for implemented biofouling monitoring projects and related experiments, and for the design of novel antifouling technologies. According to the literature (reviewed in Braithwaite and McEvoy, 2005; de Nys and Guenther, 2009; Dürr and Watson, 2010; Fitridge et al., 2012), the accumulation of biomass is the most important variable to be considered for farm management of those measured in the present study because it has the strongest effect on water flow through the net and subsequently on the fish and the equipment. The low variability in biomass between the two mesh sizes and the individual cages suggested that monitoring of a single cage may allow the extrapolation of the state of fouling cover to all cages at a farm, independent of mesh size, which may save time and equipment. However, some care is advised because there are reports on differences in fouling cover between cages and individual washing intervals (Guenther et al., 2010; and personal communication with farm personnel) probably due to variable quality of the antifouling coating, as well as farm operations and weather conditions that influence the washing schedule.

The highest abundance of biofouling occurred during the summer months (Aug–Oct). Consequently, it would be beneficial for fish farm managers to introduce their net cages and fish into the water after this main biofouling phase. Thus, their exposure to the peak biofouling pressure would be limited to a single time during a grow-out phase of 18 months. However, the flexibility of the timing of the grow-out phase may be limited as it is furthermore influenced by other factors such as fish biology, governmental regulations, market demands and company strategies.

Filter feeders, dominated by *M. edulis* and *E. larynx*, made up 97% of the sessile species biomass on the cage nets. While both species are able to consume considerable amounts of phytoplankton and zooplankton (Gili et al., 1996; Maar et al., 2008; Petersen et al., 2008), the impact of cage biofouling on the carrying capacity is, in contrast to mussel culture facilities (Crawford, 2003), not yet included in aquaculture risk assessment analyses in Norway (Taranger et al., 2013). By 2011, close to 1000 fish farms were in operation along the Norwegian coast (Norwegian Directorate of Fisheries, 2012), and estimates of a working group of The Royal Norwegian Society of Sciences and Letters and the Norwegian Academy of Technological Sciences predict that the aquaculture production may increase by a factor of five until 2050 (Olafsen et al., 2012), thereby adding a large amount of new biofouling habitats to the coastal waters. In order to assess the impact of this concentration of filter feeders on the local carrying capacity, calculations of expected biomass accumulation should be included in future risk assessments.

Finally, the considerable species richness encountered is relevant to the development of future antifouling strategies for fish cages, in particular for non-biocidal solutions that are often highly species-specific (reviewed in *e.g.* Magin et al., 2010; Ralston and Swain, 2011; Scardino and de Nys, 2011). For the same reason the differences in community composition at different sampling times and varying immersion periods should be taken into account, as the overall performance of certain antifouling agents may differ according to the season and the related pool of settling propagules.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.aquaculture.2013.09.025.

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# Appendix A:

**Table I:** Results (permutational *P*) of PERMANOVA and pairwise comparisons for the influence of sampling time ('Time'), mesh size and variability between cages ('Cage'), including respective interactions, on biomass, species richness and community composition based on dry weight or presence/absence of species. Data were analysed separately for each immersion period (1, 3, 6 and 12 months).

**Immersion period**

| **Biomass** | **Factor** | ***df*** | **Pseudo-*F*** | ***p*** |
|---|---|---|---|---|
| 1 month | Time | 10 | 112.61 | <0.001 |
| | Mesh size | 1 | 0.03 | 0.872[a] |
| | Cage | 2 | 0.43 | 0.655 |
| | Time × Mesh size | 10 | 0.50 | 0.881 |
| | Time × Cage | 20 | 0.42 | 0.992 |
| | Mesh size × Cage | 2 | 1.26 | 0.284 |
| | Time × Mesh size × Cage | 20 | 1.13 | 0.318 |
| | Residuals | 198 | | |
| 3 months | Time | 3 | 75.04 | <0.001 |
| | Mesh size | 1 | 0.04 | 0.856[a] |
| | Cage | 2 | 0.872 | 0.439 |
| | Time × Mesh size | 3 | 0.56 | 0.655 |
| | Time × Cage | 6 | 0.81 | 0.564 |
| | Mesh size × Cage | 2 | 1.74 | 0.186 |
| | Time × Mesh size × Cage | 6 | 1.18 | 0.329 |
| | Residuals | 72 | | |
| 6 months | Time | 1 | 12.95 | 0.071[a] |
| | Mesh size | 1 | 4.21 | 0.176[a] |
| | Cage | 2 | 2.77 | 0.076 |
| | Time × Mesh size | 1 | 2.39 | 0.277 |
| | Time × Cage | 2 | 1.05 | 0.355 |
| | Mesh size × Cage | 2 | 0.51 | 0.607 |
| | Time × Mesh size × Cage | 2 | 2.22 | 0.124 |
| | Residuals | 36 | | |
| 12 months | Mesh size | 1 | 3.29 | 0.214[a] |
| | Cage | 2 | 0.94 | 0.411 |
| | Mesh size × Cage | 2 | 0.56 | 0.586 |
| | Residuals | 18 | | |

Table I, continued

| **Immersion period** | | | | |
|---|---|---|---|---|
| **Species richness** | | ***df*** | **Pseudo-*F*** | ***p*** |
| 1 month | Time | 10 | 294.8 | <0.001 |
| | Mesh size | 1 | 2.28 | 0.276[a] |
| | Cage | 2 | 0.47 | 0.633 |
| | Time × Mesh size | 10 | 2.13 | 0.074 |
| | Time × Cage | 20 | 1.32 | 0.188 |
| | Mesh size × Cage | 2 | 2.98 | 0.053 |
| | Time × Mesh size × Cage | 20 | 1.66 | 0.045 |
| | Residuals | 198 | | |
| 3 months | Time | 3 | 36.71 | <0.001 |
| | Mesh size | 1 | 3.77 | 0.193[a] |
| | Cage | 2 | 2.75 | 0.065 |
| | Time × Mesh size | 3 | 1.83 | 0.244 |
| | Time × Cage | 6 | 2.92 | 0.013 |
| | Mesh size × Cage | 2 | 1.82 | 0.171 |
| | Time × Mesh size × Cage | 6 | 0.92 | 0.487 |
| | Residuals | 72 | | |
| 6 months | Time | 1 | 1239.8 | <0.001[ab] |
| | Mesh size | 1 | 12.76 | 0.073[a] |
| | Cage | 2 | 3.28 | 0.056 |
| | Time × Mesh size | 1 | 1.28 | 0.382 |
| | Time × Cage | 2 | 0.28 | 0.752 |
| | Mesh size × Cage | 2 | 0.23 | 0.796 |
| | Time × Mesh size × Cage | 2 | 0.26 | 0.771 |
| | Residuals | 36 | | |
| 12 months | Mesh size | 1 | 0.04 | 0.867[a] |
| | Cage | 2 | 0.13 | 0.871 |
| | Mesh size × Cage | 2 | 3.05 | 0.074 |
| | Residuals | 18 | | |

Table I, continued

| **Immersion period** | | | | |
|---|---|---|---|---|
| **Community composition (dry weight)** | | ***df*** | **Pseudo-*F*** | ***p*** |
| 1 month | Time | 10 | 24.93 | <0.001 |
| | Mesh size | 1 | 0.53 | 0.849[a] |
| | Cage | 2 | 3.80 | <0.001 |
| | Time × Mesh size | 10 | 0.76 | 0.901 |
| | Time × Cage | 20 | 3.40 | <0.001 |
| | Mesh size × Cage | 2 | 160 | 0.044 |
| | Time × Mesh size × Cage | 20 | 1.63 | <0.001 |
| | Residuals | 198 | | |
| 3 months | Time | 3 | 39.63 | <0.001 |
| | Mesh size | 1 | 1.47 | 0.259[a] |
| | Cage | 2 | 3.30 | <0.001 |
| | Time × Mesh size | 3 | 1.60 | 0.146 |
| | Time × Cage | 6 | 3.20 | <0.001 |
| | Mesh size × Cage | 2 | 1.55 | 0.087 |
| | Time × Mesh size × Cage | 6 | 1.53 | 0.022 |
| | Residuals | 72 | | |
| 6 months | Time | 1 | 93.84 | <0.001[a] |
| | Mesh size | 1 | 0.80 | 0.578[a] |
| | Cage | 2 | 0.88 | 0.580 |
| | Time × Mesh size | 1 | 0.89 | 0.483 |
| | Time × Cage | 2 | 0.87 | 0.591 |
| | Mesh size × Cage | 2 | 2.30 | 0.010 |
| | Time × Mesh size × Cage | 2 | 2.18 | 0.014 |
| | Residuals | 36 | | |
| 12 months | Mesh size | 1 | 0.57 | 0.694[a] |
| | Cage | 2 | 1.34 | 0.205 |
| | Mesh size × Cage | 2 | 1.31 | 0.226 |
| | Residuals | 18 | | |

Table I, continued

| **Immersion period** | | | | |
|---|---|---|---|---|
| **Community composition (pres/abs)** | | ***df*** | **Pseudo-*F*** | ***p*** |
| 1 month | Time | 10 | 89.17 | <0.001 |
| | Mesh size | 1 | 7.07 | 0.041[a] |
| | Cage | 2 | 4.47 | <0.001 |
| | Time × Mesh size | 10 | 1.62 | 0.077 |
| | Time × Cage | 20 | 2.15 | 0.001 |
| | Mesh size × Cage | 2 | 0.61 | 0.708 |
| | Time × Mesh size × Cage | 20 | 0.91 | 0.662 |
| | Residuals | 198 | | |
| 3 months | Time | 3 | 124.94 | <0.001 |
| | Mesh size | 1 | 2.10 | 0.198[a] |
| | Cage | 2 | 3.35 | <0.001 |
| | Time × Mesh size | 3 | 1.02 | 0.466 |
| | Time × Cage | 6 | 1.12 | 0.344 |
| | Mesh size × Cage | 2 | 0.76 | 0.650 |
| | Time × Mesh size × Cage | 6 | 0.71 | 0.815 |
| | Residuals | 72 | | |
| 6 months | Time | 1 | 99.55 | < 0.001[a] |
| | Mesh size | 1 | 0.22 | 0.880[a] |
| | Cage | 2 | 2.59 | 0.007 |
| | Time × Mesh size | 1 | 1.43 | 0.319 |
| | Time × Cage | 2 | 4.23 | 0.302 |
| | Mesh size × Cage | 2 | 1.30 | 0.262 |
| | Time × Mesh size × Cage | 2 | 0.91 | 0.536 |
| | Residuals | 36 | | |
| 12 months | Mesh size | 1 | 0.895 | 0.528[a] |
| | Cage | 2 | 1.612 | 0.070 |
| | Mesh size × Cage | 2 | 1.093 | 0.378 |
| | Residuals | 18 | | |

[a] pMC values instead of permutational p-values.
[b] First half > second half.

**Table II:** List of species and species groups and frequency of occurrence (presence/absence) per 24 panels

| Species/Frequency | Sum of all panels | January | February | March | April | May | June | July | August | September | October | November | December | Winter | Spring | Summer | Autumn | 1st half | 2nd half | 1 year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ciliatea* sp. 1 | **125** | 0 | 0 | 24 | 24 | 24 | 0 | | 1 | 0 | 24 | 4 | 0 | 24 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Ciliatea* sp. 2 | **230** | 0 | 0 | 24 | 24 | 24 | 24 | | 0 | 0 | 24 | 18 | 20 | 24 | 24 | 0 | 0 | 24 | 0 | 0 |
| Diatoms | **432** | 24 | 24 | 24 | 24 | 24 | 24 | | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 | 24 |
| *Licmophora* sp. 1 | **168** | 0 | 24 | 24 | 24 | 24 | 6 | | 0 | 0 | 24 | 16 | 1 | 24 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Licmophora* sp. 2 | **99** | 0 | 0 | 0 | 24 | 24 | 24 | | 0 | 1 | 24 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| Tube-dwelling diatoms | **162** | 0 | 0 | 24 | 24 | 24 | 6 | | 0 | 4 | 6 | 2 | 0 | 24 | 24 | 0 | 0 | 24 | 0 | 0 |
| Foraminifera spp. | **186** | 0 | 0 | 24 | 2 | 1 | 3 | | 10 | 20 | 2 | 6 | 0 | 24 | 4 | 24 | 1 | 24 | 17 | 24 |
| *Porifera* sp. | **5** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 3 |
| *Membranipora membranacea* | **49** | 0 | 0 | 0 | 0 | 0 | 0 | | 7 | 10 | 0 | 0 | 0 | 0 | 0 | 12 | 2 | 12 | 2 | 4 |
| *Scruparia ambigua* | **14** | 0 | 0 | 0 | 0 | 0 | 0 | | 10 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 2 |
| *Electra pilosa* | **93** | 0 | 0 | 0 | 0 | 1 | 3 | | 13 | 2 | 1 | 0 | 0 | 0 | 5 | 11 | 1 | 19 | 18 | 19 |
| *Ctenostomata* sp. | **7** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 |
| *Bryozoa* sp. 1 | **18** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 8 | 0 | 0 | 0 | 0 | 1 | 9 | 0 | 0 | 0 | 0 |
| *Bryozoa* sp. 2 | **19** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 1 | 6 | 0 | 6 |
| *Actiniaria* sp. | **23** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 4 | 9 | 0 | 2 | 5 |
| *Ectopleura larynx* | **197** | 0 | 0 | 0 | 0 | 0 | 0 | | 16 | 20 | 24 | 24 | 24 | 0 | 1 | 20 | 24 | 2 | 21 | 21 |
| *Obelia* sp. | **103** | 0 | 0 | 0 | 2 | 2 | 23 | | 16 | 6 | 0 | 6 | 1 | 0 | 22 | 0 | 0 | 24 | 0 | 1 |
| *Bougainvilla* sp. | **7** | 0 | 0 | 0 | 0 | 0 | 1 | | 1 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 1 | 0 |
| *Sarsia tubulosa* | **9** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 5 | 0 | 1 |

Table II, continued

| Species/Frequency | Sum of all panels | January | February | March | April | May | June | July | August | September | October | November | December | Winter | Spring | Summer | Autumn | 1st half | 2nd half | 1 year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Opercularella lacerata* | **40** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 21 | 0 | 0 | 19 | 0 | 0 |
| *Eudendrium* sp. | **14** | 0 | 0 | 0 | 0 | 0 | 0 | | 3 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 2 | 3 |
| *Clytia gracilis* | **5** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 4 | 0 | 0 |
| *Campanularia volubilis* | **1** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Haliclystus salpinx* | **11** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 4 | 0 | 0 |
| *Platyhelminthes* spp. | **4** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 0 |
| *Nematoda* spp. | **299** | 0 | 0 | 24 | 0 | 24 | 13 | | 14 | 24 | 24 | 1 | 7 | 24 | 24 | 24 | 24 | 24 | 24 | 24 |
| *Nemertea* sp. | **35** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 9 | 12 |
| Species indet. | **6** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 1 | 0 | 0 |
| *Capitella capitata* | **45** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 8 | 1 | 0 | 19 | 16 |
| *Nereis pelagica* | **132** | 0 | 0 | 24 | 1 | 0 | 1 | | 3 | 10 | 1 | 0 | 0 | 24 | 16 | 21 | 2 | 24 | 0 | 5 |
| *Pomatoceros triqueter* | **6** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 5 |
| *Polynoidea* sp. 1 | **72** | 0 | 0 | 0 | 0 | 0 | 0 | | 4 | 10 | 4 | 0 | 0 | 0 | 11 | 15 | 8 | 15 | 1 | 4 |
| *Polynoidea* sp. 2 | **1** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Syllidae* sp. 1 | **82** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 21 | 7 | 0 | 0 | 0 | 1 | 10 | 20 | 1 | 9 | 13 |
| *Syllidae* sp. 2 | **13** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 |
| *Phyllodocidae sp.* | **3** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| *Spionidae sp.* | **10** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 5 |
| *Spirorbis* sp. | **3** | 0 | 0 | 0 | 0 | 0 | 0 | | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| *Serpulidae* sp. | **1** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Phyllodocidae* sp. 2 | **5** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 1 |

Table II, continued

| Species/Frequency | Sum of all panels | January | February | March | April | May | June | July | August | September | October | November | December | Winter | Spring | Summer | Autumn | 1st half | 2nd half | 1 year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Polychaeta sp. 1* | **3** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Polychaeta sp. 2* | **3** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 2 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Polychaeta sp. 3* | **14** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 10 | 0 | 0 |
| *Polychaeta sp. 4* | **7** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 5 | 0 | 0 |
| *Polychaeta sp. 5* | **3** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 1 |
| *Polychaeta sp. 6* | **5** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 2 |
| *Polychaeta sp. 7* | **2** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| *Hiatella arctica* | **154** | 0 | 0 | 0 | 0 | 2 | 5 | | 19 | 21 | 0 | 0 | 0 | 0 | 9 | 24 | 15 | 11 | 24 | 24 |
| *Mytilus edulis* | **245** | 0 | 0 | 0 | 0 | 0 | 24 | | 24 | 24 | 24 | 5 | 0 | 0 | 24 | 24 | 24 | 24 | 24 | 24 |
| *Heteranomia squamula* | **78** | 0 | 0 | 0 | 0 | 0 | 0 | | 3 | 8 | 0 | 0 | 0 | 0 | 0 | 24 | 2 | 0 | 24 | 17 |
| *Pecten maximus* | **49** | 0 | 0 | 0 | 0 | 0 | 0 | | 12 | 9 | 1 | 0 | 0 | 0 | 0 | 11 | 8 | 0 | 3 | 5 |
| *Musculus marmoratus* | **1** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| *Bivalvia* sp. 1 | **3** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| *Bivalvia* sp. 2 | **2** | 0 | 0 | 0 | 0 | 0 | 0 | | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Hydrobia* sp. | **167** | 0 | 0 | 0 | 0 | 0 | 1 | | 24 | 24 | 24 | 6 | 1 | 0 | 1 | 24 | 20 | 1 | 17 | 24 |
| *Littorina* sp. | **107** | 0 | 0 | 0 | 0 | 0 | 4 | | 17 | 4 | 5 | 0 | 0 | 0 | 17 | 11 | 1 | 24 | 3 | 21 |
| *Skenea* sp. | **5** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 3 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| *Onchidorididae* sp. | **9** | 0 | 0 | 0 | 0 | 0 | 0 | | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 2 | 1 | 0 | 1 | 2 |
| *Dexiarchia* spp. | **108** | 0 | 0 | 0 | 0 | 0 | 2 | | 6 | 15 | 3 | 1 | 1 | 0 | 16 | 6 | 24 | 24 | 5 | 5 |
| *Asterias* sp. | **48** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 4 | 0 | 0 | 0 | 0 | 23 | 0 | 0 | 21 | 0 | 0 |
| *Echinacea* sp. | **3** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

Table II, continued

| Species/Frequency | Sum of all panels | January | February | March | April | May | June | July | August | September | October | November | December | Winter | Spring | Summer | Autumn | 1st half | 2nd half | 1 year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Jassa falcata* | **314** | 0 | 0 | 24 | 6 | 18 | 20 | | 21 | 24 | 24 | 23 | 8 | 24 | 20 | 24 | 17 | 24 | 17 | 20 |
| *Caprellidae* spp. *(C. mutica, C. linearis, Aeginina longicornis)* | **141** | 0 | 0 | 0 | 1 | 1 | 0 | | 15 | 24 | 24 | 24 | 6 | 0 | 0 | 24 | 10 | 2 | 5 | 5 |
| *Ciripedia* sp. 1 | **10** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 5 | 0 | 4 |
| *Ciona intestinalis* | **24** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 2 | 16 |
| Ascidia sp. 1 | **4** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| *Polysiphonia stricta* | **176** | 0 | 0 | 0 | 1 | 18 | 24 | | 10 | 7 | 6 | 10 | 0 | 24 | 22 | 11 | 2 | 16 | 10 | 15 |
| *Lomentaria clavellosa* | **103** | 0 | 0 | 0 | 0 | 0 | 24 | | 6 | 3 | 3 | 0 | 0 | 0 | 12 | 18 | 0 | 24 | 5 | 8 |
| *Saccharina latissima* | **155** | 0 | 0 | 24 | 0 | 3 | 17 | | 1 | 10 | 0 | 0 | 1 | 24 | 22 | 8 | 1 | 24 | 1 | 19 |
| *Alaria esculenta* | **23** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 22 | 0 | 1 |
| *Bangia fuscopurpurea* | **19** | 0 | 0 | 0 | 0 | 3 | 7 | | 1 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 1 | 0 | 0 |
| *Urospora pencilliformis* | **132** | 0 | 0 | 0 | 0 | 8 | 11 | | 12 | 19 | 21 | 11 | 6 | 0 | 8 | 8 | 14 | 1 | 5 | 8 |
| *Ectocarpus siliculosus* | **133** | 0 | 0 | 0 | 10 | 24 | 24 | | 20 | 0 | 4 | 0 | 1 | 0 | 24 | 0 | 0 | 24 | 0 | 2 |
| *Desmarestia viridis* | **41** | 0 | 0 | 0 | 0 | 0 | 0 | | 3 | 4 | 0 | 0 | 0 | 0 | 20 | 1 | 1 | 4 | 3 | 5 |
| *Antithamnion sp.* | **64** | 0 | 0 | 0 | 0 | 6 | 3 | | 2 | 12 | 14 | 8 | 0 | 0 | 12 | 0 | 0 | 7 | 0 | 0 |
| *Florideophyceae* sp. | **93** | 0 | 24 | 24 | 0 | 0 | 0 | | 0 | 6 | 0 | 0 | 0 | 24 | 12 | 0 | 0 | 1 | 2 | 0 |
| *Ceramium* sp. | **135** | 0 | 0 | 0 | 0 | 0 | 24 | | 20 | 11 | 20 | 2 | 0 | 0 | 21 | 16 | 1 | 16 | 4 | 0 |
| *Monostroma* sp. | **19** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 19 | 0 | 0 |
| *Cladophora* sp. | **101** | 0 | 0 | 24 | 0 | 0 | 0 | | 0 | 6 | 10 | 2 | 0 | 24 | 1 | 4 | 7 | 1 | 12 | 10 |
| *Spongomorpha* sp. | **91** | 0 | 0 | 0 | 0 | 10 | 11 | | 2 | 6 | 16 | 9 | 0 | 0 | 24 | 4 | 0 | 9 | 0 | 0 |

Table II, continued

| Species/Frequency | Sum of all panels | January | February | March | April | May | June | July | August | September | October | November | December | Winter | Spring | Summer | Autumn | 1st half | 2nd half | 1 year |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Ulvophyceae* sp. | **103** | 0 | 0 | 24 | 0 | 12 | 17 | | 0 | 0 | 0 | 0 | 0 | 24 | 22 | 0 | 2 | 0 | 0 | 2 |
| *Ectocarpus* sp. | **20** | 0 | 0 | 0 | 0 | 0 | 0 | | 1 | 5 | 9 | 2 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| *Ulothrix* sp. 1 | **118** | 0 | 0 | 24 | 24 | 24 | 7 | | 0 | 14 | 0 | 0 | 0 | 24 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Ulothrix* sp. 2 | **3** | 0 | 0 | 0 | 2 | 1 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Rhodophyceae* sp. 1 | **17** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 15 | 0 | 2 |
| *Rhodophyceae* sp. 2 | **11** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Chlorophyceae* sp. 1 | **14** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 12 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Phaeophyceae* sp. 1 | **2** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Phaeophyceae* sp. 2 | **3** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 |
| Algae sp.1 | **8** | 0 | 0 | 0 | 0 | 0 | 0 | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 |
| **Total** | **90** | **1** | **3** | **14** | **15** | **23** | **28** | | **36** | **51** | **34** | **23** | **15** | **15** | **52** | **41** | **35** | **51** | **36** | **51** |
| **Average** | | **1** | **3** | **14** | **8** | **13** | **15** | | **14** | **19** | **18** | **9** | **4** | **15** | **23** | **19** | **12** | **25** | **14** | **19** |

## Paper II

**Using stable isotopes to identify the link between fish farm nutrients and associated biofouling organisms**

Is not included due to copyright

## Paper III

**Prevalence and oxygen consumption of the fouling hydroid *Ectopleura larynx*: implications for salmon aquaculture**

Is not included due to copyright

## Paper IV

**The fouling hydroid *Ectopleura larynx*:**
**a lack of effect of next generation antifouling technologies**

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# The fouling hydroid *Ectopleura larynx*: a lack of effect of next generation antifouling technologies

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The hydroid *Ectopleura larynx* is one of the main fouling organisms on salmon aquaculture cages in Norway; this study investigated novel surface materials and microtopographies to deter its settlement. The settlement preferences of hydroid larvae for 12 materials with wettabilities ranging from hydrophobic (54°) to hydrophilic (112°) were tested in a no-choice bioassay. Although settlement differed between materials, with the highest average settlement on polytetrafluoroethylene (95%) and the lowest on untreated polyurethane (53%), no trend regarding the tested wettabilities could be found and none of the tested materials was able to reduce average settlement below 50%. Furthermore, nine high-density polyethylene (HDPE, 100–600 µm microtopographies) and seven polydimethylsiloxane (PDMS; 40–400 µm microtopographies) microtextured surfaces were tested. There was no systematic effect of microtopography on the settlement of *E. larynx* larvae. However, there was a preference for settlement in channels on PDMS microtopographies between 80 and 300 µm. Similarly, there were no preferences for any of the examined microtopographies in a 12-day field test using PDMS surfaces at a commercial fish farm. The study indicated that neither surface wettability (hydrophilicity–phobicity) nor microtopographies were effective at deterring the settlement of the hydroid *E. larynx*. The high plasticity of the aboral pole and the hydrorhiza of the hydroids may explain settlement even under unfavourable conditions, highlighting the successful colonisation traits of this dominant biofouling species.

**Keywords:** biofouling; antifouling; *Ectopleura larynx*; wettability; microtopography; attachment point theory

## Introduction

Biofouling poses serious problems for the finfish aquaculture industry worldwide due to its negative effects on cage deformation and structural fatigue, the restriction of water exchange across the net, and the reduction of water quality and subsequently fish health (reviewed in Braithwaite & McEvoy 2005; de Nys & Guenther 2009; Dürr & Watson 2010; Fitridge et al. 2012). The hydroid *Ectopleura larynx* Ellis and Solander 1786 (syn. *Tubularia larynx*) is among the most common fouling organisms (Olafsen 2006; Guenther et al. 2010) on finfish aquaculture infrastructure between South- and mid-Norway. After initial settlement by the end of June, *E. larynx* increases in abundance and forms large, almost monocultural, communities on cage nets between August and November, which may cover and occlude whole nets (Guenther et al. 2010). *E. larynx* is attached to the nets with its hydrorhiza winding around and through the threads of the net and incorporating parts of filaments into the perisarc (Carl et al. 2011). It develops hydrocauli with polyps and reaches a length of up to 17 cm (Schuchert 2010). Gonophore development is completed approximately 24 days after settlement and is followed by the release of actinula larvae over 2 weeks and the autonomisation of polyps (Pyefinch & Downing 1949). The body of the newly released larva has a spherical shape that elongates with time, while the tentacles point in every direction, giving the larva a star-shaped appearance (Figure 1(A)). When encountering a surface, the larvae first attach with their aboral tentacles by discharging nematocysts to connect to the surface (Moate 1985). They may crawl on their tentacles before settling permanently with their aboral pole (Pyefinch & Downing 1949). Since they are unable to swim, settlement occurs in the immediate vicinity of the adult colonies (Pyefinch & Downing 1949; Moate 1985).

The most common strategy in Norwegian finfish aquaculture to prevent and reduce biofouling is the use of copper-based coating on nets, combined with regular washing (Olafsen 2006). The washing is mainly performed with net cleaners, which are equipped with discs that expel high-pressure water, and is sometimes complemented by divers and remotely operated vehicles. Additionally, nets can be partly dried or exchanged after prominent fouling phases during the year (Olafsen 2006). Unfortunately, the applied antifouling (AF) strategies are

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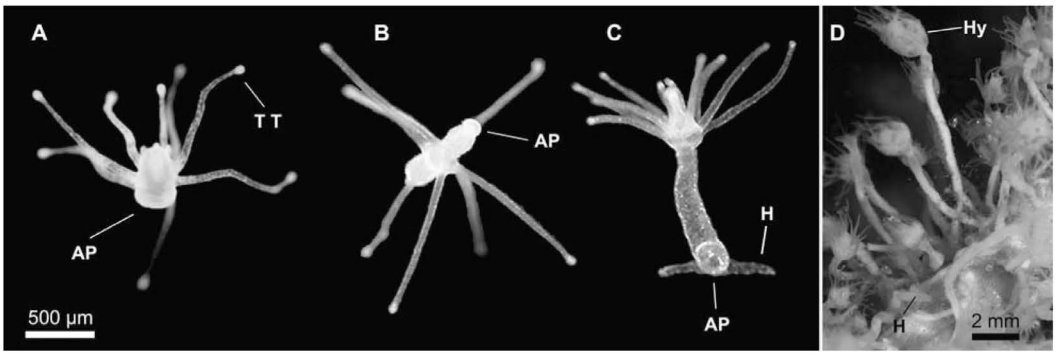


Figure 1. Larvae of *E. larynx* at different developmental stages. A: newly released larva (Photo: C. Carl; from Carl 2008), B: elongated larva, C: settled larva and D: young colony on a net. TT = tentacle tip; AP = aboral pole; H = hydrorhiza; Hy = hydranth.

unable to prevent biofouling completely. For example, some fouling organisms, such as the hydroid *E. larynx* and caprellid amphipods, have an increased resistance against copper (Pyefinch & Downing 1949; Perrett et al. 2006). Furthermore, after immersion for 6 months, copper-based coatings on nets generally lose their efficiency (Braithwaite & McEvoy 2005; Braithwaite et al. 2007), due to the leaching of copper ions and progressive wear by washing. Anecdotal evidence suggests that after washing the nets for the first time during a season, the growth of hydroids is enhanced and their presence on the nets resembles a monoculture. This phenomenon may be facilitated by the several-fold increase in larval release into the water during washing (Carl et al. 2011), which will most likely settle on the same net.

In addition to the long-term inefficiency of copper-coated nets, there are environmental concerns regarding the use of copper. Although copper does not accumulate in the liver and muscle tissues of cultured salmon (Solberg et al. 2002), other organisms, especially algae, molluscs and crustaceans, may be negatively impacted by the metal leaching into the environment surrounding the farms (reviewed in de Nys & Guenther 2009; Burridge et al. 2010). Furthermore, there is a high financial cost associated with the use of copper-coated nets (Olafsen 2006) and therefore alternative AF treatments for nets used in aquaculture are desirable. The focus is on novel techniques that do not leach potentially hazardous compounds into the environment surrounding the farms.

Settlement of marine organisms is influenced by behavioural, chemical or physical factors, or a combination of these (reviewed in Wahl 1989; Maki & Mitchell 2002; Prendergast 2010). Among these factors, surface wettability and microtopography have received increased attention in recent years (reviewed in Genzer & Efimenko 2006; de Nys et al. 2010; Ralston & Swain 2011; Scardino & de Nys 2011).

A diversity of typical fouling species has been tested for their settlement preferences on materials ranging from low wettability (hydrophobic, low-energy surfaces) to high wettability (hydrophilic, high-energy surfaces). Low surface energy between 20 and 30 mN cm$^{-1}$ are widely effective in reducing the attachment and facilitating the removal of epibionts (Baier 1972; reviewed in Genzer & Efimenko 2006). In bioassays, the barnacle *Balanus amphitrite* (Rittschof & Costlow 1989) and the blue mussel *Mytilus edulis* (Aldred et al. 2006) avoid surfaces with low wettability. Conversely, the green alga *Ulva linza* (Callow et al. 2000), the bryozoan *Bugula neritina* (Rittschof & Costlow 1989) and the barnacle *Balanus improvisus* (Dahlström et al. 2004) avoided surfaces with high wettability. In some cases, there is little or no preference across the spectrum of wettability (Roberts et al. 1991; Holm et al. 1997; Carl et al. 2012).

The influence of surface microtopography on settlement has been observed for a large number of fouling species, including algal spores, diatoms, tubeworms, bryozoans and barnacle cyprids. In general, larval settlement is reduced when the number of possible contact points between the organism and the substratum is low (reviewed in Scardino & de Nys 2011). According to the 'attachment point theory', the settlement of active settling propagules and larvae is lowest on microtopographies which are smaller than the width of the settler, and highest where the microtopography is wider than the settling organism, allowing them to attach to the substratum with as much body surface area as possible. This concept has been successfully employed in the design of the bioinspired Sharklet$^{TM}$ microtopography, which reduced the settlement of zoospores of the ubiquitous green alga *U. linza* by 85% (Carman et al. 2006). The algal spores, with an average diameter of 5 µm, could not settle in channels with a topography 2 µm wide and 2 µm high, and settlement on the 2 µm wide ridges was only

possible by bridging the ridge and thereby minimising the body surface in contact with the substratum. In comparison, topographies with channels 5 µm wide increased settlement by 150% (Carman et al. 2006). A similar Sharklet™ microtopography designed with 40 µm high, 20 µm wide ridges and crevices to repel the barnacle *B. amphitrite*, whose cyprid larvae have a diameter of ~500 µm, reduced the settlement of the larvae by 97% (Schumacher et al. 2007). However, designing topographies that repel more than one species remains complex because the targeted organisms cover a wide size range (Schumacher et al. 2007; reviewed in Magin et al. 2010; Callow & Callow 2011; Scardino & de Nys 2011). Furthermore, the selection of a suitable settlement site is not always determined by the size of the organism itself but the scale of the structures involved in the sensing apparatus that examines the surface prior to settlement (reviewed in Callow & Callow 2011). In addition, the likelihood of the removal of organisms from the surface plays an important role for the choice of substratum (Aldred et al. 2010).

The effect of wettability has not been specifically examined for *E. larynx* and previous research on the settlement preferences of this organism is limited to a comparison of four scales of surface roughness between 0 and 1000 µm (Nellis & Bourget 1996) with reduced settlement on microtopographies between 250 and 500 µm. Therefore, the aim of this study was to identify the settlement preferences of *E. larynx* in response to surface wettability and microtopography using both laboratory and field experiments in order to contribute to the development of novel AF materials.

## Materials and methods

### *Collection of larvae*

Colonies of the hydroid *E. larynx* were collected from two Atlantic salmon farm sites in SW (Austevoll: 60° 05.500′N, 05°16.129′E) and mid-Norway (Roan: 64° 11.172′N, 10°07.373′E). The colonies were transported to the laboratory in 10 l buckets, placed into fresh, sand-filtered seawater and aerated overnight. *E. larynx* larvae were collected with a glass pipette using a dissecting microscope and placed into 0.45 µm filtered seawater.

### *Surface wettability*

The settlement preferences of *E. larynx* larvae in response to surface wettability were tested by comparing the number of settled larvae on 12 surfaces with varying wettability. The 12 materials ranged from 54° (hydrophilic) to 112° (hydrophobic; for detailed contact angles see Figure 2) were: (1) epoxy (R180 epoxy resin mixed 5:1 with H180 standard hardener, Nuplex Industries); (2) nylon (Type 6, Quadrant EPP); (3) polyethylene tetraphthalate (PET; Bayer Material Science); (4) polycarbonate (PC; Bayer Material Science); (5) polystyrene (PS; Dow Corning); (6) empty polystyrene 6-well plates (PS well plates; Greiner bio-one); (7) high-density polyethylene (HDPE; Simona); (8) thermoplastic polyurethane (PU; Colex International Ltd); (9) polypropylene (PP; Röchling); (10) heat treated thermoplastic PU (Colex International Ltd, hot pressed for 5 min in a 4-tonne hydraulic press at 155 °C to flatten the material); (11) polytetrafluoroethylene (PTFE; Fluoro Pacific); and (12) polydimethylsiloxane (PDMS; Sylgard 184, Dow Corning). Contact angle data were taken from Carl et al. (2012) with the exception of the untreated PU, which was analysed by the static drop method (20 µl drops of Milli-Q water, $n = 8$) using a goniometer (DSA 100, Drop Shape Analysis Systems; Krüss, Germany). The material was prepared as described in Carl et al. (2012) and the compatibility of the data was ensured by comparative measurements of heat-treated PU and PDMS, which showed similar values to those given in Carl et al. (2012).

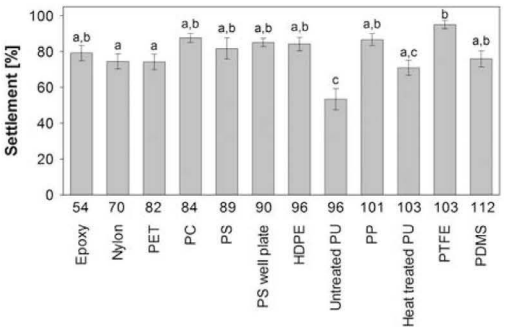


Figure 2. Settlement (% ± SE) of *E. larynx* on the 12 test materials with varying wettability ($n = 12$). Superscripts indicate significant differences (Tukey's HSD multiple comparison test, $\alpha = 0.05$). Materials are ranked according to increasing hydrophobicity from left to right. Water contact angles [°] are given below the bars and were measured by Carl et al. (2012), with the exception of untreated PU.

For the assays, the surfaces were cut into discs and fitted into the wells (35 mm in diameter) of 6-well plates. For cleaning, surfaces were submerged in distilled water for at least 24 h, rinsed and then air-dried. Well assays were used for the settlement experiments, in preference to drop assays, as the tentacles of *E. larynx* larvae readily attach to the pipettes, making the accurate placement of multiple larvae in single drops of a set volume less reliable. Ten larvae were added to each well filled with 5 ml of 0.45 µm filtered seawater ($n = 12$). The well plates were kept in darkness for 72 h at a constant temperature of 12 °C to allow the larvae to settle. Settled and non-settled larvae were then counted using a dissecting microscope. Larvae were considered as settled

when they were irreversibly attached to the surface via the aboral pole, and their tentacles were raised. For PS, PTFE and HDPE, it was necessary to dye the settled larvae with Lugol's solvent to gain sufficient contrast to the white surface to be able to count the white larvae. The material colour was not expected to influence experiments as they were conducted in the dark and furthermore, *E. larynx* does not show a preference for colours (Guenther et al. 2009). Larvae that had settled on the edge of the disc were not counted, as the surface microtopography on the edge was modified when the discs were cut and handled.

### *Surface microtopography*

Prior to experiments, the aboral poles and tentacle tips of 20 newly released, 40 elongated yet not attached, and 47 settled *E. larynx* larvae were measured to identify a suitable range of microtopographies to be tested. The standard errors of the measurements were well below 0.1 µm and are therefore not reported. The aboral pole of newly released larvae had an average diameter of 143 µm, while those of elongated and newly settled larvae had diameters of 139 and 194 µm, respectively. The tentacle tips of unsettled (both newly released or elongated) and newly settled larvae had a length of 65 and 83 µm, respectively, and a width of 55 and 57 µm, respectively (Figure 1). Therefore, a minimum of 40 µm, which was below the size of the tentacle tips, and a maximum of 600 µm, well above the diameter of the aboral pole, were chosen for the microtopographies to be tested. PDMS and HDPE were selected for the surface microtopography experiments, because HDPE is a commonly used material in aquaculture and PDMS is similar in material properties to many foul-release coatings. The materials differed not only in wettability (Figure 2), but also in elasticity and colour, with the black HDPE having a lower wettability and elasticity than the clear PDMS.

The effect of surface microtopography on hydroid settlement was tested in a laboratory and a field experiment. In the laboratory no-choice assay, the settlement of *E. larynx* larvae on seven PDMS surfaces (microtopographies: 40, 60, 80, 100, 200, 300, 400 µm, aspect ratio [width:depth of flat-bottomed channels, separated by equally sized ridges] = 1:1) and nine HDPE surfaces (microtopographies: 100, 150, 200, 250, 300, 350, 400, 500, 600 µm, aspect ratio = 1:1) and a smooth control (polystyrene 6-well plates, Greiner bio-one) was analysed ($n = 10$). The size range of both microtopographies overlaps and allows for a direct comparison of materials. However, as PDMS is more amenable to production of smaller microtopography sizes, PDMS was utilized to extend the minimum microtopography size to 40 µm. Detailed manufacturing procedures for the microtextured PDMS are described elsewhere (Carl et al. 2012), and briefly, involves casting Sylgard 184 PDMS over polymer templates (Ciba ST-43 and ST-92) microtextured via photolithography. HDPE was microtextured by hot pressing 3 mm thick HDPE sheets (Simona), 150 × 210 mm, along with a nickel template (CSIRO, Australia) containing the desired microtexture. The sample was pressed for 2.5 min at 129 °C in a 4-tonne prewarmed hydraulic press and then allowed to cool under pressure until the temperature was below 60 °C to minimise warping after removal from the press.

To conduct the microtopography assays, the same methods as for the wettability assays were used. In addition, the preference of the larvae regarding settlement either in channels or on ridges was analysed. The 40 and 60 µm PDMS microtopographies had to be excluded from this analysis, because it was not possible to identify the exact location of initial settlement.

For the field assay, seven PDMS panels of 60 × 60 mm ($n = 6$) with a textured (40, 60, 80, 100, 200, 300 and 400 µm, aspect ratio = 1:1) and a smooth side were attached to a PVC frame that was freely rotating and submerged at a depth of 5 m outside a commercial salmon cage at Korsneset (63°08.565′N, 08°13.496′E). After immersion for 12 days, the number of settled larvae on the textured and the smooth side of every panel was counted and compared.

### *Statistical analysis*

All statistical analyses were performed with SPSS Statistics version 19. The assumptions of homogeneity and normality of variance were checked with Levene and Shapiro Wilk tests, respectively. The results are reported as means ± 1 standard error (SE). The results of the wettability assay were analysed with one-factor analysis of variance (ANOVA), followed by Tukey's HSD multiple comparison test ($\alpha = 0.05$). A regression analysis was used to test for a correlation between settlement and wettability of the materials.

The results of the microtopography assays using PDMS and HDPE surfaces were analysed separately with one-factor ANOVA, followed by Tukey's HSD multiple comparison test ($\alpha = 0.05$) because the tested microtopographies did not match over the full size range. Although the PDMS data were not normally distributed, it was analysed untransformed, because transformations did not improve the data and ANOVA is robust against the violation of assumption of normality if sample sizes are equal (Underwood 1981). The preference of larvae to settle on either ridges or in channels was analysed with one-factor ANOVA followed by a polynomial contrast analysis. The PDMS data were arcsine transformed to meet the assumptions of the analysis. The effects of surface microtopography on the settlement of *E. larynx* in the field were also analysed with one-factor ANOVA, followed by Tukey's HSD multiple comparison test ($\alpha = 0.05$). The preference of the hydroids to settle on the

textured or the smooth side was tested with a paired sample *t*-test for each microtopography.

## Results

### *Surface wettability*

When testing the settlement responses of the hydroid *E. larynx* to 12 materials with different wettabilities, settlement differed significantly between the materials ($F_{(11,132)} = 6.55$, $p < 0.001$, Figure 2). Settlement was highest on PTFE (95 ± 2%) and differed significantly from settlement on nylon (74 ± 4%), PET (74 ± 4%) and PU (both heat treated (71 ± 4%) and untreated (53 ± 6%)). The latter, untreated PU, had the lowest settlement which differed significantly from all other materials except heat-treated PU. However, there was no correlation between wettability and settlement.

### *Surface microtopography*

In the laboratory assays, the settlement of larvae of *E. larynx* did not differ significantly between any of the tested microtopographies, either on HDPE ($F_{(9,98)} = 1.41$, $p = 0.196$) or on PDMS ($F_{(7,76)} = 2.01$, $p = 0.064$, Figure 3). Although the average diameter of the aboral pole was 194 µm, settlement occurred in channels as small as 80 µm. When comparing settlement in channels and on ridges, there was a preference for settlement in channels and this decreased significantly with the increasing size of the microtopography for both materials, following a linear trend (one-way ANOVA, HDPE: $F_{(8,81)} = 2.87$, $p = 0.007$; PDMS: $F_{(4,45)} = 15.82$, $p < 0.001$, Figure 4). However, the $R^2$ values for the HDPE surfaces were very low (regression analysis, $R^2$: HDPE = 0.07, PDMS = 0.54).

In the field assay using PDMS panels, surface microtopography did not have a significant effect on the settlement of *E. larynx* ($F_{(6,35)} = 1.02$, $p = 0.43$, Figure 5). Furthermore, there was no preference for the textured or the smooth side for any of the microtopography sizes, except for the 300 µm surface, where the larvae settled preferentially on the textured surface ($t_{(5)} = 8.22$, $p$(2-tailed) < 0.001, Figure 5). The settlement was generally low on all panels with an average of 3.5 ± 0.4 larvae per panel.

The survival of settled *E. larynx* larvae after 72 h, on all surfaces in both wettability and microtopography assays including the controls, was uniformly low with an average survival between 0 and 2% in the wettability assays, and between 0 and 4% in the laboratory microtopography assays. Although dead larvae disintegrated, it was still possible to recognise larvae that had settled before dying, because the newly developed hydrorhiza or the attached and still upright hydrocaulus were clearly visible. Given the consistently low survival, the data were not formally analysed and are not presented. In the microtopography field assay survival was 100%.

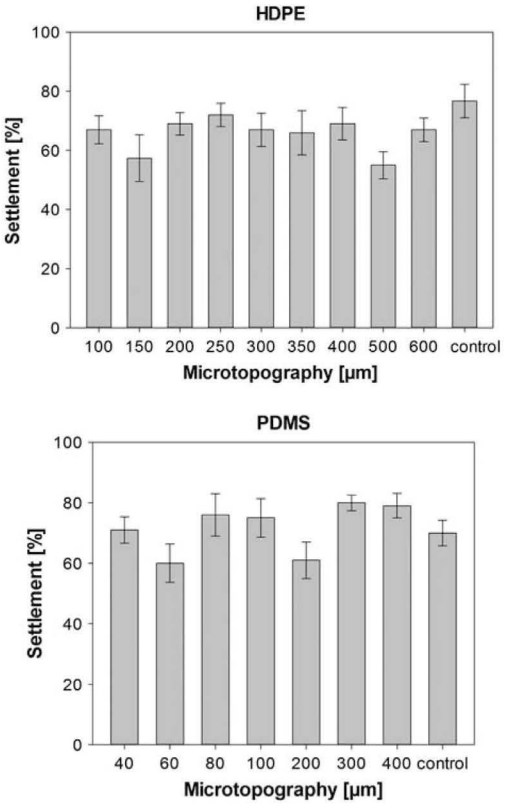


Figure 3. Mean settlement (% ± SE) of *E. larynx* larvae on various surface microtopographies of HDPE and PDMS during laboratory assays ($n = 10$). There were no significant differences in settlement.

## Discussion

### *Surface wettability*

This study showed that neither the tested surface wettabilities nor microtopographies were effective in deterring the settlement of the fouling hydroid *E. larynx*, either in laboratory assays or in the field. In contrast to other studies, where fouling species showed a clear preference for, or repulsion from, a certain surface wettability (eg Rittschof & Costlow 1989; Callow et al. 2000; Dahlström et al. 2004; Aldred et al. 2006; Hung et al. 2008), there were no marked aversive reactions of *E. larynx* across the tested wettability range. Although there was a statistical difference in settlement between the tested materials, none of these materials reduced average settlement below 50%. Moreover, both the most preferred and the least preferred materials had similar water contact angles (untreated PU: 96°, PTFE: 103°), suggesting that the differences in settlement between materials were not due to wettability. These results concur with those from

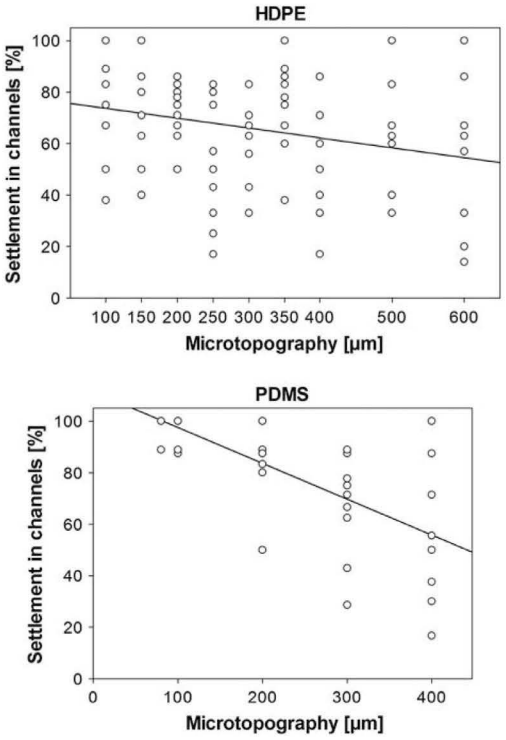


Figure 4. Settlement of *E. larynx* larvae in channels of the textured surfaces (as opposed to on the ridges) in relation to the size of the microtopography ($n = 10$). Linear regression lines are shown (HDPE: channel settlement = $-0.38 \times$ microtopography size + 77.5, $R^2 = 0.07$; PDMS: channel settlement = $-0.14 \times$ microtopography size + 111.36, $R^2 = 0.54$).

previous studies investigating the settlement of hydroid communities, consisting mainly of *Ectopleura* spp. and *Obelia* spp. in short-term field assays (3 days; Roberts et al. 1991), and *Ectopleura crocea* (syn. *Tubularia crocea*), *Eudendrium carneum*, *Pennaria tiarella* and *Obelia* spp. in long-term (30 days; Holm et al. 1997) field assays on silanised glass rods with surface wettabilities ranging from low (trimethylsilyl) to high (glass). In both studies, no differences in the settlement of hydroid larvae could be found, while concurrently settled barnacle and bryozoan larvae showed clear preferences in the short-term field assay. Although surface charge was not quantified, this study suggests that *E. larynx* is not affected by surface charge, a recently identified key driver for settlement in *B. amphitrite* (Petrone et al. 2011) given that the range of polymers used to test the effect of surface energy should also provide a range of surface charges.

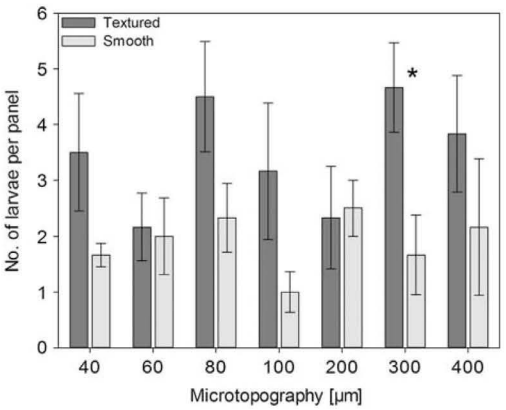


Figure 5. Mean number of larvae (±SE) of *E. larynx* settled on the textured and the smooth side of the PDMS panels ($n = 6$) submerged for 12 days outside a salmon cage. * = significant differences (paired sample *t*-test, $p < 0.05$).

### *Surface microtopography*

The attachment point theory predicts settlement on microtopographies in relation to the size of settling propagules or body structures involved in the attachment process. Actively settling organisms attach preferentially to microtopographies that are slightly larger than their body size and avoid those that are below this size, or the size of their sensing apparatus (Callow & Callow 2002, 2011; Carman et al. 2006; Schumacher et al. 2007; Scardino et al. 2008), thereby securing optimal attachment with a maximum area of attachment.

Based on the measurements of the aboral pole of *E. larynx*, reduced settlement was expected on microtopographies <200 µm. However, no differences in settlement occurred on any of the tested microtopographies in either the laboratory or field assays. These results are in contrast to Nellis and Bourget (1996) who found significantly lower settlement of *E. larynx* on sand-coated panels with heterogeneity levels of 250 and 500 µm than on 1000 µm and smooth surfaces. Nellis and Bourget (1996) explain this observation on the basis of the size of the aboral pole of the larvae, which they measured to be approximately 1000 µm and therefore too large for attachment of the whole aboral pole on the 250 and 500 µm topographies. However, this reported diameter of the aboral pole differs from the measurements in this study (194 µm) and the measurements by Pyefinch and Downing (1949), where the diameter of the aboral pole of newly settled larvae was ~280 µm.

Köhler et al. (1999) observed similar settlement preferences to those of Nellis and Bourget (1996) using unidentified hydroids and five different levels of roughness, produced from glass beads of various sizes

(smooth, 100, 500, 1000 and 5000 µm). In two out of three field assays, settlement increased with the size of the microtopography. Furthermore, there was a decreasing preference for pits with increasing size of microtopography (Köhler et al. 1999). This is also the case in the present study where settlement in channels was preferred on smaller microtopographies of the PDMS surfaces, while a very low $R^2$ value of 0.07 suggests a statistically, but not biologically relevant, effect for HDPE. However, while Köhler et al. (1999) report a preference for pits for both 500 and 1000 µm microtopographies, and no preference for 5000 µm, in the present study the larvae settled in higher numbers in channels between 80 and 300 µm PDMS microtopographies, while settlement was equally distributed over channels and ridges on 400 µm PDMS microtopographies. The differences in settlement between these studies may be explained by differing hydroid species. The lack of preference on the larger microtopographies may occur because when topographies are distinctly wider than the body of the larvae they may no longer be able to detect the presence of differences in microtopography (Nellis & Bourget 1996; Scardino et al. 2006).

### *Larval survival*

Larval survival was low in all laboratory experiments, but not in the field assay. Similarly, Nellis and Bourget (1996) report low survival in laboratory experiments with *E. larynx* larvae. This is attributed to the lack of nutritional reserves that force the larvae to immediately feed once settled. Since no feed was provided during the experiments, the larvae starved. As in this study, Nellis and Bourget (1996) were also able to analyse settlement based on the attached hydrocauli and hydrorhiza of larvae that had settled before dying. In contrast, in the conducted field trials, where feed was available, no remains of dead larvae were found on the panels.

### *Possible reasons for the lack of a deterrent effect of both surface wettability and microtopography*

While the lack of avoidance of *E. larynx* of specific surface wettability and microtopography appears consistent with passive settlement, there is substantial evidence that *E. larynx* settles actively. Non-motile organisms that settle passively, such as the spores of the red alga *Centroceras clavulatum*, are not affected by surface microtopography and the associated number of attachment points (Scardino et al. 2008). Similar to the results of the present study, Bourget et al. (1994) reported a lack of preference of *E. larynx* when analysing distributions of fouling organisms in field assays on PVC panels with various degrees of complexity derived from combinations of crevices between 0, 1, 10 and 100 mm. They concluded that the uniform distribution of the hydroids was a result of passive settlement, since concurrently settled mussels (*Mytilus edulis*, *Hiatella arctica* and *Anomia simplex*) showed preferences that could not be explained by mere water movement, but required active choice of settlement location. However, larvae of *E. larynx* crawl on their tentacles before permanently attaching to a surface (Pyefinch & Downing 1949), which could be an exploratory behaviour to examine the substratum for a suitable settlement location. Similar crawling behaviour occurred in *E. larynx* in choice assays investigating preferences for colour (Guenther et al. 2009). Although some larvae did not move and settled on their initial point of contact, most larvae crawled and settled at a different site. Moreover, active settlement of the closely related *E. crocea* is assumed. Walters and Wethey (1996) tested the settlement of the hydroid on panels with uniformly distributed 5 mm pillars in field assays. When significantly more larvae attached to the base of the pillars, resulting in a distinct non-random distribution, it was concluded that the settlement of *E. crocea* had to be active. Furthermore, Lemire and Bourget (1996) performed field settlement assays, similar to Bourget et al. (1994), on surfaces with 0, 1, 10 and 100 mm crevices. Although there was no preference for one of the tested crevice sizes, overall settlement was significantly higher on the most complex panels and the most exposed locations. From these preferences and the distribution of larvae on the panels, it was concluded that *E. crocea* explores the surface actively prior to attachment and is capable of active planktonic behaviour at a short distance and active benthic behaviour after initial contact. In the present study, the preference for channels on PDMS surfaces with microtopographies between 80 and 300 µm also suggests a choice made by the hydroids. Although this was not found in a similar distinct pattern on HDPE microtopographies, it can be concluded that *E. larynx* is in principle capable of active settlement, which is, however, not substantially affected by the tested wettability or microtopography range. In this study, only microtopographies with channels were tested, which in experiments with algal spores are less effective than more complex geometries such as the Sharklet™ topography (Schumacher et al. 2007). Hydroids may react differently to more complex topography geometries or shapes. However, the key to developing surfaces that may be effective is to understand the main factors driving hydroid settlement so that these can be manipulated to affect the deterrence of settlement. To gain more insight into the drivers of the settlement process, the pre-settlement behaviour of the larvae should be investigated more closely using video analysis to identify potential selection behaviour of *E. larynx* associated with their choice of a final settlement site.

Independently of the mode of settlement, the lack of avoidance of *E. larynx* of the tested surface wettability

and microtopography may be enabled by its stolonial growth form. Many of the fouling species with strong preferences for specific wettability and microtopography that settle in accordance with attachment point theory (Scardino et al. 2006, 2008) differ in their growth form from *E. larynx* (Ralston & Swain 2011). The arborescent bryozoan *Bugula neritina*, which attaches to a surface with a single holdfast (Rittschof & Costlow 1989; Roberts et al. 1991), shows strong preferences in terms of both wettability (Roberts et al. 1991) and microtopography (Walters & Wethey 1996; Scardino et al. 2008). Similarly, barnacles that have only one attachment point, such as *B. amphitrite* and *B. improvisus*, show equally strong preferences (Rittschof & Costlow 1989; Dahlström et al. 2004; Schumacher et al. 2007). These organisms rely on a single attachment point and therefore have to choose their settlement location with care (Roberts et al. 1991; Walters & Wethey 1996). In contrast, the high plasticity of the aboral pole and the fast-growing hydrorhiza may make the larvae of *E. larynx* less affected by the position and number of potential points of attachment. In the present study, the aboral pole, with an average diameter of 194 µm when attached to a smooth surface, fitted into channels as small as 80 µm and the hydrorhiza grew in any channel of the tested microtopographies. The latter developed immediately once the aboral pole was permanently attached to the substratum (Figure 1(C)) and thereby maximised the number of attachment points. They mostly grew parallel to the channel/ridge (Moate 1985; and personal observations). However, perpendicular growth also occurred in the form of miniature subbranches perpendicular to the main axis. Furthermore, on three-dimensional structures such as nets, *E. larynx* larvae are able to wind their hydrorhiza around the threads and through gaps to further strengthen the attachment (Carl et al. 2011). Thereby, the hydrorhiza probably take over the main attachment load from the aboral pole and extend the mode of attachment from a mainly chemical, adhesive-based attachment of the aboral pole (Pyefinch & Downing 1949) by an additional mechanical component that is less susceptible to surface chemistry. This extensive stolonal network, growing at a speed of more than 3 mm $day^{-1}$ (Pyefinch & Downing 1949; Moate 1985), may enable the hydroid to attach to even the most unfavourable substratum and make it independent and less selective in terms of the wettability and microtopography of any encountered surface. Therefore, the choice of a specific attachment site or the avoidance of certain surface characteristics regarding wettability and microtopography might be of less importance to hydroid larvae than to other fouling species (Roberts et al. 1991; Walters & Wethey 1996).

The key objective of testing a broad range of materials and topographies is to identify the main drivers of larval settlement for specific organisms, to then manipulate to either enhance, or in this case, deter settlement. Material properties such as wettability and structure have limited effects on *E. larynx*. This does not infer that surface manipulation is broadly ineffective as microtopographies effectively deter specific organisms (Magin et al. 2010; Scardino & de Nys 2011). However, it does confirm the importance of understanding the fundamental biological basis of settlement and selection of settlement sites, and consequently developing targeted materials based on surface chemistries and topographies, be they at nano, micro or multiple hierarchical scales, to maximise efficacy.

In conclusion, given the flexible and adaptive colonisation strategy of *E. larynx*, its resistance to copper (Pyefinch & Downing 1949) and the broad spectrum of new generation materials tested here, there is a clear need to think beyond conventional AF technologies. This requires careful consideration of the settlement process and identifying a specific settlement and colonisation processes that can be selectively interfered with to deliver a disruptive AF technology.

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**Doctoral theses in Biology**
**Norwegian University of Science and Technology**
**Department of Biology**

| Year | Name | Degree | Title |
|---|---|---|---|
| 1974 | Tor-Henning Iversen | Dr. philos Botany | The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism |
| 1978 | Tore Slagsvold | Dr. philos Zoology | Breeding events of birds in relation to spring temperature and environmental phenology |
| 1978 | Egil Sakshaug | Dr.philos Botany | "The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton" |
| 1980 | Arnfinn Langeland | Dr. philos Zoology | Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake |
| 1980 | Helge Reinertsen | Dr. philos Botany | The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton |
| 1982 | Gunn Mari Olsen | Dr. scient Botany | Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana* |
| 1982 | Dag Dolmen | Dr. philos Zoology | Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation |
| 1984 | Eivin Røskaft | Dr. philos Zoology | Sociobiological studies of the rook *Corvus frugilegus* |
| 1984 | Anne Margrethe Cameron | Dr. scient Botany | Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats |
| 1984 | Asbjørn Magne Nilsen | Dr. scient Botany | Alveolar macrophages from expectorates – Biological monitoring of workers exosed to occupational air pollution. An evaluation of the AM-test |
| 1985 | Jarle Mork | Dr. philos Zoology | Biochemical genetic studies in fish |
| 1985 | John Solem | Dr. philos Zoology | Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains |
| 1985 | Randi E. Reinertsen | Dr. philos Zoology | Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds |
| 1986 | Bernt-Erik Sæther | Dr. philos Zoology | Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach |
| 1986 | Torleif Holthe | Dr. philos Zoology | Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna |
| 1987 | Helene Lampe | Dr. scient | The function of bird song in mate attraction and |

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|---|---|---|---|
| | | Zoology | territorial defence, and the importance of song repertoires |
| 1987 | Olav Hogstad | Dr. philos Zoology | Winter survival strategies of the Willow tit *Parus montanus* |
| 1987 | Jarle Inge Holten | Dr. philos Botany | Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway |
| 1987 | Rita Kumar | Dr. scient Botany | Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium* |
| 1987 | Bjørn Åge Tømmerås | Dr. scient. Zoolog | Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction |
| 1988 | Hans Christian Pedersen | Dr. philos Zoology | Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care |
| 1988 | Tor G. Heggberget | Dr. philos Zoology | Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure |
| 1988 | Marianne V. Nielsen | Dr. scient Zoology | The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*) |
| 1988 | Ole Kristian Berg | Dr. scient Zoology | The formation of landlocked Atlantic salmon (*Salmo salar* L.) |
| 1989 | John W. Jensen | Dr. philos Zoology | Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth |
| 1989 | Helga J. Vivås | Dr. scient Zoology | Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces* |
| 1989 | Reidar Andersen | Dr. scient Zoology | Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation |
| 1989 | Kurt Ingar Draget | Dr. scient Botany | Alginate gel media for plant tissue culture |
| 1990 | Bengt Finstad | Dr. scient Zoology | Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season |
| 1990 | Hege Johannesen | Dr. scient Zoology | Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung |
| 1990 | Åse Krøkje | Dr. scient Botany | The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test |
| 1990 | Arne Johan Jensen | Dr. philos Zoology | Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmion (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams |
| 1990 | Tor Jørgen Almaas | Dr. scient Zoology | Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues |
| 1990 | Magne Husby | Dr. scient | Breeding strategies in birds: Experiments with the |

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| | | Zoology | Magpie *Pica pica* |
| 1991 | Tor Kvam | Dr. scient Zoology | Population biology of the European lynx (*Lynx lynx*) in Norway |
| 1991 | Jan Henning L'Abêe Lund | Dr. philos Zoology | Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular |
| 1991 | Asbjørn Moen | Dr. philos Botany | The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands |
| 1991 | Else Marie Løbersli | Dr. scient Botany | Soil acidification and metal uptake in plants |
| 1991 | Trond Nordtug | Dr. scient Zoology | Reflctometric studies of photomechanical adaptation in superposition eyes of arthropods |
| 1991 | Thyra Solem | Dr. scient Botany | Age, origin and development of blanket mires in Central Norway |
| 1991 | Odd Terje Sandlund | Dr. philos Zoology | The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism |
| 1991 | Nina Jonsson | Dr. philos | Aspects of migration and spawning in salmonids |
| 1991 | Atle Bones | Dr. scient Botany | Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase) |
| 1992 | Torgrim Breiehagen | Dr. scient Zoology | Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher |
| 1992 | Anne Kjersti Bakken | Dr. scient Botany | The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.) |
| 1992 | Tycho Anker-Nilssen | Dr. scient Zoology | Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica* |
| 1992 | Bjørn Munro Jenssen | Dr. philos Zoology | Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks |
| 1992 | Arne Vollan Aarset | Dr. philos Zoology | The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans. |
| 1993 | Geir Slupphaug | Dr. scient Botany | Regulation and expression of uracil-DNA glycosylase and $O^6$-methylguanine-DNA methyltransferase in mammalian cells |
| 1993 | Tor Fredrik Næsje | Dr. scient Zoology | Habitat shifts in coregonids. |
| 1993 | Yngvar Asbjørn Olsen | Dr. scient Zoology | Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels ans some secondary effects. |
| 1993 | Bård Pedersen | Dr. scient Botany | Theoretical studies of life history evolution in modular and clonal organisms |
| 1993 | Ole Petter Thangstad | Dr. scient Botany | Molecular studies of myrosinase in Brassicaceae |
| 1993 | Thrine L. M. Heggberget | Dr. scient Zoology | Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra*. |
| 1993 | Kjetil Bevanger | Dr. | Avian interactions with utility structures, a biological |

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| | | scient. Zoology | approach. |
| 1993 | Kåre Haugan | Dr. scient Bothany | Mutations in the replication control gene trfA of the broad host-range plasmid RK2 |
| 1994 | Peder Fiske | Dr. scient. Zoology | Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek |
| 1994 | Kjell Inge Reitan | Dr. scient Botany | Nutritional effects of algae in first-feeding of marine fish larvae |
| 1994 | Nils Røv | Dr. scient Zoology | Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo* |
| 1994 | Annette-Susanne Hoepfner | Dr. scient Botany | Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.) |
| 1994 | Inga Elise Bruteig | Dr. scient Bothany | Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers |
| 1994 | Geir Johnsen | Dr. scient Botany | Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses |
| 1994 | Morten Bakken | Dr. scient Zoology | Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes* |
| 1994 | Arne Moksnes | Dr. philos Zoology | Host adaptations towards brood parasitism by the Cockoo |
| 1994 | Solveig Bakken | Dr. scient Bothany | Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply |
| 1994 | Torbjørn Forseth | Dr. scient Zoology | Bioenergetics in ecological and life history studies of fishes. |
| 1995 | Olav Vadstein | Dr. philos Botany | The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions |
| 1995 | Hanne Christensen | Dr. scient Zoology | Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision* |
| 1995 | Svein Håkon Lorentsen | Dr. scient Zoology | Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition |
| 1995 | Chris Jørgen Jensen | Dr. scient Zoology | The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity |
| 1995 | Martha Kold Bakkevig | Dr. scient Zoology | The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport |
| 1995 | Vidar Moen | Dr. scient Zoology | Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations |
| 1995 | Hans Haavardsholm Blom | Dr. philos Bothany | A revision of the *Schistidium apocarpum* complex in Norway and Sweden |
| 1996 | Jorun Skjærmo | Dr. scient | Microbial ecology of early stages of cultivated marine |

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| | | Botany | fish; inpact fish-bacterial interactions on growth and survival of larvae |
| 1996 | Ola Ugedal | Dr. scient Zoology | Radiocesium turnover in freshwater fishes |
| 1996 | Ingibjørg Einarsdottir | Dr. scient Zoology | Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines |
| 1996 | Christina M. S. Pereira | Dr. scient Zoology | Glucose metabolism in salmonids: Dietary effects and hormonal regulation |
| 1996 | Jan Fredrik Børseth | Dr. scient Zoology | The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics |
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| 1997 | Jon Arne Grøttum | Dr. scient. Zoology | Physiological effects of reduced water quality on fish in aquaculture |
| 1997 | Per Gustav Thingstad | Dr. scient. Zoology | Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher |
| 1997 | Torgeir Nygård | Dr. scient Zoology | Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as Biomonitors |
| 1997 | Signe Nybø | Dr. scient. Zoology | Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway |
| 1997 | Atle Wibe | Dr. scient. Zoology | Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry |
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| 1997 | Kåre Magne Nielsen | Dr. scient Botany | An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius* |
| 1997 | Jarle Tufto | Dr. scient Zoology | Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models |
| 1997 | Trygve Hesthagen | Dr. philos Zoology | Population responces of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters |

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| 1997 | Trygve Sigholt | Dr. philos Zoology | Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet |
| 1997 | Jan Østnes | Dr. scient Zoology | Cold sensation in adult and neonate birds |
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| 1998 | Thor Harald Ringsby | Dr. scient Zoology | Variation in space and time: The biology of a House sparrow metapopulation |
| 1998 | Erling Johan Solberg | Dr. scient. Zoology | Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment |
| 1998 | Sigurd Mjøen Saastad | Dr. scient Botany | Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity |
| 1998 | Bjarte Mortensen | Dr. scient Botany | Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro |
| 1998 | Gunnar Austrheim | Dr. scient Botany | Plant biodiversity and land use in subalpine grasslands. – A conservtaion biological approach |
| 1998 | Bente Gunnveig Berg | Dr. scient Zoology | Encoding of pheromone information in two related moth species |
| 1999 | Kristian Overskaug | Dr. scient Zoology | Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach |
| 1999 | Hans Kristen Stenøien | Dr. scient Bothany | Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts) |
| 1999 | Trond Arnesen | Dr. scient Botany | Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway |
| 1999 | Ingvar Stenberg | Dr. scient Zoology | Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos* |
| 1999 | Stein Olle Johansen | Dr. scient Botany | A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis |
| 1999 | Trina Falck Galloway | Dr. scient Zoology | Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.) |
| 1999 | Marianne Giæver | Dr. scient Zoology | Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gradus morhua*) in the North-East Atlantic |
| 1999 | Hans Martin Hanslin | Dr. scient Botany | The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus* |
| 1999 | Ingrid Bysveen Mjølnerød | Dr. scient Zoology | Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques |
| 1999 | Else Berit Skagen | Dr. scient | The early regeneration process in protoplasts from |

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|---|---|---|---|
| | | Botany | *Brassica napus* hypocotyls cultivated under various g-forces |
| 1999 | Stein-Are Sæther | Dr. philos Zoology | Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe |
| 1999 | Katrine Wangen Rustad | Dr. scient Zoology | Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease |
| 1999 | Per Terje Smiseth | Dr. scient Zoology | Social evolution in monogamous families: mate choice and conflicts over parental care in the Bluethroat (*Luscinia s. svecica*) |
| 1999 | Gunnbjørn Bremset | Dr. scient Zoology | Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions |
| 1999 | Frode Ødegaard | Dr. scient Zoology | Host spesificity as parameter in estimates of arhrophod species richness |
| 1999 | Sonja Andersen | Dr. scient Bothany | Expressional and functional analyses of human, secretory phospholipase A2 |
| 2000 | Ingrid Salvesen | Dr. scient Botany | Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture |
| 2000 | Ingar Jostein Øien | Dr. scient Zoology | The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race |
| 2000 | Pavlos Makridis | Dr. scient Botany | Methods for the microbial econtrol of live food used for the rearing of marine fish larvae |
| 2000 | Sigbjørn Stokke | Dr. scient Zoology | Sexual segregation in the African elephant (*Loxodonta africana*) |
| 2000 | Odd A. Gulseth | Dr. philos Zoology | Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard |
| 2000 | Pål A. Olsvik | Dr. scient Zoology | Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway |
| 2000 | Sigurd Einum | Dr. scient Zoology | Maternal effects in fish: Implications for the evolution of breeding time and egg size |
| 2001 | Jan Ove Evjemo | Dr. scient Zoology | Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species |
| 2001 | Olga Hilmo | Dr. scient Botany | Lichen response to environmental changes in the managed boreal forset systems |
| 2001 | Ingebrigt Uglem | Dr. scient Zoology | Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.) |
| 2001 | Bård Gunnar Stokke | Dr. scient Zoology | Coevolutionary adaptations in avian brood parasites and their hosts |
| 2002 | Ronny Aanes | Dr. scient | Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*) |
| 2002 | Mariann Sandsund | Dr. scient Zoology | Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses |
| 2002 | Dag-Inge Øien | Dr. scient Botany | Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway |

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| 2002 | Frank Rosell | Dr. scient Zoology | The function of scent marking in beaver (*Castor fiber*) |
| 2002 | Janne Østvang | Dr. scient Botany | The Role and Regulation of Phospholipase $A_2$ in Monocytes During Atherosclerosis Development |
| 2002 | Terje Thun | Dr.philos Biology | Dendrochronological constructions of Norwegian conifer chronologies providing dating of historical material |
| 2002 | Birgit Hafjeld Borgen | Dr. scient Biology | Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth |
| 2002 | Bård Øyvind Solberg | Dr. scient Biology | Effects of climatic change on the growth of dominating tree species along major environmental gradients |
| 2002 | Per Winge | Dr. scient Biology | The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and the Ral GTPase from *Drosophila melanogaster* |
| 2002 | Henrik Jensen | Dr. scient Biology | Causes and consequenses of individual variation in fitness-related traits in house sparrows |
| 2003 | Jens Rohloff | Dr. philos Biology | Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control |
| 2003 | Åsa Maria O. Espmark Wibe | Dr. scient Biology | Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L. |
| 2003 | Dagmar Hagen | Dr. scient Biology | Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach |
| 2003 | Bjørn Dahle | Dr. scient Biology | Reproductive strategies in Scandinavian brown bears |
| 2003 | Cyril Lebogang Taolo | Dr. scient Biology | Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana |
| 2003 | Marit Stranden | Dr.scient Biology | Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*) |
| 2003 | Kristian Hassel | Dr.scient Biology | Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum* |
| 2003 | David Alexander Rae | Dr.scient Biology | Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments |
| 2003 | Åsa A Borg | Dr.scient Biology | Sex roles and reproductive behaviour in gobies and guppies: a female perspective |
| 2003 | Eldar Åsgard Bendiksen | Dr.scient Biology | Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo Salar* L.) parr and smolt |
| 2004 | Torkild Bakken | Dr.scient Biology | A revision of Nereidinae (Polychaeta, Nereididae) |
| 2004 | Ingar Pareliussen | Dr.scient Biology | Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar |
| 2004 | Tore Brembu | Dr.scient Biology | Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana* |
| 2004 | Liv S. Nilsen | Dr.scient | Coastal heath vegetation on central Norway; recent |

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| | | Biology | past, present state and future possibilities |
| 2004 | Hanne T. Skiri | Dr.scient Biology | Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*) |
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| 2004 | Linda Dalen | Dr.scient Biology | Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming |
| 2004 | Lisbeth Mehli | Dr.scient Biology | Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea* |
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| 2005 | Sten Karlsson | Dr.scient Biology | Dynamics of Genetic Polymorphisms |
| 2005 | Terje Bongard | Dr.scient Biology | Life History strategies, mate choice, and parental investment among Norwegians over a 300-year period |
| 2005 | Tonette Røstelien | ph.d Biology | Functional characterisation of olfactory receptor neurone types in heliothine moths |
| 2005 | Erlend Kristiansen | Dr.scient Biology | Studies on antifreeze proteins |
| 2005 | Eugen G. Sørmo | Dr.scient Biology | Organochlorine pollutants in grey seal (*Halichoerus grypus*) pups and their impact on plasma thyrid hormone and vitamin A concentrations |
| 2005 | Christian Westad | Dr.scient Biology | Motor control of the upper trapezius |
| 2005 | Lasse Mork Olsen | ph.d Biology | Interactions between marine osmo- and phagotrophs in different physicochemical environments |
| 2005 | Åslaug Viken | ph.d Biology | Implications of mate choice for the management of small populations |
| 2005 | Ariaya Hymete Sahle Dingle | ph.d Biology | Investigation of the biological activities and chemical constituents of selected *Echinops* spp. growing in Ethiopia |
| 2005 | Anders Gravbrøt Finstad | ph.d Biology | Salmonid fishes in a changing climate: The winter challenge |
| 2005 | Shimane Washington Makabu | ph.d Biology | Interactions between woody plants, elephants and other browsers in the Chobe Riverfront, Botswana |
| 2005 | Kjartan Østbye | Dr.scient Biology | The European whitefish *Coregonus lavaretus* (L.) species complex: historical contingency and adaptive radiation |
| 2006 | Kari Mette Murvoll | ph.d Biology | Levels and effects of persistent organic pollutans (POPs) in seabirds |

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| | | | Retinoids and α-tocopherol – potential biomakers of POPs in birds? |
| 2006 | Ivar Herfindal | Dr.scient Biology | Life history consequences of environmental variation along ecological gradients in northern ungulates |
| 2006 | Nils Egil Tokle | ph.d Biology | Are the ubiquitous marine copepods limited by food or predation? Experimental and field-based studies with main focus on *Calanus finmarchicus* |
| 2006 | Jan Ove Gjershaug | Dr.philos Biology | Taxonomy and conservation status of some booted eagles in south-east Asia |
| 2006 | Jon Kristian Skei | Dr.scient Biology | Conservation biology and acidification problems in the breeding habitat of amphibians in Norway |
| 2006 | Johanna Järnegren | ph.d Biology | Acesta Oophaga and Acesta Excavata – a study of hidden biodiversity |
| 2006 | Bjørn Henrik Hansen | ph.d Biology | Metal-mediated oxidative stress responses in brown trout (*Salmo trutta*) from mining contaminated rivers in Central Norway |
| 2006 | Vidar Grøtan | ph.d Biology | Temporal and spatial effects of climate fluctuations on population dynamics of vertebrates |
| 2006 | Jafari R Kideghesho | ph.d Biology | Wildlife conservation and local land use conflicts in western Serengeti, Corridor Tanzania |
| 2006 | Anna Maria Billing | ph.d Biology | Reproductive decisions in the sex role reversed pipefish *Syngnathus typhle*: when and how to invest in reproduction |
| 2006 | Henrik Pärn | ph.d Biology | Female ornaments and reproductive biology in the bluethroat |
| 2006 | Anders J. Fjellheim | ph.d Biology | Selection and administration of probiotic bacteria to marine fish larvae |
| 2006 | P. Andreas Svensson | ph.d Biology | Female coloration, egg carotenoids and reproductive success: gobies as a model system |
| 2007 | Sindre A. Pedersen | ph.d Biology | Metal binding proteins and antifreeze proteins in the beetle *Tenebrio molitor*<br>- a study on possible competition for the semi-essential amino acid cysteine |
| 2007 | Kasper Hancke | ph.d Biology | Photosynthetic responses as a function of light and temperature: Field and laboratory studies on marine microalgae |
| 2007 | Tomas Holmern | ph.d Biology | Bushmeat hunting in the western Serengeti: Implications for community-based conservation |
| 2007 | Kari Jørgensen | ph.d Biology | Functional tracing of gustatory receptor neurons in the CNS and chemosensory learning in the moth *Heliothis virescens* |
| 2007 | Stig Ulland | ph.d Biology | Functional Characterisation of Olfactory Receptor Neurons in the Cabbage Moth, (*Mamestra brassicae* L.) (Lepidoptera, Noctuidae). Gas Chromatography Linked to Single Cell Recordings and Mass Spectrometry |
| 2007 | Snorre Henriksen | ph.d Biology | Spatial and temporal variation in herbivore resources at northern latitudes |
| 2007 | Roelof Frans May | ph.d Biology | Spatial Ecology of Wolverines in Scandinavia |
| 2007 | Vedasto Gabriel Ndibalema | ph.d Biology | Demographic variation, distribution and habitat use between wildebeest sub-populations in the Serengeti |

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| | | | National Park, Tanzania |
| 2007 | Julius William Nyahongo | ph.d Biology | Depredation of Livestock by wild Carnivores and Illegal Utilization of Natural Resources by Humans in the Western Serengeti, Tanzania |
| 2007 | Shombe Ntaraluka Hassan | ph.d Biology | Effects of fire on large herbivores and their forage resources in Serengeti, Tanzania |
| 2007 | Per-Arvid Wold | ph.d Biology | Functional development and response to dietary treatment in larval Atlantic cod *(Gadus morhua* L.) Focus on formulated diets and early weaning |
| 2007 | Anne Skjetne Mortensen | ph.d Biology | Toxicogenomics of Aryl Hydrocarbon- and Estrogen Receptor Interactions in Fish: Mechanisms and Profiling of Gene Expression Patterns in Chemical Mixture Exposure Scenarios |
| 2008 | Brage Bremset Hansen | ph.d Biology | The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and its food base: plant-herbivore interactions in a high-arctic ecosystem |
| 2008 | Jiska van Dijk | ph.d Biology | Wolverine foraging strategies in a multiple-use landscape |
| 2008 | Flora John Magige | ph.d Biology | The ecology and behaviour of the Masai Ostrich (Struthio camelus massaicus) in the Serengeti Ecosystem, Tanzania |
| 2008 | Bernt Rønning | ph.d Biology | Sources of inter- and intra-individual variation in basal metabolic rate in the zebra finch, (*Taeniopygia guttata*) |
| 2008 | Sølvi Wehn | ph.d Biology | Biodiversity dynamics in semi-natural mountain landscapes. - A study of consequences of changed agricultural practices in Eastern Jotunheimen |
| 2008 | Trond Moxness Kortner | ph.d Biology | "The Role of Androgens on previtellogenic oocyte growth in Atlantic cod (*Gadus morhua*): Identification and patterns of differentially expressed genes in relation to Stereological Evaluations" |
| 2008 | Katarina Mariann Jørgensen | Dr.Scient Biology | The role of platelet activating factor in activation of growth arrested keratinocytes and re-epithelialisation |
| 2008 | Tommy Jørstad | ph.d Biology | Statistical Modelling of Gene Expression Data |
| 2008 | Anna Kusnierczyk | ph.d Bilogy | *Arabidopsis thaliana* Responses to Aphid Infestation |
| 2008 | Jussi Evertsen | ph.d Biology | Herbivore sacoglossans with photosynthetic chloroplasts |
| 2008 | John Eilif Hermansen | ph.d Biology | Mediating ecological interests between locals and globals by means of indicators. A study attributed to the asymmetry between stakeholders of tropical forest at Mt. Kilimanjaro, Tanzania |
| 2008 | Ragnhild Lyngved | ph.d Biology | Somatic embryogenesis in *Cyclamen persicum.* Biological investigations and educational aspects of cloning |
| 2008 | Line Elisabeth Sundt-Hansen | ph.d Biology | Cost of rapid growth in salmonid fishes |

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| 2008 | Line Johansen | ph.d Biology | Exploring factors underlying fluctuations in white clover populations – clonal growth, population structure and spatial distribution |
| 2009 | Astrid Jullumstrø Feuerherm | ph.d Biology | Elucidation of molecular mechanisms for pro-inflammatory phospholipase A2 in chronic disease |
| 2009 | Pål Kvello | ph.d Biology | Neurons forming the network involved in gustatory coding and learning in the moth *Heliothis virescens:* Physiological and morphological characterisation, and integration into a standard brain atlas |
| 2009 | Trygve Devold Kjellsen | ph.d Biology | Extreme Frost Tolerance in Boreal Conifers |
| 2009 | Johan Reinert Vikan | ph.d Biology | Coevolutionary interactions between common cuckoos *Cuculus canorus* and *Fringilla* finches |
| 2009 | Zsolt Volent | ph.d Biology | Remote sensing of marine environment: Applied surveillance with focus on optical properties of phytoplankton, coloured organic matter and suspended matter |
| 2009 | Lester Rocha | ph.d Biology | Functional responses of perennial grasses to simulated grazing and resource availability |
| 2009 | Dennis Ikanda | ph.d Biology | Dimensions of a Human-lion conflict: Ecology of human predation and persecution of African lions (*Panthera leo*) in Tanzania |
| 2010 | Huy Quang Nguyen | ph.d Biology | Egg characteristics and development of larval digestive function of cobia (*Rachycentron canadum*) in response to dietary treatments -Focus on formulated diets |
| 2010 | Eli Kvingedal | ph.d Biology | Intraspecific competition in stream salmonids: the impact of environment and phenotype |
| 2010 | Sverre Lundemo | ph.d Biology | Molecular studies of genetic structuring and demography in *Arabidopsis* from Northern Europe |
| 2010 | Iddi Mihijai Mfunda | ph.d Biology | Wildlife Conservation and People's livelihoods: Lessons Learnt and Considerations for Improvements. Tha Case of Serengeti Ecosystem, Tanzania |
| 2010 | Anton Tinchov Antonov | ph.d Biology | Why do cuckoos lay strong-shelled eggs? Tests of the puncture resistance hypothesis |
| 2010 | Anders Lyngstad | ph.d Biology | Population Ecology of *Eriophorum latifolium*, a Clonal Species in Rich Fen Vegetation |
| 2010 | Hilde Færevik | ph.d Biology | Impact of protective clothing on thermal and cognitive responses |
| 2010 | Ingerid Brænne Arbo | ph.d Medical technology | Nutritional lifestyle changes – effects of dietary carbohydrate restriction in healthy obese and overweight humans |
| 2010 | Yngvild Vindenes | ph.d Biology | Stochastic modeling of finite populations with individual heterogeneity in vital parameters |
| 2010 | Hans-Richard Brattbakk | ph.d Medical technology | The effect of macronutrient composition, insulin stimulation, and genetic variation on leukocyte gene expression and possible health benefits |
| 2011 | Geir Hysing Bolstad | ph.d Biology | Evolution of Signals: Genetic Architecture, Natural Selection and Adaptive Accuracy |
| 2011 | Karen de Jong | ph.d | Operational sex ratio and reproductive behaviour in |

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| | | Biology | the two-spotted goby (*Gobiusculus flavescens*) |
| 2011 | Ann-Iren Kittang | ph.d Biology | *Arabidopsis thaliana* L. adaptation mechanisms to microgravity through the EMCS MULTIGEN-2 experiment on the ISS:– The science of space experiment integration and adaptation to simulated microgravity |
| 2011 | Aline Magdalena Lee | ph.d Biology | Stochastic modeling of mating systems and their effect on population dynamics and genetics |
| 2011 | Christopher Gravningen Sørmo | ph.d Biology | Rho GTPases in Plants: Structural analysis of ROP GTPases; genetic and functional studies of MIRO GTPases in *Arabidopsis thaliana* |
| 2011 | Grethe Robertsen | ph.d Biology | Relative performance of salmonid phenotypes across environments and competitive intensities |
| 2011 | Line-Kristin Larsen | ph.d Biology | Life-history trait dynamics in experimental populations of guppy (*Poecilia reticulata*): the role of breeding regime and captive environment |
| 2011 | Maxim A. K. Teichert | ph.d Biology | Regulation in Atlantic salmon (*Salmo salar*): The interaction between habitat and density |
| 2011 | Torunn Beate Hancke | ph.d Biology | Use of Pulse Amplitude Modulated (PAM) Fluorescence and Bio-optics for Assessing Microalgal Photosynthesis and Physiology |
| 2011 | Sajeda Begum | ph.d Biology | Brood Parasitism in Asian Cuckoos: Different Aspects of Interactions between Cuckoos and their Hosts in Bangladesh |
| 2011 | Kari J. K. Attramadal | ph.d Biology | Water treatment as an approach to increase microbial control in the culture of cold water marine larvae |
| 2011 | Camilla Kalvatn Egset | ph.d Biology | The Evolvability of Static Allometry: A Case Study |
| 2011 | AHM Raihan Sarker | ph.d Biology | Conflict over the conservation of the Asian elephant (*Elephas maximus*) in Bangladesh |
| 2011 | Gro Dehli Villanger | ph.d Biology | Effects of complex organohalogen contaminant mixtures on thyroid hormone homeostasis in selected arctic marine mammals |
| 2011 | Kari Bjørneraas | ph.d Biology | Spatiotemporal variation in resource utilisation by a large herbivore, the moose |
| 2011 | John Odden | ph.d Biology | The ecology of a conflict: Eurasian lynx depredation on domestic sheep |
| 2011 | Simen Pedersen | ph.d Biology | Effects of native and introduced cervids on small mammals and birds |
| 2011 | Mohsen Falahati-Anbaran | ph.d Biology | Evolutionary consequences of seed banks and seed dispersal in *Arabidopsis* |
| 2012 | Jakob Hønborg Hansen | ph.d Biology | Shift work in the offshore vessel fleet: circadian rhythms and cognitive performance |
| 2012 | Elin Noreen | ph.d Biology | Consequences of diet quality and age on life-history traits in a small passerine bird |
| 2012 | Irja Ida Ratikainen | ph.d Biology | Theoretical and empirical approaches to studying foraging decisions: the past and future of behavioural ecology |
| 2012 | Aleksander Handå | ph.d Biology | Cultivation of mussels (*Mytilus edulis):*Feed requirements, storage and integration with salmon (*Salmo salar*) farming |
| 2012 | Morten Kraabøl | ph.d | Reproductive and migratory challenges inflicted on |

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| | | Biology | migrant brown trour (*Salmo trutta* L) in a heavily modified river |
| 2012 | Jisca Huisman | ph.d Biology | Gene flow and natural selection in Atlantic salmon |
| 2012 | Maria Bergvik | ph.d Biology | Lipid and astaxanthin contents and biochemical post-harvest stability in *Calanus finmarchicus* |
| 2012 | Bjarte Bye Løfaldli | ph.d Biology | Functional and morphological characterization of central olfactory neurons in the model insect *Heliothis virescens*. |
| 2012 | Karen Marie Hammer | ph.d Biology. | Acid-base regulation and metabolite responses in shallow- and deep-living marine invertebrates during environmental hypercapnia |
| 2012 | Øystein Nordrum Wiggen | ph.d Biology | Optimal performance in the cold |
| 2012 | Robert Dominikus Fyumagwa | Dr. Philos. | Anthropogenic and natural influence on disease prevalence at the human –livestock-wildlife interface in the Serengeti ecosystem, Tanzania |
| 2012 | Jenny Bytingsvik | ph.d Biology | Organohalogenated contaminants (OHCs) in polar bear mother-cub pairs from Svalbard, Norway Maternal transfer, exposure assessment and thyroid hormone disruptive effects in polar bear cubs |
| 2012 | Christer Moe Rolandsen | ph.d Biology | The ecological significance of space use and movement patterns of moose in a variable environment |
| 2012 | Erlend Kjeldsberg Hovland | ph.d Biology | Bio-optics and Ecology in *Emiliania huxleyi* Blooms: Field and Remote Sensing Studies in Norwegian Waters |
| 2012 | Lise Cats Myhre | ph.d Biology | Effects of the social and physical environment on mating behaviour in a marine fish |
| 2012 | Tonje Aronsen | ph.d Biology | Demographic, environmental and evolutionary aspects of sexual selection |
| 2012 | Bin Liu | ph.d Biology | Molecular genetic investigation of cell separation and cell death regulation in *Arabidopsis thaliana* |
| 2013 | Jørgen Rosvold | ph.d Biology | Ungulates in a dynamic and increasingly human dominated landscape – A millennia-scale perspective |
| 2013 | Pankaj Barah | ph.d Biology | Integrated Systems Approaches to Study Plant Stress Responses |
| 2013 | Marit Linnerud | ph.d Biology | Patterns in spatial and temporal variation in population abundances of vertebrates |
| 2013 | Xinxin Wang | ph.d Biology | Integrated multi-trophic aquaculture driven by nutrient wastes released from Atlantic salmon (*Salmo salar*) farming |
| 2013 | Ingrid Ertshus Mathisen | ph.d Biology | Structure, dynamics, and regeneration capacity at the sub-arctic forest-tundra ecotone of northern Norway and Kola Peninsula, NW Russia |
| 2013 | Anders Foldvik | ph.d Biology | Spatial distributions and productivity in salmonid populations |
| 2013 | Anna Marie Holand | ph.d Biology | Statistical methods for estimating intra- and inter-population variation in genetic diversity |
| 2013 | Anna Solvang Båtnes | ph.d Biology | Light in the dark – the role of irradiance in the high Arctic marine ecosystem during polar night |
| 2013 | Sebastian Wacker | ph.d | The dynamics of sexual selection: effects of OSR, |

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| | | Biology | density and resource competition in a fish |
| 2013 | Ragnhild Pettersen | ph.d | Identification of marine organisms using |
| | | Biology | chemotaxonomy and hyperspectral imaging |
| 2013 | Angela Mwakatobe | ph.d | Human-Wildlife Interaction in the Western Serengeti: |
| | | Biology | Crop Raiding, Livestock Depredation and Bushmeat Utilisation |