Master's thesis

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An investigation of biofouling and its management in Norwegian salmon aquaculture, and the potential effects on cleaner fish behaviour

Master's thesis in Ocean Resources

Supervisor: Kjell Inge Reitan (NTNU) and Nina Blöcher (SINTEF)

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Mai-Louise K. Bouwman

Abstract

The high-frequency net cleaning regime in Norwegian salmon aquaculture, which is highly driven by ensuring cleaner fish delousing efficacy, has detrimental effects on the cage environment, fish health & welfare, and farm economy. There is currently no standard on maximum recommended biofouling conditions on cage nets, and there is conflicting evidence on the actual impact of biofouling on cleaner fish behaviour in terms of habitat usage. The aims of this study were to characterise biofouling conditions at the time of net cleaning, the effect of spatial factors on biofouling, and investigate current management practices. Also, the potential impacts of biofouling abundance on cleaner fish behaviour in terms of proximity to net walls was investigated to subsequently develop a threshold of recommended biofouling cover on net walls.

Five sampling events were conducted at four unique salmon farms from September through November 2019. This study quantified % biofouling abundance at the time of net cleaning at 1, 5 and 10m depth on four transects (North, East, South, West) within one or two cages at each site. Data was collected through vertical filming of net walls and subsequent image analysis. The presence/absence of lice skirt was also linked to each image. To determine the effect of net fouling on cleaner fish habitat usage, net walls were filmed in horizontal transects and resulted in cleaner fish counts per sampled minute.

'Maximum' biofouling conditions (i.e., biofouling abundance on cage nets at the day of net cleaning) differed significantly between sampling events and varied between 10.4% and 58%. Algae and hydroids were identified on image samples, and the dominance of the species groups varied between sampling events. The variability between cages, depth, and cardinal direction all influenced total biofouling abundance and algae abundance. Hydroids were not affected by cardinal direction. There was significantly more biofouling in samples of the first meter below the lice skirt edge than in samples from the last meter still protected by lice skirts. The abundance of the two species groups responded inversely to lice skirt cover, where hydroid abundance was significantly higher in the selected samples from below skirt edge than above, and algae abundance was higher above than below.

Across all sampled cages, cleaner fish numbers observed before net cleaning were significantly higher than after net cleaning. Although cleaner fish numbers showed a strong positive relationship with increasing biofouling abundance on cage nets, the relationship was only true for cleaner fish observed before net cleaning took place. When data of cleaner fish observed before and after net cleaning were analysed together, said relationship was much weaker but still significant.

This study demonstrated that there is no standard in maximum biofouling conditions on net walls before net cleaning is conducted. Spatial factors influenced biofouling abundance and community composition, but not in a consistent pattern, indicating that within-farm knowledge is vital. Although cleaner fish abundance along net walls was greater before net cleaning than after, and the relationship between cleaner fish and increasing biofouling was significant, the results were weak, indicating that the effect of biofouling may not be as great as fish farmers have formerly believed. Regardless, based on the current findings, a biofouling threshold of 40% may be reasonable with regards to cleaner fish numbers observed in close proximity to net walls. However, if a standard is to be developed, regardless of the effect on cleaner fish or cage environment and fish welfare, a simple and accurate way to monitor biofouling abundance *in situ* must be developed for farmers.

Sammendrag

Det høyfrekvente notvaskregimet i norsk lakseoppdrett, som er sterkt drevet av å sikre avlusningseffekten og appettitten for lus hos rensefisk, har store negative konsekvenser for merdmiljøet, fiskehelse & velferd og økonomi. Det er foreløpig ingen utviklet standard for maksimal anbefalt groe på nøter, og det er motstridende bevis om den faktiske effekten av groe på rensefiskadferd med tanke på habitatvalg. Målet med denne studien var å karakterisere groetilstanden ved tidspunktet for notvask, effekten av romlige faktorer på groen, og å kartlegge dagens groeforvaltning. I tillegg ble den potensielle påvirkningen av groe på rensefiskadferd med tanke på nærhet til notvegg undersøkt, for deretter å kunne utvikle en terskel for anbefalt groe på notvegg basert på dette.

Fem prøvetakninger ble gjennomført på fire forskjellige oppdrettsanlegg fra september til november 2019. Studien kvantifiserte % groeforekomst på 1, 5 og 10m dybde i fire transekter (Nord, Øst, Sør, Vest) i én eller to merder ved hvert anlegg. Data ble samlet inn gjennom filming av notvegg og påfølgende bildeanalyser. Tilstedeværelse av luseskjørt ble også knyttet til hvert enkelt bilde. For å bestemme effekten av groe på rensefisk ble notveggen filmet i vertikale transekter og resulterte i antall fisk observert per minutt filmet.

'Maksimal' groeforekomst (dvs. groe på notvegg ved tidspunktet for notvask) skilte seg betydelig mellom prøvetakningene, og varierte mellom 10.4% og 58%. Alger og hydroider ble identifisert på bildeprøver, og dominansen til de to artsgruppene varierte mellom prøvetakningene. Variasjonen mellom lokaliteter, merder, dybde og kardinalpunkt påvirket total groeforekomst og algeforekomst. Kun variasjonen mellom lokaliteter, merder og dybde hadde en effekt på hydroider, som ikke ble påvirket av retning. Det var betydelig mer groe på prøver fra den første meteren under luseskjørtkanten enn fra den siste meteren som fremdeles var beskyttet av skjørt. Forekomsten av de to artsgruppene reagerte motsatt på luseskjørtbeskyttelse, hvor hydroideforekomst var signifikant høyere under skjørtkanten enn over, og algaeforekomsten var høyere over enn under.

Blant alle merdene filmet var antall observerte rensefisk per minutt før notvask betydelig høyere enn etter notvask. Selv om antall rensefisk viste en sterkt positiv korrelasjon med økende mengde groe på notvegg, var dette bare tilfellet før notvask hadde funnet sted. Når data fra både før og etter notvask ble lagt sammen var nevnte korrelasjon mye svakere, men fortsatt signifikant.

Denne studien demonstrerte at det ikke finnes noen standard i maksimal groeforekomst på notvegger før notvask blir utført. Romlige faktorer påvirket groeforekomst og fordelingen av arter, men ikke i et konsistent mønster, noe som indikerer at kunnskap om groebildet innen hvert enkelt oppdrettsanlegg er viktig. Selv om antall rensefisk langs notveggen var høyere før notvask enn etter, og at forholdet mellom rensefisk og økende groe var signifikant, var resultatene svake. Dette indikerer at effekten av groe på rensefiskadferd ikke er så stor som fiskeoppdrettere tidligere har trodd. Til tross for dette, basert på de nåværende funnene kan en groeforekomst på 40% være rimelig med hensyn til antall rensefisk observert langs notvegg. Hvis en standard skal utvikles, uavhengig om det er grunnet effekten på rensefisk eller merdmiljø og fiskevelferd, må det imidlertid utvikles en enkel og nøyaktig måte å vurdere groeforekomst på for oppdretterne.

Table of Contents

List of Figures

List of Tables

Abbreviations

BF	Biofouling
DO	Dissolved Oxygen
FAO	Food and Agriculture Organization of the United Nations
Mt	Million tonnes
ROV	Remotely Operated Vehicle

Chapter 1

Introduction

1.1 Global and Norwegian aquaculture

Aquaculture is the fastest-growing major food production sector in the world, with a global production of 110.2 Mt in 2016, worth US$243.5 (FAO, 2018). This included 54.1 Mt of finfish (US$138.5), 30.1 million tonnes of aquatic plants (US$11.7), and 17.1 Mt of molluscs (US$29.1). According to the UN, the world's population continues to grow and the total global population is expected to reach 9.7 billion by 2050 (United Nations, 2019). Food demand in many regions of the world will increase and put considerable pressure on global food production. It has been projected that to maintain a fish consumption of 15-20 kg^{-yr} per capita, annual fish production (both from fisheries and aquaculture) must be between 125 and 210 Mt^{-yr} (Merino et al., 2012). Garcia and Rosenberg (2010) estimated the need for a 50% production increase from the 2006 production level (144 Mt) to supply a 2050 population with a minimum of 20% dietary protein. In Norway, aquaculture production mainly consists of Atlantic salmon (*Salmo salar*) and Rainbow trout (*Oncorhynchus mykiss*), making up 99% of total aquaculture production nationally. In 2016, Norwegian salmon sales reached 1.23 million tonnes (Directorate of Fisheries, 2019*a*) – 54.7% of total Atlantic salmon production globally (FAO, 2018).

However, many barriers hold constraints on further growth, and biofouling is one of the main restrictions to ensure profitable and sustainable production in both finfish, seaweed, and mollusc aquaculture worldwide (Hodson et al., 1997; Dürr and Watson, 2010; Bannister et al., 2019).

1.2 Biofouling in the marine environment

In the marine environment, biofouling is the dynamic settlement of unwanted biological organisms on a surface exposed to seawater (Carve et al., 2019). The first known mention of fouling was by Aristoteles in the 4th century BCE, connecting it with the suckerfish 'remora', whose genus name *Echeneis* comes from the Greek for 'to hold a ship' (WHOI, 1952). Biofouling as a concept was first documented around CE 99 by the Greek historian and philosopher Plutarch, distinguishing that "weeds, ooze and filth" made ships go slower, identifying biofouling as the cause of slowing (WHOI, 1952).

Biofouling communities are made up of a succession of species, where biofoulers are characterized as microfoulers (bacteria and diatoms) and macrofoulers (e.g., bryozoans, hydroids, barnacles, tubeworms, tunicates, mussels, and macroalgae) (Dobretsov, 2010; Prendergast, 2010). More than 4000 fouling organisms have been recorded (Lewis, 1998), and common for most

macrofouling species is their attached and sessile lifestyle, and affinity for unoccupied space, being a key resource for settlement (Jenkins and Martins, 2010). Scientists have spent several decades trying to understand the succession process and initiation cues of fouling species (Prendergast, 2010), using this knowledge to develop methods to prevent unwanted accumulation on marine structures (Wahl, 1989).

Biofouling has become a significant nuisance in many industries with submerged structures worldwide, creating challenges in shipping, power stations, desalination plants, pipes, cooling systems, sensors, oil platforms, and mariculture (Davidson et al., 2016; Finnie and Williams, 2010; Henderson, 2010; Page et al., 2010; Fitridge et al., 2012). Biofouling in maritime industries is mostly discussed in context with commercial ships, boats, and shipping vessels. Major biofouling growth on ship hulls increases surface roughness and thereby hydrodynamic drag due to more frictional resistance (Schultz, 2007). This leads to increased power requirement and higher fuel consumption. The economic impact of biofouling on ship hulls has been estimated to be $56M for the entire DDG-51 class of the US Navy (Baxter et al., 2012). Methods to prevent fouling has been spoken about since ancient times (reviewed in WHOI (1952)), and the first official use of copper as an antifoulant was as early as in 1625.

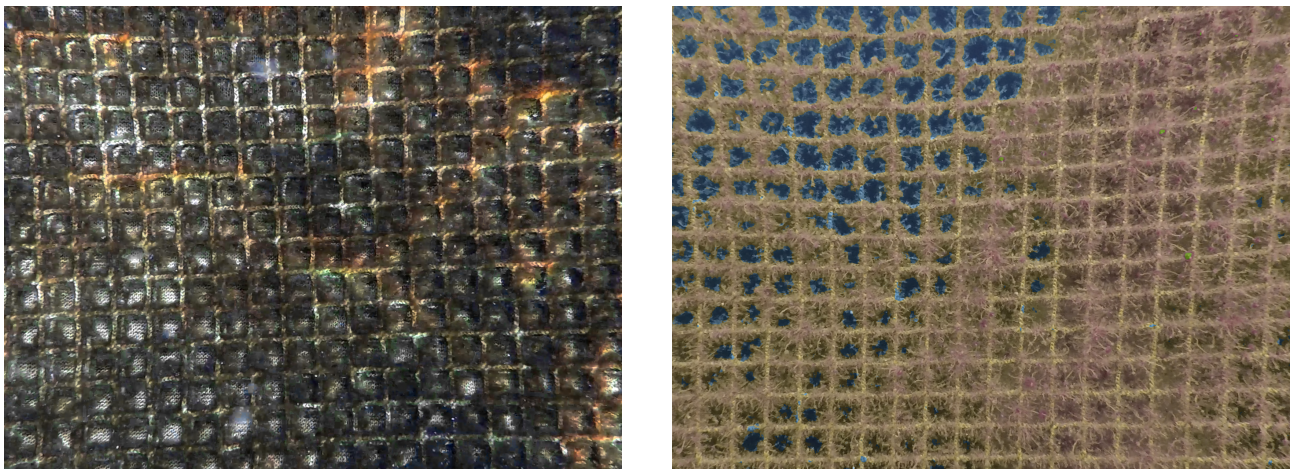
Besides being a significant challenge in many industries, biofouling species may pose substantial threats to indigenous marine flora and fauna if they are introduced beyond their native ranges. The introduction of non-native species may have severe consequences for ecosystem functions and biodiversity, often resulting in habitat destruction (Lewis and Coutts, 2010). The main vectors of non-native species introductions are assumed to be shipping vessels and aquaculture practices (Coutts and Dodgshun, 2007; Hopkins and Forrest, 2010; Gollasch, 2002). Mariners' motivation for antifouling product development has mainly been driven by cost reduction in terms of fuel consumption, and has not been in favour of environmental concerns. However, biosecurity management has, in recent years, become a more established sector, highlighting the need to focus on invasion ecology (Davidson et al., 2016).

Although biofouling is regarded as a pest, several processes are benefiting from this phenomenon. Seaweed and mussel cultivation is dependent on the species' ability to foul rope for seedling (Braithwaite and McEvoy, 2004), a process making up a significant part of global aquaculture production (FAO, 2018). Growth on artificial reefs, as a tool in the conservation and management of coastal ecosystems, is also an excellent example of desired biofouling (Terlizzi and Faimali, 2010; Oren and Benayahu, 1997; Reed et al., 2006).

1.3 Biofouling in marine finfish aquaculture

Marine finfish aquaculture in western countries is primarily made up of fish culturing in cage structures with netting-bags suspended from a floating frame. The multifilament cage netting material used is a model substrate for fouling organisms as it has a high surface-to-volume ratio and many crevices that allow for entrapment and protection of sessile organisms (Hodson et al., 2000). Intensive fish farming practices create an ideal environment for opportunistic suspension feeders such as sponges, barnacles, mussels, ascidians and hydroids due to the availability of uneaten fish feed and faecal particulates (reviewed in Braithwaite and McEvoy (2004) and Dürr and Watson (2010)), but also for algae, as elevated levels of inorganic nutrients released by fish stimulates algal growth (Chopin et al., 1999; Wang et al., 2012).

There are several spatio-temporal variations in biofouling communities. Seasonality is the primary driver of temporal community changes throughout the year, where the time of year and seasonal variations in environmental conditions may impact recruitment, establishment, survival, periods of growth and periods of dormancy or regression (Jenkins and Martins, 2010; Fitridge et al., 2012). In temperate and polar waters, biofouling on artificial surfaces has a far



(a) Patches of algae growing on cage net (lice skirt in background)

(b) Heavily fouled net occluded by *E. larynx*

Figure 1.1: Examples of cage net biofouling in Norwegian salmon aquaculture

greater growth rate in warmer months than in colder months, where many marine organisms cease or slow growth (Poloczanska and Butler, 2010). There is also a significant variation in species diversity between temperate and tropical waters, with a latitudinal diversity cline, showing evident regional differences (Canning-Clode and Wahl, 2010). However, within-fish farm variation is mainly driven by light availability and water flow, where depth and cage placement play a significant role in both abundance and species composition (Cronin et al., 1999; Madin et al., 2010).

Bloecher, Olsen and Guenther (2013) identified 90 species and multi-species categories during a 1-year field study at a salmon farm in mid-Norway, where the most common sessile species were the common biofouling hydroid, *Ectopleura larynx* (syn. *Tubularia larynx*), the blue mussel *Mytilus edulis*, the amphipod *Jassa falcata* and the algae species *Polysiphonia stricta* and *Saccharina latissima*. *Mytilus edulis* and *E. larynx* contributed most to the biomass. There are, however, both temporal and spatial variations, where biofouling type and peak fouling period vary between farms and geographical location (Olafsen, 2006). Despite large variations, fish farmers generally find *E. larynx* (Figure 1.1b), accompanied by *M. edulis* and various algae species (Figure 1.1a), as the most problematic foulers (Olafsen, 2006).

1.3.1 Effects of biofouling in cage aquaculture

There is a consensus among fish farmers and scientists that biofouling in cage aquaculture has detrimental effects on cage environment, farm structures, and fish health & welfare (Figure 1.2a). In addition, it is widely believed that net fouling impacts cleaner fish efficacy, and this is discussed in detail below. Oxygen depletion in fish farm cages is a well-known problem, where low dissolved oxygen (DO) levels adversely impact feeding and growth (Remen et al., 2012) and induce stress (Sundh et al., 2010; Oppedal et al., 2011; Remen et al., 2012; Hvas and Oppedal, 2019), leading to compromised fish health & welfare (Segner et al., 2012). Oxygen demand of fish (Cronin et al., 1999), stocking density (Johansson et al., 2006), and water temperature (Davis, 1975; Deutsch et al., 2015) all contribute to DO levels within the cage. A steady water flow through the cage ensures waste removal and oxygen replenishment, maintaining an optimal cage environment. Biofouling occlusion on cage nets rapidly constricts water flow throughout the cage, and Madin et al. (2010) encountered a water velocity reduction of 79% after two weeks of cage immersion and up to 91% with further biofouling development. High net occlusion will, therefore, cause disruptions in water flow, and combined with oxygen demands of both fish and fouling organisms, subsequently lead to critically low DO levels (Cronin et al., 1999).

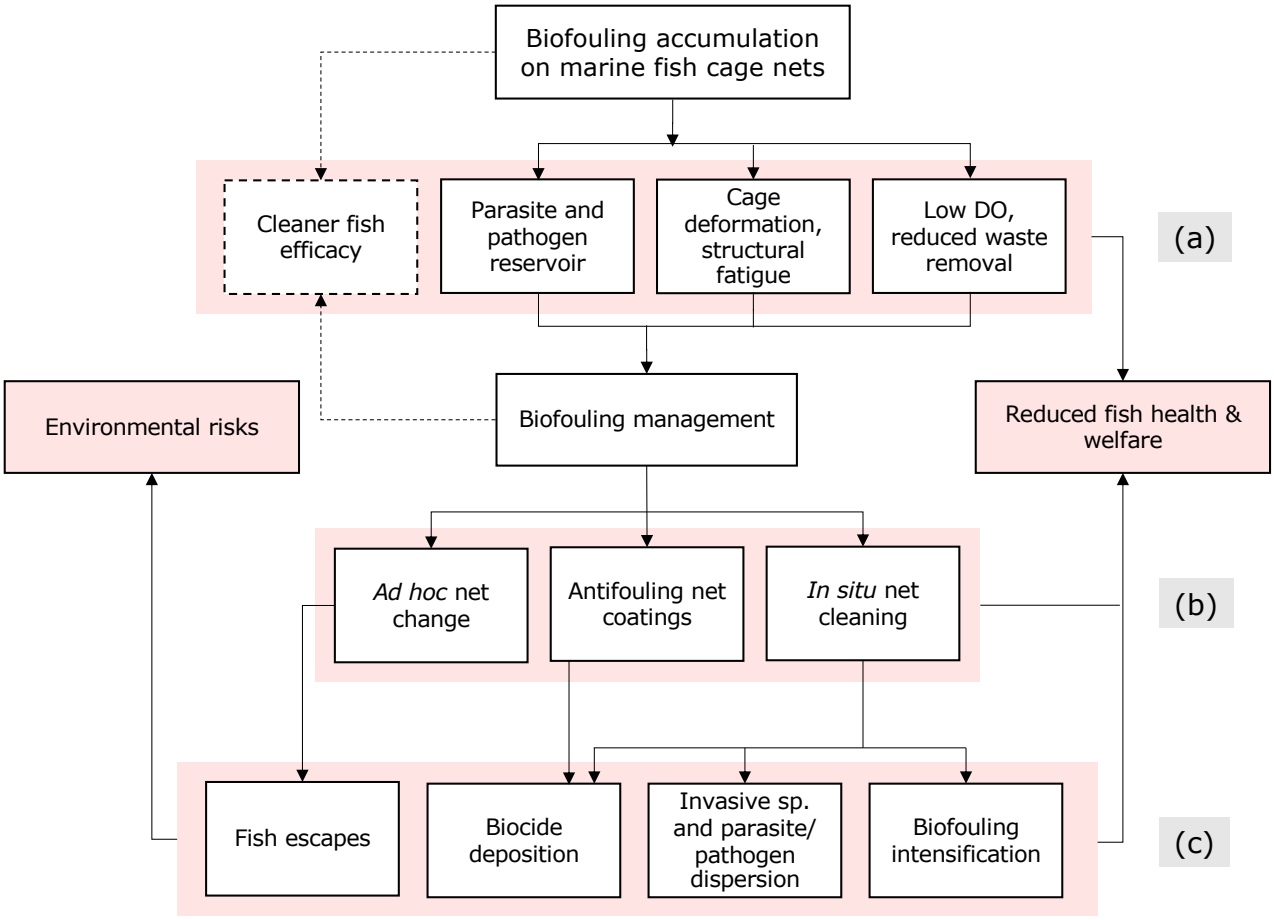


Figure 1.2: Biofouling accumulation on marine fish cage nets impacts cage environment and fish health & welfare (a). To prevent or remove biofouling organisms on cage nets, a range of methods are commonly used in management practices (b), but all methods are proven to have several adverse effects (c), subsequently affecting both the surrounding environment and fish health & welfare.

Heavy fouling can alter hydrodynamic forces on nets, where increased net occlusion can severely deform cages while adding structural strains on moorings. Most Norwegian fish farms experience currents around 0.5-1m s^{-1}. Current speeds of 0.35 m^{-1} can reduce cage volume up to 40% (Lader et al., 2008), and major biofouling accumulation can increase the hydrodynamic load ten times that of a clean net (Bi et al., 2018), showing the potential of severe cage deformities. Reduced cage volume has serious impacts on the cage environment, where crowding will simulate increased stocking density, leading to higher DO consumption and ammonia production per unit volume (Lader et al., 2008; De Nys and Guenther, 2009). Crowding may further increase fish stress levels, compromising growth and fish welfare (Turnbull et al., 2005).

Biofouling communities also have the potential to act as a reservoir for parasitic and pathogenic organisms harboured by macro- or microbial fouling species, posing health risks for farmed fish (Figure 1.2. Shellfish can accumulate and hold viral finfish pathogens for long periods, and pathogenic bacteria causing disease in fish are commonly found in shellfish tissues (De Nys and Guenther, 2009; Fitridge et al., 2012). The amoeba causing amoebic gill disease (AGD) in Atlantic salmon, *Paramoeba perurans*, is associated with several common biofouling organisms (e.g., bryozoans, mussels, and hydroids) during AGD outbreaks, but it is still unclear if they act as a reservoir (Hellebø et al., 2017). Several other parasites can use biofouling organisms as an intermediate host before reaching its final host (reviewed in (Fitridge et al., 2012) and (Bannister et al., 2019)). Net fouling can also pose direct health risks to farmed fish. Nematocysts (stinging cells) of *E. larynx* can harm salmon by causing gill and skin damage upon direct contact (Baxter et al., 2012).

1.3.2 Antifouling strategies in cage aquaculture and their implications

In fish cage aquaculture, farmers usually combine several methods to prevent or remove the accumulation of biofouling organisms on cage nets, including the use of antifouling net coatings, *in situ* net cleaning, and *ad hoc* net change (Bannister et al., 2019) (Figure 1.2b). Unfortunately, most methods pose significant threats to fish health and the surrounding environment (Figure 1.2c).

Cage nets are often coated with antifouling coatings containing biocides, such as copper, to prevent the settlement of biofoulers (Guardiola et al., 2012). Braithwaite et al. (2006) tested a commercial copper coating on cage net panels over ten months. They found that fouling on treated nets was repressed for 150 days longer than on non-treated nets. Today, however, we see a failure of commercial copper coatings already after 2 months (Bloecher and Floerl, 2020). Copper coatings are, unfortunately, a biological hazard. Through net cleaning and leaking, copper is released into the cage and surrounding environment. Dissolved copper may have detrimental effects on target species by acting as a neurotoxin in many salmonids (reviewed in Tierney et al. (2010), including Atlantic salmon (Bjerselius et al., 1993), but also by causing gill, liver, kidney and skin damage (Al-Bairuty et al., 2013). It may also adversely affect non-target organisms (Katranitsas et al., 2003; Guardiola et al., 2012; Kiaune and Singhasemanon, 2011), in addition to altering seabed chemistry (Loucks et al., 2012; Nikolaou et al., 2014). Despite these damaging effects, copper-based coatings are the most commonly used coatings in aquaculture today. Efforts are being made into developing coatings with reduced copper content or alternative biocides, and many are available today. Still, no suitable alternative measuring up to the efficacy of copper has been found to date (Bloecher and Floerl, 2020). Therefore, the use of copper-based antifouling coatings prevails on most Norwegian salmon farms (Bloecher and Floerl, 2020) due to its high antifouling efficacy, regardless of the well-known consequences mentioned above. Some farmers use copper-coated nets with net-change the first year in the sea in response to fish size, then change to uncoated nets and continue with *in situ* net cleaning until slaughter (Olafsen, 2006). Although copper coatings significantly reduce fouling (Guenther et al., 2009; Bloecher et al., 2015), copper-coated nets still need to be cleaned fortnightly during peak biofouling season, if not more frequently. In 2016, an estimated amount of 1088 tonnes of copper was discharged into Norwegian coastal waters from fish farms, making up 85% of total copper used on farms (Skarbøvik et al., 2017).

Conversely, there is a growing demand for biocide-free alternatives. While mostly being due to an increasing number of farmers wanting to obtain specific certification standards with antifouling coatings restrictions (e.g., Aquaculture Stewardship Council (ASC) Salmon Standard (Aquaculture Stewardship Council, 2019)), the reduction of biocide loads should be assumed to have a positive effect on both the environment and fish health. Biocide-free coatings are often water-based (e.g., NetCoating PLUS by Steen-Hansen) and functions to provide a smooth and durable surface area for easier net cleaning and higher durability against mechanical wear and tear. Despite biocide-free or copper-reduced coatings being a better solution for the environment and biocide-related welfare issues, biofouling pressure on cage nets will increase. Unfortunately, this requires a more intense cleaning regime or net exchange frequency, posing further risks to coating integrity and fish health & welfare.

Most *in situ* net cleaning operations are based on systematic cleaning of cage nets with a remotely operated vehicle (ROV) steered by a crew on a support vessel or from cage walkways. The most common net cleaning ROVs are equipped with specialized rigs with rotating discs expelling high-pressure water (Floerl et al., 2016; Bannister et al., 2019). Other methods, such as systems based on cavitation and suction, are also used but considered rare. Although *In situ* net cleaning is relatively efficient and semi-automatic, it does have disadvantages. Frequent net cleaning leads to abrasion of antifouling coatings (Bloecher et al., 2019), reducing its efficiency. The process also releases all of the cleaning waste, containing fragments of biofouling organisms,

into the cage environment. Nematocysts of *E. larynx* remain active following high-pressure net cleaning treatment and can damage the gills of Atlantic salmon, even in a fragmented state (Baxter et al., 2012; Bloecher et al., 2018). It can also trigger the release of gametes, leading to rapid and extensive recolonization (Carl et al., 2011). Complete removal may be impossible, as leftover fragments of attached hydroids may initiate re-growth rapidly and extensively, requiring another cleaning event soon after (Carl et al., 2011). *In situ* net cleaning may also open for further distribution to other cages, farm structures, and downstream farms (Floerl et al., 2016). This may thereby increase connectivity between farms and the environment, opening for further invasions and potential disease spread to both farmed and wild fish. Also, there may be non-indigenous species amongst biofoulers, and net cleaning can aid in their further distribution. Furthermore, the net cleaning process is also reported to instigate stressful behaviour and reduced appetite in salmon, compromising their health & welfare (Floerl et al., 2016; Bannister et al., 2019).

In Norway, most fish farmers operate with weekly or fortnightly net cleaning events during peak biofouling season, and there may be up to 20 cleaning events per farm annually, releasing significant amounts of cleaning waste (Floerl et al., 2016). Other farm structures, such as moorings (Figure 1.3), feed tubes, and feed barges, are cleaned at much lower rates and are largely unmanaged compared to cage nets (Bloecher et al., 2015). It is speculated that these structures may function as reservoirs, driving biofouling recruitment further (Bloecher et al., 2015).

In Norwegian salmon farming, direct economic costs related to biofouling management can make up to 5.5% of total production costs and is one of the most resource-consuming factors in the sector (Olafsen, 2006). In 2018, an average of 3826 net cages were used to stock Atlantic salmon and rainbow trout (Directorate of Fisheries, 2018), and a typical Norwegian farm consists of 50 000 m^2 of submerged artificial surface area, providing a significant amount of substrate for settlement of fouling organisms. There is, however, no common antifouling strategy in the industry (Olafsen, 2006). Some areas in Canada have discontinued *in situ* net cleaning due to the release of large amounts of organic materials, whereas biocidal coatings have been banned in Australia and New Zealand due to environmental concerns (Bannister et al., 2019). However, in most major salmon-producing nations, copper coatings and *in situ* net cleaning prevails. In Norway, net cleaning is primarily conducted by external service providers and on a set schedule without regarding biofouling abundance on nets. Considering net cleaning is hazardous both

Figure 1.3: Heavy biofouling on cage moorings. Moorings and other farm structures are largely unmanaged and are cleaned at a much lower rate than cage nets. This can, in addition to add hydrodynamic load on the moorings, aid in driving biofouling recruitment beyond farm borders.

for the environment and the fish, the call for more knowledge on biofouling communities and better antifouling strategies is pressing. Furthermore, *in situ* net cleaning in Norway is not subject to governmental regulations beyond the regulation of released pollutants, allowing fish farmers to determine the necessity of cleaning frequency themselves. There is clearly a need for alternative biofouling management methods, both in terms of net cleaning, waste collection, and environmentally friendly coatings.

1.4 Cleaner fish and biofouling

The prevalence of salmon lice (*Lepeophtheirus salmonis* (Krøyer, 1857)) is one of the most significant drivers of economic loss in Norwegian salmon aquaculture today and has prevented further sector growth for several years. Salmon lice is a common, highly pathogenic ectoparasite of salmonids in the marine environment (Tucker et al., 2002). Infections of salmon lice lead to erosion of the epidermis, and may in severe cases, expose skeletal muscle, becoming a large welfare issue (Torrissen et al., 2013). Sea lice management methods, including chemical and mechanical delousing, are applied as acute, last-resort treatments, and the direct and associated costs of these methods were estimated to be 2.6 billion NOK in 2016 (Iversen et al., 2017).

However, as a pro-active, continuous, and biological delousing method, lice-consuming cleaner fish are deployed into the cages. At present, cleaner fish such as lumpfish (*Cyclopterus lumpus*), goldsinny wrasse (*Ctenolabrus rupestris*) and ballan wrasse (*Labrus bergylta*) (Figure 1.4) are commonly used as a biological delousing method in Norwegian salmon and rainbow trout aquaculture (Skiftesvik et al., 2013, 2014; Powell et al., 2018). In 2018, Norwegian salmon farmers deployed 50 million cleaner fish with a total value of 1.04 billion NOK into salmon cages (Directorate of Fisheries, 2019*b*). In addition, the costs associated with holding cleaner fish in sea cages was estimated to be 200 million NOK in 2016 (Iversen et al., 2017), and are presumably higher today.

Besides cleaning salmon by consuming salmon lice, lumpfish and wrasse species also forage on other feed sources if available. They show a strong opportunistic feeding behaviour and do not necessarily discriminate between feed sources: they actively forage on crustaceans, mussels, zooplankton, algae, salmon feed, and hydrozoans if available (Deady et al., 1995; Kvenseth, 1996; Imsland et al., 2014, 2015; Eliasen et al., 2018). It is, however, unclear which feed source is preferred and if the preference is related to the ease of acquisition. It is widely believed amongst fish farmers that cleaner fish actively forage on net biofouling if present, compromising their appetite and interest for sea lice, especially with regards to wrasse species. Ballan wrasse

Figure 1.4: A ballan wrasse swimming along a cage net on a salmon farm in mid-Norway.

is known to reduce mussel spat abundance on cage nets considerably, and Kvenseth (1996) reported that the use of ballan wrasse as biofouling control reduced net cleaning event frequency with 50%. Deady et al. (1995) found goldsinny wrasse to spend most of their time swimming and foraging at the net prior to net change and significantly less time swimming along cage net after net change, presumably due to the lack of fouling organisms. Imsland et al. (2014) recorded feeding behaviour over time in lumpfish and found that they spent 4.7% of total foraging time (60%) feeding on net fouling, and only 0.4% cleaning or inspecting salmon. However, lice infestation was still significantly lower in cages with lumpfish than cages without. Despite these studies indicating that cleaner fish forage on net fouling, several controversies exist. Using passive-acoustic telemetry, Leclercq et al. (2018) tracked 9 individual lumpfish and 9 wild-caught ballan wrasse for two months in a commercially operated Scottish salmon cage. They reported no significant variations in behavioural parameters such as depth distribution, activity, and habitat use of both lumpfish and ballan wrasse before or after the removal of net biofouling. Even more controversial, through gastric lavage, Eliasen et al. (2018) analysed over 5500 lumpfish stomachs over a two-year period, and found that lumpfish who had foraged biofouling organisms were also more likely to have consumed sea lice. This indicates that net fouling does not reduce the cleaning efficacy of lumpfish, but instead has a positive influence on sea lice grazing.

In Norway, the main driver of the intense net cleaning regime is indeed to remove net fouling as a potential feed resource and distraction for cleaner fish (Figure 1.2) (Iversen et al., 2015, 2017). The above studies show that conclusive knowledge of the effect of net fouling on cleaner fish delousing activity is uncertain, and most fish farmers' perceptions are based on conflicting anecdotal evidence. Considering the adverse impacts of net cleaning on salmon health & welfare, and the significant amount of resources going into the process, it is crucial to identify if these efforts are justified to indeed secure cleaner fish performance.

1.5 Aims and hypotheses of this study

As mentioned, cleaner fish efficacy is the main driver for net cleaning in Norway. During peak biofouling season cleaning schedules are driven by pre-bookings rather than through visual assessment of biofouling conditions on the net. Therefore, we currently do not even know how much biofouling is present on nets when they are washed/exchanged, and if the amount is enough to have a potential impact on cleaner fish behaviour. So, there is a clear need to identify the actual impacts of biofouling on cleaner fish efficacy, if there are any. Also, to investigate options for improving biofouling management practices, we need to know more about the biofouling in general, currently applied management methods, and their effects on cleaner fish. There are no known studies that have correlated cleaner fish abundance situated along net walls with biofouling abundance, and it is therefore highly relevant to investigate how the presence of different grades of biofouling abundance impacts cleaner fish behaviour, which potentially could lead to determining a threshold of maximum biofouling allowed.

The aim of this thesis was to investigate biofouling and current management practices in Norwegian salmon aquaculture, and the potential effects of biofouling on cleaner fish behaviour.

The overall aim was divided into sub-aims, specified as follows:

1. Characterise biofouling conditions and 'maximum biofouling' before net cleaning events with regards to total abundance and spatial variation
2. Assess how the spatial parameters depth and cardinal direction, and the presence/absence of lice skirts may impact biofouling abundance and community composition
3. Investigate the impact of biofouling abundance on cleaner fish behaviour in terms of location in cage

4. Investigate current biofouling management practices at Norwegian fish farms through surveying

Based on the above aims, the following hypotheses were tested:

1. There is a significant variation in 'maximum biofouling' and community composition between sampling events and between cages within sampling events
2. Spatial factors and the presence/absence of lice skirts will have an effect on biofouling abundance and community composition
3. There will be a higher amount of cleaner fish observed close to net walls before net cleaning than after net cleaning
4. Increasing biofouling abundance will have a positive effect on cleaner fish numbers situated in close proximity to net walls

Chapter 2

Materials and methods

2.1 Study sites

The sampling events were carried out at four salmon farms situated off the coast of mainland Trøndelag, Central Norway (Figure 2.1, see coordinates in Table 2.1). All farms are operated on a commercial scale by Salmar ASA. Buholmen, Tristeinen, and Rataren locations are SINTEF ACE full-scale aquaculture test sites, allowing researchers to conduct practical experiments on-site (SINTEF, 2017). Gjæsingen is co-operated with Benchmark Genetics Norway AS, having both on-growing and broodstock licenses. Further details about each sampling event and environmental/farm conditions can be seen in Table 2.1. All locations can be defined as off-coast farming (Holmer, 2010). Rataren is situated 5 km from the mainland and is the farm closest to Norway's maritime boundary, but has many neighbouring farms within a 4 km radius. Tristeinen is the most isolated location, located 4 km from the mainland, but the closest nearby farm is 7.5 km away. Buholmen and Gjæsingen are situated relatively close to each other, and both are semi-exposed, situated 6.5 km and 3.7 km from the mainland, respectively. The production cycle at Tristeinen was close to the end, and slaughter was to commence in January 2020. Rataren, Buholmen, and Jæsingen production cycles started in 2019, in weeks 15, 28, and 30, respectively; thus, cages at those locations held younger salmon (see 'Biomass' in Table 2.1).

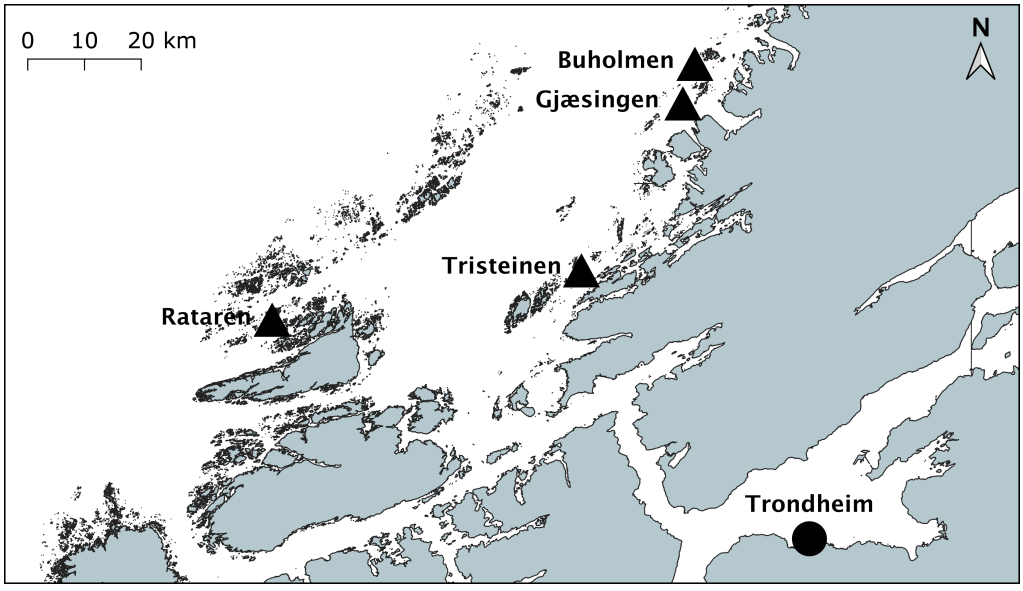
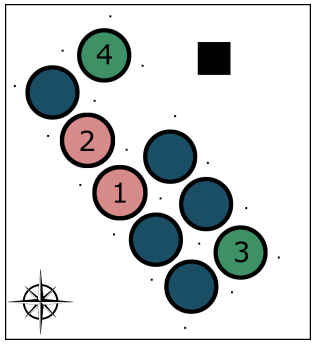
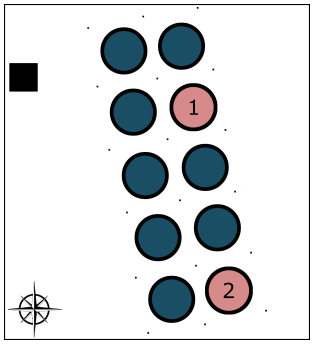
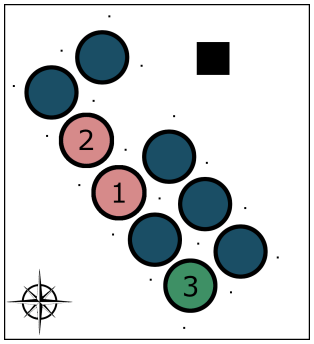
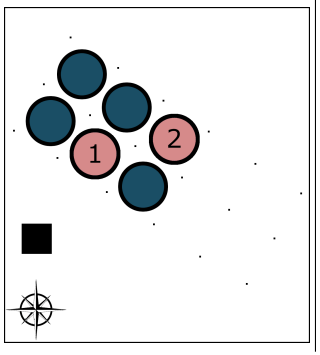
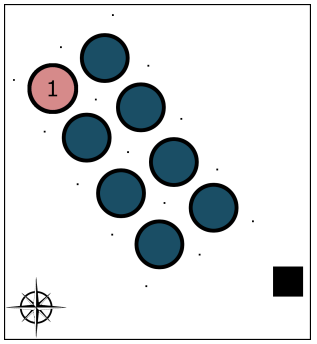


Figure 2.1: Map of geographical locations of sampling sites used in this study.

Table 2.1: Detailed overview of study locations and cage arrangements during each sampling event. Empty slots did not hold any cages. Circle = cage in operation. Pink circle = Cage used for biofouling sampling and cleaner fish sampling before net cleaning. Green circle = Additional cage used for cleaner fish sampling after net cleaning if different from 'before' cage. Black square = Feed barge.

Sampling event	**Buholmen 1**	**Tristeinen**	**Buholmen 2**	**Gjæsingen**	**Rataren**
					
Coordinates	64°09'23.2"N 10°00'34.6"E	63°52'02.4"N 9°36'59.9"E	64°09'23.2"N 10°00'34.6"E	64°07'04.2"N 9°58'50.2"E	63°46'45.1"N 8°31'11.4"E
Sampling date	24.09.19	26.09.19	07.10.19	15.10.19	12.11.19
Air temperature	8°C	15°C	8°C	7°C	3°C
Water temperature	12.7°C	12.6°C	11.3°C	10.7°C	11°C
Main current dir.	North-East	South	North-East	Unknown	North-East
Cage circumference	157 m	157 m	157 m	157 m	157 m
Lice skirt	Below 5 m	Below 6 m	Below 5 m	Below 5 m	Below 5 m
Salmon biomass at site	744 tonnes	4679 tonnes	744 tonnes	226 tonnes	5100 tonnes
Days since last net cleaning event	14 days	7 days	7 days	8 days	10 days
Cleaner fish type	Wrasse	Lumpfish & wrasse	Wrasse	Lumpfish	Lumpfish & Wrasse
# depl. CF cage 1	10800 wrasse	8700 wrasse 9000 lumpfish	10800 wrasse	6500 lumpfish	5500 lumpfish 1800 wrasse
# depl. CF cage 2	10800 wrasse	5600 wrasse 14000 lumpfish	10800 wrasse	10000 lumpfish	– – –

2.2 Biofouling sampling and analysis

2.2.1 Data collection

To determine biofouling abundance, community composition, and spatial variation at the time of net cleaning, each sampling event was conducted on the same day as nets were cleaned. Two sampling cages were chosen in accordance with farm and cleaner service schedules. To obtain biofouling abundance data, net walls inside cages were filmed in vertical transects with a GoPro Hero 5 Black action camera mounted on a custom-made 1 m tall steel frame. The camera had a fixed distance of 40 cm from the net, and with a medium field-of-view setting, giving approximately 70 cm views from left to right. The frame was loaded with 2.5 kg weights on either side and deployed inside the cage along the net wall, held up with a rope. The rope was marked with 1-meter increments.

The camera was filming continuously during immersion. The frame was lowered slowly, and held still for 20 seconds at three points around 1, 5, and 10 m depths each, giving three replicates per depth. Four replicate depth transects were filmed per cage, where deployment locations were at the southern, eastern, northern, and western cardinal points of the cage (n_{total} per depth/cage = 12 and n_{total} per cage = 36). During the first sampling event, the procedure was repeated after net cleaning to confirm that the net cleaning operation removed the net fouling in its entirety. An illustration of the sampling design can be seen in Figure 2.2. Two cages were sampled during each sampling event, except for Rataren, where only one cage was sampled due to farm logistics (Table 2.1).

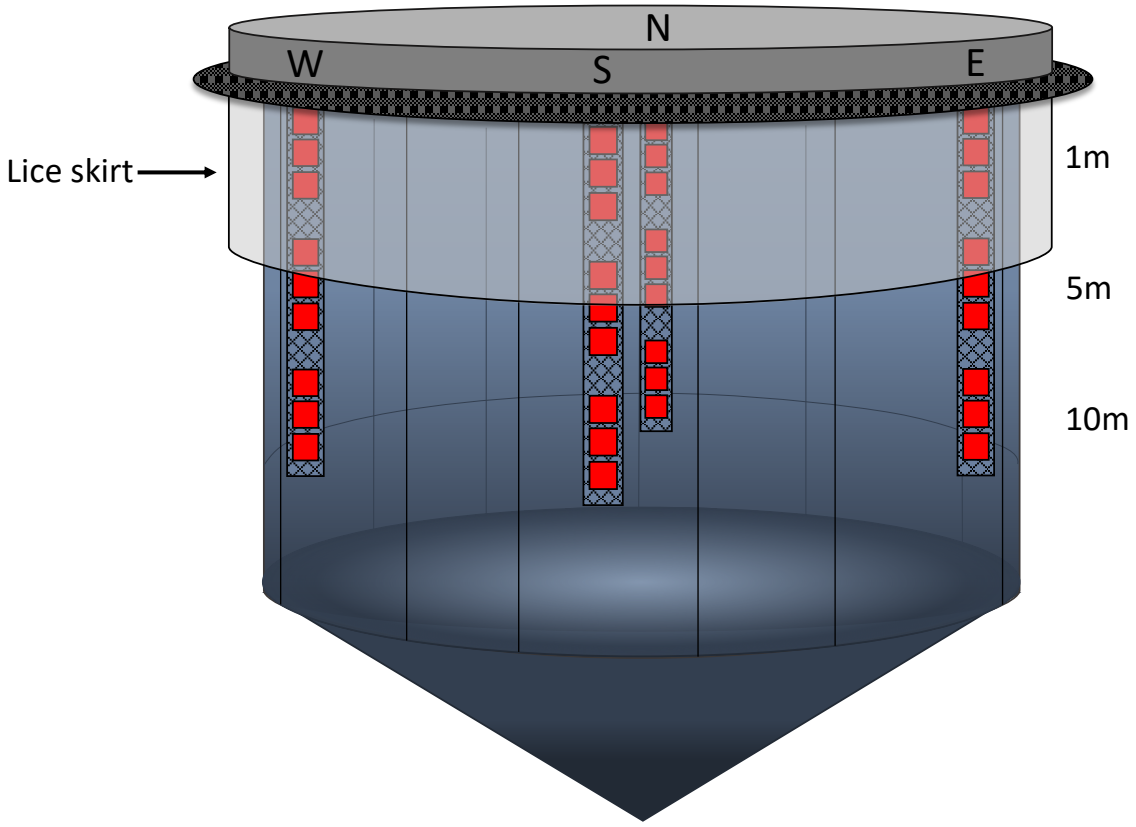


Figure 2.2: Illustration of a net cage showing the biofouling cover sampling method. Four vertical transects (N, E, S, W), three depths (1m, 5m, 10m) and three replicates per depth were registered. The camera frame was held still for 20 seconds at each red square, and each red square was defined as a sample. For each cage, this resulted in 9 samples per transect, 12 samples per depth, and a total of 36 samples per cage.

2.2.2 Biofouling cover analysis

Several methods for determining biofouling abundance are documented, including image analysis and wet-weight analysis. Both ways are applied to assess the efficacy of antifouling treatments (Braithwaite et al., 2006; Guenther et al., 2011; Bloecher, de Nys, Poole and Guenther, 2013; Bloecher and Floerl, 2020), whereas when investigating biofouling development, imaging is most often used (Braithwaite et al., 2006; Guenther et al., 2010). Percentage Net Occlusion can be determined through image processing of binary images and analysis algorithms. This method, although rapid and semi-automatic, is limited to assess biofouling abundance, but does not allow for ascertaining community composition or distinguish between biofouling types. Therefore, a more manual approach was taken.

The screenshot function in VLC Media Player (VideoLan Association, 2019) was used to obtain still images from the videos, where one picture was taken each time the frame was held still. Each picture was regarded as one sample and given a unique sample ID. The images were loaded into GIMP (The GIMP Development Team, 2020) for post-processing and analysis. A standard grid of lines was created as a separate layer, and the grid was used for all images as an overlay. Biofouling cover was estimated through analysis of minimum 60 intersecting points on the net, defined by the intersections of vertical net strands with X horizontal lines and the intersection of horizontal net strands with Y vertical lines placed over the net image (Figure 2.3). To calculate the total biofouling net coverage, the number of intersecting points, and the number of intersecting points with an organism attached was entered into a counting table (Table 2.2). If larger organisms hindered the visibility of the counting point, the point was regarded as 'point not visible'. The total % biofouling cover was calculated for each image using the following formula:

$$\text{Biofouling cover (\%)} = \left(\frac{\text{Algae + Hydroids}}{\text{Points on net - Points not visible}} \right) \text{x } 100$$

Due to questionable image quality, distance from net, and lack of contrast between cage net and the lice skirt directly behind the net, biofouling organisms were not identified to species level, but rather categorised as major biofouling groups such as algae and hydroids. The same calculation as the one above was also used to calculate % coverage of major biofouling groups separately. Each image was also linked to the following criteria describing possible impact factors of the observed biofouling: Cage number (1 and 2), depth (1 m, 5 m, and 10 m), presence or absence of lice skirt (yes/no), and cardinal point in cage (north, east, south, west). These factors were later used to investigate patterns and/or significant differences in the biofouling growth/community.

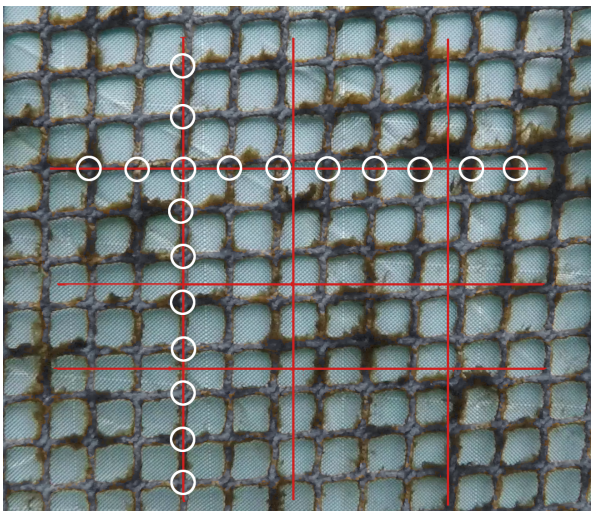


Figure 2.3: Example of biofouling analysis in GIMP. White circles indicate intersecting points for one vertical and one horizontal line where biofouling was analysed.

Table 2.2: Counting table with exemplary values where total % cover of relevant fouling organisms is calculated based on the number of intersecting points.

			Points covered with biofouling		
Sample ID	**Points on net**	**Points not visible**	**Algae**	**Hydroids**	**% cover**
TRI_C7_5-1	72	0	4	28	44%

2.3 The effect of net biofouling on cleaner fish behaviour

2.3.1 Behavioural observations

To assess behavioural changes in cleaner fish with or without the presence of net biofouling, net walls were filmed before and after net cleaning. Filming was conducted using farm-owned Blueye underwater drones (Blueye Robotics, Trondheim, Norway). The Blueye is equipped with a wide-angle camera, shooting in full HD (1080p/130fps). Automatic heading and depth can be used, ensuring stable filming conditions. The drone is remotely controlled from the cage walkway through a hand-held controller and cellphone/tablet application. The Blueye is equipped with both top and side thrusters, allowing for both horizontal and vertical movement, and has a depth gauge, displaying the current depth on the mobile device application screen.

Net walls were filmed in horizontal transects at 1 and 3 meter depths. By using the drones side thrusters, filming could be conducted sideways at a relatively even pace. A camera-net-distance of approximately half a meter was maintained during filming, giving a 1m x 1.5m field of view. The drone was deployed at a pre-determined point in the cage, and this was regarded as the reference point during filming. Due to battery and tether length logistics, cages were filmed in half-circles (Figure 2.4). The reference point was the starting point of the right-hand 1 meter transect. When the first half was filmed, the drone was descended to 3 meters depth and the first 3 meter transect was filmed returning to the reference point. From the reference point, the other half of the cage was filmed at 3 meter depth, and when the half-way point was reached the drone was ascended to 1 meter depth and the second 1 meter transect was filmed returning to the reference point.

At each sampling event, two cages were filmed both before and after net cleaning, with the exception of Rataren where only one cage was sampled. The cages sampled before net cleaning were the same as the cages used for the biofouling abundance analysis above, but due to cleaning schedules and farm logistics, cages filmed after cleaning were sometimes different cages where nets had been cleaned a few hours earlier. An overview of sampled cages can be seen in Table 2.1.

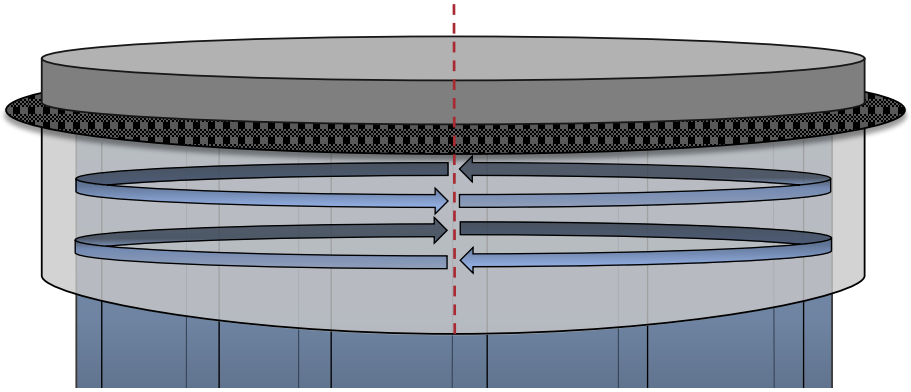


Figure 2.4: Filming was conducted along horizontal transects and at two depths. The red line indicates the deployment point, which was the start point of one depth and endpoint of the other. Cages were filmed in halves.

2.3.2 Video analysis

The Blueye videos were analysed using VLC Media Player (VideoLan Association, 2019). During playback, fish present in screen window (close to net wall) were counted per minute – i.e., the number of fish present in minute number one, minute number two, etc. If any disruptions occurred, e.g., if salmon bumped into the camera, the time was paused and resumed when the camera was back in position. Parameters included in the video analysis were sampling event, cage number, when (before or after net cleaning), depth (1m or 3m), minute number (1, 2, ..., n) and count (i.e., number of fish counted during the defined minute) (Table 2.3). Due to differences in cleaner fish types between sampling events in this study, and because the aim of this part of the study was to see if the presence of net biofouling had a general effect on cleaner fish behaviour, cleaner fish observed were not separated into different species but were pooled and regarded as one collective cleaner fish. The effect of currents was not included in the analysis due to all cages having lice skirts protecting the upper 5-6 meters. Lice skirts will significantly reduce horizontal flow inside a cage (Frank et al., 2014), and currents were therefore not taken into consideration.

Due to challenging conditions during filming, such as poor connectivity between drone and screen, and issues with invalid files at transfer to computer, filming length varied considerably between sites. As a result of this, 1 meter and 3 meter observations were pooled, disregarding the depth factor, but still analysed as separate time points. Also, it was not expected to see a large variation in fish counts between the two depths, as earlier studies mostly detected differences between deeper depths (Leclercq et al., 2018). An overview over sampling data is shown in (Table 2.4).

Table 2.3: Table used for registration of cleaner fish during video analysis with exemplary values. 'Cage' refers to cage number as seen in Table 2.1. 'When' indicates if sample is from before or after net cleaning. 'Minute' refers to which minute counting took place, and 'count' is the number of cleaner fish observed during the previously defined minute.

Sampling event	Cage	When	Depth	Minute	Count
Buholmen 1	Cage 1	Before	1m	1	20
Buholmen 1	Cage 1	Before	1m	2	13
.	.	.	.	.	.
.	.	.	.	.	.
.	.	.	.	.	.
Buholmen 1	Cage 1	After	3m	20	5
Buholmen 1	Cage 1	After	3m	21	12

Table 2.4: Overview over sampling data from cleaner fish videos. Cage numbers 1 and 2 are complementary to cages used in BF sampling. Cage 3 and 4 are marked in green in farm overviews in Table 2.1.

	Buholmen 1	Tristeinen	Buholmen 2	Gjæsingen	Rataren
Before net cleaning	**Cage 1** depth | min 1 m | 2 3 m | X	**Cage 1** depth | min 1 m | 2 3 m | 6	**Cage 1** depth | min 1 m | 8 3 m | 8	**X**	**Cage 1** depth | min 1 m | 25 3 m | 19
	Cage 2 depth | min 1 m | 3 3 m | 5	**Cage 2** depth | min 1 m | 2 3 m | 5	**Cage 2** depth | min 1 m | 10 3 m | 11	**X**	**X**
After net cleaning	**Cage 3** depth | min 1 m | 3 3 m | 4	**X**	**Cage 3** depth | min 1 m | 6 3 m | 4	**Cage 1** depth | min 1 m | 9 3 m | 10	**Cage 1** depth | min 1 m | 12 3 m | 8
	Cage 4 depth | min 1 m | 6 3 m | 7	**X**	**X**	**X**	**X**

2.3.3 Assessing the effect of net fouling on cleaner fish location in the cage

To assess the effect net fouling had on cleaner fish behaviour, observations before and after net cleaning were compared. The average number of cleaner fish observed along net walls per minute was compared before net cleaning (i.e., with fouled nets) and after net cleaning (i.e., no fouling present). For the statistical comparison of 'fouled' vs. 'clean' nets, only cages with substantial biofouling (> 30%) were included in the analysis. As filming took place in 1 and 3 m depth, biofouling cover from 1 and 5 m was used as closest reference.

Finally, to investigate if increasing biofouling abundance had a positive effect on cleaner fish abundance, average cleaner fish numbers per sampled cage were correlated with mean biofouling abundance of 1m and 5m depths determined in subsection 2.2.2. Here, only samples from before net cleaning were used, as we want to detect the potential effect of increasing biofouling abundance.

2.4 Survey of biofouling management practices

To investigate current biofouling management practices at Norwegian salmon farms, a number of salmon farmers across the country were asked to participate in a survey. The survey was created in Google Forms and included 9 questions about biofouling management and cleaner fish (Table 2.5). The survey contained questions with binary answers, lists of options, and for the most part, free-form answer questions. Therefore, to be able to compare answers, some answers were generalised. For example, the question involving net coating types used received answers with coating names, and were therefore grouped into main coating types. If a question was not applicable to the farmer, or if no answer was recorded, it was classified as N/A. An option to add additional comments about the topics was also included.

An invite to participate in the survey with a link to the questionnaire was distributed via e-mail through the NTNU R&D project 'Taskforce Sea Lice', and was sent to farm managers at salmon farms involved in the project. It was also distributed to salmon farms in Lerøy Midt, in addition to the farms used for data sampling in this master's project. All responding farm managers were asked to supply an e-mail address to ensure the possibility to ask follow-up questions. All data collected for this survey was handled confidentially.

Table 2.5: Questions included in survey used to investigate biofouling management practices in Norwegian salmon aquaculture.

	Questions
General	**1)** Are cage nets cleaned or exchanged at your farm? **2)** How often are nets cleaned during peak BF season? **3)** How often are nets cleaned in winter? **4)** Which type of net coating is used at your farm?
Assessing BF ab.	**5)** Do you assess BF abundance before booking cleaning services? **6)** When assessing BF abundance, do you use a guideline? **7)** How do you assess BF abundance?
Other	**8)** What are the main drivers for net cleaning at your farm? **9)** Do you see an increase in cleaner fish mortality after net cleaning? ***10)*** *What are the estimated costs for net cleaning per production cycle at your farm? (Follow-up question)*

2.5 Statistical analyses

A permutational analysis of variance (PERMANOVA) was used to test for factors explaining variations in biofouling abundance. This analysis was performed based on Euclidean distance with 9999 unrestricted permutations of residuals under a reduced model and a significance level of 5% using the PERMANOVA+ routine in PRIMERv7.0 (Plymouth Routines in Multivariate Ecological Research, UK). The analysis used the factors Site (5; random), Cage (2, except Rataren where only 1 cage was sampled; random, nested in Site), Direction (4; fixed), and Depth (3; fixed). Pseudo *F* statistics were calculated for each term using direct analogues to univariate expectations of mean squares (EMS). In a PERMANOVA, the highest significant interaction is to be regarded, as the focus of the interpretation lies on the highest order of significant terms (Anderson et al., 2008).

While the structure of the data (variability between cages and sites are random factors) limits the use of post-hoc tests, some of the data was further explored for potential trends. All further statistical analyses were carried out using R-studio (Version 1.2.1335), and all analyses were performed at the 95% confidence level ($p < 0.05$) throughout. P-values below 0.001 were reported as such.

Normal distribution of data was tested using Shapiro-Wilk tests of normality, and equality of variance was analysed by Levene's test for Homogeneity of Variance.

Differences between two groups of normal and non-normal distributed data were tested using unpaired t-tests and Mann-Whitney U tests (Mann and Whitney, 1947), respectively. Kruskall-Wallis one-way ANOVA on ranks was used to compare distributions of more than two groups of non-normally distributed numerical data. It was thereafter followed by pairwise Wilcoxon and Dunn's post-hoc tests, where the latter was used in cases of unequal sample sizes. For correlations, Spearman's Rank Correlation was executed if variables were non-normally distributed.

The potential effect of lice skirt on biofouling abundance and community was analysed by pooling all image data from the last meter still protected by the lice skirt and the first meter below the skirt edge from all sites ($n_{total} = 36$ per condition) and comparing the two groups.

When comparing cleaner fish numbers on cage nets before and after net cleaning, all observations across all sampled cages were pooled into the two groups. No direct comparison from the same cage was possible due to the above described constraints in the sampling regime.

Detailed information about the statistical tests performed are displayed in Table 2.6.

All results are presented as mean ± 95% confidence interval (CI). All graphs were made in R.

Table 2.6: Overview over statistical tests used to detect significant differences or effects in data with certain criteria. Notations in italics indicate tests used to investigate trends regardless of PERMANOVA results. CF = Cleaner fish.

Analysis	Factor(s)	Levels	Measure(s)	Global test	Post-hoc test
Variability in BF ab. between all factors	Site Cage(Site) Depth Cardinal direction	5 - Random 9 - Random 3 (1m, 5m, 10m) - Fixed 4 (N, E, S, W) - Fixed	Abundance Algae ab. Hydroid ab.	PERMANOVA	-
Differences in BF ab. between sites	*Sites*	*5 - Random*	*Abundance*	*PERMANOVA*	*Dunn test*
Effect of lice skirt on BF abundance	Lice skirt	2 (Above/below) - Fixed	Abundance	Mann-Whitney U test	-
Effect of lice skirt on BF composition	Lice skirt BF type	2 (Yes/no) - Fixed 2 (BF type) - Fixed	Abundance per BF type	Kruskal-Wallis test by rank	Pairwise-Wilcoxon test
Difference in observed cleaner fish before/ after net cleaning	BF conditions When (before/after)	2 (Before/after) - Fixed	CF count/minute	Mann-Whitney U test	-
Relationship between cleaner fish and BF abundance	CF count/min BF abundance	-	Spearman’s rho	Spearman’s Rank Correlation rho	-

2.6 Sampling and data analysis cooperation

During this study, all sampling events were conducted in cooperation with co-student, Tormod Stenersen. Tormod was in charge of controlling the BluEye underwater drone during cleaner fish data sampling (section 2.3.1), in addition to analysing the videos post-sampling (section 2.3.2). Additionally, the survey in section 2.4 also included questions regarding cleaner fish management. Those questions were used by Tormod in his thesis, but disregarded in this study.

Chapter 3

Results

3.1 Biofouling conditions on net walls at time of net cleaning

During the five sampling events (hereafter referred to as 'sites'), 'maximum' biofouling abundance (i.e., abundance on net walls the day of net cleaning) varied between 10.4±2.9% (Mean±CI) and 58±5.9%. Algae and hydroids were to two main species groups identified on 324 image samples. In average, algae abundance was 12.54±2.2% and hydroid abundance was 18.1±2.88% across all sites. Of all biofouling registered, algae made up 41% and hydroids 59%, respectively.

Biofouling showed strong spatial variation, with Site, Cage, Cardinal Direction, and Depth contributing to the largest components of variation to the overall model (Table 3.1). Biofouling abundance differed significantly between depth and direction, but not in consistent patterns between cages, hence the full four-way interaction term 'Cage(Site) x Direction x Depth' ($F_{15;216} = 1.795$, $p < 0.05$). When running the same analysis only based on algal biofouling, the same full interaction between all factors was observed, where cage, site, cardinal direction, and depth had an effect on algae abundance, but did not follow the same pattern (Cage(Site) x Direction x Depth; $F_{15;216} = 3.36$, $p < 0.001$; Table 3.1). In contrast, when looking only at the distribution of hydroid biofouling, no effect of cardinal direction could be found (Cage(Site) x Depth; $F_{11;216} = 3.38$, $p < 0.001$; Table 3.1). Therefore, the distribution of hydroids was not influenced by the cardinal direction on the cage, while algae fouling did differ between directions.

Table 3.1: Results (permutational *P*) of PERMANOVA for main effects for the influence of sites, cardinal direction ('Direction'), depth and variability between cages nested in site ('Cage(Site)'), including respective interactions, on average BF abundance, algae abundance, and hydroid abundance. Bold entries indicate relevant significant results for the individual variables. For details see Table A.1 and Table A.2 in appendix.

Factor	*df*	Average BF	Algae	Hydroids
Site	4	0.011	0.367	0.078
Direction	3	0.484	0.288	0.566
Depth	2	0.973	0.001	0.030
Cage(Site)	4	0.001	<0.001	<0.001
Site x Direction	12	0.145	0.434	0.014
Site x Depth	8	0.347	0.156	0.006
Direction x Depth	6	0.097	0.06	0.656
Cage(Site) x Direction	12	0.022	<0.001	0.91
Cage(Site) x Depth	8	<0.001	<0.001	**<0.001**
Site x Direction x Depth	24	0.75	0.899	0.091
Cage(Site) x Direction x Depth	24	**0.018**	**<0.001**	0.935
Residuals	216			

3.1.1 Biofouling cover on net wall at time of net cleaning

Average biofouling abundance (Figure 3.1) is reflecting the difference in biofouling at the time of net cleaning between the five sites. When taking a closer look at the differences between sites indicated by the PERMANOVA analysis, Buholmen1, which had been last cleaned 14 days ago due to bad weather, had the highest abundance of all sites with 58.2±5.9%. The remaining sites all had significantly lower abundance. All groups differed from each other, with the exception of Tristeinen and Buholmen2 (Dunn test, $p > 0.05$), and Gjæsingen and Rataren (Dunn test, $p > 0.05$). Number of days since last cleaning event ranged from 14 days earlier at Buholmen1 to 7 days earlier at Tristeinen and Buholmen2.

There were large differences in species composition between sites, and on average, algae abundance ranged from 2.8±2.3% (Rataren) to 19.2±6.2% (Tristeinen), whereas the highest hydroid abundance was observed at Buholmen1 (41.8±9.0%) and the least was observed at Gjæsingen (3.7±1.2%) (Figure 3.1). The dominating species group was not the same at all sites. Hydroids dominated at Buholmen1, Buholmen2, and Rataren, whereas algae was the dominating species group at Tristeinen and Gjæsingen.

Taking a closer look at the cages sampled (Figure 3.2), the differences in biofouling abundance between cages indicated by the main analysis were significant at some or all sites. The two sites with the largest differences between cages and largest variations within cages were Buholmen1 and Buholmen2. At Buholmen1, mean biofouling abundance in cage 1 was 61.5±8.7% and 54.6±8.3% in cage 2, respectively. At Buholmen2, biofouling abundance varied between 26±8% (Cage 1) and 34.6±7.6% (Cage 2).

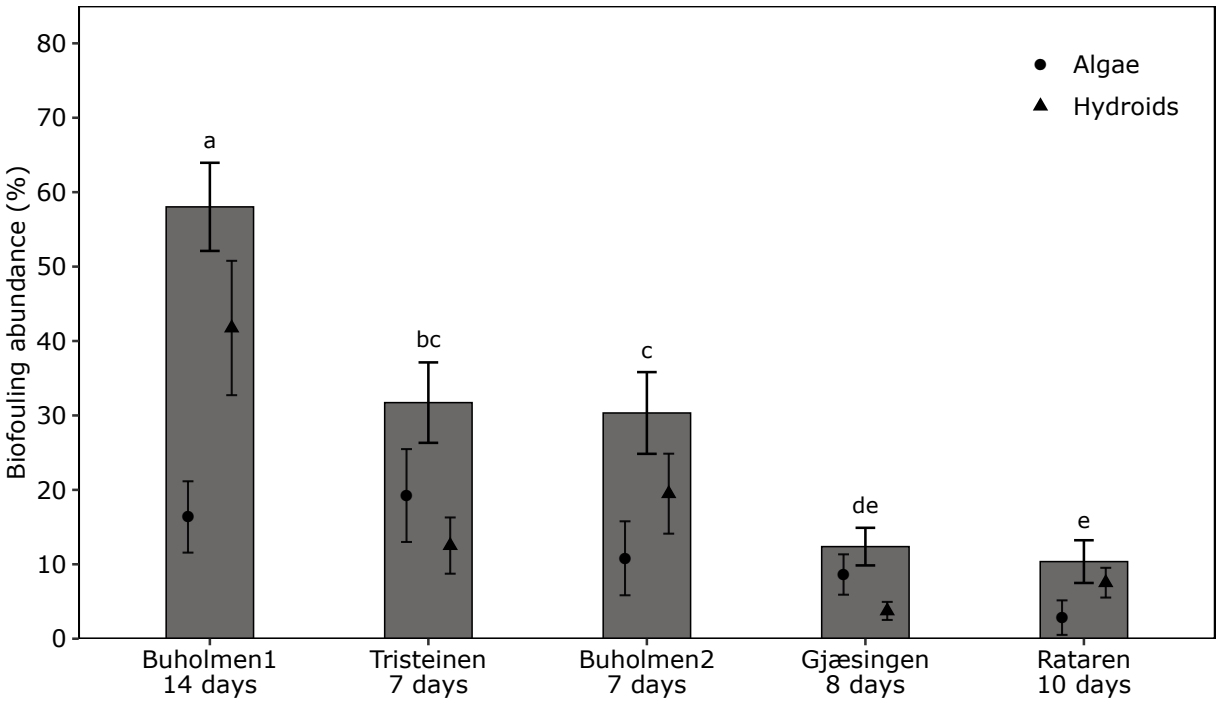


Figure 3.1: Average biofouling abundance (±CI), including mean algae and hydroid abundance (points), at the day of net cleaning. Two cages were sampled at each site ($n_{total} = 72$), with the exception of Rataren ($N_{total} = 36$). Notations of 'X days' below site name indicates number of days since last cleaning event. Same superscripts indicate non-significant differences between sites ($p > 0.05$).

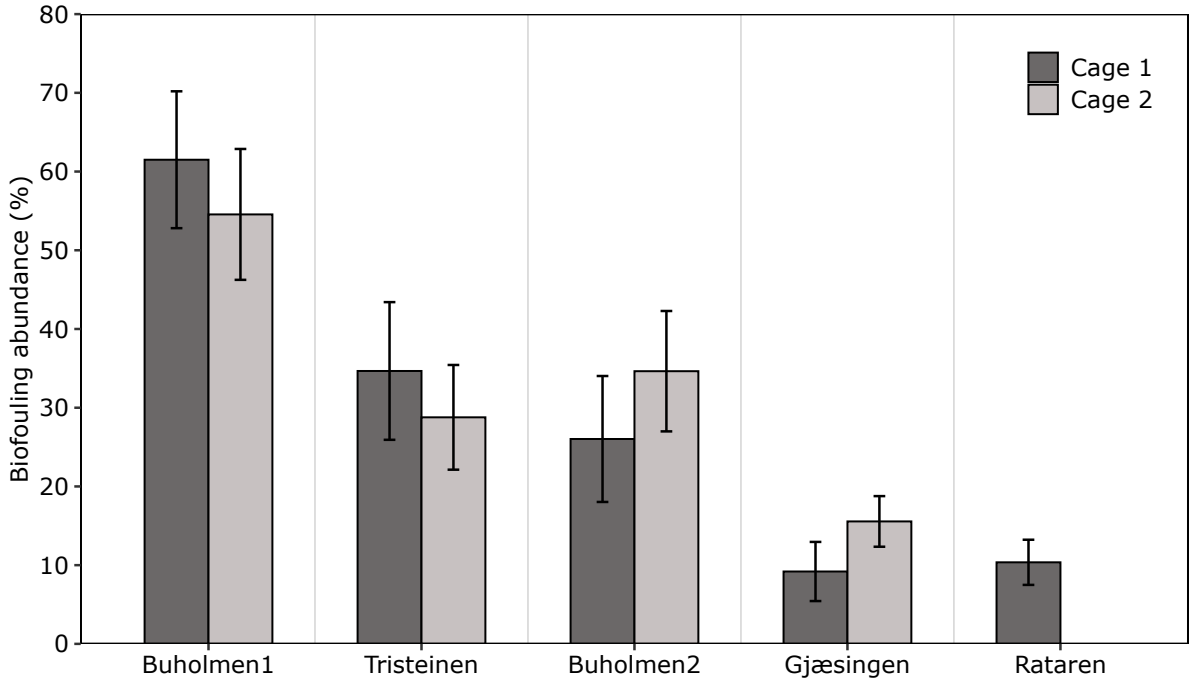


Figure 3.2: Average biofouling abundance (±CI) of all sampled cages at the day of net cleaning (n_{cage}=36).

3.1.2 Spatial variation – Depth

Biofouling abundance

Biofouling abundance varied between depths, and as indicated by the significant interaction term in the original analysis, the effect of depth was not consistent at all sites (Table 3.1). The results are reflected in Figure 3.3, which shows that biofouling growth at most sites responded differently to decreasing depth. Much of the variation within depths was, according to the analysis, also found within sampled cages, which can be investigated in detail in Figure A.1.

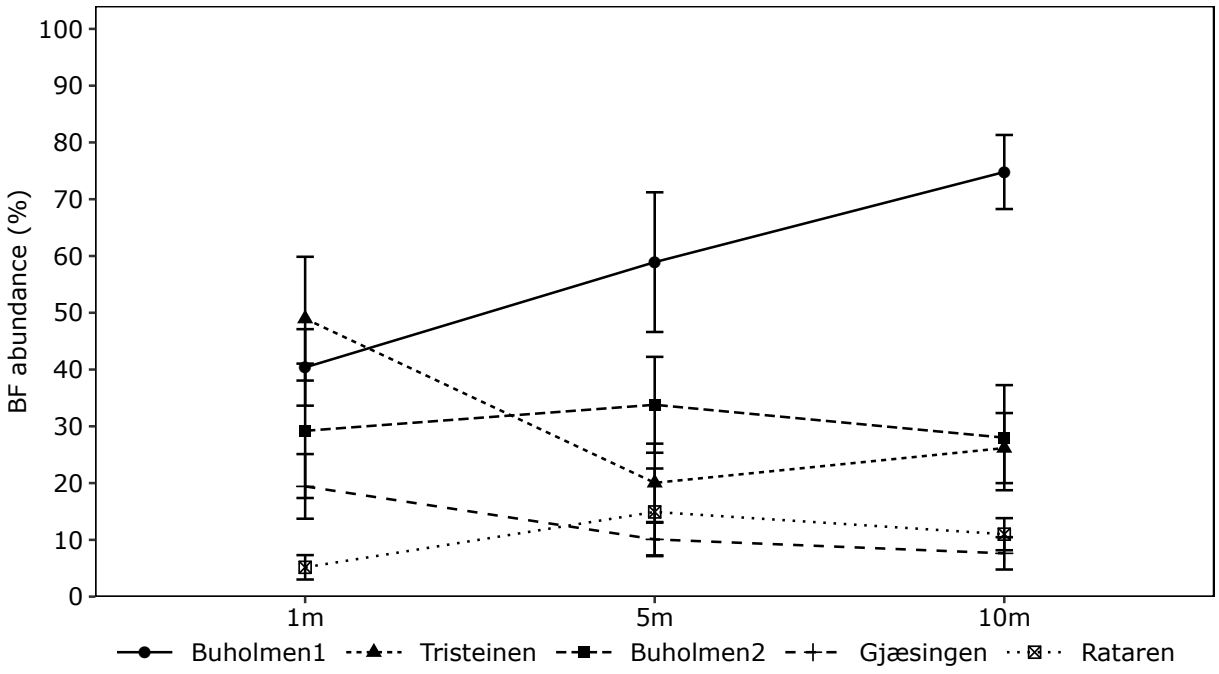


Figure 3.3: Average biofouling abundance (±CI) at three sampling depths for five sampling sites. N_{depth} = 24 (Rataren: N_{depth} = 12).

Biofouling community

Both algae and hydroid abundance varied between depths. Figure 3.4 shows the variation in biofouling abundance of algae and hydroids at the three sampled depths for each site. Algae clearly dominated at 1m depth, and showed an obvious decreasing trend with depth. Conversely, hydroids dominated at 5m and 10m depth, and few hydroids were observed in 1m samples. Looking closer at the variation between each site, there were large variations in hydroid abundance with depth. At Buholmen2 and Rataren, hydroid abundance levelled out below 5 meters, whereas at the remaining sites, it increased to nearly twice the amount between 5m and 10m. The more dramatic decrease in algae abundance with depth therefore implies that algae are more limited by depth than hydroids.

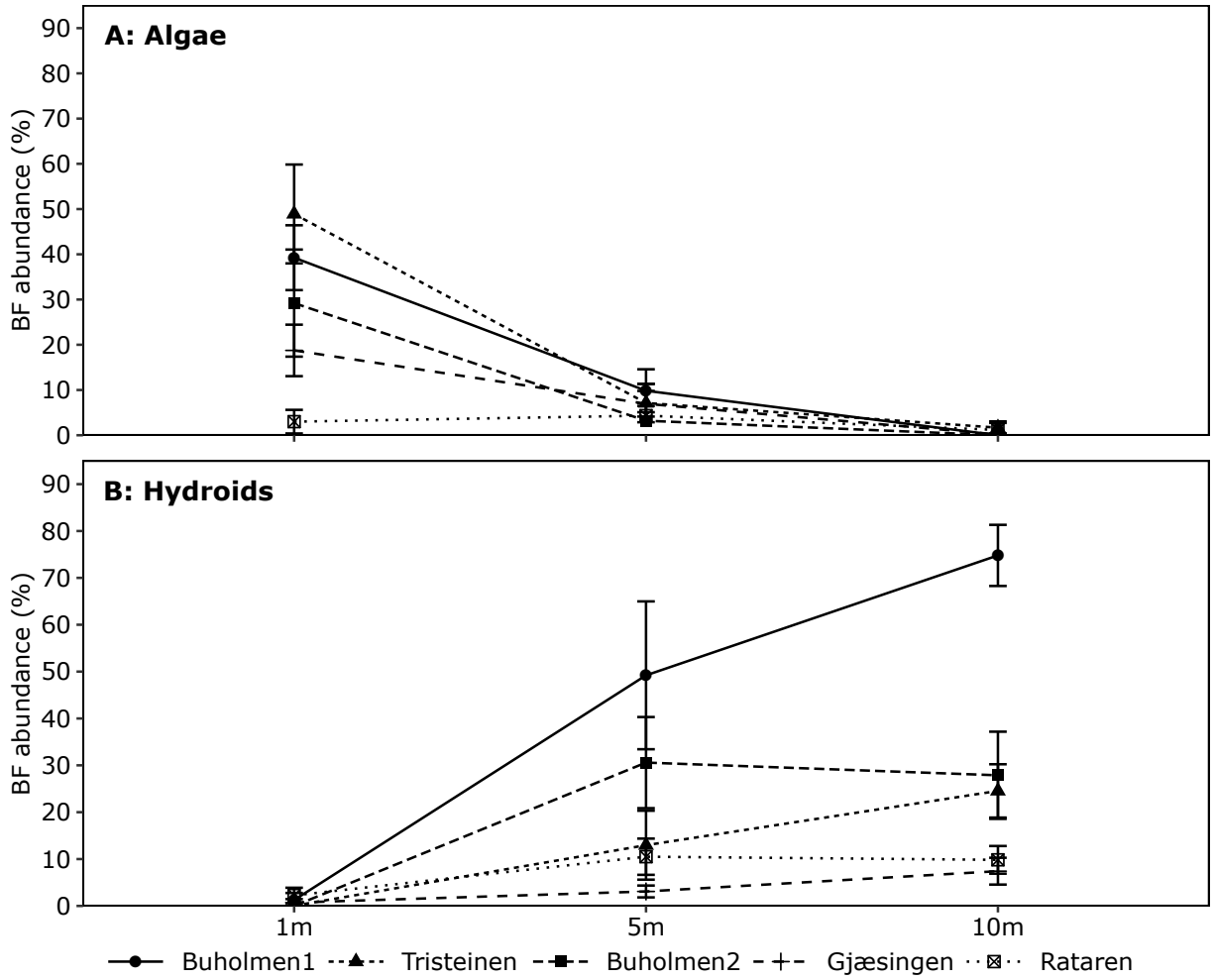


Figure 3.4: Average biofouling abundance (±CI) of algae (A) and hydroids (B) at 3 sampling depths for five sampling sites. N = 24 per depth (N = 12 per depth at Rataren).

3.1.3 Spatial variation – Cardinal direction

Biofouling abundance

Biofouling abundance varied between cardinal points, but not in a consistent pattern at all sites, as it interacted with the other spatial factors (PERMANOVA; Table 3.1). There was a lot of variation throughout the system (Figure 3.5), explaining the lack of a general trend on biofouling abundance related to cardinal direction. Similar trends in biofouling abundance were, however, seen at Buholmen1 and Buholmen2, which may imply that the behaviour of biofouling growth may be similar within farms. Also, according to the analysis, much of the variation seen was explained by variation between cages, which can be investigated in detail in Figure A.1.

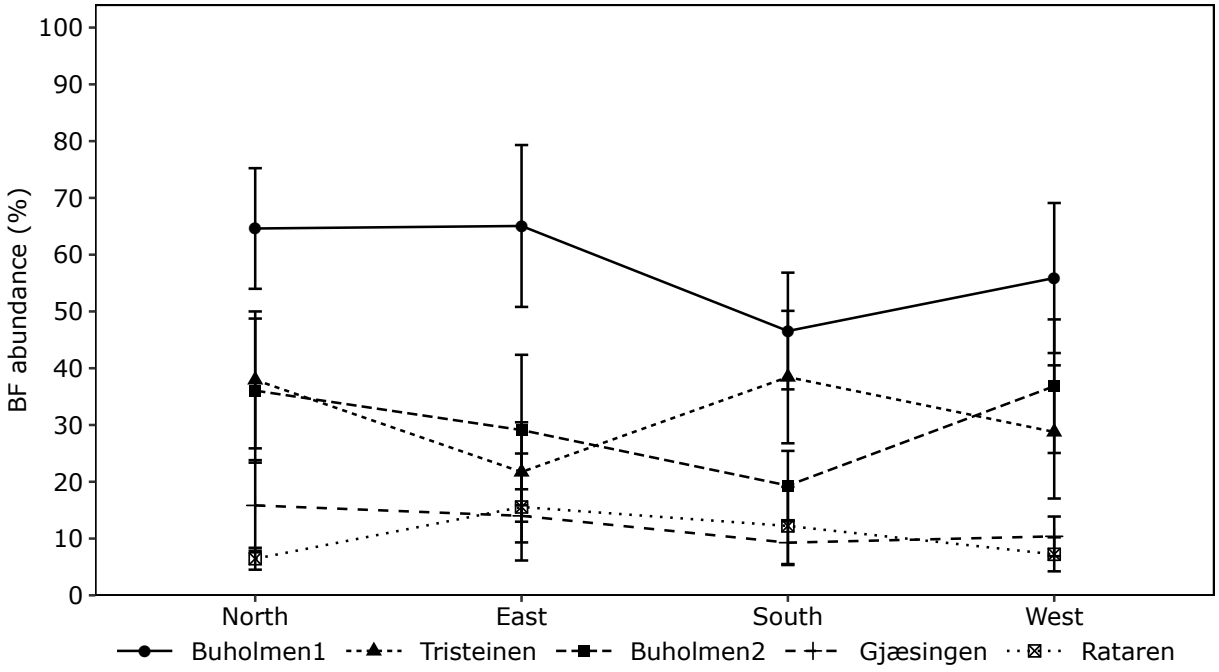


Figure 3.5: Average biofouling abundance (±CI) at the four sampled cardinal points for five sampling sites. $N_{cardinal\ point} = 18$ per site (Rataren: $N_{cardinal\ point} = 9$).

Biofouling community

As mentioned earlier, algae abundance varied between all factors, including cardinal direction, but again, not in a consistent pattern (Table 3.1). Hydroids were not impacted by cardinal direction within the cage.

The trends seen in Figure 3.6 highlights the results of the analyses. Algae abundance varied between cardinal points at most sites. Although hydroid growth at Buholmen1 and Buholmen2 showed some differences between cardinal points, there were large within-direction variations at both sites.

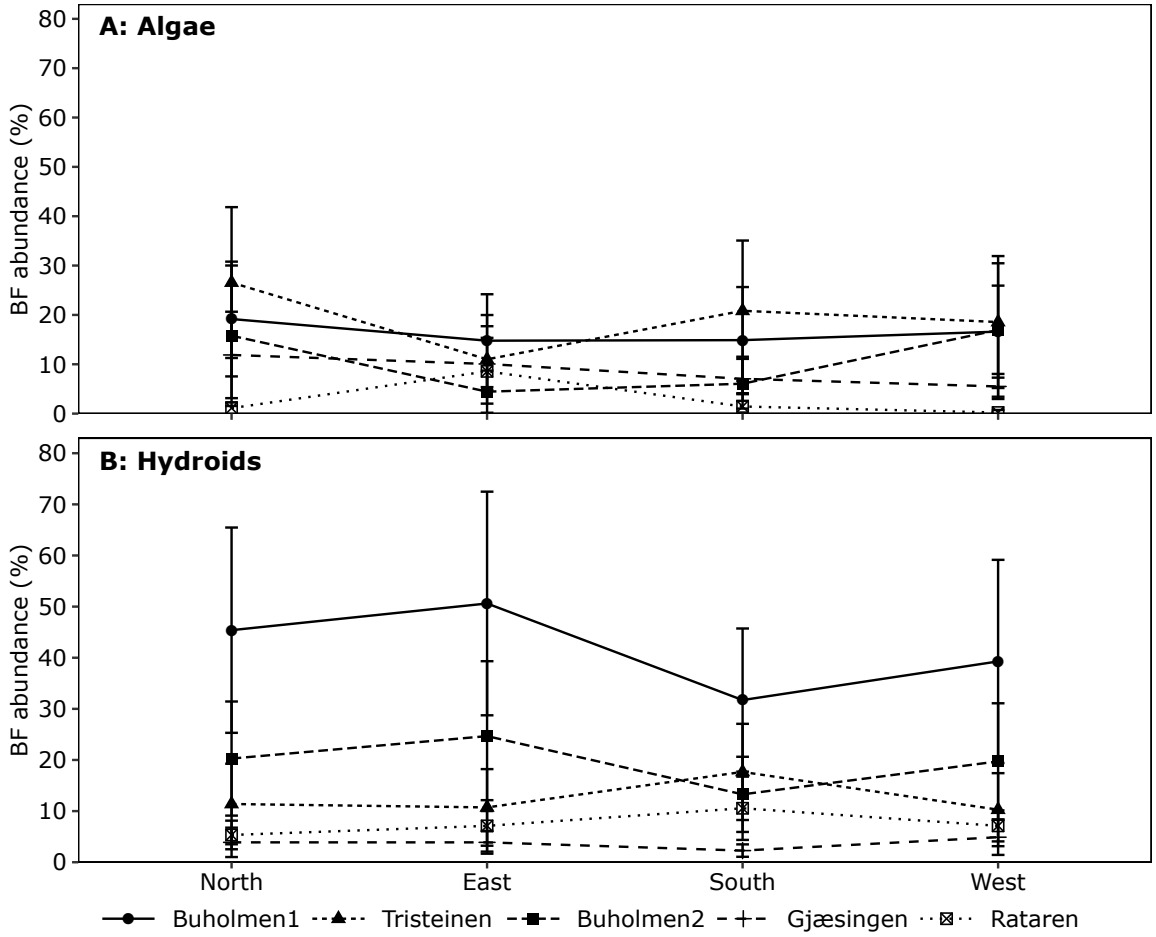


Figure 3.6: Average biofouling abundance (±CI) of algae (A) and hydroids (B) at 4 cardinal directions within the cages for five sampling sites. N = 18 per cardinal point (N = 9 per cardinal point at Rataren).

3.1.4 The effect of lice skirts on biofouling abundance

Looking at the biofouling abundance around the lice skirt edges, there was significantly less biofouling on the last meter still protected by the lice skirt ('above') than on the first meter below the skirt edge ('below') (Mann-Whitney U test; $W = 371.5$, $p < 0.001$). The variation between the two groups can be seen in Figure 3.7, where biofouling abundance above lice skirt edge was 14.9±4.3%, and 31.7±9.2% below lice skirt edge, respectively.

At a closer look at the specific species composition there was significantly less algae growth in samples from the first meter below the skirt edge than the last meter still protected by the skirt (Mann-Whitney U test; $W = 989.5$, $p < 0.001$). 'Above' skirt edge samples had an algae abundance of 11.6±3.5% while 4.5±1.9% were present on the first meter below the lice skirt edge, respectively. Similarly, lice skirt protection also had a significant effect on the presence of hydroids (Mann-Whitney U test; $W = 168$, $p < 0.001$), where hydroid abundance on the last meter still protected by a lice skirt was 3.0±2% compared to 27.9±9.4% on the first meter below the skirt edge, implying that the abundance of the two groups respond inversely, and are highly impacted by the presence of lice skirts (Figure 3.8).

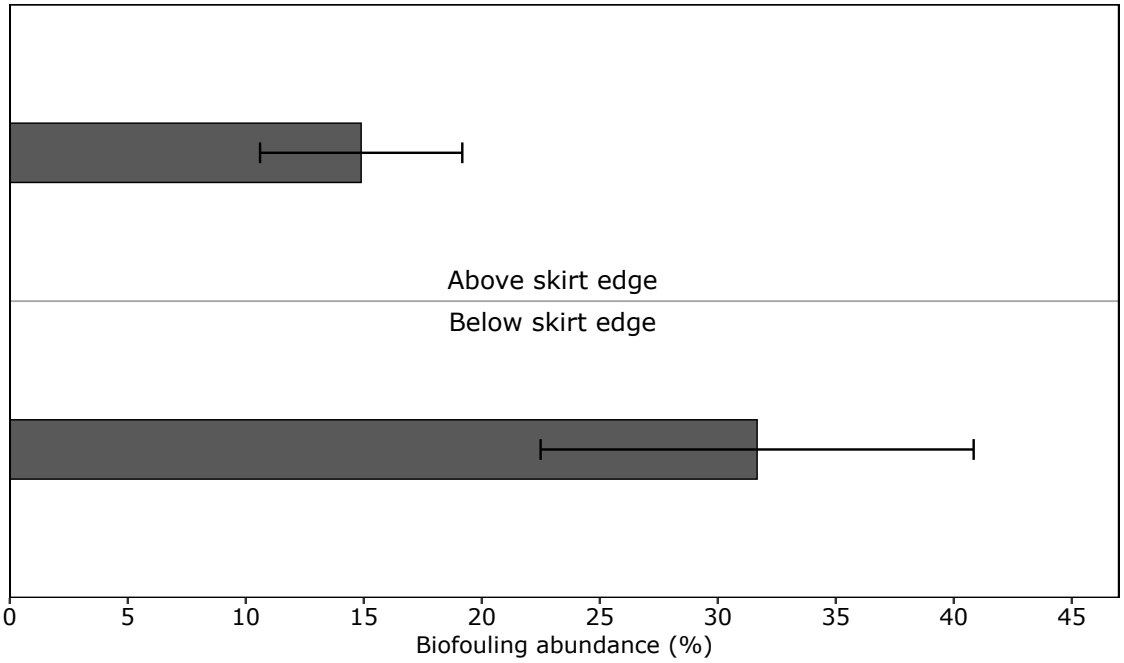


Figure 3.7: Average biofouling abundance (±CI) on samples from the last meter still protected by lice skirt (n = 36) and the first meter below the lice skirt edge (n = 36).The distance between 'above' samples and 'below' samples was approximately 1m.

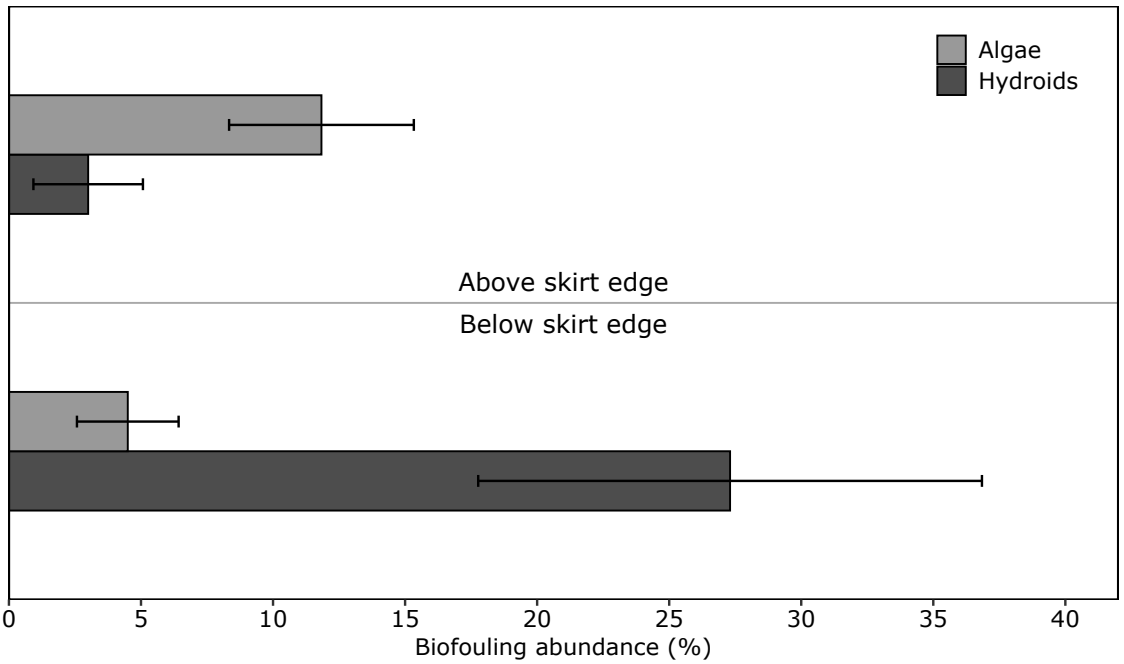


Figure 3.8: Average biofouling abundance (±CI) of the two BF species groups. Samples are from the last meter still protected by lice skirt (n = 36) and the first meter below the lice skirt edge (n = 36).The distance between 'above' samples and 'below' samples was approximately 1m.

3.2 The effect of biofouling on cleaner fish distribution in cage

The following analyses were based on the number of cleaner fish observed in close proximity to net walls at a given time. Complete details of observed cleaner fish per time unit for each site can be seen in Appendix B. Results from the pilot study showed that cleaning successfully removed all biofouling from net walls. Hence, 'after net cleaning' biofouling abundance was therefore regarded as 0%.

Figure 3.9 shows the distribution of cleaner fish observed per minute before ($Mdn = 27.5$) and after ($Mdn = 13$) net cleaning events. Significantly more cleaner fish were observed close to net walls with >30% biofouling cover (i.e., before net cleaning) than after net cleaning events, where nets were assumed to be clean (Mann-Whitney U test; $W = 2667.5$, $p < 0.05$).

As visualised in Figure 3.10, the number of cleaner fish observed in close proximity to the net walls had a weak positive correlation with biofouling abundance (Spearman's Rank Correlation rho; $r_s = 0.18$, $p < 0.05$). When considering only instances before net cleaning was conducted, a stronger correlation was found ($r_s = 0.79$, $p < 0.001$).

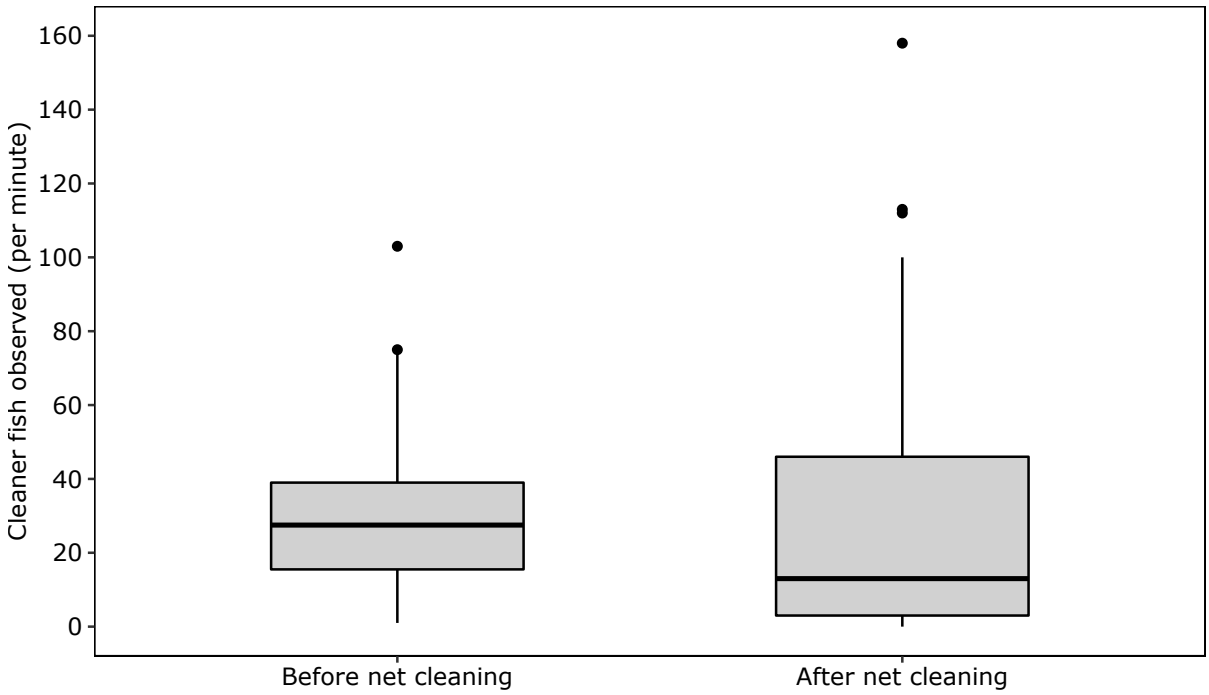


Figure 3.9: Distribution of cleaner fish observed in close proximity to net walls before (observations with BF abundance >30%) and after net cleaning.There was a significant difference between cleaner fish observed per minute before net cleaning (*Mdn* = 27.5) compared to after net cleaning (*Mdn* = 13).

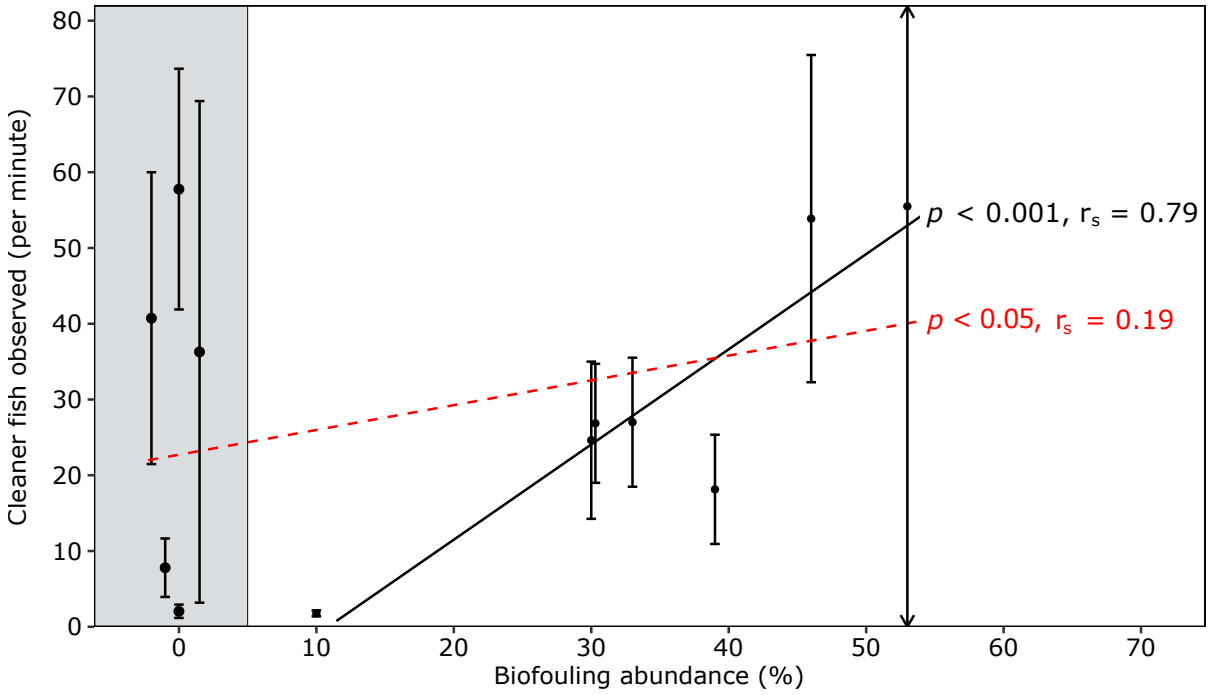


Figure 3.10: Spearman's Rank correlations between cleaner fish observed per minute (Mean±CI) and BF abundance. Black line = Correlation of observations before net cleaning was conducted. Red line: Correlation of all observations (before and after net cleaning). Each point represents a sampled cage and was correlated against the average BF cover on net walls at 1m and 5m depths of sampled cage. Values within grey area are fish observed in cages after net cleaning was conducted, where BF abundance was assumed to be zero.

3.3 Survey of biofouling management practices

Twenty-five farm managers from 11 different companies, spanning from Stavanger in south-western Norway to Finnmark in the north, responded to the survey.

A follow-up e-mail was sent to all responding farm managers, requesting information about annual costs of biofouling management at the respective farms. Nine farmers responded to the request. Annual costs of net cleaning ranged between 200,000 and 250,000 NOK per cage. However, there were two farmers who estimated the costs per cage to be closer to 400,000 NOK per year. Most farms pay approximately 15,000 NOK per cage at each cleaning event, but farms with their own equipment estimate this cost to be 5,000 NOK less, thus 10,000 NOK per cage per cleaning event.

Other comments registered from responses were as following:

> ”Our farms with lumpfish have a lower threshold of allowed biofouling abundance before net cleaning is commenced”
>
> ”We try to clean nets when biofouling abundance is low. We can then wash with lower pressure and avoid abrasion of coatings”
>
> ”We need to focus more on copper content in coatings and copper levels in the sediments”
>
> ”Clean cage nets increase fish welfare, growth, and cleaner fish efficacy”
>
> ”The difference in cleaner fish consuming net fouling organisms between fouled and clean nets is not as great as we have formerly believed”
>
> ”Many cleaner fish also die during net exchange”
>
> ”We see increased mortality if the washing rig operators are inattentive or drive too fast as they hit the fish”
>
> ”When nets are heavily fouled, mortality is high due to cleaner fish feeding on nets when washing rigs are in operation”

General

When asked which biofouling removal method was used (Figure 3.11), the majority of farms conducted *in situ* net cleaning, with one net change per production cycle (68%). Only 4% of respondents used net change as the sole biofouling removal method, and the reminding 28% used *in situ* net cleaning throughout the year.

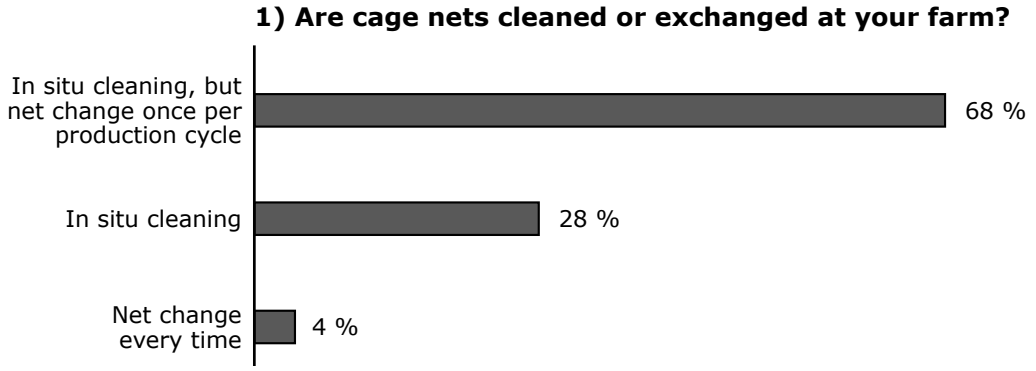


Figure 3.11: Percentages of respondents who implement either *in situ cleaning* with net change, *in situ* cleaning only, or net change every time. Results are based on 25 respondents.

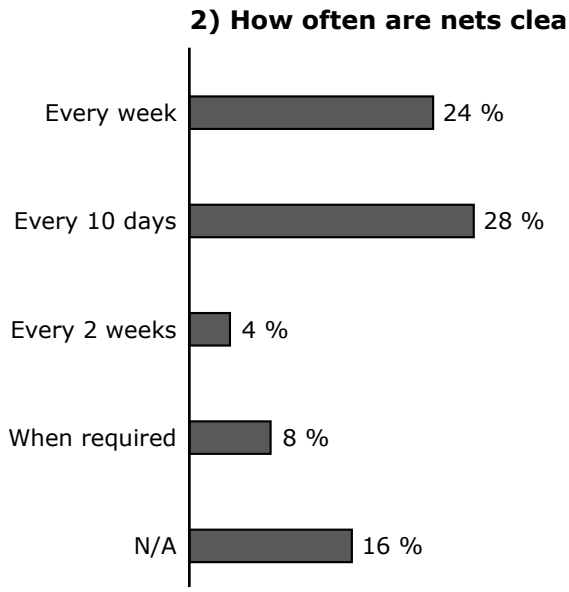


Figure 3.12: Percentages of respondents who, during peak BF season, implement net wall cleaning every week, every 10 days, every 2 weeks, or when required. N/A reflects respondents who did not answer or where question was non-relevant. Results are based on 25 respondents.

During peak biofouling season, the majority of responding fish farmers clean nets every week or every 10 days (Figure 3.12). 6% of responders clean when required, and only 4% clean every two weeks.

There was a relatively even distribution in responses of how often nets were cleaned in winter (Figure 3.13). When providing specific intervals, most farmers clean nets specifically every 2 (20%) or 4 weeks (24%), respectively. However, the majority of farmers clean nets when required, suggesting biofouling conditions are assessed prior to net cleaning.

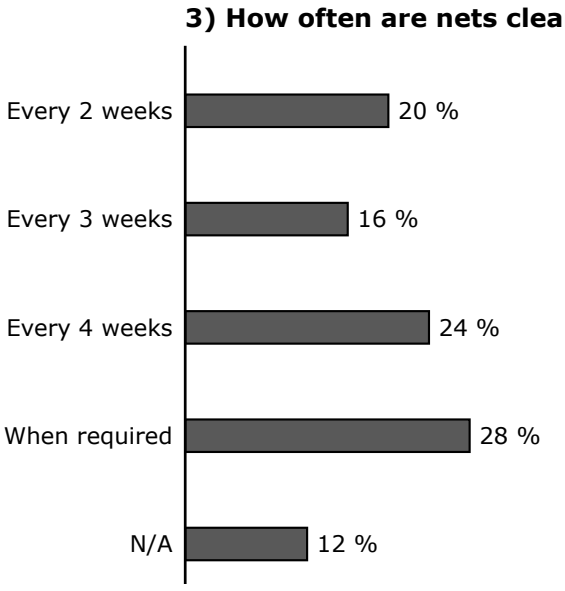


Figure 3.13: Percentages of respondents who, during winter, implement net wall cleaning every 2 weeks, every 3 weeks, every 4 weeks, or when required. N/A reflects respondents who did not answer or where question was non-relevant. Results are based on 25 respondents.

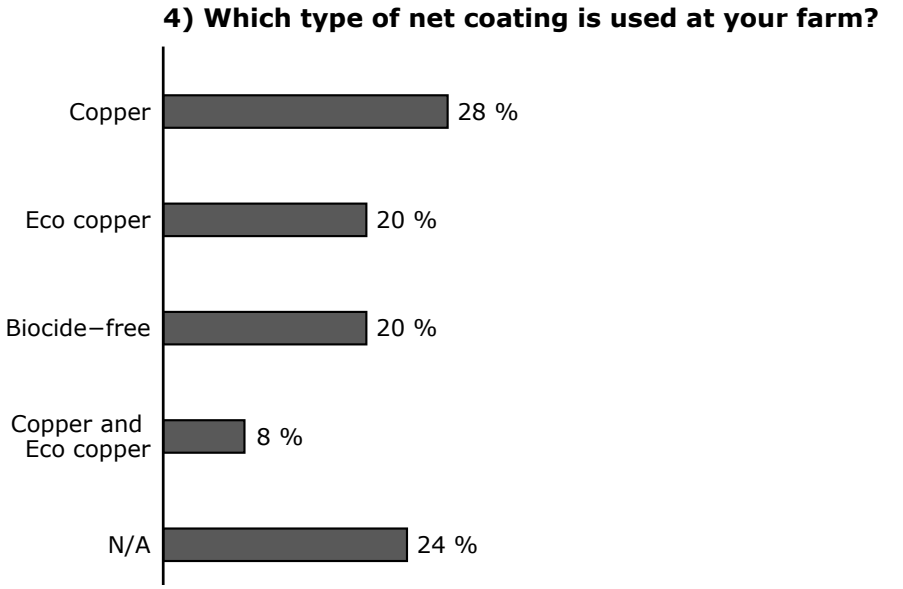


Figure 3.14: Percentages of respondents who use either Copper, Eco copper (= coating with lower copper content and approved for organic farming), Biocide-free, or both Copper and Eco copper coatings on nets. N/A reflects respondents who did not answer or where question was non-relevant. Results are based on 25 respondents.

The use of net coatings and types at survey respondents' farms can be seen in Figure 3.14. The most frequently used antifouling net coating is copper (28%), but both Eco-copper (i.e., coating with reduced copper content and approved for organic farming) and Biocide-free coatings are readily used (both 20%). Only 8% of farmers used both Copper and Eco copper at their farms, but highlighted that they were in a transition period of changing all nets to Eco-copper.

Assessing biofouling abundance

When asked if biofouling conditions on net walls are assessed before booking cleaning services (Figure 3.15), 44% respondents claimed that they only practise this outside biofouling season, and 24% never assess, meaning that net cleaning at the respective farms is entirely driven by pre-bookings at times of no assessment taking place.

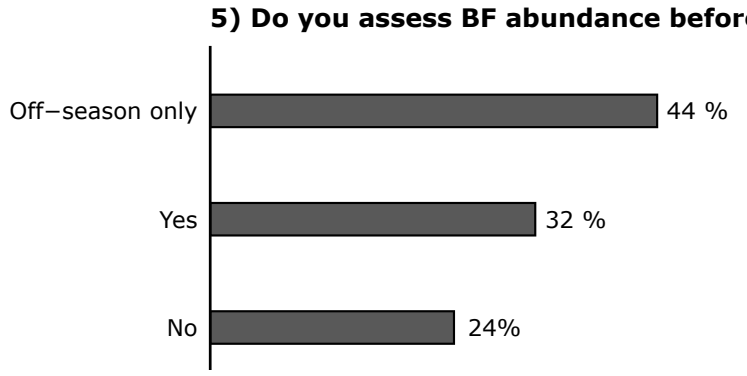


Figure 3.15: Percentages of respondents who assess BF conditions only during Off-season (i.e winter), Always (Yes), or never (No). Results are based on 25 respondents.

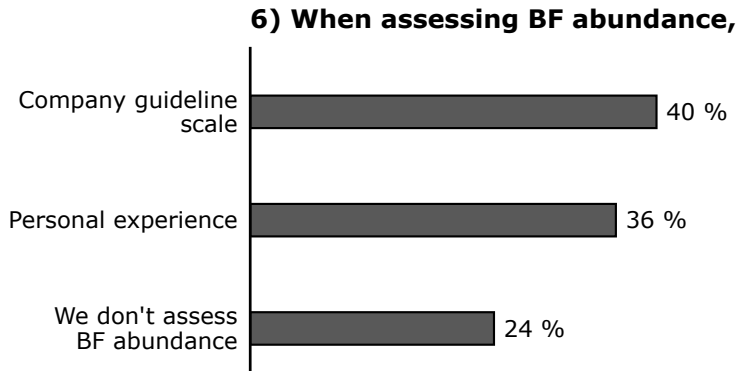


Figure 3.16: Percentages of respondents who use a company guideline or personal experience when assessing BF conditions. 'We don't assess' reflects respondents who answered 'No' in Figure 3.15. Results are based on 25 respondents.

Survey participants were asked if a guideline was used when assessing biofouling conditions (Figure 3.16). With the exception of farmers never assessing, 40% use a guideline scale developed within the company, and the remaining 26% assess based on personal experience.

The proportions of farmers using different methods for obtaining information about biofouling abundance on net walls can be seen in Figure 3.17. Most farms use combinations of two methods to assess biofouling, where visual assessment from cage walkway in combination of feed cameras is most used (24%). 16% of respondents implement visual assessment from cage walkway and SCUBA, and the same amount use ROV's.

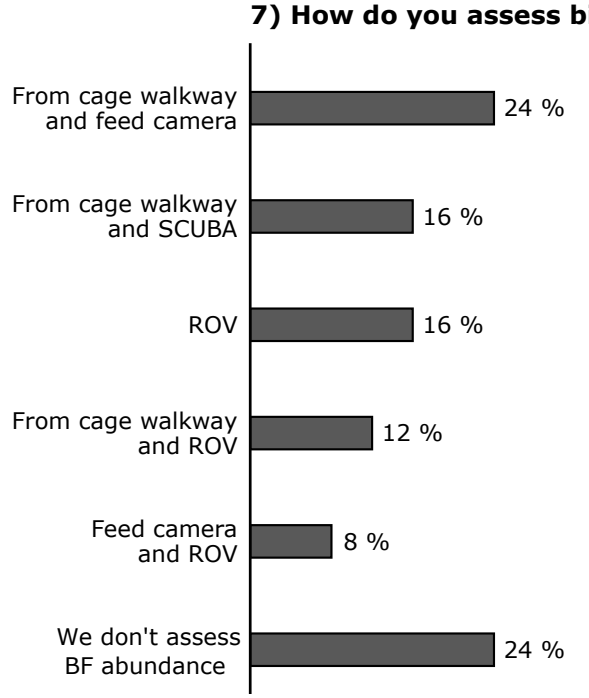


Figure 3.17: Percentages of respondents who assess BF conditions through visial assessment from cage walkway, through feed camera, through SCUBA divers, with ROV's, or through combinations of mentioned methods. 'We don't assess' reflects respondents who answered 'No' in Figure 3.15. Results are based on 25 respondents.

Other

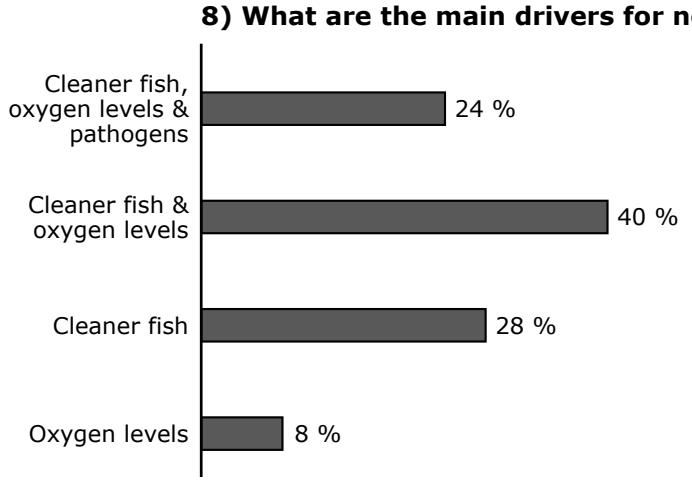


Figure 3.18: Percentages of respondents who's main driver for net cleaning is either ensuring cleaner fish efficacy, maintaining oxygen levels, preventing pathogen dispersion, or combinations of the three. Results are based on 25 respondents.

Survey participants were asked to tick off which factors were the main driver for net cleaning at their farms (Figure 3.18). Most cleaning regimes were driven by the need to ensure cleaner fish efficacy and ; Table 3.1 levels (40%), and 28% said cleaner fish efficacy was the only driver. 24% also included pathogen dispersion as a factor, in addition to cleaner fish and DO levels. Only 8% of cleaning regimes were driven by only DO levels within the cage.

Responses from farmers regarding increased mortality in cleaner fish after net cleaning were evenly distributed (Figure 3.19). 40% of farmers experienced higher mortality during net cleaning events, and 40% did not see a difference. Only 4% did not know if there were any changes in mortality.

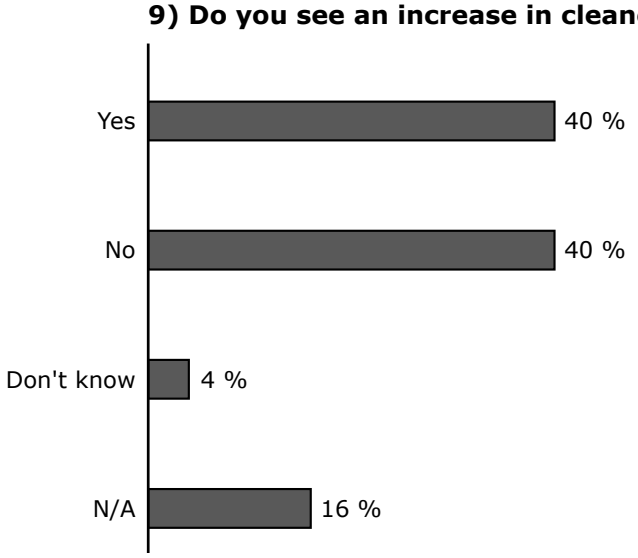


Figure 3.19: Percentages of respondents who responded to observing increased mortality in cleaner fish after net cleaning. N/A reflects respondents who did not answer or where question was non-relevant. Results are based on 25 respondents.

Chapter 4

Discussion

The main aims of the biofouling fieldwork in this study was to quantify and document 'maximum' biofouling conditions (i.e., biofouling amount present on cage nets at the time of net cleaning) at several salmon farms. Also, to further understand the behaviour of biofouling on net walls, spatial variation and potential patterns were investigated, also with regards to biofouling composition. There were significant differences in maximum biofouling abundance on cage nets at the time of net cleaning between the different sites, and there were significant spatial variations within sites, where biofouling abundance depended on both depth and cardinal direction. Differences between cardinal directions were due to differences in algae abundance, while hydroid abundance did not differ between the four directions. The presence of lice skirts was found to potentially limit biofouling growth in general, but specifically hydroid growth.

The abundance of cleaner fish in the vicinity of net walls was assessed. The subsequent goal was to recommend a certain threshold of biofouling abundance present on cage nets before net cleaning would commence with regards to cleaner fish numbers observed per minute. It was, however, shown that although cleaner fish numbers showed a strong relationship with increasing biofouling abundance on cage nets, the relationship was only true for cleaner fish observed before net cleaning took place. When data of cleaner fish observed before and after net cleaning were analysed together, said relationship was much weaker.

4.1 Biofouling conditions at the time of net cleaning

4.1.1 'Maximum' biofouling abundance

'Maximum' biofouling abundance (i.e., abundance on net walls the day of net cleaning) varied significantly between most of the sites investigated in this study and ranged from 10.4% to 58%. This confirms earlier statements that cleaning regimes today are mostly carried out independent of biofouling amounts on net walls (Bloecher, de Nys, Poole and Guenther, 2013; Bloecher et al., 2015). Biofouling abundance also differed amongst cages within some or all sites. This is consistent with Svane et al. (2006), who found significant differences in biofouling abundance with depth in two out of five test months when testing two antifouling treatments in three replicate cages. However, Bloecher, Olsen and Guenther (2013) did not find any differences in biomass between six replicate cages when investigating biofouling development on cage nets at a Norwegian salmon farm. Although currents, placement of cages, and fish biomass may explain the variation between the cages (Madin et al., 2010), another explanation may be that net walls were not accurately cleaned during the previous cleaning event.

When salmon farmers were asked if biofouling abundance is assessed before booking cleaning services, nearly half of the farmers said that they only assess biofouling abundance in the off-season (winter), and 24% never assess, regardless of season. External cleaning vessels are in high demand during peak biofouling season, and it is therefore necessary to pre-book their services to ensure that biofouling is removed before reaching critical amounts, this being the reason why biofouling abundance is not assessed in summer. Also, when asked about how biofouling is assessed, 36% said they use personal experience to determine biofouling abundance, whereas 40% have access to a company guideline. Furthermore, the equipment used to assess fouling varied. Many farmers assess from the cage walkway in combination with either SCUBA divers, feed cameras, or ROV's. This calls for substantial differences in assessments, considering only the first meter is visible from the cage walkway, and that many feed cameras have a limited range. All these factors may contribute to the large discrepancies in biofouling amount on net walls at the time of net cleaning.

Only two biofouling species groups (algae and hydroids) were registered in this study. However, earlier studies have documented a range of biofouling organisms present on cage nets in Norwegian aquaculture (Guenther et al., 2010; Bloecher, Olsen and Guenther, 2013; Bloecher and Floerl, 2020). Although compromised image quality may have disguised individuals of other species, it can further be explained by the continuous disturbance of fouling communities due to frequent net cleaning, where the biofouling does not have time to grow to a climax community (Bloecher, Olsen and Guenther, 2013). The *in situ* cleaning is preventing the natural succession seen in other studies where nets or net panels have been left undisturbed for a longer time (Guenther et al., 2010). This is also in accordance with Valdivia et al. (2005), who determined that high disturbance frequencies allow for re-emergence of sub-ordinate species. The abundance of each species group also varied between sites, where some sites experienced more algae than hydroids, and vice versa. However, across all sites, hydroids were the dominant species and made up 59% of total cover. Although Guenther et al. (2010) found hydroids to be the most dominant species at their studied farm, this study highlights that biofouling composition may be significantly different at various sites. Species composition also varied between cages but did not vary in the same pattern. This is consistent with Bloecher, Olsen and Guenther (2013)'s findings, who found differences in species composition between 6 cages but not in consistent patterns. Fish biomass may have influenced algae growth through inorganic nutrient loading (Wang et al., 2012), and zooplankton availability may have supported hydroid growth (Bloecher, 2013).

Although all the mentioned studies have documented biofouling growth, they have only involved single locations. This study, however, reinforces that there are large variations between and within sites, highlighting the need for within-farm knowledge of biofouling growth patterns. If this is obtained, biofouling prevention could be tailored to individual farms, thereby maximising cost efficiency and maintaining healthier stocks if net cleaning events are reduced, or if net coatings can be optimised for different biofouling species.

4.1.2 Spatial variability of biofouling

In addition to differing between sites and cages, as mentioned above, biofouling abundance also differed with both depth and cardinal direction. However, the effects were interacting and did not act in a consistent pattern at all sites. When studying biofouling growth on tuna cage nets in Australia, Cronin et al. (1999) also found that the biomass of the fouling community differed with depth, depending on species. However, the inconsistency seen between depths can be validated by Guenther et al. (2010)'s study, who also found a significant effect of depth on biofouling abundance; but whilst the upper depths displayed the highest abundance before net cleaning, the trend reversed after net cleaning events were conducted, changing to displaying the highest abundance at deeper depths. Considering all sites in this study normally underwent cleaning events every 7-10 days at the time of sampling, the biofouling communities experienced

frequent disturbances, promoting the large variability. Variation in biofouling is also known to be driven by orientation. Cronin et al. (1999) also found differences between cardinal locations within cages, and connected the differences with light availability, physical disturbance, and competition for space. However, due to the interactions found in this study, the effect of depth, nor cardinal direction, could be decoupled from the other, highlighting the complexity of biofouling development on net walls, which makes it difficult to predict future growth patterns.

The spatial behaviour of algae and hydroids are not streamlined, and there are large variations throughout the entire net cage systems. The results indicate that algae and hydroid abundance was driven by variations between sites, cages, and depth. Additionally, algae growth was also affected by cardinal location. Algae were the dominating species at 1m, and although present at 5m and 10m, the abundance rapidly declined. Algae abundance also declined with later sampling events, i.e., when sampling happened closer to the winter period. Naturally, as light availability is a limiting factor for photosynthetic organisms, the extinction of the photic zone would determine algae growth. Guenther et al. (2010) found algae depths down to the deepest measured depth of 15 meters in august samples but was limited to above 5m and 10m at later dates, indicating that shorter days and lower sun position may have an effect on algae growth. Also, Cronin et al. (1999) found that algae dominated the shallower depths in the cage system, but also compromised a small component at depths of 8-12m. However, they measured sufficient light for algae growth down to 16 meters and concluded that faster-growing heterotrophs outcompeted algae at the deeper depths. Moreover, there was a significant impact of cardinal direction on algal abundance. This is in accordance with Cronin et al. (1999) findings, who found that sides exposed to direct sunlight had the greatest photosynthetic biomass. However, no obvious trends in algae growth and cardinal direction were seen between sites in this study, which could be explained by the weather and the time of year, as the sun lies relatively low during October/November. In turn, hydroid growth within sites was not driven by cardinal location, suggesting that hydroid growth does not rely on sunlight.

It is important to notice, during image analysis, some transects had dramatically more biofouling cover than the others. In some of those, other heavily fouled fish farm structures, such as moorings, were in close proximity to the net wall (e.g., BF abundance 'East', Rataren; Figure A.1). Observations like these strengthen the theory of Bloecher et al. (2015), who believe such structures may drive biofouling recruitment, and stresses the need to increase biofouling removal of very under-managed farm structures.

As a novel method to prevent sea lice infestations, 'snorkel' sea cage technology is emerging (Oppedal et al., 2017). This involves the concept of salmon being held deep via a net-roof, preventing salmon from swimming in the upper 10 meters of the cage, but are allowed to re-fill their bladder going through an impermeable tube. Here, fish can be held at up to 40m depth, if not deeper (Wright et al., 2017). This will require more knowledge on biofouling development at deeper depths, and if the patterns of algae and hydroid abundance with depth found in this study and others (e.g., (Guenther et al., 2010)) are applicable, hydroids may be the sole concern in these specific cage environments. Current antifouling methods do not work well on hydroids. Although copper coatings may reduce hydroid growth for a limited amount of time (Guenther et al., 2009), it cannot prevent hydroid settlement over a longer time period (Bloecher and Floerl, 2020). Also, *in situ* net cleaning releases large amounts of still viable hydroid polyps who shed propagules, inducing re-settlement Carl et al. (2011). This indicates that other methods are needed to efficiently prevent hydroid growth and remove specimens in its entirety. If hydroids are the sole problems at deeper depths, antifouling methods can be specifically dedicated to prevent and remove said species.

4.1.3 The effect of sea lice skirt on biofouling

There was significantly less biofouling on samples from the last meter still protected by lice skirts ('above skirt edge') than the first meter below the skirt edge ('below skirt edge'). Also, there was significantly more algae present above the skirt edge than below, and vice versa with regards to hydroids. Considering the samples were from 5m depth, as discussed above, light could be a limitation for growth, explaining the low algae abundance. However, the fact that there was a dramatic jump in hydroid abundance above the skirt edge to below could be explained by the lice skirt itself, as hydroids are opportunistic and rapidly colonise free space (Guenther et al., 2010) in addition to not being limited by light (Cowie, 2010). Guenther et al. (2010) did a similar study in cages without lice skirts, investigating biofouling abundance at several depths, and found that hydroids dominated throughout the entire water column within the cage from September and onwards. Similarly, Cronin et al. (1999) did not see a consistent pattern in non-photosynthetic biomass with depth. In contrast, during this study, very few hydroids were observed in samples from 1 meter, and although present, fewer were found in the samples from 5 meters which were still protected by lice skirts than in samples from 5 meters not protected by the skirt. The reason for this is unclear, but one possible explanation could be that, during net cleaning, hydroid waste is transported out of the cage with horizontal flow; caused by both currents and movement of fish inside the cage (Frank et al., 2014), and thereby little may reach shallower depths for re-colonisation. A more likely possibility may be that algae outcompete hydroids at shallower depths if sufficient light is available and at deeper depths, where algae suffer from loss of light, hydroids, who are not dependent on light outcompete algae. Additionally, as the main purpose of lice skirts is to prevent the salmon lice (zooplankton) from entering the cage (Næs et al., 2012), hydroids may be of disadvantage, as their main diet consists of zooplankton (Bloecher, 2013), and thereby experiencing a nutritional limitation within lice skirt zones.

These results indicate that the protection of a lice skirt or equivalent may have a somewhat restrictive effect on hydroid growth on cage nets. However, this pattern may not be similar during winter months, where very little light is available for algae growth, especially in mid and northern Norway. Regardless, this knowledge can be used to potentially tailor antifouling methods if, in the future, nets can be coated differently at different depths, targeting individual species groups.

4.2 Cleaner fish in relation to BF abundance

There was a significant difference in cleaner fish observations along net walls before and after net cleaning. Cleaner fish are known to forage on biofouling organisms, which has been documented several times (Deady et al., 1995; Imsland et al., 2014, 2015). Only Leclercq et al. (2018) has compared the behaviour of wrasse and lumpfish with or without net fouling. Lumpfish preferred cage edges and corners as habitat during the day, whereas wrasse preferred said locations during the night. They did, however, not see a significant difference in depth distribution, swimming activity or habitat use with or without net fouling in either species. This may indicate that although cleaner fish forage on net fouling, the time spent doing this is not significantly higher than time spent in other locations within the cage. Considering the dispersion in cleaner fish numbers was large, especially in the 'after net cleaning' group where cleaner fish numbers ranged from 0 to almost 160 fish per minute, a reliable and certain conclusion on the observed differences cannot be drawn.

Assuming that cleaner fish actively forage or search for food on net wall fouling, the goal of this study was, therefore, to determine a threshold for biofouling abundance before net cleaning would have to be conducted. A strong positive relationship between cleaner fish numbers on net walls and increasing biofouling abundance was found, but this was only true for observations before net cleaning was conducted. When observations of cleaner fish observed after net cleaning was added

to the correlation, the relationship was weak. The 'after net cleaning' counts could be explained by cleaner fish inspecting nets due to disturbed habitat or availability of free-floating biofouling organisms, such as caprellid shrimps, which may be easier to consume. However, in Leclercq et al. (2018)'s study, net change was used as biofouling control, a process that does not release large amounts of cleaning waste. It may seem that, since the relationship found in this study was weak, cleaning waste availability may have an effect on counts, but not in its entirety, if compared with the previous study. Additionally, Imsland et al. (2014) compared lumpfish behaviour in cages with and without salmon, and found that lumpfish spent less time feeding on net fouling in cages with salmon present. This means that the effect of net fouling on cleaner fish location in the cage may not be as large as fish farmers have previously thought. One could also argue that observing 10-20 fish per minute swimming along cage nets is a very small proportion of the 10,000-20,000 cleaner fish present in the cage. Furthermore, considering Eliasen et al. (2018) found a positive association between lice consumption and biofouling consumption in lumpfish, the small numbers of fish observed could be benefited by the potential biofouling consumed. However, to validate these findings, more data would be needed in terms of more grades of biofouling abundance, both in cages with lower (< 30%) and higher (> 50%) net wall biofouling abundance. Regardless, based on the current findings, a biofouling threshold of 40% may be reasonable with regards to cleaner fish numbers. The average number of cleaner fish observed cages with 30-40% biofouling was below 30 per minute, which makes out a very low proportion of the closer to 20,000 cleaner fish deployed in the cages.

During the preparation of a novel guideline for efficacy testing of antifouling cage net coatings for the Norwegian Environmental Agency and a new biocide directive coming from the EU, the need for more knowledge on maximum biofouling tolerance at Norwegian fish farms was highlighted (N. Bloecher, SINTEF, pers. comm.). If the current dominating view on biofouling and cleaner fish is to continue, maximum biofouling on net walls will need to be determined with regards to cleaner fish behaviour. However, the current results indicate that cleaner fish may not be as affected as previously thought, other parameters (oxygen and fish health) must be used to determine the extent of biofouling that can be tolerated on cage nets.

Biofouling management methods at Norwegian salmon farms have both prevailed and changed during the past 14 years. Previously, the main driver for removing net fouling was to assure good growth, good fish health & welfare, and good economy (Olafsen, 2006). Since then, the number of cleaner fish deployed into sea cages has increased with a ten-fold (Directorate of Fisheries, 2019*b*). Today, according to question 8 in our survey, the main driver of net cleaning is largely to ensure cleaner fish efficacy in addition to maintaining sufficient oxygen levels. However, at the sites examined in this study, there was no difference in dissolved oxygen levels before and after net cleaning with the current amount of biofouling. Based on this, one could therefore assume that the high-frequency net cleaning seen today is solely driven by the need to remove fouling to ensure cleaner fish efficacy. Indeed, in our survey, farmers commented that sites with lumpfish had a lower threshold of biofouling abundance before net cleaning was initiated, and some displayed the standard views that 'clean nets increase fish welfare, growth, and cleaner fish efficacy'.

Although cleaner fish have been mentioned in the annual fish health report from the Norwegian Veterinary Institute for several years in terms of diseases, their welfare has only become a point of focus in recent years. However, net cleaning operations have not been documented as a cause in their welfare issues until this year, where mortality was linked to net cleaning operations but was not recognised as a major cause of overall mortality (Stien et al., 2020). In our survey, nearly half of the farm managers observed increased mortality after *in situ* net cleaning, and a farmer also commented that mortality was high during net exchange. Cleaner fish are mutilated if physically connecting with certain cleaning rigs (pers. obs.). Farmers commented that if cleaning rigs were driven too fast or if nets were heavily fouled during net cleaning, leading to more fish feeding on net fouling, mortality would be higher. More information is needed on how cleaner

fish mortality is linked to net cleaning, i.e., how the net cleaners induce mortality. This will aid in the development of more fish-friendly net cleaners, which is a clear necessity if the current net cleaning regime is to continue.

4.3 Comments on biofouling management practices

The survey results indicate that the most common method of net biofouling removal is *in situ* net cleaning. Several farms change nets once per production cycle, but this is mostly in response to fish size, rather than being a specific biofouling management method. The earlier method of drying nets was not registered in this survey and is most likely an outdated method. There has, however, been a shift in the use of antifouling coatings. Previously, the use of copper coatings was the obvious norm (Olafsen, 2006). Although copper is still mostly used, many farmers use either coatings with reduced copper content (Eco-copper) or biocide-free coatings. A farmer commented through the survey that there is a need for a larger focus on copper levels in sediments, indicating that although copper is used, farmers understand the related issues. Regardless of this, during peak biofouling season, most farms clean nets every 7-10 days, but during winter, it ranges from every 14 days to every four weeks or whenever required. As discussed, his coincides with the large discrepancies of how often nets are cleaned during on- and off-season between farmers show that there is no common standard in cleaning frequency, especially during the off-season.

Evidently, biofouling management does, indeed, make up a high cost for salmon farmers. The variations in annual costs could, indeed, be explained by the lack of a maximum biofouling threshold, and considering the differences in cleaning frequencies between farms, the variations may also be due to farmers' different tolerances to allowed biofouling. A farmer commented that they try to clean nets early, allowing for *in situ* net cleaning with lower water pressure, and thereby avoiding abrasion of copper coatings. If this is a strategy to prevent biocide deposition or to save costs of re-coating nets is unknown, but it is clearly a strategy that requires a high-frequency cleaning regime. As mentioned earlier, net cleaning events are controlled by pre-bookings and available service providers. To control net cleaning frequency, farms may invest in personal cleaning equipment. Survey responses indicated that the cost of cleaning per cage was decreased with a third if using personal equipment. However, purchasing the actual cleaning robot is a large investment, and does also require additional staff, boat space, and servicing. In turn, possessing equipment may possibly reduce cleaning events significantly, which will reduce costs in the long run.

4.4 Challenges and limitations

Field work

In this study, data was only sampled during autumn and early winter (September through November). Biofouling is known to vary temporally (Bloecher, Olsen and Guenther, 2013), and it would be beneficial to see if the patterns found in this study differed between seasons. Also, the findings could have been strengthened by sampling additional cages at each site. However, farm logistics and time did not allow for this during sampling days.

All cages studied had lice skirts reaching down to 5-6m depths. As seen in the results, lice skirts may impact both biofouling abundance and community composition, meaning that the effects seen here may not be the same at farms who do not use lice skirts.

The cleaner fish study experienced challenges related to sampling methodology and technical difficulties. Due to farm logistics, it was not always possible to film the same cage after net

cleaning as before. Also, recurring technical issues with the underwater drone resulted in large variations of sampled minutes at all sites. Although the goal was to set a threshold in biofouling abundance with regards to cleaner fish, limited sample sizes did not lead to any conclusive answers, but findings opened up for further studies.

Results of survey responses were affected by responders not answering all questions. When follow-up questions were sent, few e-mails were responded to. This resulted in moderate amounts of no answer (N/A) proportions of the total answers, and some survey results may, therefore, not reflect the current situations completely.

Data analysis

Images extracted from the GoPro videos were of limited quality. Due to challenging contrast differences between cage net and lice skirt in the upper 5 meters in addition to the movement of the skirts, biofouling abundance, and community compositions estimates could have been somewhat compromised. The GoPro was used due to its simplicity and accessibility, but cannot be recommended for similar future studies. The methodology of determining biofouling abundance is solid but is reliant on good image quality for accurate analysis.

Due to data loss during cleaner fish data sampling, there are large limitations in the data set, hence the decision to combine cages and species. During the last sampling event, we were finally prepared for any potential issues, which resulted in many successful sampled minutes. However, the current results give a clear indication of trends, and further studies may strengthen observations found during this thesis.

4.5 Future work and prospects

The results in this thesis open up for several interesting questions and further work. There is clearly a need to develop a 'maximum recommended biofouling' threshold, not only because of the associated costs with regards to management but also with regards to salmon and cleaner fish health & welfare. The frequent net cleaning regime practiced today poses great threats to salmon skin and gill health, and cleaner fish are impaled if hit by cleaning ROVs.

If snorkel cages are to be implemented at a commercial scale in the future, it will be crucial to investigate biofouling at deeper depths. Currently, depths down to 15m have been investigated (Guenther et al., 2010), but considering snorkel cages may reach even deeper, knowledge about biofouling patterns at deeper depths is needed.

More data with biofouling abundance and cleaner fish reaction to different abundances of biofouling may allow for developing said threshold (= maximum recommended biofouling abundance before net cleaning is conducted). This will, however, require a simple but relatively accurate way to determine biofouling abundance at respective farms. Here, the concept of Precision Fish Farming (Føre et al., 2018) is highly relevant, where technology and automation systems potentially could determine biofouling through e.g., feed cameras or ROVs. Obtaining similar data as in this study, but from several consecutive days after net cleaning events, would give us more confidence in the relationship found in this study. This could also show if the relatively large amount of cleaner fish present directly after net cleaning is indeed due to biofouling waste suspended in the water, leading to cleaner fish searching for food. Also, considering sampling happened at 1 and 3 meters, it would be beneficial to replicate the study at deeper depths, where other biofouling species may be encountered, as algae were dominating at the shallower depths during this study.

If net cleaning frequency is to be reduced as a result of more accurate assessment or development

of thresholds, it will be important to ensure that external net cleaning providers show more flexibility than today. However, this will require additional boats and equipment, leading to, for example, increased fuel consumption and the release of fossil fuels, and the costs of services may increase. Regarding maintaining a healthy fish stock and environment, farmers may benefit from investing in personal cleaning equipment, which will allow for complete control in net cleaning frequency.

Chapter 5

Conclusions

The results of this study show that there is no standard in 'maximum' biofouling abundance on net walls at the fish farms. 'Maximum' biofouling, algae, and hydroid abundance varied between sampling events and within cages, and spatial factors influenced this variation.

There were large variations throughout the system, where the variability in site, cage, depth, and cardinal direction all influenced total biofouling abundance, but the effects could not be compartmentalised. Biofouling growth did not behave in a consistent way, and the effects were not the same at all farms, indicating that within-farm knowledge of biofouling behaviour is vital. Additionally, both algae and hydroid variability were driven by site, cage, and depth, where algae abundance dominated at shallower depths, and hydroids clearly dominated in deeper sections. Moreover, algae growth was also driven by cardinal direction, but not in a consistent way between sites. Hydroids were not affected by cardinal direction. Lice skirts affected biofouling abundance in general, with significantly less growth on the last meter still protected by lice skirt than the first meter below the skirt edge. Also, the protection of lice skirts highly affected biofouling composition in an inverse manner: algae abundance was greater above the edge than below, and hydroid abundance was significantly greater below than above. Considering the large between-site variations, it is highly relevant to obtain knowledge about biofouling behaviour and patterns at each site to ensure proper antifouling management in terms of cost efficiency and a fish welfare perspective.

There were significantly more cleaner fish observations along net walls before than after net cleaning, but we cannot say with full certainty that this is a reliable conclusion due to large dispersions. The relationship between cleaner fish numbers and biofouling abundance was significant. The correlation was strong if only including observations with fouled nets, but when including observations from after net cleaning (i.e., clean nets), the correlation was weak. The results do indicate that there is an effect of net fouling on cleaner fish, but it may not be as great as fish farmers have formerly believed. Regardless of the limitations in samples, based on the current findings, a biofouling threshold of 40% may be reasonable with regards to cleaner fish numbers. Also, if a threshold is to be set, an easy and accurate way of determining biofouling abundance within farms must be developed.

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Appendix A

Appendix for BF Abundance

A.1 Details of PERMANOVA partitionings

Total biofouling abundance

Table A.1: Results (permutational *P*) of PERMANOVA for main effects for the influence of site, cardinal direction ('Direction'), depth and variability between cages nested in site ('Cage(Site)'), including respective interactions, on biofouling abundance. Bold entries indicate significant results.

Factor	***df***	**Pseudo-*F***	***p***
Site	4	26.13	**0.011**
Direction	3	0.85	0.484
Depth	2	0.02	0.973
Cage(Site)	4	4.43	**0.001**
Site x Direction	12	1.88	0.145
Site x Depth	8	1.35	0.347
Direction x Depth	6	2.01	0.097
Cage(Site) x Direction	12	2.04	**0.022**
Cage(Site) x Depth	8	12.83	**<0.001**
Site x Depth x Direction	24	0.75	0.75
Cage(Site) x Direction x Depth	24	1.79	**0.018**
Residuals	216		

Algae and hydroid abundance

Table A.2: Results (permutational *P*) of PERMANOVA for main effects for the influence of site, cardinal direction ('Direction'), depth and variability between cages nested in site ('Cage(Site)'), including respective interactions, on algae and hydroid abundance. Bold entries indicate significant results.

		Algae		**Hydroids**	
Factor	***df***	**Pseudo-*F***	***p***	**Pseudo-*F***	***p***
Site	4	1.46	0.367	6.0	0.078
Direction	3	1.4	0.288	0.7	0.566
Depth	2	12.12	**0.001**	4.91	**0.030**
Cage(Site)	4	18.25	**0.0001**	12.8	**0.0001**
Site x Direction	12	1.12	0.434	3.74	**0.014**
Site x Depth	8	2.07	0.156	6.68	**0.006**
Direction x Depth	6	2.27	0.06	0.68	0.656
Cage(Site) x Direction	12	3.18	**0.0006**	0.49	0.91
Cage(Site) x Depth	8	10.09	**0.0001**	3.38	**0.0007**
Site x Depth x Direction	24	0.59	0.899	1.73	0.091
Cage(Site) x Direction x Depth	24	3.36	**0.0001**	0.58	0.935
Residuals	216				

A.2 Difference in BF abundance between sampled cages

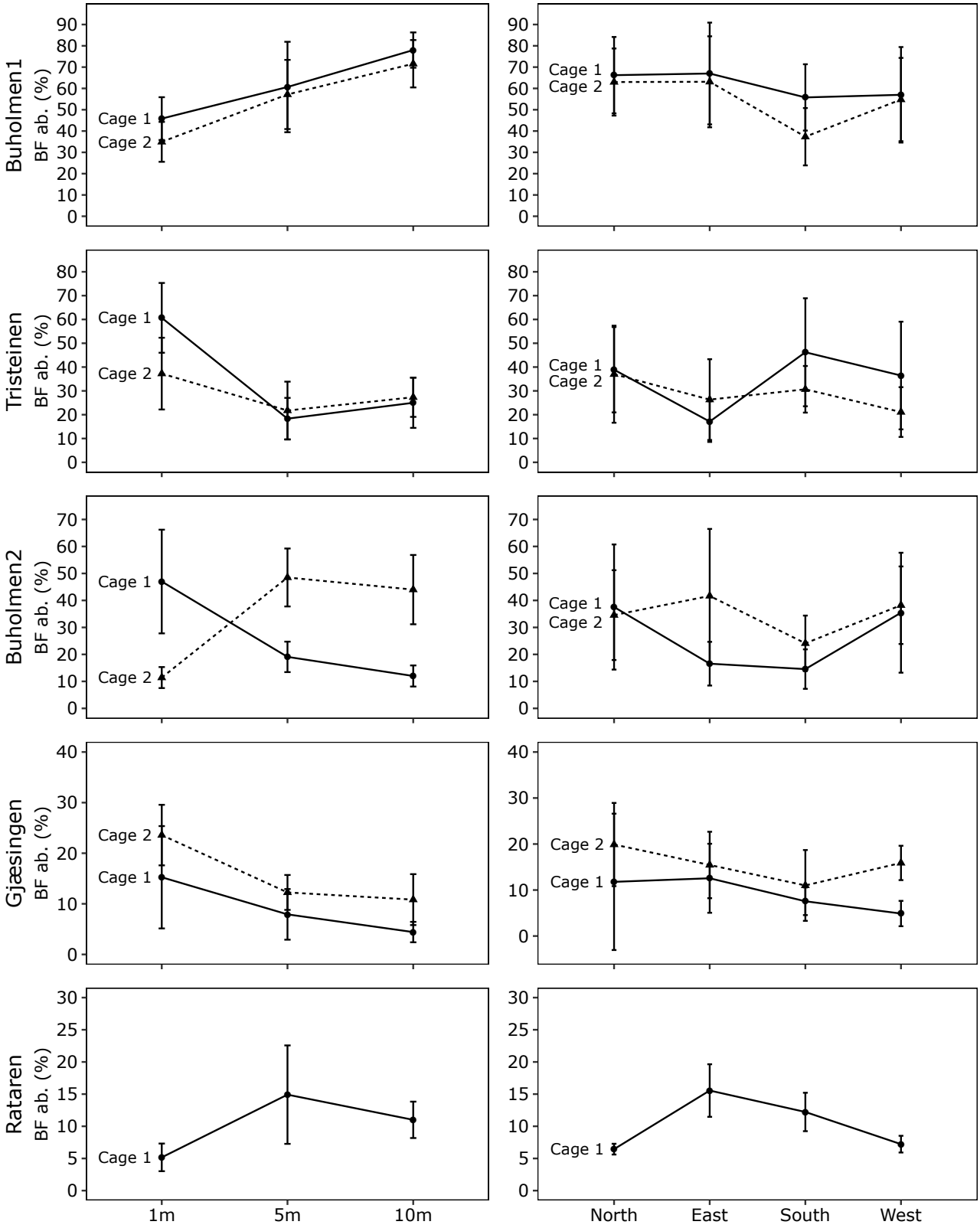


Figure A.1: Biofouling abundance (Mean±CI) of all sampled cages at each depth and cardinal direction. Note the differences in scales between sites.

Appendix B

Appendix for Cleaner Fish Data Sampling

B.1 Cleaner fish observations at each sampling event

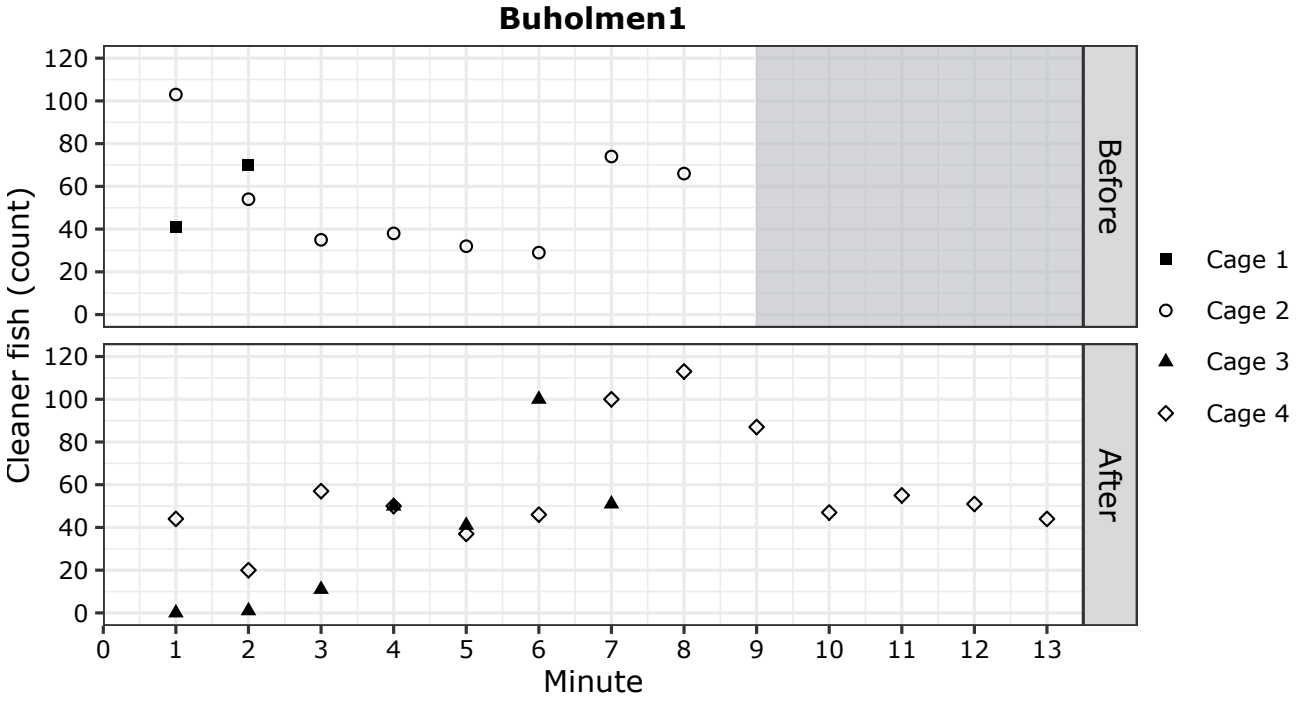


Figure B.1: Timeline of cleaner fish counted per minute filmed before and after (i.e., no fouling present on net wall) net cleaning was conducted at Buholmen1. Cages correspond with cages shown in farm layout in Table 2.1. Grey shadings indicate minutes not recorded.

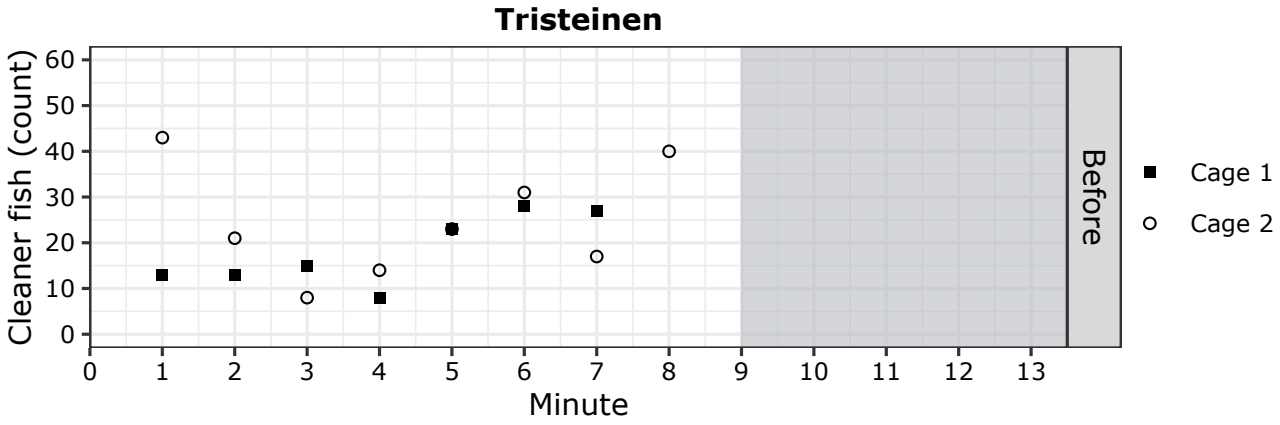


Figure B.2: Timeline of cleaner fish counted per minute filmed before and after (i.e., no fouling present on net wall) net cleaning was conducted at Tristeinen. Cages correspond with cages shown in farm layout in Table 2.1. Grey shadings indicate minutes not recorded.

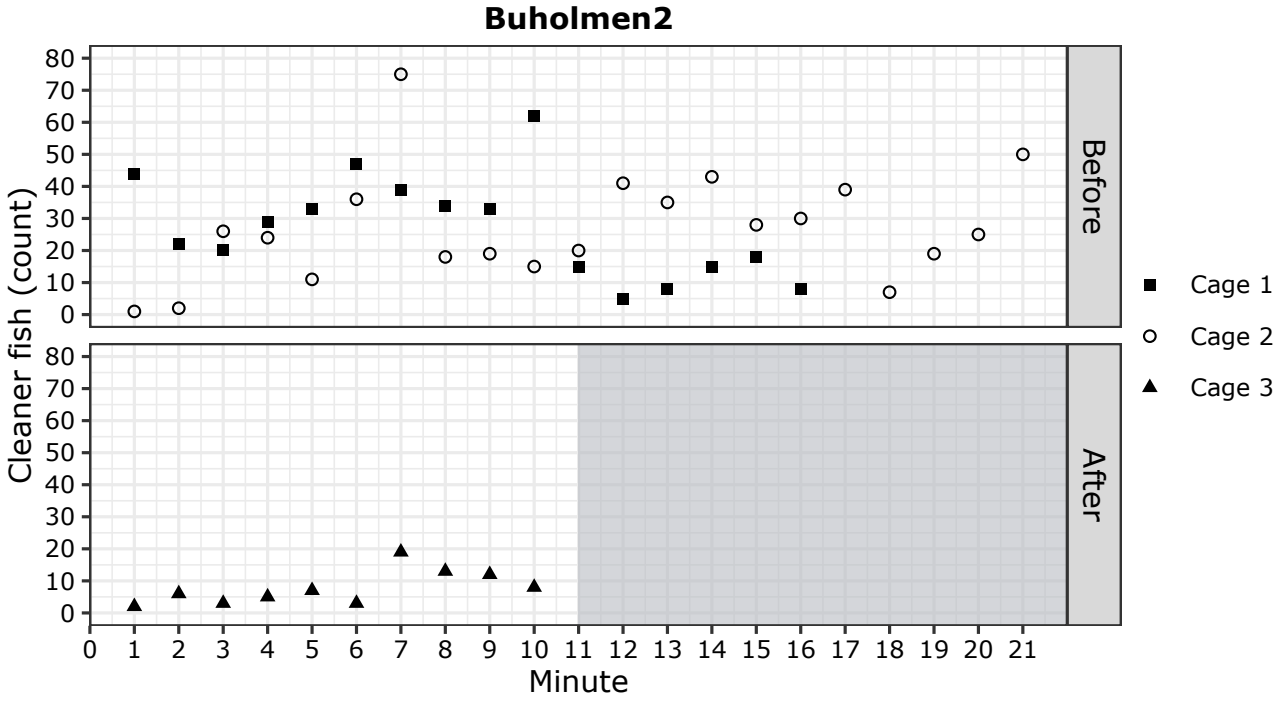


Figure B.3: Timeline of cleaner fish counted per minute filmed before and after (i.e., no fouling present on net wall) net cleaning was conducted at Buholmen2. Cages correspond with cages shown in farm layout in Table 2.1. Grey shadings indicate minutes not recorded.

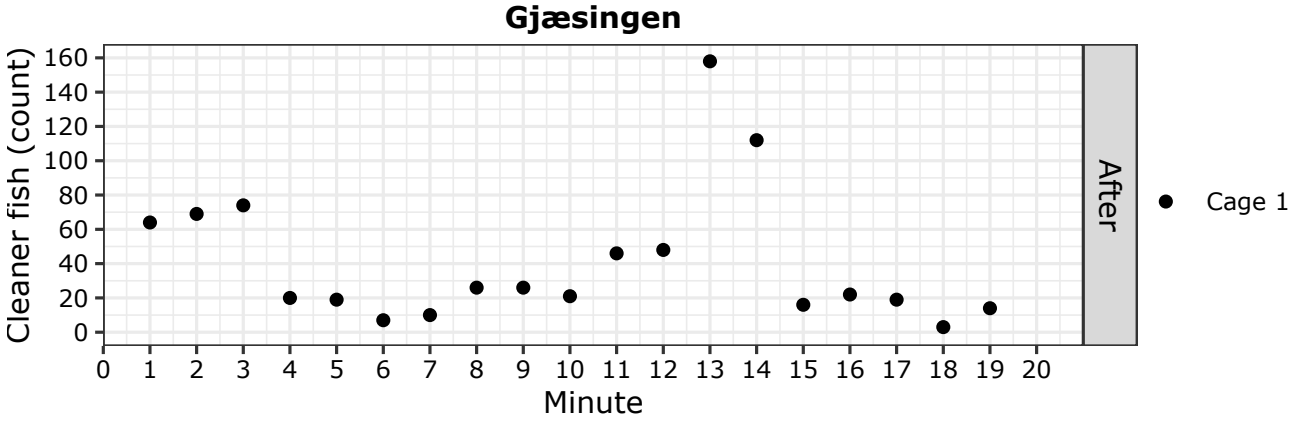


Figure B.4: Timeline of cleaner fish counted per minute filmed before and after (i.e., no fouling present on net wall) net cleaning was conducted at Gjæsingen. Cages correspond with cages shown in farm layout in Table 2.1.

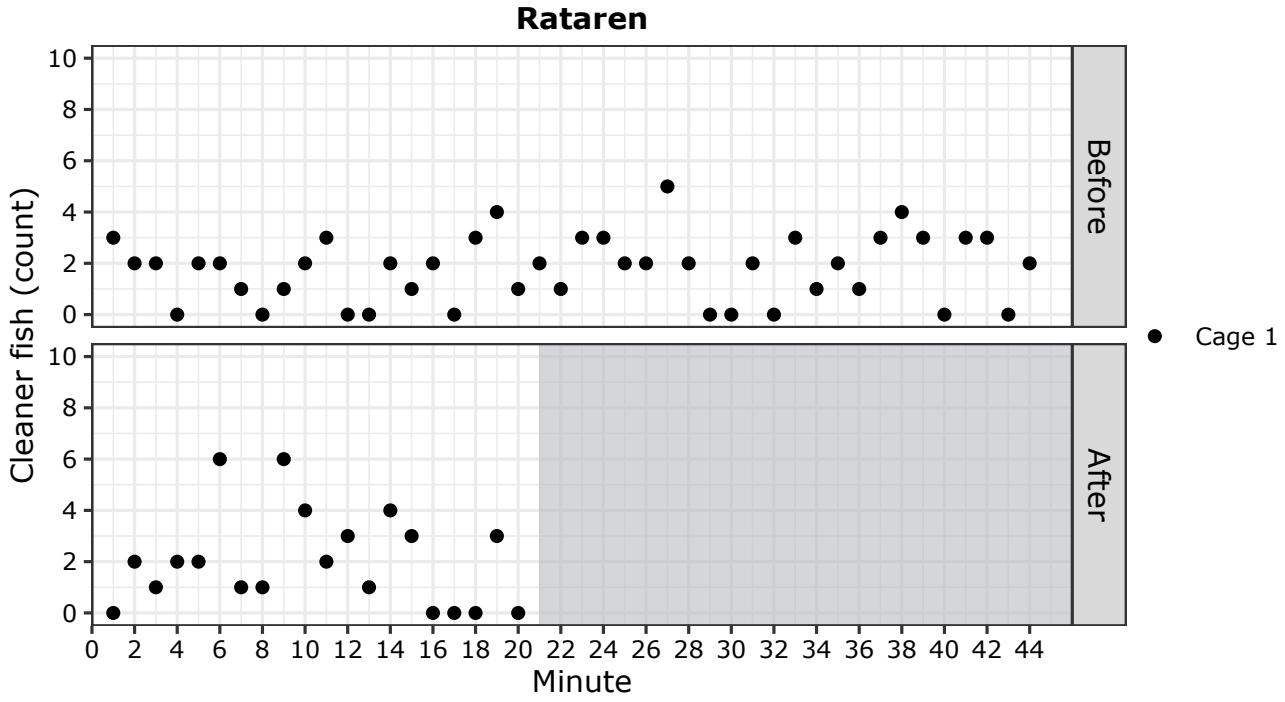
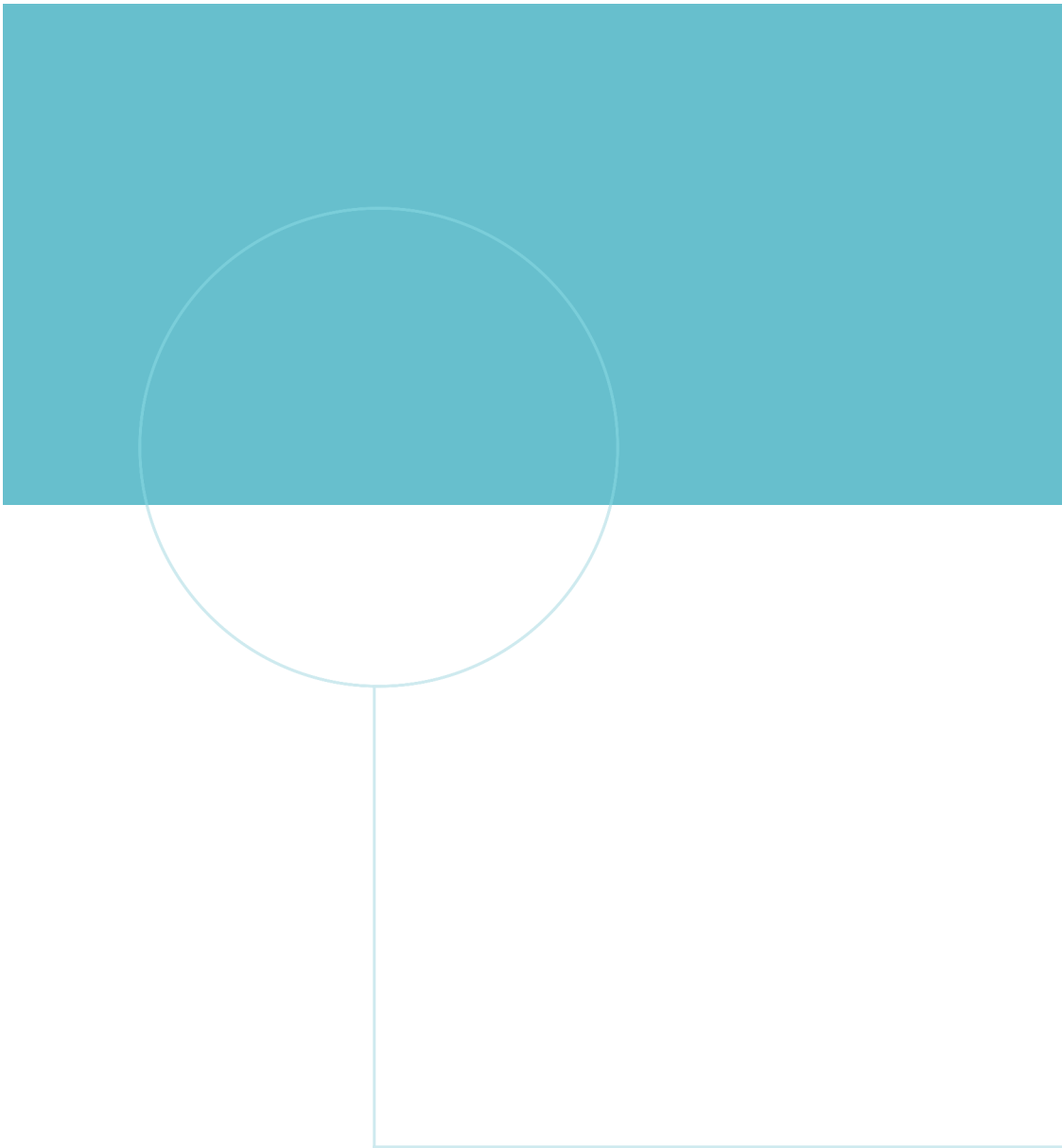


Figure B.5: Timeline of cleaner fish counted per minute filmed before and after (i.e., no fouling present on net wall) net cleaning was conducted at Rataren. Cages correspond with cages shown in farm layout in Table 2.1. Grey shadings indicate minutes not recorded.



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