UiT The Arctic University of Norway

Department of Arctic and Marine Biology

Mapping and assessing Eelgrass (*Zostera marina*) distribution and growth parameters in two Ramsar-sites

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Mapping and assessing Eelgrass (*Zostera marina*) distribution and growth parameters in two Ramsar-sites.

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Abstract

Eelgrass (*Zostera marina*) is a keystone species in coastal ecosystems that provides several crucial ecosystem services such as, habitat formation, carbon sequestration, and coastal protection. This thesis aims to map and assess the distribution of eelgrass and the performance parameters, eelgrass coverage, epiphyte coverage, and shoot height at two Ramsar sites: Sørkjosleira, and Kobbevågen in Balsfjord, Troms, Norway. A Blueye ROV was used to do the mapping. Additionally, this thesis aims to investigate if the eelgrass meadow at Sørkjosleira is negatively impacted by Tine dairy plant, by measuring nutrient concentrations. And to investigate if the eelgrass depth limit at Sørkjosleira is affected by the sediment grain size.

Results showed a reduction of eelgrass area at Sørkjosleira of 28.7% since 2009. At Kobbevågen there was a 2.8-fold increase in eelgrass area. Nutrient levels were higher at Sørkjosleira. This may be caused by comparatively more agriculture in the vicinity than at Kobbevågen. Eelgrass coverage and shoot height were lower at Sørkjosleira, but epiphyte coverage was higher compared to Kobbevågen. Grain size was similar above and below the depth limit of eelgrass, indicating that the depth limit is not affected by the physical substrate composition. The higher nutrient levels at Sørkjosleira may negatively affect eelgrass health.

Keywords: Eelgrass (*Zostera marina*) – Ramsar site – Eutrophication – Ecosystem monitoring – Dairy plant – Blueye

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August Kristiansen

Contents

1 Introduction

Seagrasses are important organisms in coastal marine ecosystems. They perform many ecological functions of critical importance. With examples such as carbon sequestration, provision of habitat and nursery grounds for many species, and strengthening resilience for coastal ecosystems against detrimental effects caused by climate change. As seagrass ecosystems face threats from anthropogenic influences and environmental change, monitoring and conservation efforts are of vital importance. This thesis aims to contribute to the monitoring efforts and quantified eelgrass (*Zostera marina)* abundance and shoot length at two protected marine areas. Furthermore, investigate whether there is an indication of detrimental effects on eelgrass caused by the dairy plant at one of the sites.

1. 1 Seagrass and its importance

Seagrasses are marine angiosperms, vascular flowering plants. They are a foundation species in coastal marine ecosystems, due to transforming the seabed and creating habitat that supports species that would not otherwise be present (Hughes et al., 2009). They accomplish this by increasing habitat heterogeneity, supplying multiple ecological niches for species that live attached to, associated with, and in the root system of seagrass. Due to this, they support the resilience (the ability of an ecosystem to recover from disturbances caused by environmental changes, extreme weather events and anthropogenic impacts) of the marine coastal realm, as resilience is dependent on biodiversity (Oliver et al., 2015). Seagrasses are also considered to be one of the most productive marine ecosystems in the world (Hemminga & Duarte, 2000).

Eelgrass (*Zostera marina*), the focus species of this thesis (Fig. 1), is one of the most abundant seagrass species, in the northern hemisphere it is the most widespread marine plant (Krause-Jensen et al., 2005). It can be found along the entire mainland coast of Norway, as large meadows with high density in southern Norway, and smaller meadows in northern Norway (Olsen et al., 2013; Dybsland et al., 2021). While the preferred habitat for eelgrass is sheltered and shallow coastal areas, it has a broad

Figure 1: Eelgrass (Zostera marina), Sørkjosleira (Kristiansen, 2023).

range of habitat, ranging from coarse sand in the more exposed regions of the coastline to fine grained mud in protected bays (Short & Coles, 2001). In-culture experiments that tested the effects of salinity and temperature showed that eelgrass performs similarly for parameters such as mortality, photosynthetic capacity, and growth across a salinity range of 10 - 35‰ (Nejrup & Pedersen, 2008). However, lower levels of salinity (2.5 and 5 ‰) have multiple negative effects for eelgrass, such as a 3-6-fold increased mortality, reduced photosynthetic capacity and reduced growth of 50-60% (Nejrup & Pedersen, 2008). Additionally, the preferred temperature range for eelgrass is broad, with low (0-5%) mortality in the temperature range of 5 to 20°C (Nejrup & Pedersen, 2008). The depth limit, the maximum depth at which eelgrass occurs, is one of the important eelgrass performance parameters. Depth limits are dependent on water quality and clarity, which is closely tied to turbidity and eutrophication levels (Krause-Jensen et al., 2005).

Eelgrass provides humans with many important ecosystem services (Waycott et al., 2009). These services range from the previously mentioned habitat building, and primary production that supports fisheries and ecological diversity, to other services such as nutrient cycling, which helps to improve water quality, and sediment stabilization to protect coasts from erosion. Globally it is estimated that seagrass ecosystems provide ecosystem services that are valued at 1.9 trillion dollars per year (Waycott et al., 2009).

1. 2 Threats and developments

Globally

The effects of climate change, such as ocean warming and sea level rise, can lead to several negative effects for seagrasses (Tang & Hadibarata 2022). First, ocean warming can have direct negative effects on seagrasses, as increasing temperatures are related to shoot mortality and reduced growth. It can also have indirect negative effects caused by the increased spread of invasive species which can have harmful effects for seagrass communities. Second, rising sea levels can cause negative effects through physical disturbance by increasing wave energy received by seagrass communities. With increasing sea levels more wave energy can propagate to the sheltered areas that seagrass inhabit, as deep-water waves are not slowed down by friction with the seabed, and proportionally more waves will be deep water waves with rising sea levels. Additionally, sea level rise will reduce light availability caused by increasing water depth, and although seagrass could follow higher on the shore, the substrate may not be favourable for such.

The global development of seagrass performance is concerning. Waycott et al. (2009) estimated a 29% decrease in areal extent of seagrass since 1879. A major cause of historical decline of eelgrass is the wasting disease, which occurred in the 1930s when it was estimated that 90% of eelgrass in the North Atlantic Ocean was wiped out (Muehlstein, 1989). Eelgrass meadows recovered in the years after, but in more recent decades has seen an increasing decline. The rate of loss has accelerated since 1990, before 1940 the areal loss per year was 0.9% but after 1990 the yearly loss was 7% (Waycott et al., 2009). Seagrass development in Europe from 1869 to 2016 was investigated across 737 sites in 25 different countries (Santos et al., 2019). They showed that eelgrass has lost 57% of its assessed area, but also that the rates of decline slowed down in recent decades, as well as some recovery since the 2000s.

Regionally

Regional threats to eelgrass are also related to anthropogenic impact. The input of nutrients may cause negative impacts on the performance of eelgrass. One big source of nutrient input into marine ecosystems comes from agricultural run-off. In Norway, for instance, 149,000 t of nitrogen and 20,400 t of phosphor was used as components of fertilizer used in agriculture in 2022 (Bjørlo, 2024). Industrial activities that discharge wastewater into marine ecosystems also contribute to pollution. The dairy industry is one of the biggest contributors and its wastewaters can elevate nitrogen and phosphor levels (Shete & Shinkar, 2013; Slavov, 2017). Elevated nutrient levels have both direct and indirect effects on the performance of eelgrass. An experimental study showed that nitrate enrichment in low water exchange environments leads to reduced eelgrass growth and survival unrelated to reduced light caused by algae (Burkholder et al., 1992). Another experimental study testing the response of morphological and physiological traits under nitrate enrichment at different light conditions showed that that the seagrass *Zostera noltii* has increased survival under nitrate enrichment at high light levels, but at low light levels the nitrate enrichment turns toxic (Jiménez-Ramos et al., 2022). Increasing nitrogen input leads to indirect negative effects for eelgrass caused by increased algal overgrowth that causes light-limitation of eelgrass (Hauxwell et al. 2003; Short & Burdick, 1996). Additionally, direct mechanical damage is also a threat to eelgrass meadows. An example of direct mechanical damage is bottom dredging, which leads to negative effects on seagrass communities through effects such as the direct removal of seagrasses and through increased sedimentation and turbidity (Erftemeijer & Lewis III, 2006).

Eelgrass have been mapped previously in the Troms County region in northern Norway. Historically it has been recorded at 24 different sites and 19 of these sites were revisited in a more recent mapping project from 2008 to 2011 (Jørgensen & Bekkby, 2013). This project showed that eelgrass at 8 of the earlier mapped locations had disappeared, but that eelgrass was also found at 7 new sites.

1.3 Monitoring and conservation

Due to the role of eelgrass as a foundation species supporting species diversity and the services it provides, monitoring and conservation efforts are important. Especially as a decrease in eelgrass can lead to declines in organisms associated with it (Hughes et al., 2009). Another important function of eelgrass that highlights the need for monitoring and conservation efforts is that eelgrass can be used as an indicator of water quality since the depth limit of eelgrass is largely regulated by water quality (Krause-Jensen et al., 2005). Water quality affects water clarity, and eelgrass grow deeper with better water clarity (Duarte, 1991). Restoration efforts of eelgrass can lead to an increase in ecosystem services provided, as observed in a large-scale project in the mid-western Atlantic where restoration of seagrass meadows lead to improvements in services such as carbon sequestration, biodiversity support, and revival of commercially valuable fishery (Orth et al., 2020).

The establishment of marine protected areas can contribute to conservation efforts by limiting what type of human activities are allowed. Both study sites in this thesis are protected areas, and additionally they are designated as Ramsar-sites. The Ramsar-convention, also known as the convention on wetlands, is an international agreement made with the purpose of conserving important wetland areas (Convention on Wetlands Secretariat, 2023).

1.4 Aims

Eelgrass has been mapped at several sites across Norway in relation to the national coastal ecosystem monitoring program “Økokyst” (Akvaplan-niva, 2022). This program seeks to document how costal ecosystems in Norway are responding to climate change, pollution, and eutrophication. This master thesis will contribute to this effort by mapping the eelgrass meadows in Sørkjosleira and Kobbevågen, two marine protected areas and Ramsar-sites in

Balsfjord, Troms County in accordance with the methodology used in Økokyst and described in the classification guide "Klassifiseringsveileder 02:2018" (Direktoratsgruppen vanndirektivet, 2018). The parameters being measured are, seagrass area, eelgrass coverage, epiphyte coverage, shoot height, and depth limits. The distribution of eelgrass at these sites were mapped in 2009 (Jørgensen & Bekkby, 2013), and this thesis investigates how the vertical eelgrass distribution has developed since. Additionally, this work seeks to provide insight to whether the dairy plant at Storsteinnes (town next to Sørkjosleira) has a negative effect on the eelgrass meadows due to nutrient discharge. This will be done by investigating if there is a nutrient gradient going from the dairy plant discharge point and through the eelgrass meadow, and to see if there is any corresponding change in eelgrass parameters. The nutrients measured are, nitrate, nitrite, and phosphate. Lastly, to determine whether eelgrass at this site can be used as a reliable indicator for water quality, this work seeks to answer if the depth limit of eelgrass at this site is affected by the physical characteristics of the substrate, specifically the sediment grain size. This work cannot establish any form of causality but can provide further insight into the questions stated above. The following objectives and hypotheses will be investigated:

1.5 Hypotheses

H.1: Eelgrass performance parameters are lower at Sørkjosleira than at Kobbevågen, except for epiphyte coverage which is higher at Sørkjosleira.

H.2: Nutrient levels and river input at Sørkjosleira are higher than at Kobbevågen.

H.3: Nutrient levels are positively correlated with epiphyte coverage, and negatively with eelgrass coverage and height.

H.4: Nutrient levels decrease with increasing distance from Tine dairy plant, with a corresponding decrease in epiphyte coverage, but an increase in eelgrass coverage and height.

H.5: There is a significant difference in grain size between depths where eelgrass is present and depths where eelgrass is absent.

2 Methods:

2.1 Study sites:

The survey sites are Sørkjosleira (69.23172 N, 19.26873 E) and Kobbevågen (69.49329 N, 18.85994 E) nature reserves in Balsfjord, Troms, northern Norway (Fig. 2). Sørkjosleira is in the bay where the small town Storsteinnes is. Tine dairy plant is located here, and there is widespread agriculture in the area that has runoff into rivers that empty into Sørkjosleira. Kobbevågen is an area in Balsfjord. This site will likely have lower nutrient input due to less agricultural and human activities.

Figure 2: Map of Norway. The red square inlet shows a part of Balsfjord in Troms northern Norway where the sites are located (Naturbase, 2023).

Although eelgrass distribution in these sites has previously been mapped (Jørgensen & Bekkby, 2013), these sites were chosen due to the county governors' interest in having them more thoroughly mapped in accordance with similar methodology as used in the more recent national ecosystem monitoring program: "Økokyst-delprogram Norskehavet Nord 2021".

2.2 Eelgrass survey:

The survey was done by using a Blueye underwater drone (hereafter ROV) operated from a boat from 14 to 28 August 2023. Video was recorded at several locations on one long transect going through the eelgrass meadow and parallel to the shoreline. From locations on that transect, more transects were surveyed going towards the coastline, ie. the upper growth boundary as well as towards the sea, ie. the lower growth boundary. Additional random locations across the meadow were also sampled. GPS-coordinates and time were registered at each location when eelgrass growth parameters were measured. Depth was registered at each location where the ROV was used, and depth measurements were adjusted relative to

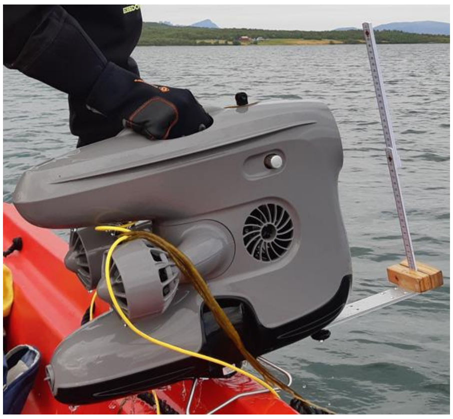


Figure 3: The Blueye ROV with the attached ruler.

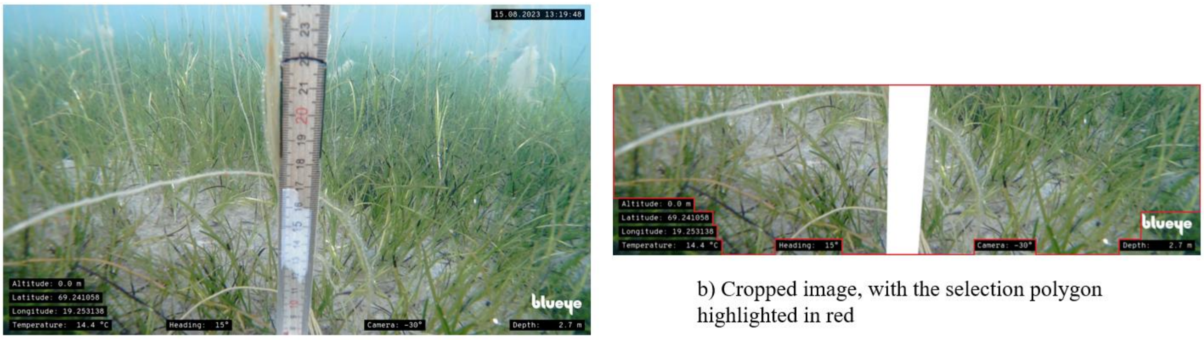
lowest astronomical tide. The total depth range sampled was from -1.2 to 12 m. The total amount of stations sampled for eelgrass in this thesis was 1027 (Appendix – Fig. 31 & 32

Survey times with a boat and the use of the ROV were limited to a 4-hour period ranging from two hours before to two hours after high tide. The reason for this limitation was to reduce possible disturbance of the wildlife using the area. The intertidal zone was surveyed on foot at low tide, from -1.2 m to 0 m depth. Photos were taken in intervals of less than 50 m. The upper growth boundary of the eelgrass meadow at Sørkjosleira was determined on foot in the field, GPS-coordinates of the upper growth boundary were recorded at intervals of less than 50 meters.

Stills were taken from the video recordings to be analysed for different eelgrass parameters such as percent coverage of eelgrass, percent coverage of epiphytes and shoot height. To measure shoot height, a ruler was attached to the bottom of the ROV (Fig. 3.). The ruler was perpendicular to the seabed (Fig. 4a.). Using the ROV inbuilt measure of altitude above the seabed in combination with the ruler allowed for estimations of plant height.

Analysis of ROV imagery was done both with a visual estimation of coverage parameters and by using the program ImageJ (version 1.54f). Two analysis approaches in ImageJ were tested on 30 images, as well as a visual estimation.

For the first approach, stills were cropped (parts of the image was cut out) to only include the bottom half (Fig. 4b.). This was done due to the upper half of the images often containing open water and eelgrass at longer distances away from the camera with different light conditions and colour compared to the bottom half of the images. Doing this ensured that the light conditions in the images were more homogeneous which improved the programs' ability to identify eelgrass, as the colour thresholding was not confused. Additionally, the ruler was cropped out, and a selection polygon that excluded the black boxes with ROV sensor information (coordinates, depth, temperature) was overlain (Fig. 4b.).

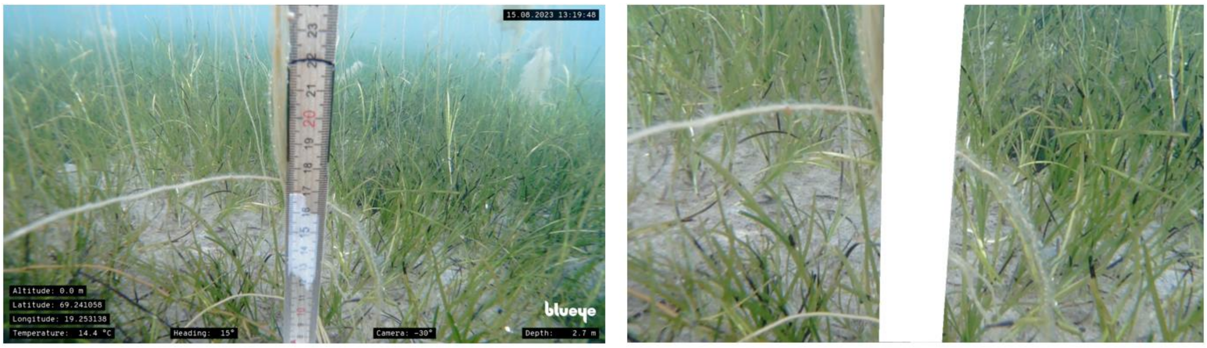


b) Cropped image, with the selection polygon highlighted in red

a) Original image

Figure 4: ROV Image. a) Original still from a video recording. b) Image after it has been cropped, with a selection polygon.

For the second approach, stills were cropped to only include a central square of the original image (Fig. 5b.). This was done for the same reason as for the first approach.

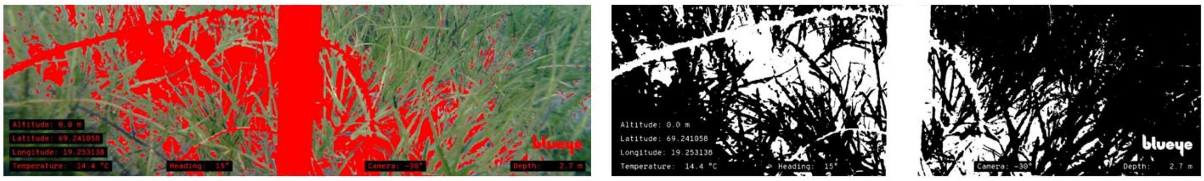


a) Original image

b) Cropped image

Figure 5: ROV Image. a) Original still from a video recording. b) Image after it has been cropped.

Following this, colour thresholding was used to mark eelgrass (Fig. 6a.) before the image were changed to binary (Fig. 6b.), where all marked eelgrass is black, and the rest of the image is white. This allowed for easy measurement of area fractions of black and white and gave percent coverage measurements of eelgrass. Percent coverage of epiphytes was estimated visually, due to the colour composition being very heterogeneous and colour thresholding not being able to accurately mark epiphytes. For the purposes of this thesis, the term ‘Epiphyte’ includes macroalgae.



a) Color thresholding applied b) Image converted to binary

Figure 6: Cropped ROV Image. a) Shows the mask applied with colour thresholding. b) Shows the image when converted to binary.

As a Shapiro-wilk test indicated that the data (percent coverage estimates) was not normally distributed (W = 0.96, p = 0.00), a Kruskal-Wallis rank sum test (chi-sq = 0.55, p = 0.90) was used which indicated no significant difference between both approaches in ImageJ, and the visual estimates (table 1). ImageJ approach one was chosen to be used for the analysis.

Table 1: Shows the difference in mean and standard deviation (SD) of eelgrass coverage between each analysis approach.

Method 1	**Method 2**	**Mean difference**	**SD difference**
ImageJ 1	Visual 1	0.52	9.11
ImageJ 2	Visual 2	1.68	8.41
ImageJ 1	ImageJ 2	-3.16	11.01

To test whether experience with adjusting colour thresholding settings led to different measures of eelgrass coverage throughout the analysis process, the first 20 images were analysed again, after all images had been analysed. As the normality assumption was not met, a Mann-Whitney U Test was done (W = 183, p = 0.65) which indicated no significant difference in eelgrass cover estimates occurred between the start and end of the analysis.

2.3 Nutrient samples:

Water samples to be used for measuring the concentration of nitrate, nitrite and phosphate were collected regularly throughout July and August at both sites, 8 times in total. Each sampling campaign at Sørkjosleira, I collected one sample at nine locations spread throughout the eelgrass meadows to be used for investigating if there was a nutrient gradient between distance from Tine dairy plant. Furthermore, one sample each was taken in the mouth of the two largest rivers, as well as one location halfway between the river mouths and eelgrass meadows (Fig. 7).

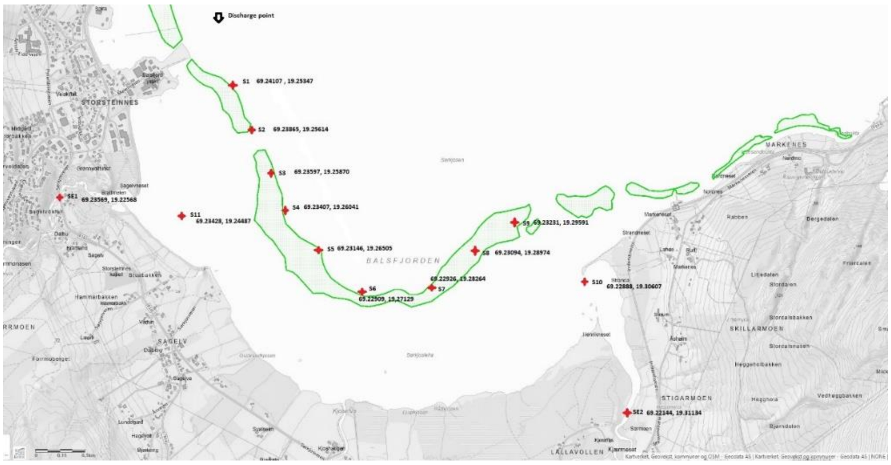


Figure 7: Map of Sørkjosleira, shows the water sampling stations in red and previously mapped eelgrass distribution in green.

At Kobbevågen, I collected 5 water samples spread throughout the eelgrass meadows and in the two rivers emptying into the site (Fig. 8). Samples were manually collected with 50 ml falcon tubes, and a kayak was used to get to the sampling locations. Water samples were filtered and frozen for storage before they were analysed in an autoanalyzer by a technician and measured for the concentrations of dissolved nitrite, nitrate, and phosphate. The concentration ranges suitable for the autoanalyzer was 0.006 – 8 µmol/L for phosphate, 0.03 – 4 µmol/L for nitrite, and 0.5 – 64 µmol/L for nitrate.

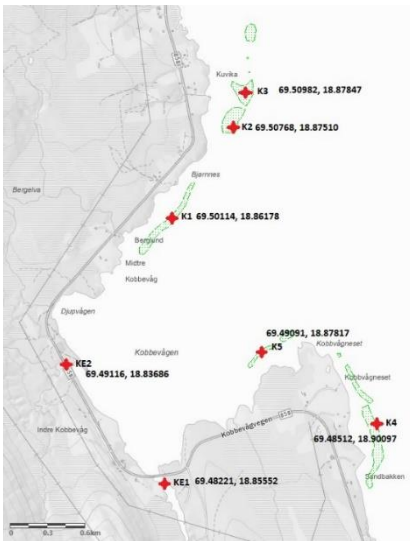


Figure 8: Map of Kobbevågen, shows the water sampling stations in red, and previously mapped eelgrass distribution in green.

2.4 Sediment samples:

At Sørkjosleira, 6 sediment samples were collected in each of 3 transects. Each transect started below the eelgrass depth limit and was directed towards the coastline to include the eelgrass depth limit and different depths within the eelgrass meadow. Sampling was done by a small (15x15cm) van veen grab-sampler from a boat. The samples were put in labelled plastic bags and brought to a lab at the university for analysis of grain size distributions.

To remove calcium carbonate and organic matter before grain size analysis, the samples had to be treated with the following procedure. A subsample of each sample was taken (approximately 60 g) and freeze dried to remove all moisture. Then a subsample of >2 g was taken out of each previous 60 g subsample and put in a test tube and treated with enough 20% HCl to cover the subsamples and left under a fume hood for 24 hours. Afterwards the subsamples were centrifuged for 4 minutes at 4000 rpm, and any liquid in the test tubes was discarded. To flush away remaining HCl from the samples, distilled water was then added to the test tubes and slightly shaken to ensure that the sediment was slightly mixed in the water before it was centrifuged again for 4 minutes at 4000rpm. This step was repeated twice.

Afterwards enough 20% H_2O_2 was added to the test tubes to cover the samples. Then the test tubes were covered with aluminium foil and placed in a hot water bath at 85 centigrade for 2 hours. Afterwards, test tubes were centrifuged for 4 minutes at 4000rpm, and any liquid in the test tubes was discarded. Distilled water was then added to the test tubes and slightly shaken to ensure that the sediment was slightly mixed in the water before it was centrifuged again for 4 minutes at 4000 rpm. This step was repeated twice.

Any remaining liquid were discarded, and the samples were transferred to small beakers using a spray bottle with distilled water. The samples were then air dried in a fume hood. Then a subsample of 0.2-0.5g were transferred to new beakers and added 20 ml distilled water before they were put on a shaker table for 24 hours. Afterwards, 2 drops of a dispersant (Calgon) were added to the samples before the samples were sonicated for 5 minutes. Samples were then analysed using a Laser Diffraction Particle Size Analyzer (Beckman Coulter LS 13 320). This measured the grain size distribution of the samples, and the average grain size of each sample was used for statistical analysis.

2.5 Mapping:

All maps were created in the open-source geographical information system QGIS (version 3.34.1). Point data were imported and used to define the distribution of eelgrass meadows at the study sites. Every point with percent coverage ≥ 10% was included in the distribution polygons, this was done to ensure compatibility with the monitoring program this thesis supplements where a meadow is defined as <10% eelgrass coverage (Økokyst).   A polygon is a two-dimensional shape used to mark the geographical positioning of a feature, in this case eelgrass distribution. The distribution polygons were filled with a colour gradient based on both eelgrass and epiphyte coverage measurements to better visualize how coverage changed across the spatial distribution of eelgrass.

2.6 Statistical analysis.

Every statistical analysis has been done in RStudio (2023.12.1 Build 402). The different assumptions (normality, heterogeneity, linearity, outliers, and homoscedasticity) to the statistical analyses used have been tested prior to each analysis. A Shapiro-wilk test was used to test for normality, a Levene's test was used to test for homoscedasticity. Welch Two-sample t-test have been used to compare eelgrass parameters between each site. Regression analysis, both linear and polynomial, were used to test for relationships between several variables: Nutrients vs. distance to the dairy plant discharge point, eelgrass, and epiphyte coverage vs. distance to the dairy plant discharge point, eelgrass, and epiphyte coverage vs. distance to Sagelva/Tømmerelva, eelgrass coverage vs. mean grain size, and eelgrass and epiphyte coverage as well as shoot height vs. water depth. Pearsons's correlation was used to test how strong correlations were between nutrient levels and eelgrass parameters. One-way ANOVA was used to investigate differences in nutrient input of selected rivers draining into the study sites. Two-way ANOVA was used to investigate how nutrient levels changed throughout the sampling period across both sites. If a significant difference was found, a Tukey post-hoc test was used.

3 Results

3.1 Eelgrass survey

Distribution

Sørkjosleira

There was a 28.7% reduction in eelgrass area since 2009 at Sørkjosleira (Fig. 9). In the previous mapping the eelgrass meadows area covered 290 525 m^2, now it covers 210 025 m^2. The general shape of the meadow remains similar. However, the western part of the meadow extends less towards the shore, but further towards the ocean. The eastern part of the meadow extends less towards both the shore and the ocean.

Figure 9: Map of Sørkjosleira showing the distribution of eelgrass. The red outline shows the 2023 distribution, the green outline shows the 2009 distribution. Scale in the bottom left, and north arrow in the top left corner. Satellite photo: Google earth.

Kobbevågen

There was a 2.8-fold increase in eelgrass area since 2009 at Kobbevågen (Fig. 10 & 11). The previous distribution had an area measurement of 102,862 m^2, while the new distribution has an area measurement of 292,392 m^2.

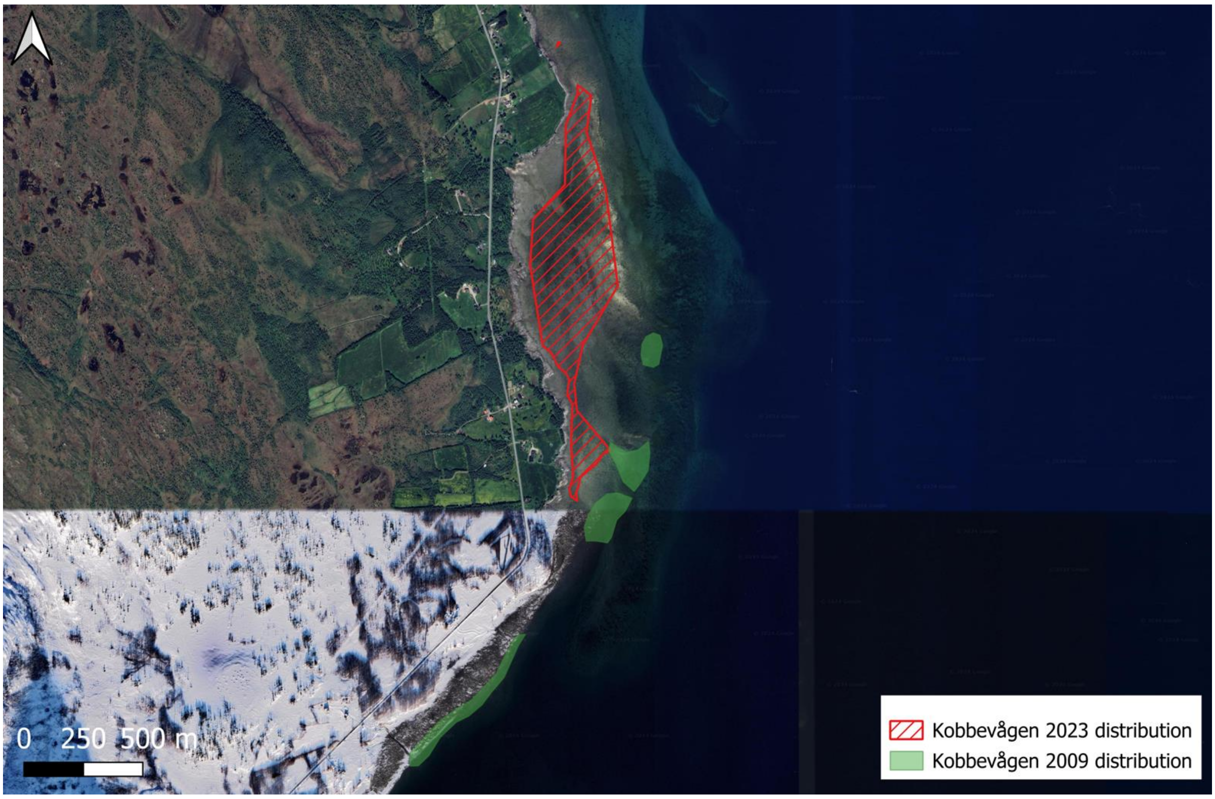


Figure 10: Map of the northern part of Kobbevågen showing the distribution of eelgrass. The red outline shows the 2023 distribution, the green outline shows the 2009 distribution. Scale in the bottom left, and north arrow in the top left corner. Satellite photo: Google earth.

No eelgrass above 10% coverage was found in the previously mapped areas of the northern part of Kobbevågen, but a large new patch that lies further in towards the coastline has been discovered (Fig 10.).

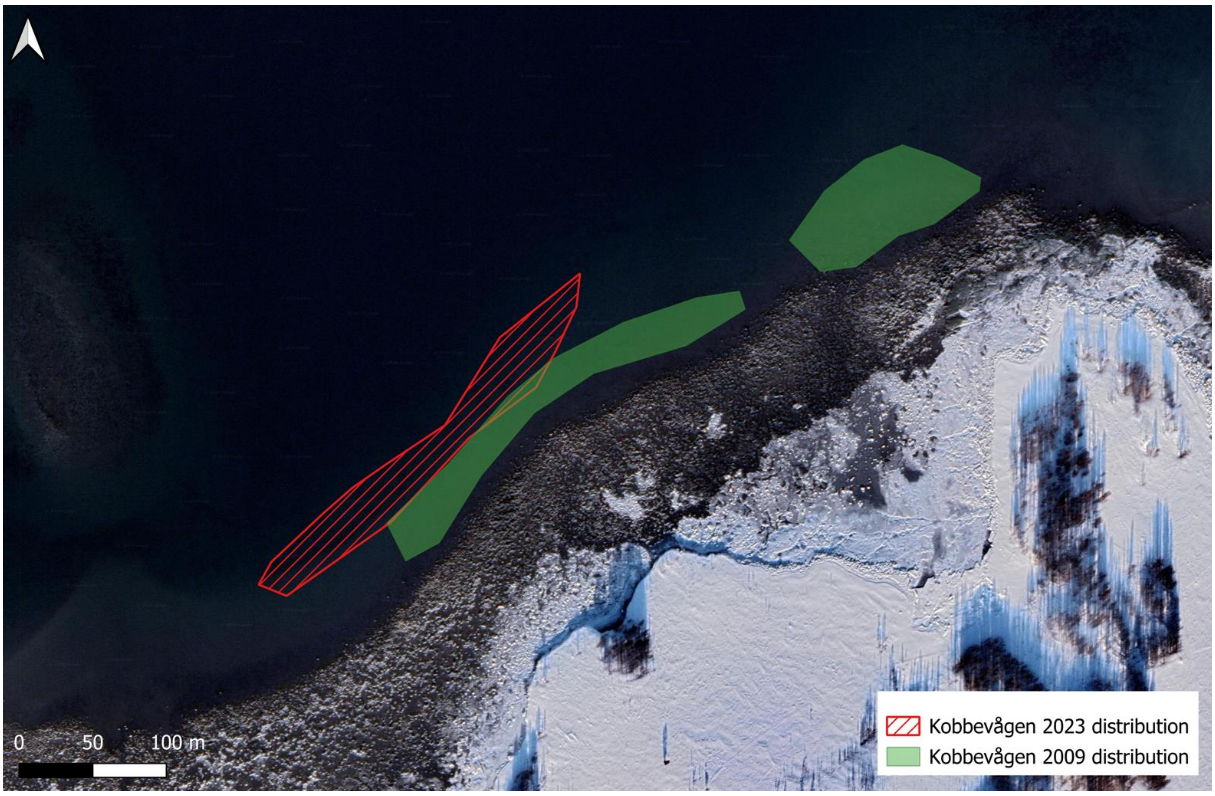


Figure 11: Map of the southern part of Kobbevågen showing the distribution of eelgrass. The red outline shows the 2023 distribution, the green outline shows the 2009 distribution. Scale in the bottom left, and north arrow in the top left corner. Satellite photo: Google earth.

In the southern part of Kobbevågen there is a 4.7% overlap between the previous and the current distribution of eelgrass. The eelgrass meadow in the southern part of Kobbevågen is now further away from the coastline compared to 2009.

Eelgrass performance parameters.

Sørkjosleira

Eelgrass coverage

The mean coverage of eelgrass at Sørkjosleira is 43% with a 95% confidence interval of 39.4% to 46.7%. The meadow has varying coverage across its spatial distribution (Fig 12.), with no apparent pattern.

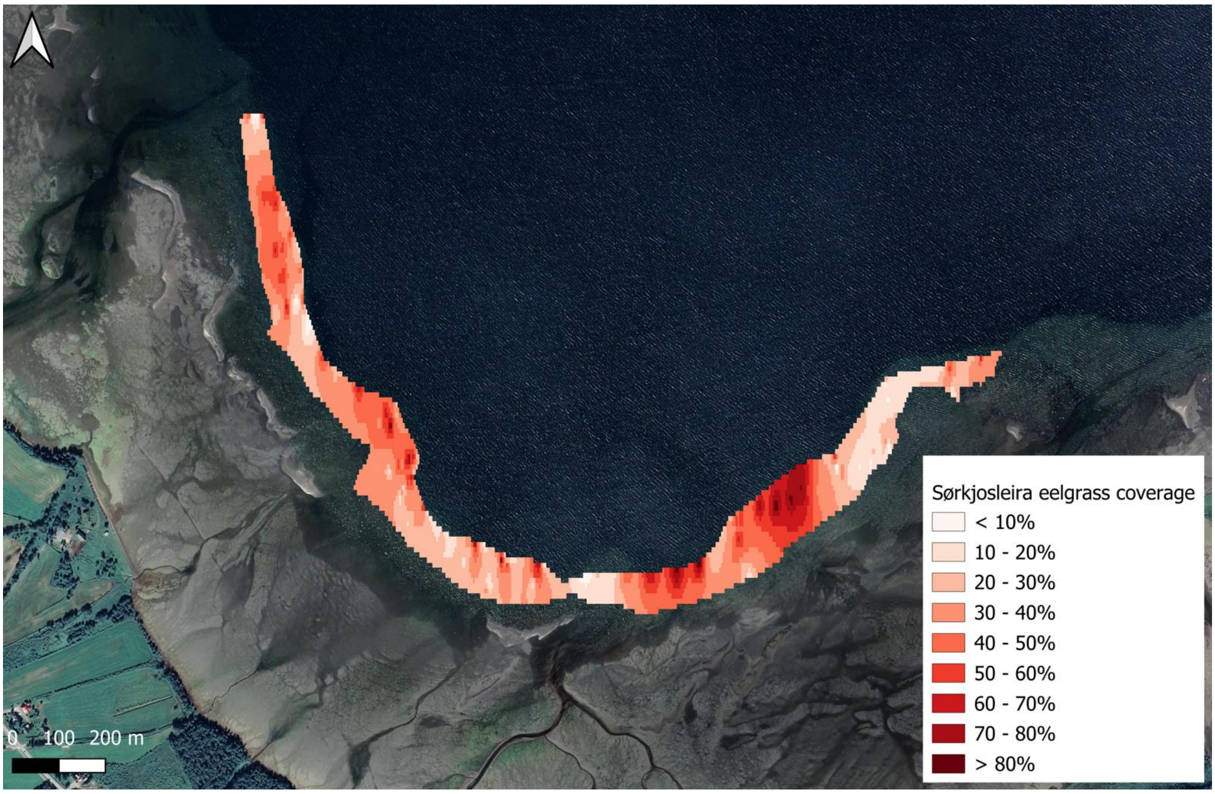


Figure 12: Map of Sørkjosleira showing eelgrass coverage across its spatial distribution. Scale in the bottom left, and north arrow in the top left corner. Satellite photo: Google earth.

Epiphyte coverage

The mean coverage of epiphytes on eelgrass at Sørkjosleira is 27%, with a 95% confidence interval of 22.6% to 31.4%. The meadow has varying coverage of epiphytes (Fig 13.), with a higher coverage of epiphytes on the eastern side.

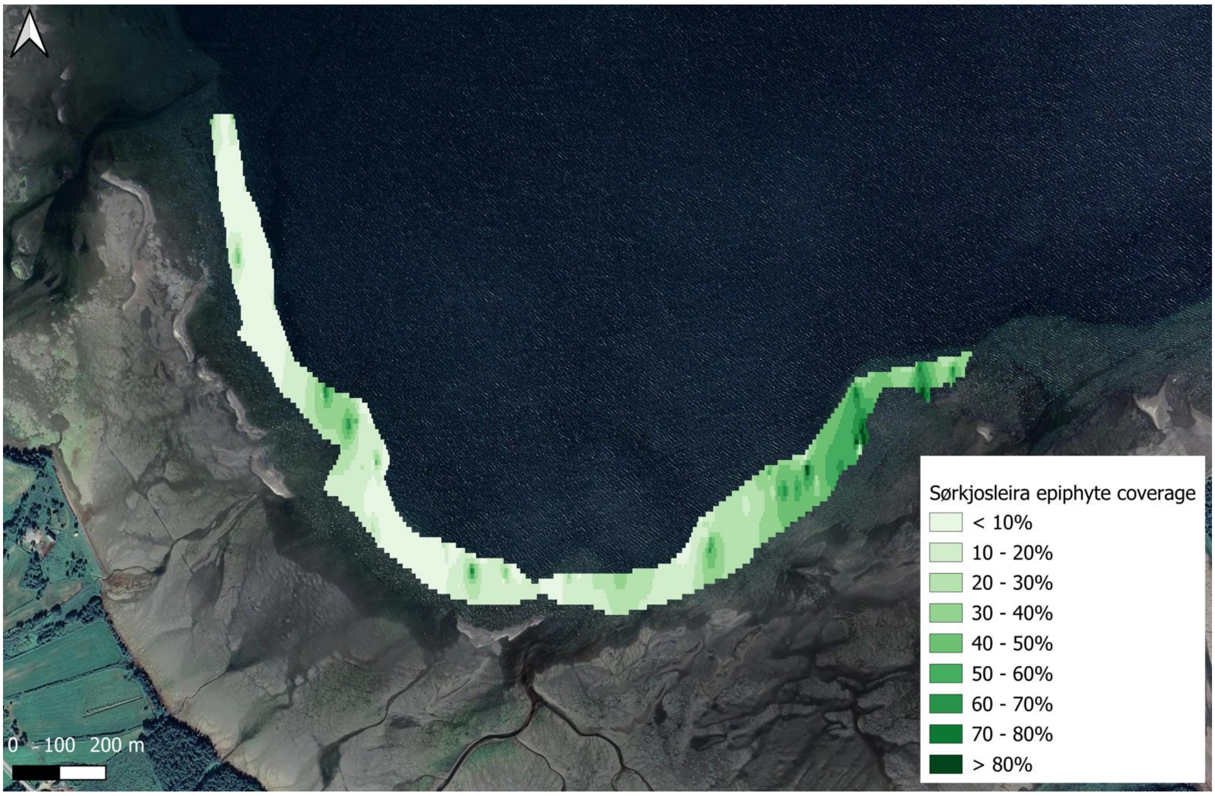


Figure 13: Map of Sørkjosleira showing epiphyte coverage on eelgrass shoots across the spatial distribution of eelgrass. Scale in the bottom left, and north arrow in the top left corner. Satellite photo: Google earth.

Height and growth boundaries.

The mean height of eelgrass at Sørkjosleira is 25.3 cm, with a 95% confidence interval of 22.7 to 27.9. The lower growth boundary for eelgrass at Sørkjosleira is at a water depth of 3.99 m, while the upper growth boundary is -1.2 m. As such, parts of the meadow are not submerged for at least some time at low tide.

Eelgrass parameters vary depending on depth (Fig 14.). Eelgrass coverage shows a unimodal relationship with depth, described by the equation $43.006 + 63.361x – 138.778x^2$ ($R^2 = 0.29$, $p = 8.3*10^{-12}$). Eelgrass coverage is lower in the shallowest regions where it is not submerged during low tide. It is highest at around 1 meter depth, and then decreases with increasing depth. Epiphyte coverage shows a negative unimodal relationship with depth, described by the equation $25.056 - 130.704x + 37.672x^2$ ($R^2 = 0.16$, $p = 5.3*10^{-6}$). Epiphyte coverage decreases with increasing depth. Eelgrass height shows an almost linear relationship, described by the equation $25.3142 + 109.1304x – 10.7302x^2$ ($R^2 = 0.45$, $p = 4.4*10^{-13}$). The length of eelgrass shoots increases, on average, with water depth.

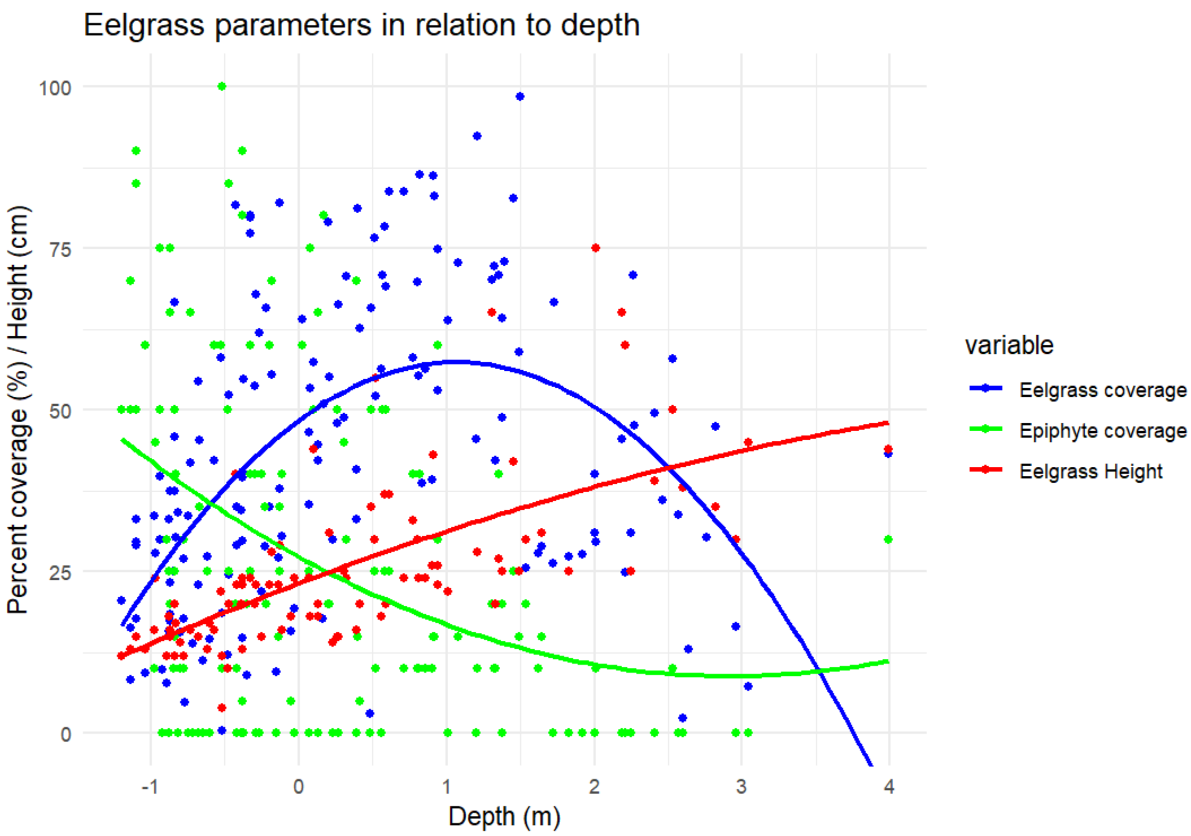


Figure 14: Shows the relationship between depth and eelgrass coverage, epiphyte coverage, and height at Sørkjosleira.

Kobbevågen

Eelgrass coverage

The mean coverage of eelgrass at Kobbevågen is 52%, with a 95% confidence interval of 44.3% to 59.7%. The meadows have varying coverage across their spatial distribution (Fig 15. & 16.), with no apparent pattern.

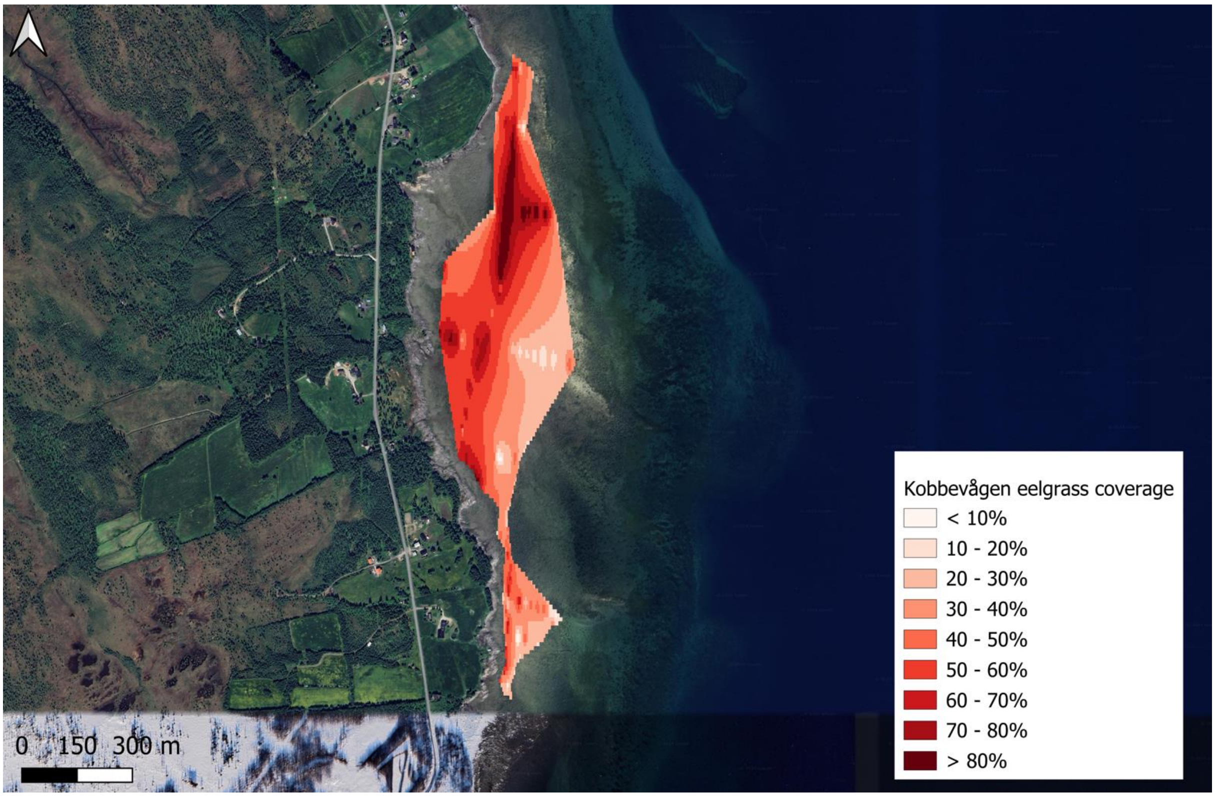


Figure 15: Map of the northern part of Kobbevågen showing eelgrass coverage across its spatial distribution. Scale in the bottom left, and north arrow in the top left corner. Satellite photo: Google earth.

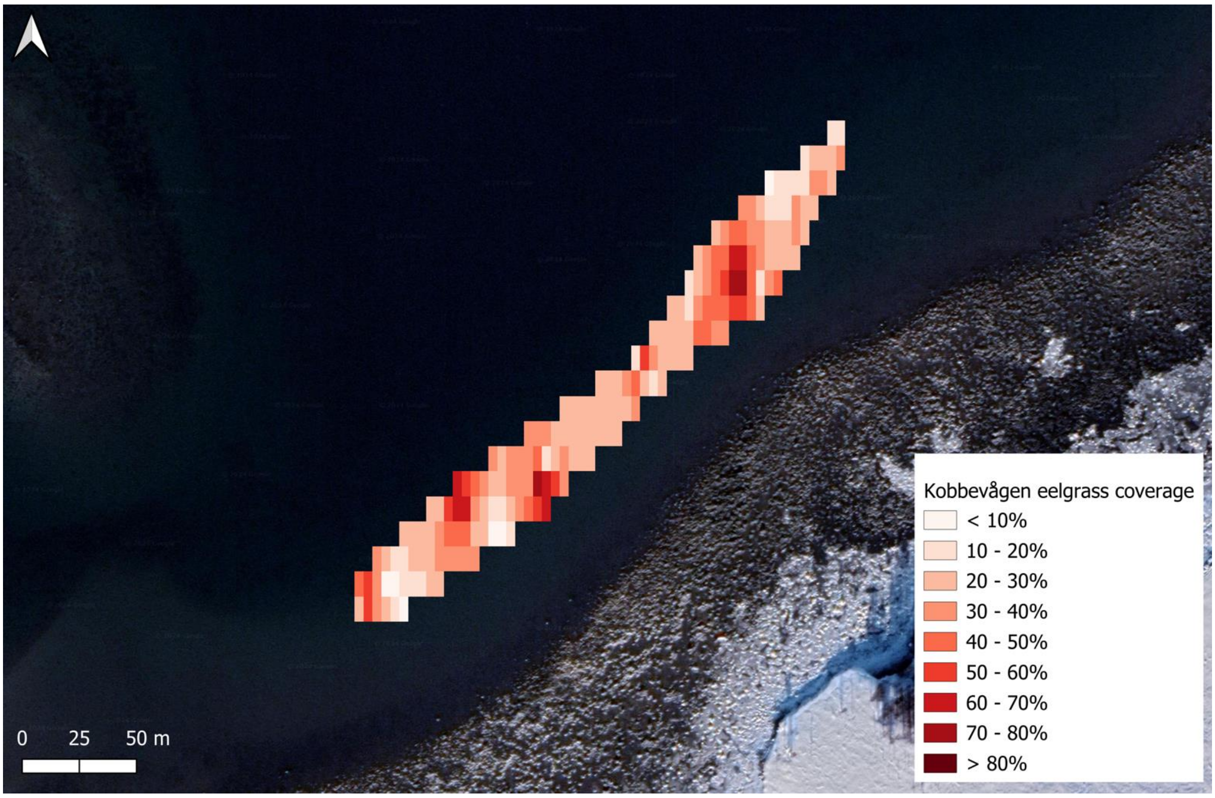


Figure 16: Map of the southern part of Kobbevågen showing eelgrass coverage across its spatial distribution. Scale in the bottom left, and north arrow in the top left corner. Satellite photo: Google earth.

Epiphyte coverage

Epiphytes cover, on average, 10.4 % of individual seagrass shoot surfaces at Kobbevågen, with a 95% confidence interval of 7.8% to 13%. There is no apparent pattern in the amount of epiphyte coverage across the eelgrass meadow at Kobbevågen (Fig. 17).

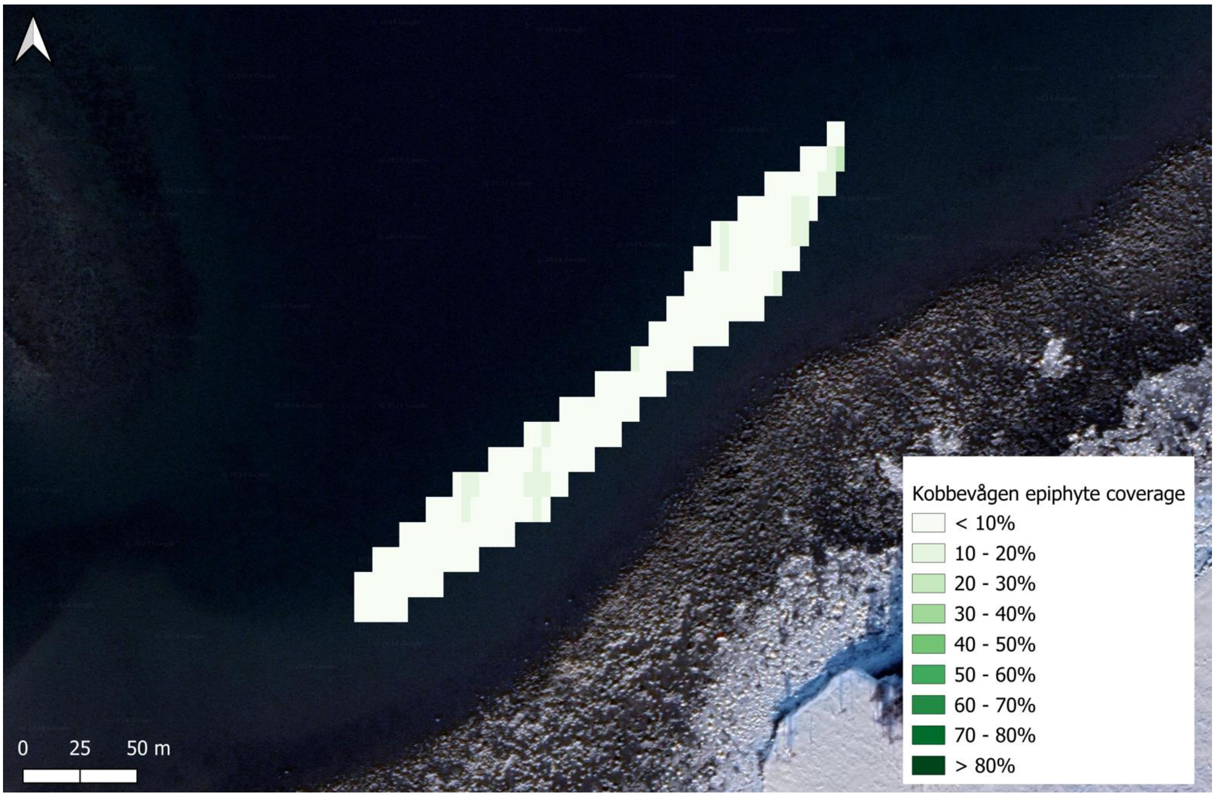


Figure 17: Map of the southern part of Kobbevågen showing epiphyte coverage across the spatial distribution of eelgrass. Scale in the bottom left, and north arrow in the top left corner. Satellite photo: Google earth.

Height and growth boundaries.

The mean height of eelgrass at Kobbevågen is 30.2 cm, with a 95% confidence interval of 27.6 to 32.9. The lower growth boundary for eelgrass at Kobbevågen is 1.29 m, while the upper growth boundary is -0.97 m.

Some eelgrass parameters in Kobbevågen change depending on depth (Fig 18.). Eelgrass shows a unimodal relationship with depth, described by the equation 51.997 – 23.92x + 102.819x^2 (-R^2 = 0.41, p = 2.3*10^{-5}). Eelgrass coverage is low in the intertidal. It has a peak at around 0.25 m water depth and then decreases with increasing depth. Epiphyte coverage appears to be relatively constant across water depth (R^2 = 0.04, p = 0.43). Eelgrass height shows a unimodal relationship, described by the equation $30.357 + 30.003x - 26.791x^2$ (R^2 = 0.26, p = 0.01).

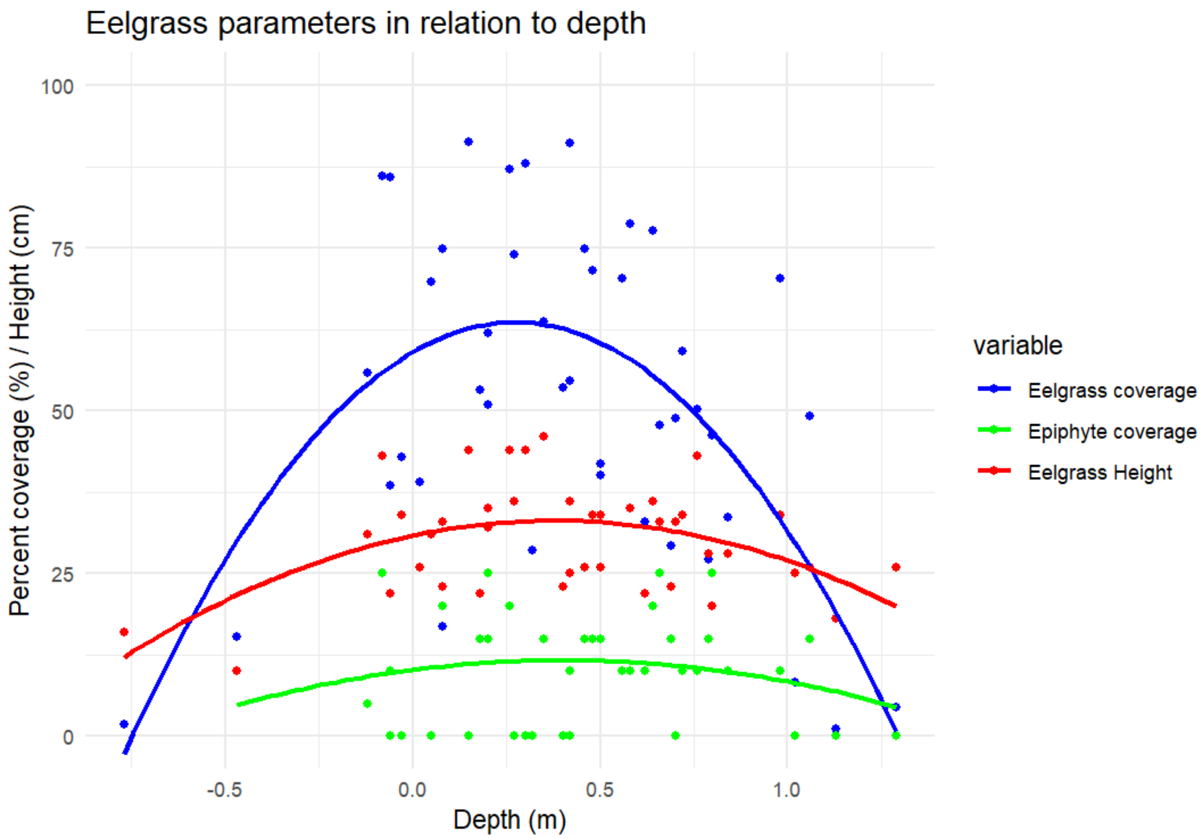


Figure 18: Shows the relationship between depth and each of the three response variables eelgrass coverage, epiphyte coverage, and height at Kobbevågen.

Comparison of eelgrass performance parameters between both study sites.

There are differences in performance parameters between both sites (Fig 19.). Eelgrass coverage is significantly different (Welch two-sample t-test: $t_{(64.7)} = -2.12$, p = 0.03), The meadow at Sørkjosleira, are, on average, 20.9% less covered with eelgrass than at Kobbevågen. Epiphyte coverage of individual eelgrass shoots is also significantly different between both study sites (Welch two-sample t-test: $t_{(182.2)} = 6.47$, p = $8.6*10^{-10}$), with, on average, 61.48% more cover at Sørkjosleira than Kobbevågen. Additionally, height of eelgrass shoots is significantly different between both study sites (Welch two-sample t-test: $t_{(113.1)} = -2.69$, p = 0.00), with eelgrass shoots being, on average, 17.5% shorter at Sørkjosleira. The depth range of where eelgrass occurs at Kobbevågen is less than half compared to Sørkjosleira. Eelgrass was encountered both higher and lower on the shore at Sørkjosleira, and the depth limit was 3.1 times larger than at Kobbevågen. While the shape of the relationship between water depth and eelgrass cover is similar between both sites, there are differences for the other parameters. Epiphyte coverage at Sørkjosleira was decreasing with increasing depth, while at Kobbevågen epiphyte coverage was relatively constant with changing depth. Eelgrass height at Sørkjosleira was increasing with increasing water depth, while at Kobbevågen it showed a unimodal pattern.

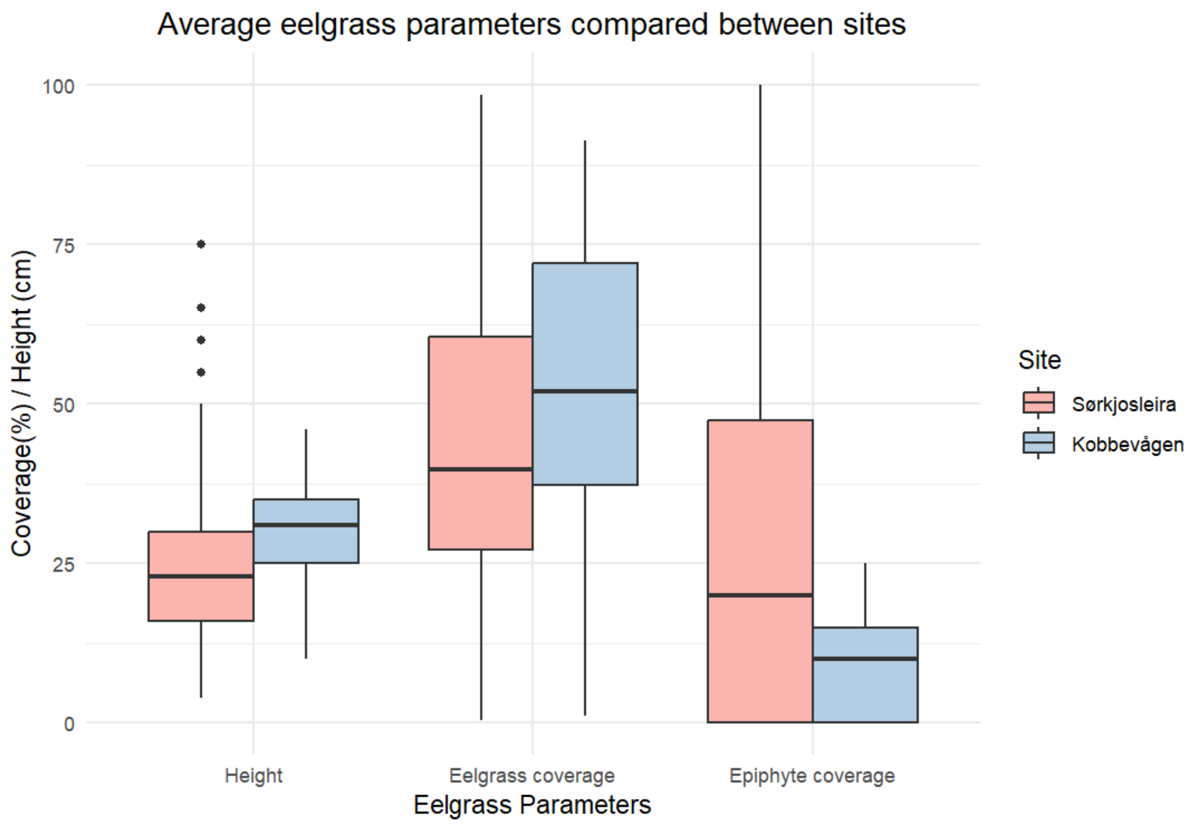


Figure 19: Shows a comparison in the averages of eelgrass coverage, epiphyte coverage, and eelgrass height between Sørkjosleira and Kobbevågen.

3.2 Nutrient survey.

Changes in nitrate concentration during the sampling period.

At Sørkjosleira, nitrate concentrations ranged from 0.0523 to 0.346 µmol/L (mean 0.154 µmol/L) during the sampling period, while at Kobbevågen it ranged from 0.0497 to 0.211 µmol/L with a mean of 0.115 µmol/L (Fig. 20). There is a significant difference in nitrate concentration between both sites (two-factorial ANOVA, $F_{(1,94)}$ = 14.544, p = 0.00), with 34 % higher concentrations at Sørkjosleira than Kobbevågen. Additionally, significant variation in nitrate concentration across the sampling period was observed (two-factorial ANOVA, $F_{(7,94)}$ = 6.506, p = $2.9*10^{-6}$). Missing of a significant time x site interaction (two-factorial

ANOVA, $F_{(7,94)} = 1.329$, $p = 0.24$) shows that the variation in nitrate concentration between both sites was similar throughout the sampling period. No significant differences in phosphate and nitrite concentration between the sites was observed (Appendix – Fig. 33 & 34).

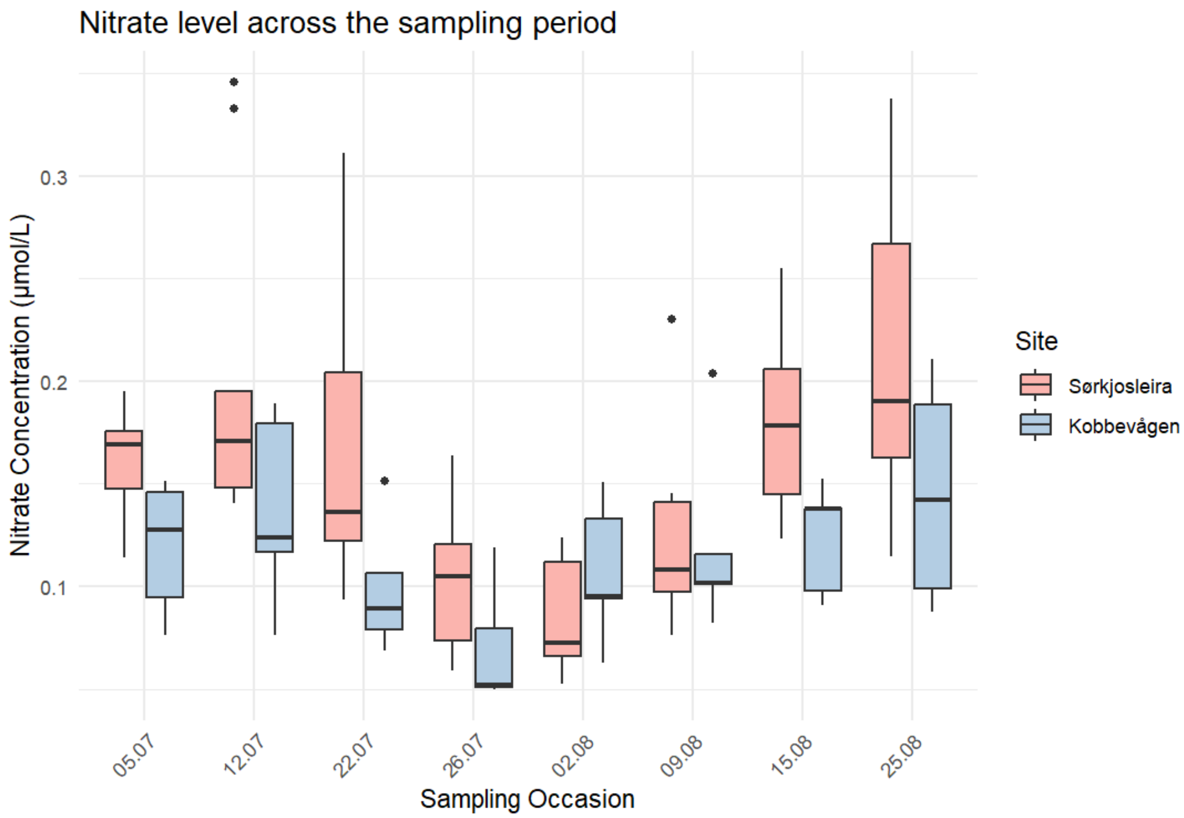


Figure 20: Shows how nitrate levels changes for both Sørkjosleira and Kobbevågen across the entire sampling period.

Nutrient correlation

A correlation test between nutrient levels and performance parameters (Fig. 21.), showed that none of the relationships between nutrient levels and performance parameters are statistically significant ($p>0.05$).

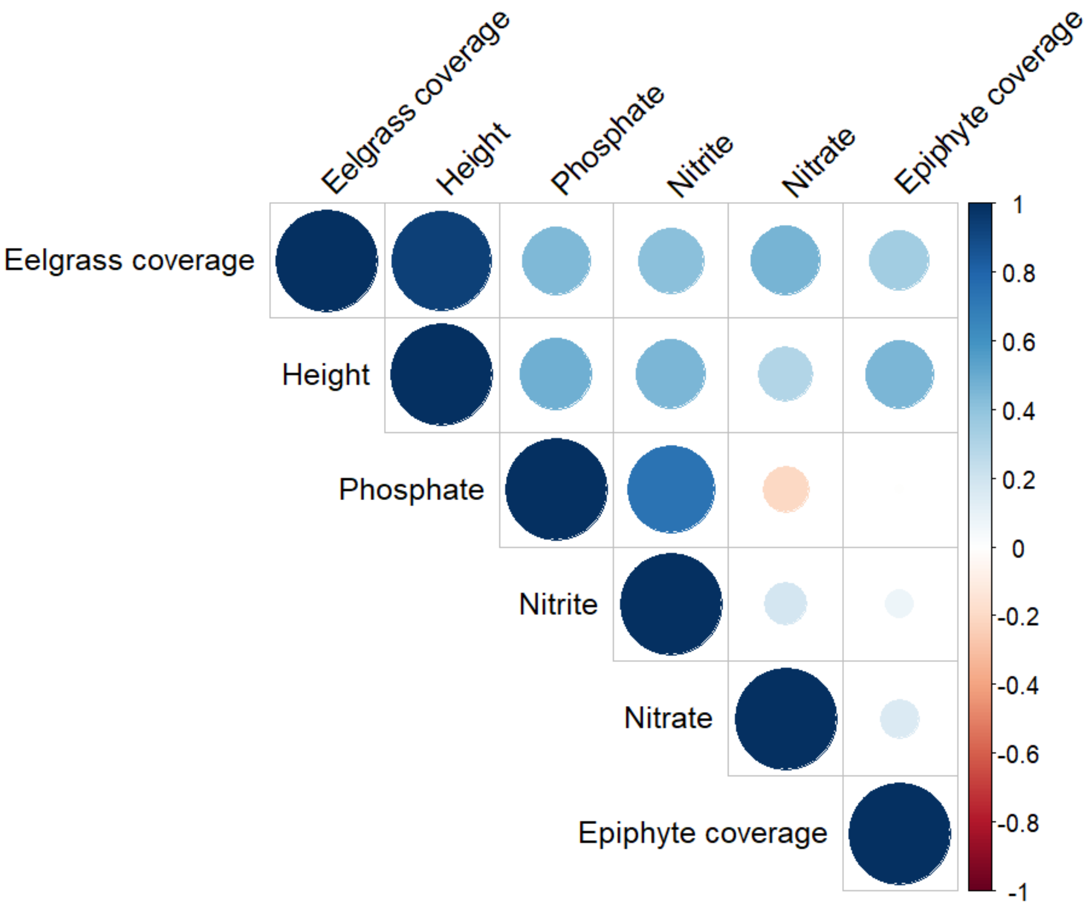


Figure 21: Correlation matrix. Correlation coefficients between two variables are represented in each cell by coloured circles, where size indicates the magnitude of the correlation and colour indicates both magnitude and direction of the correlation.

Correlation coefficients between the relationships of interest can be seen in table 2. There is a moderate positive correlation between phosphate concentration and both eelgrass coverage (0.44) and shoot height (0.49), and negligible positive correlation between phosphate and epiphyte coverage (0.01). Similarly, nitrate concentration was positively correlated with eelgrass coverage (0.47), shoot height (0.29), and epiphyte coverage (0.15). Nitrite showed a positive moderate correlation with eelgrass coverage (0.42) and shoot height (0.46), and a low positive correlation with epiphyte coverage (0.08).

Table 2: Shows the correlation coefficients of the relationships between eelgrass performance parameters and nutrient levels.

	Eelgrass coverage	Epiphyte coverage	Height

Phosphate	0.44	0.01	0.49
Nitrate	0.47	0.15	0.29
Nitrite	0.42	0.08	0.46

River input.

Nitrate concentration in the rivers entering Sørkjosleira ranged from 0.66 to 1.98 µmol/L and at Kobbevågen the range was from 0.08 to 0.38 µmol/L. There is a significant difference in nitrate input from different rivers (one-way ANOVA, $F_{(3,32)} = 55.13$, $p = 9.7*10^{-13}$, Fig. 22). While nitrate concentration was not significantly different between Indreelva and Ytterelva (both draining into Kobbevågen), the nitrate concentration of Sagelva and Tømmerelva was significantly higher, compared to the average nitrate concentration of Indreelva and Ytterelva. The average nitrate levels from rivers draining into Sørkjosleira was 1.23 µmol/L, which is 5.86 times more than the average nitrate levels in the rivers draining into Kobbevågen which was 0.21 µmol/L. Phosphate concentrations differed between the rivers. Indreelva had significantly higher phosphate concentrations compared to the other rivers (Appendix – Fig. 35). No significant difference in nitrite concentrations between different rivers were observed (Appendix – Fig.36).

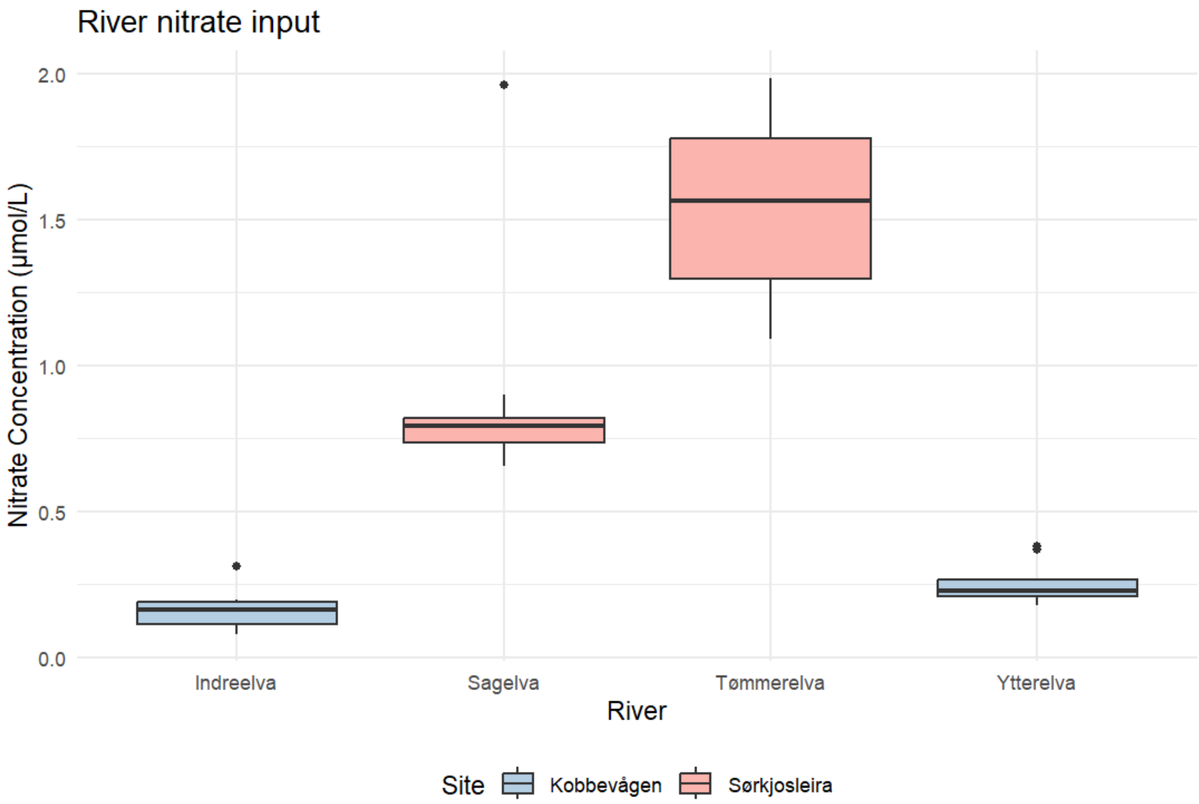


Figure 22: Shows the river nitrate input for all rivers sampled.

3.3 Is the eelgrass meadow at Sørkjosleira affected by Tine?

There is no significant relationship between nitrate concetration in water samples taken at different distances from Tine discharge point (linear regression: $F = 0.87$, $p = 0.35$, Fig. 23). There is also no significant relationship between phosphate and nitrite concentration in water samples taken at different distances from Tine discharge point (Appendix – Fig. 37 & 38).

Figure 23: Nitrate concentration at different distances from Tine dairy plant discharge point (= distance 0m).

There is no significant relationship between eelgrass coverage and distance to Tine discharge point (linear regression: $F = 0.60$, $R^2 = 0.00$, $p = 0.44$, Fig. 24)).

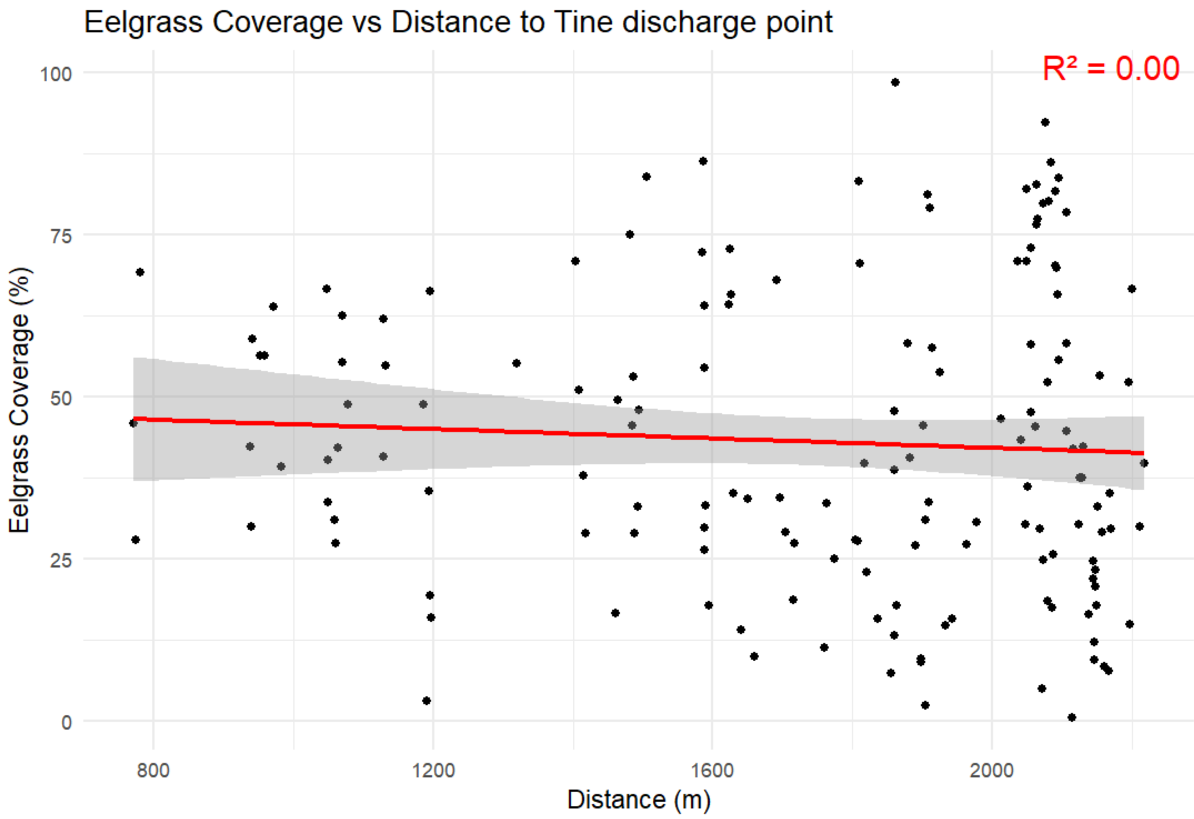


Figure 24 Shows how eelgrass coverage changes with distance to Tine dairy plant discharge point.

Epiphyte coverage shows a significant negative unimodal relationship with distance to Tine dairy plant discharge point (polynomial regression: $F = 31.52$, $p = 5*10^{-12}$, $R^2 = 0.31$, Fig. 25). The relationship is described by the equation $27.08 + 144.8x + 106.6x^2$. This indicates that the relationship changes with increasing distance. Initially, epiphyte coverage decreases with distance, before it increases.

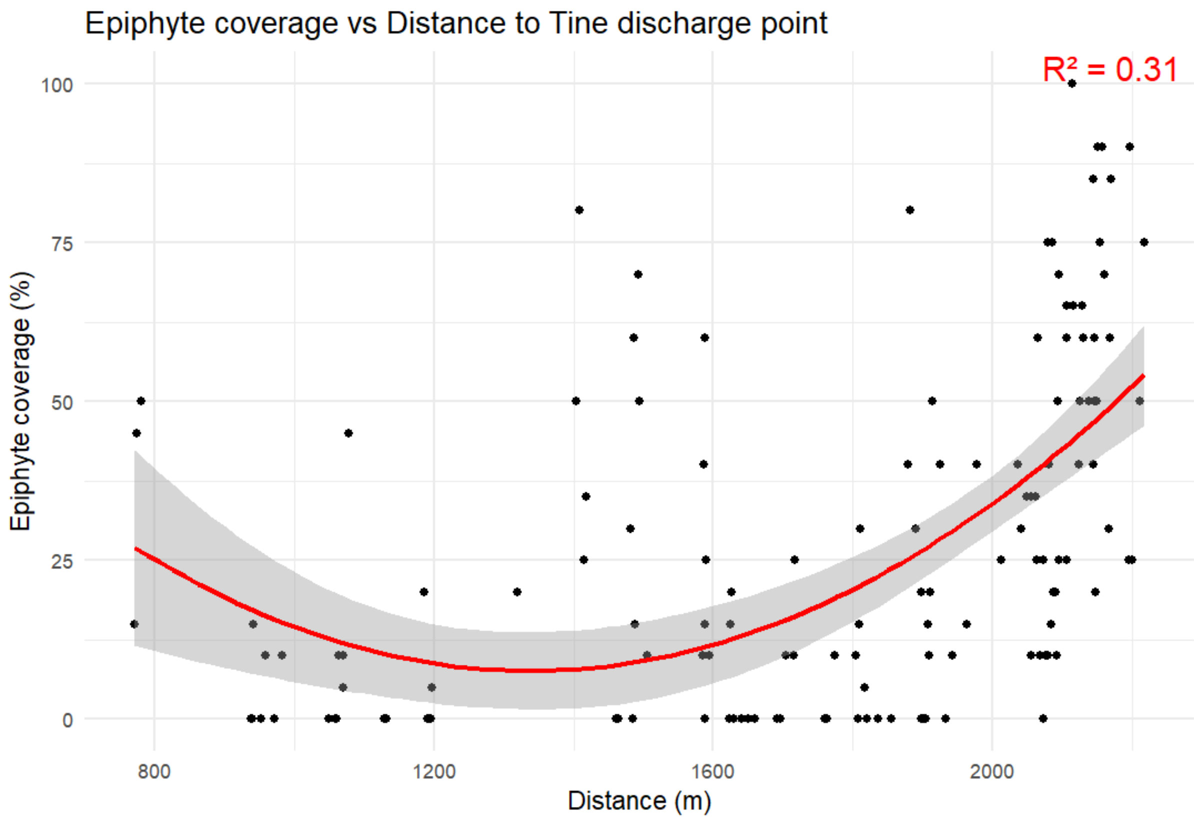


Figure 25: Shows how epiphyte coverage changes with distance to Tine discharge point.

Sagelva

There is no linear relationship between eelgrass coverage and distance to Sagelva (linear regression: F = 0.27, R^2 = 0.00, p = 0.6, Fig. 26).

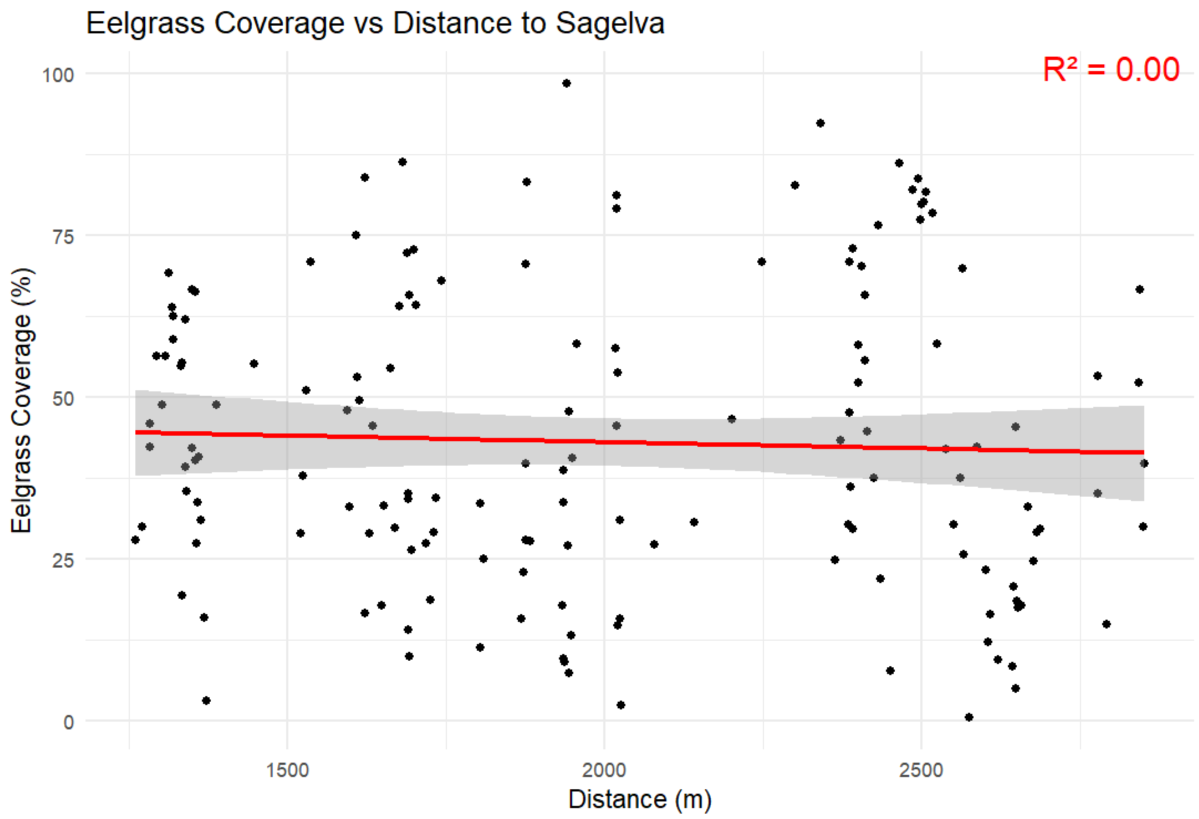


Figure 26: Shows how eelgrass coverage changes with distance to Sagelva.

Using linear regression, a significant positive relationship between epiphyte coverage and distance to Sagelva was determined (F-statistic = 66.71, $R^2 = 0.32$, $p<0.05$, Fig. 27.). The relationship is described by this equation -34.36 + 0.03x. Epiphyte cover increased with 3% per 100-meter increasing distance from Sagelva.

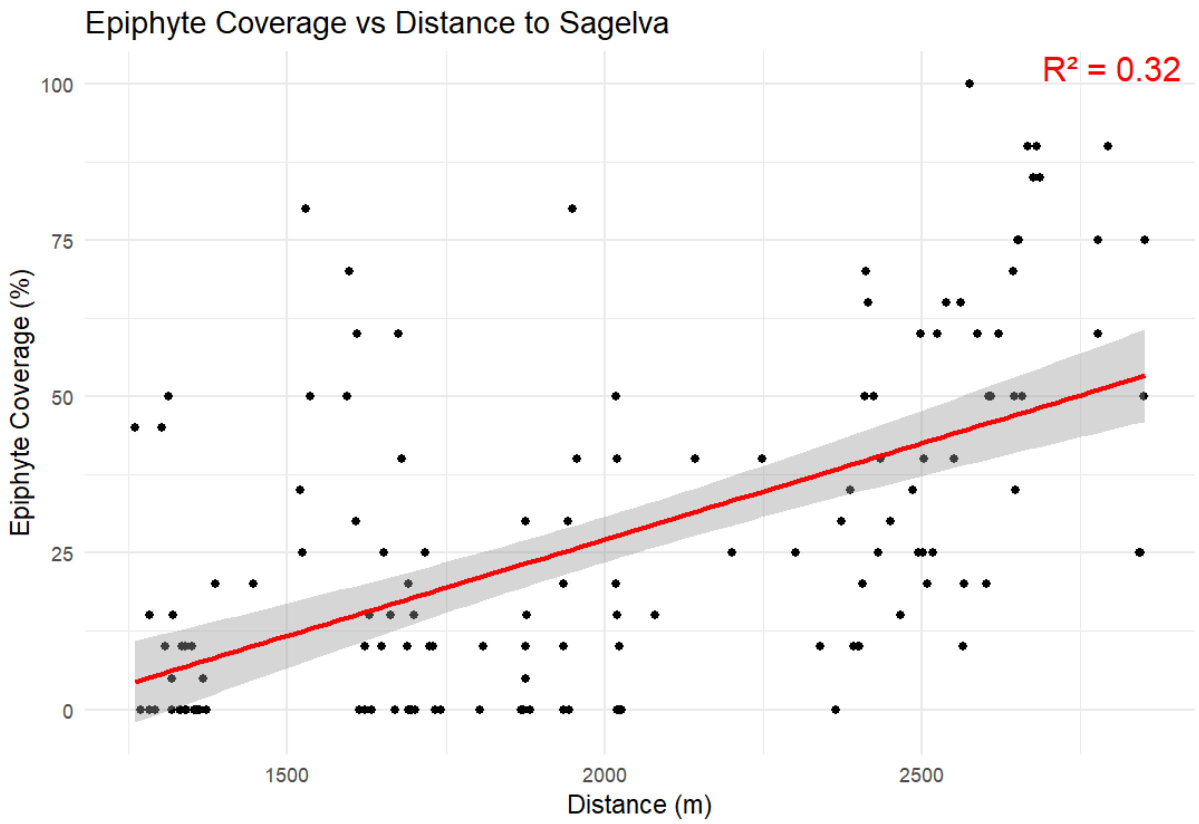


Figure 27: Shows how epiphyte coverage changes with distance to Sagelva.

Tømmerelva

Linear regression showed no relationship between eelgrass coverage and distance from Tømmerelva (F-statistic = 0.16, R^2 = 0.00, p = 0.69, Fig. 28.)

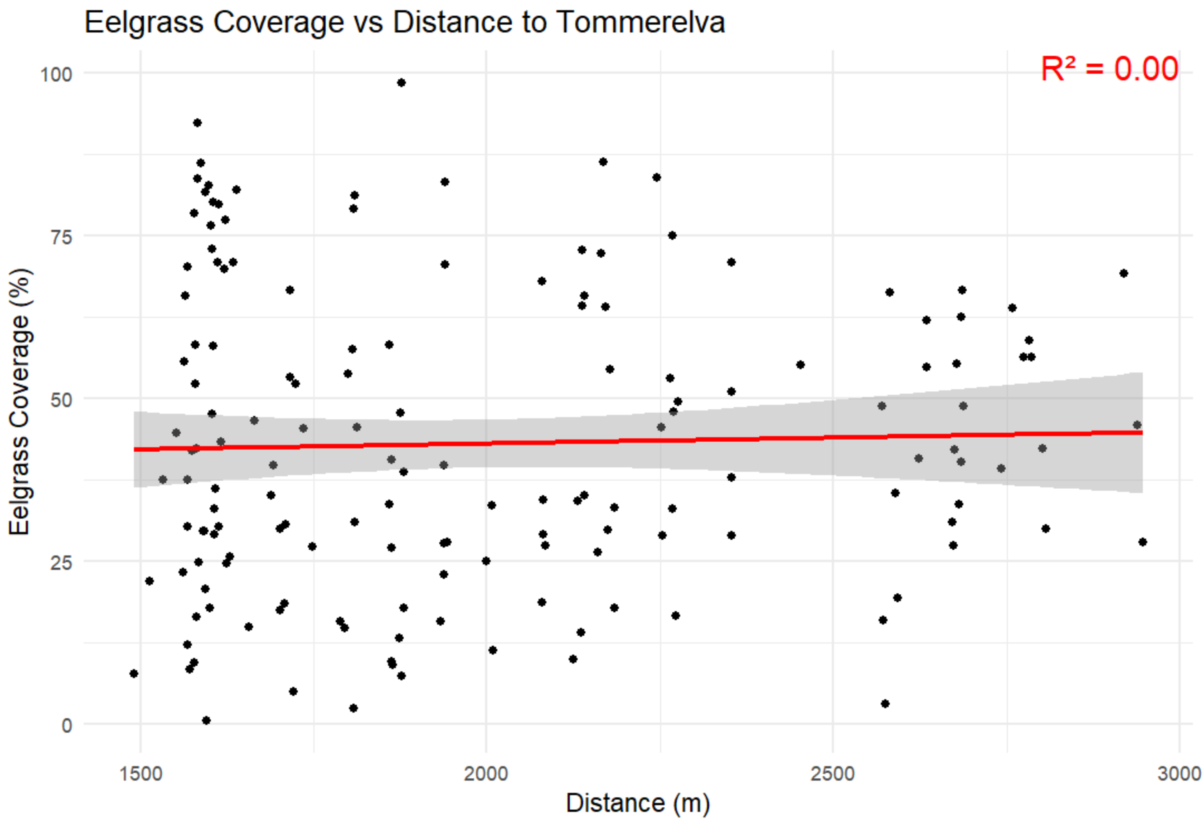


Figure 28: Shows how eelgrass coverage changes with distance to Tømmerelva.

Using linear regression, a significant relationship between epiphyte coverage and distance to Tømmerelva was determined (F-statistic = 32.28, $R^2 = 0.19$, $p = 7.4*10^{-8}$, Fig. 29.), described by the following equation 82.63 – 0.03x. The relationship shows a negative trend, with a 3% reduction in epiphyte coverage per 100-meter increase in distance.

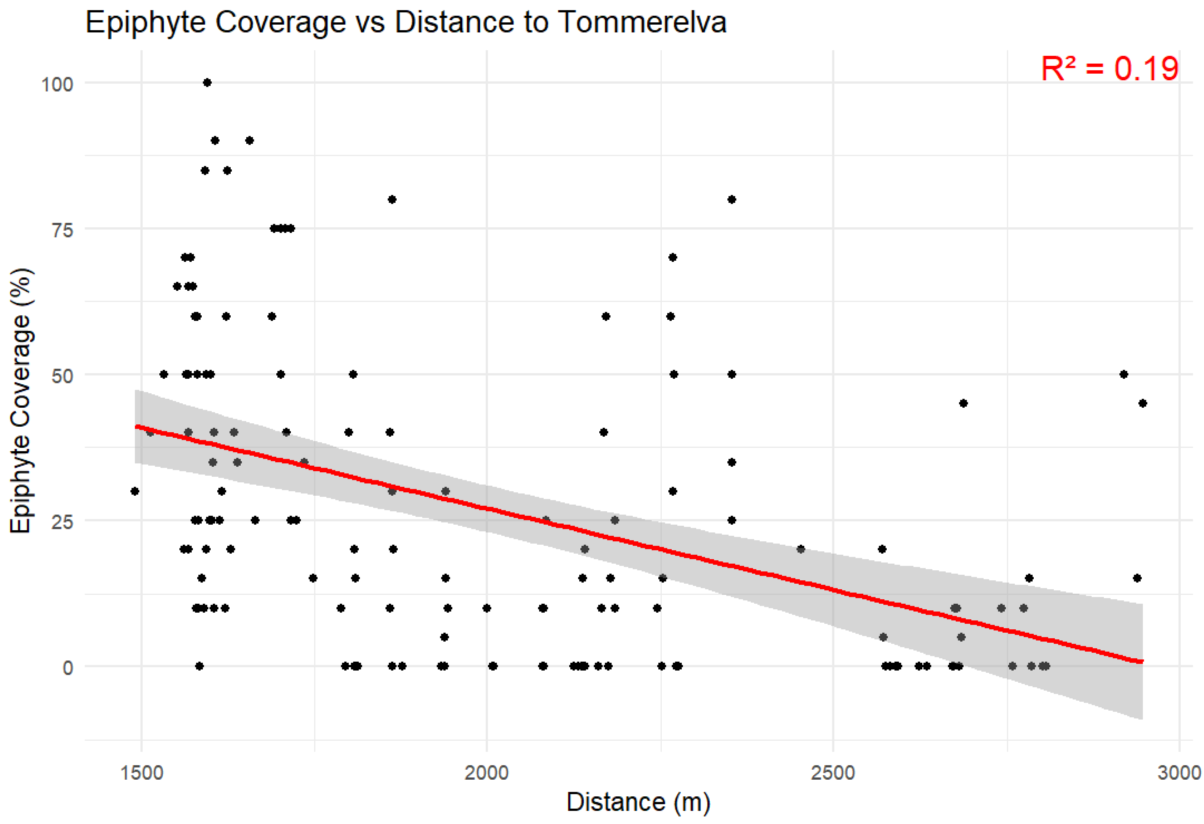


Figure 29: Shows how epiphyte coverage changes with distance to Tømmerelva.

3.4 Eelgrass coverage and grain size

At Sørkjosleira, eelgrass cover was slightly positively, but non-significantly correlated with grain size (F-statistic = 0.78, p = 0.38, R^2 = 0.05, Fig 30.). Additionally, there was no significant difference in grain size at water depths where eelgrass was present compared to areas below the maximum depth at which eelgrass occurred (Welch two-sample t-test: $t_{(10)}$ = -1.47, p = 0.17).

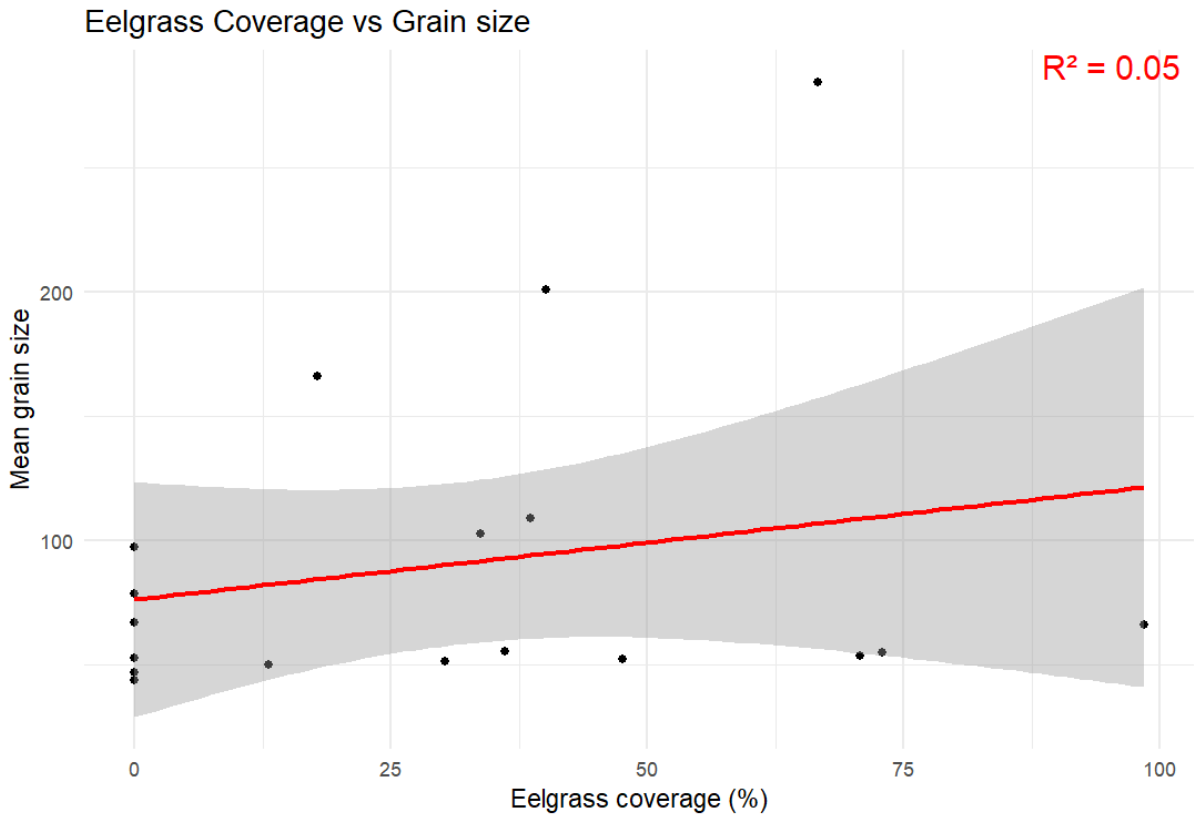


Figure 30: Shows how eelgrass coverage changes with variations in grain size.

4 Discussion

4.1 General

At Sørkjosleira the nitrate concentration during the sampling period was on average 34% higher than at Kobbevågen. The average nitrate concentration in rivers terminating in Sørkjosleira during the sampling period was 5.86 times higher than at Kobbevågen. Nitrate input from rivers at Sørkjosleira being much higher than at Kobbevågen was as hypothesized. This is possibly caused by run-off from the more widespread agriculture in the area surrounding Sørkjosleira compared to less agricultural activity close to Kobbevågen, agriculture run-off is one of the major drivers of eutrophication (Withers et al., 2014; Johnson et al., 2016). The larger nitrate river input likely contributes to the higher nitrate levels observed at Sørkjosleira. Although there are differences in phosphate concentrations between the rivers, the phosphate concentration did not significantly differ between the sites. Additionally, there were no differences in either nitrite river concentrations or nitrite concentrations between the sites. This may indicate potential impacts from phosphate and nitrite are consistent for both sites.

Epiphyte coverage at Sørkjosleira was on average significantly higher. While eelgrass coverage and height were significantly lower at Sørkjosleira. These results are as hypothesized. Comparatively more epiphyte coverage at Sørkjosleira is as expected considering the significantly higher nutrient levels in the meadow and from the river input. Nutrient loading is a known cause of increases in epiphytes (Bricker et al., 2008). Lower eelgrass coverage consequently is expected, reduction in eelgrass coverage may happen because of increased nutrient loading (Heuvel et al., 2019). Eelgrass may also recover if there are reductions in nutrient loading (Vaudrey et al., 2010). Shoot height being lower at Sørkjosleira could be due to the higher epiphyte coverage, which may cause light limitation for eelgrass. Light limitation is known to cause detrimental effects for eelgrass shoot height (Bertelli & Unsworth, 2018).

While no statistically significant correlation was found between nutrient levels and eelgrass performance parameters. There was moderate positive correlation between nutrient levels and eelgrass coverage and height, indicating that the tendency is that eelgrass coverage and shoot height will increase with increasing nutrients. The low positive correlation between nutrient levels and epiphyte coverage indicates that epiphyte coverage will weakly increase with increasing nutrients. Eelgrass may benefit from moderate nutrient enrichment due to increased nutrient availability, but excessive nutrient enrichment may cause detrimental effects due to increased competition from epiphytes (Burkholder et al., 2007).

4.2 Does Tine impact the eelgrass meadow at Sørkjosleira?

There was no significant relationship between either nitrate, phosphate, or nitrite levels and distance from Tine. Additionally, there was no significant relationship between eelgrass coverage and distance from Tine. This absence of a pattern indicates that the eelgrass meadow might not be affected by discharge from Tine, as it is expected that nutrients decrease with increasing distance from a source due to processes such as nutrient uptake by organisms or dilution (He et al., 2023). Epiphyte coverage increased significantly with increasing distance from Tine, which is opposite of the expected pattern. Although, this is probably caused by the large nutrient input from Tømmerelva which is on the opposite side of the meadow compared to Tine, as epiphyte coverage decreased significantly with increasing distance from Tømmerelva. The increased coverage of epiphytes close to the outlet of Tømmerelva is also visible (Fig. 13). Despite significant variation in nitrate levels across the sampling period, this variation was consistent for both sites. This suggest that the factors that influences nitrate concentration are consistent for both sites.

4.3 Is the depth limit of eelgrass at Sørkjosleira affected by physical characteristics of the substrate?

There was no significant relationship between eelgrass coverage and grain size mean at Sørkjosleira. Additionally, the average grain size was not significantly different in areas occupied by eelgrass compared to areas without eelgrass. These results indicate that the depth limit for eelgrass at Sørkjosleira may not be limited by the physical characteristics of the substrate, but that the depth limit is controlled by other factors. Light availability is known as one of the primary factors that affect eelgrass depth limits, eelgrass may receive less light due to increased turbidity caused by sedimentation, or shading by epiphytes (Dennison, 1986).

4.4 Changes in distribution since 2009.

At Sørkjosleira there was a 28.7% reduction in eelgrass area since 2009, while at Kobbevågen there was a 2.8-fold increase. As these areas are marine protected areas, they are protected against human caused physical disturbance such as dredging and trawling which are causes for eelgrass decline (Erftemeijer & Lewis III, 2006). But there are other possible contributing factors causing this development. Firstly, competition for nutrients and light between eelgrass and epiphytes. Epiphytes growing on and over eelgrass reaches nutrients in the water column faster, and they also reduce the amount of light that reaches eelgrass. As the epiphyte coverage at Sørkjosleira is comparatively higher than at Kobbevågen, this indicates that eelgrass experiences more competition at Sørkjosleira. Secondly, other biotic interactions such as direct grazing on eelgrass may contribute to reductions in eelgrass (Valentine & Duffy, 2006 (from Larkum 2006 book)). Differences in mapping procedure between this survey and the 2009 survey may affect the conclusions of how eelgrass development has been, due to possible inconsistencies in the eelgrass distribution data.

The horizontal distribution of eelgrass differs between the sites. Sørkjosleira has a larger horizontal distribution than Kobbevågen, and a higher depth limit. The larger depth limit at Sørkjosleira could indicate better water clarity, allowing for light to penetrate deeper, and consequently allowing for eelgrass to grow to greater depths. Or it may indicate that the eelgrass depth limit at Kobbevågen is limited by other factors. The substrate below the depth limit at Kobbevågen could be unsuitable for eelgrass growth.

4.5 Implications for monitoring and conservation

The findings in this thesis carries implications for the monitoring and conservation efforts of the eelgrass meadows at Sørkjosleira and Kobbevågen. To detect changes in eelgrass distribution and performance parameters, as well as environmental factors continuous monitoring is recommended. This may help guide conservation efforts to be as effective as possible. Decreasing nutrient input at Sørkjosleira could be done by reducing fertilizer use in agriculture, and improving waste management, allowing for possible recovery of eelgrass (Vaudrey et al., 2010). To promote recovery of eelgrass in areas where its absent, restoration efforts such as replanting may be beneficial (Eriander et al., 2016).

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6 Appendix

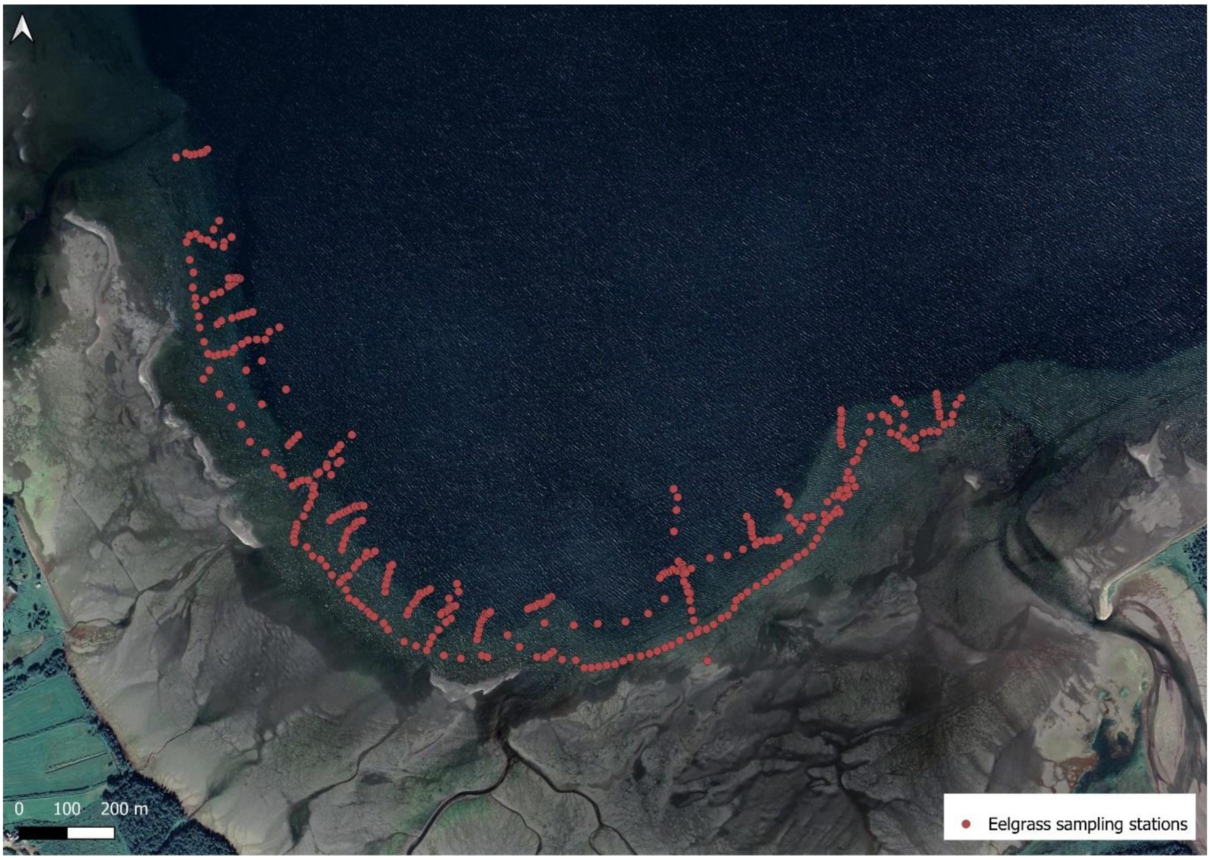

Figure 31: Map of Sørkjosleira showing all stations where eelgrass parameters was measured.

Figure 32: Map of Kobbevågen showing all stations where eelgrass parameters was measured.

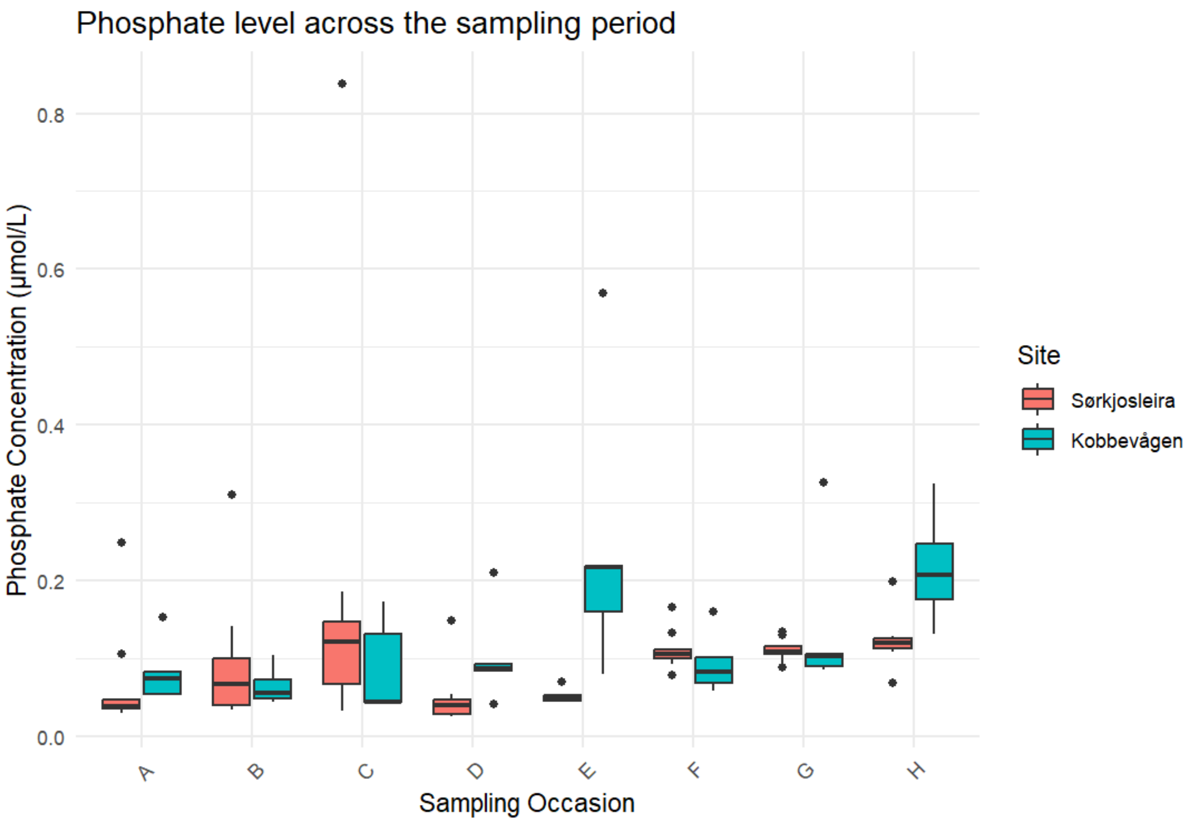

Figure 33: Shows how phosphate levels changes for both Sørkjosleira and Kobbevågen across the entire sampling period.

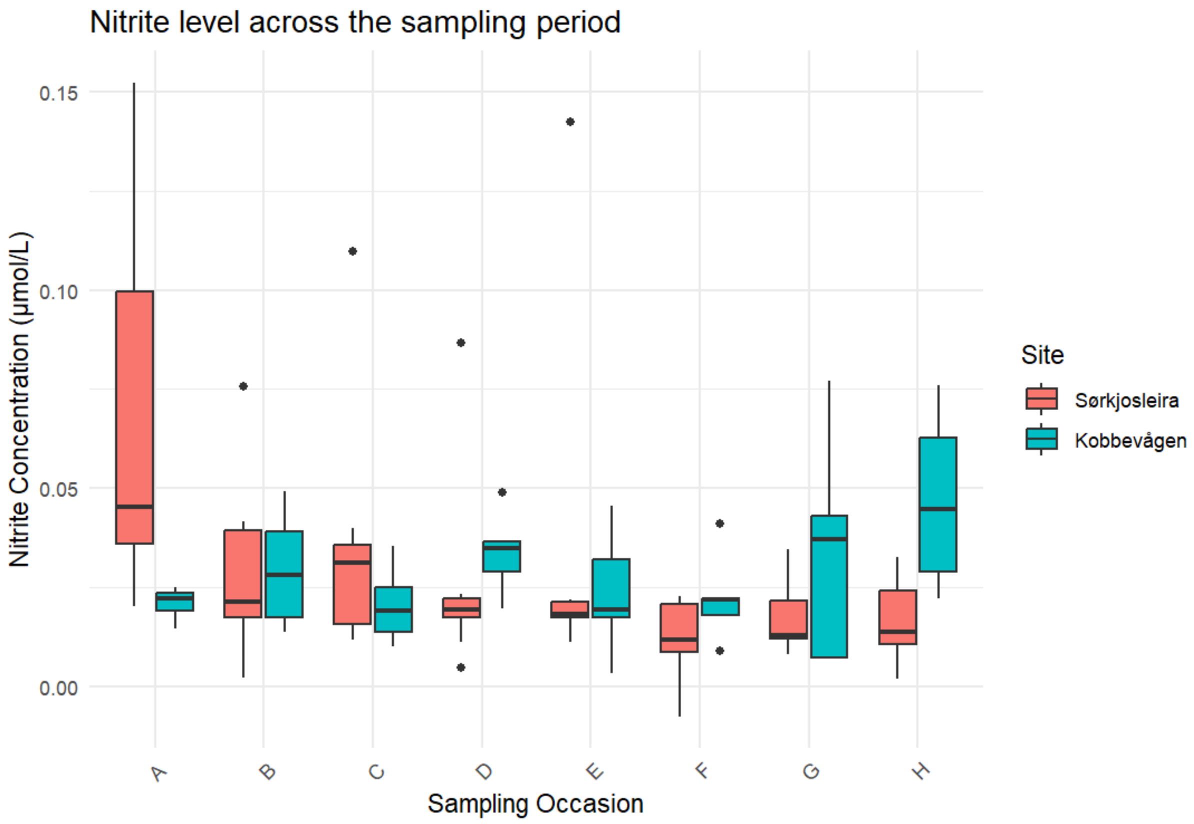

Figure 34: Shows how nitrite levels changes for both Sørkjosleira and Kobbevågen across the entire sampling period.

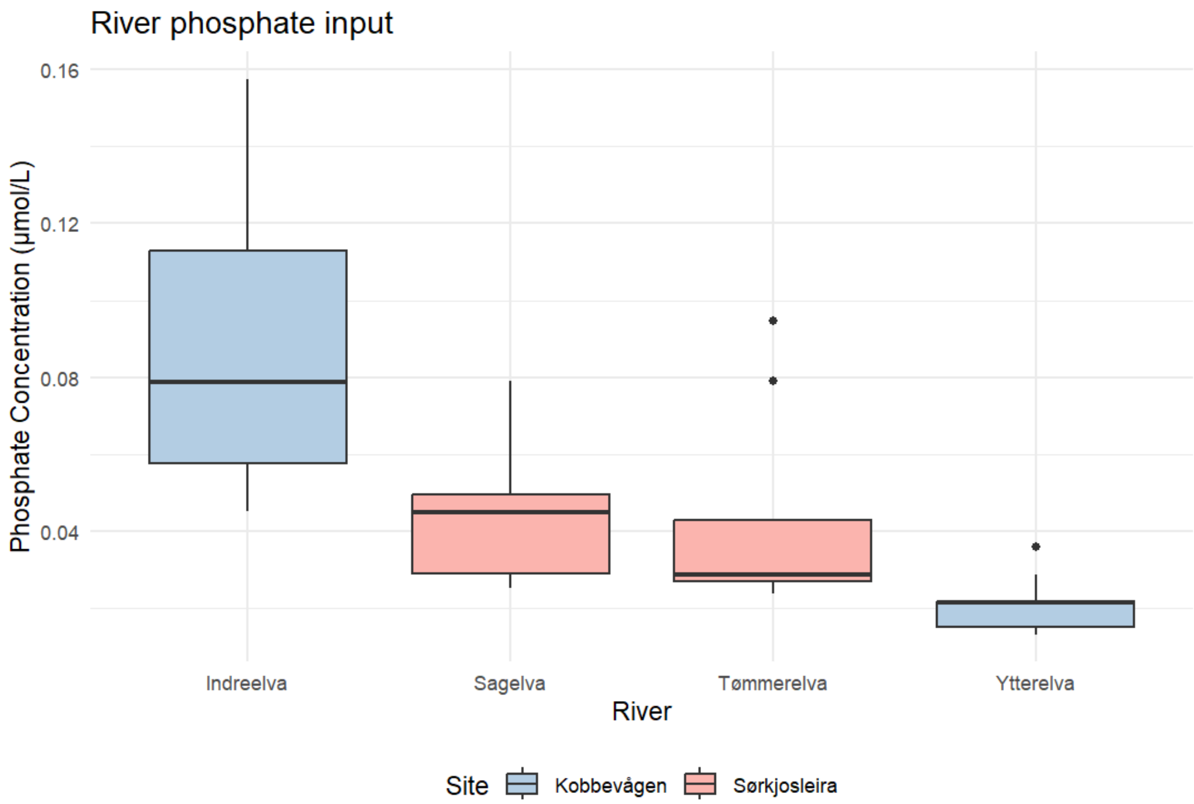

Figure 35: Shows the river phosphate input for all rivers sampled.

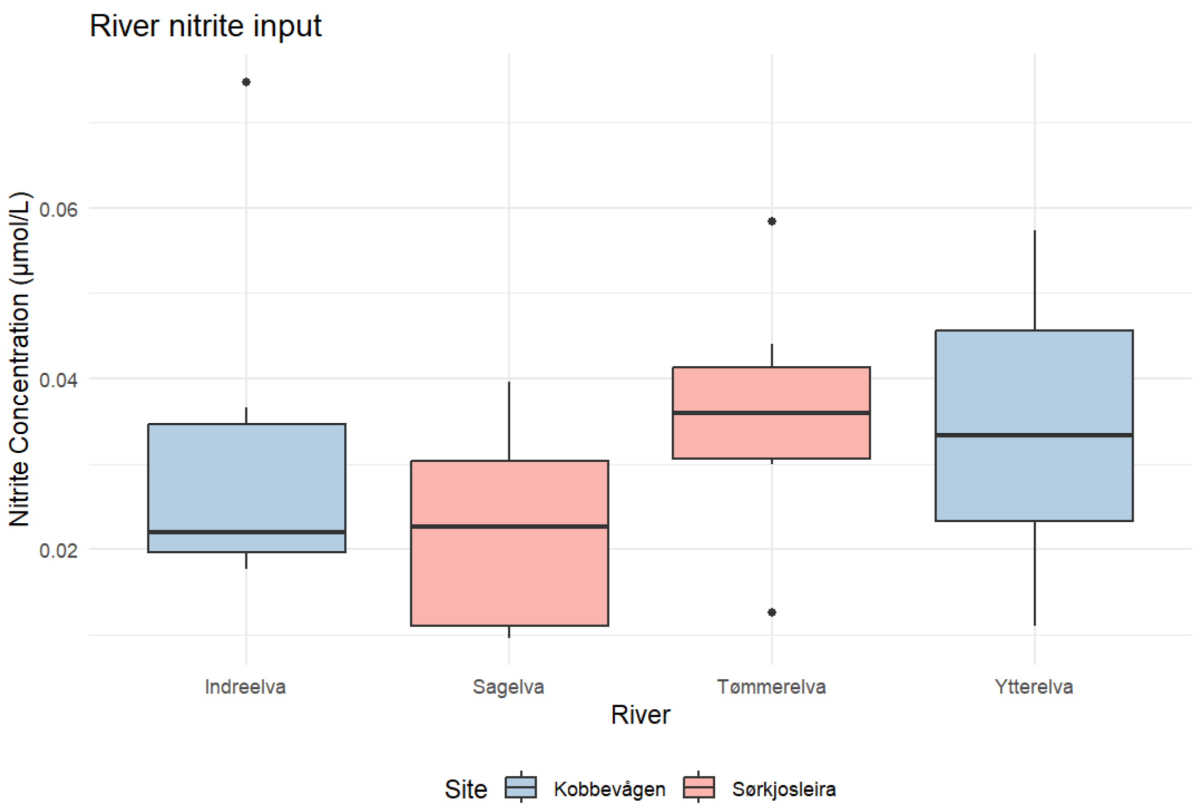

Figure 36: Shows the river nitrite input for all rivers sampled.

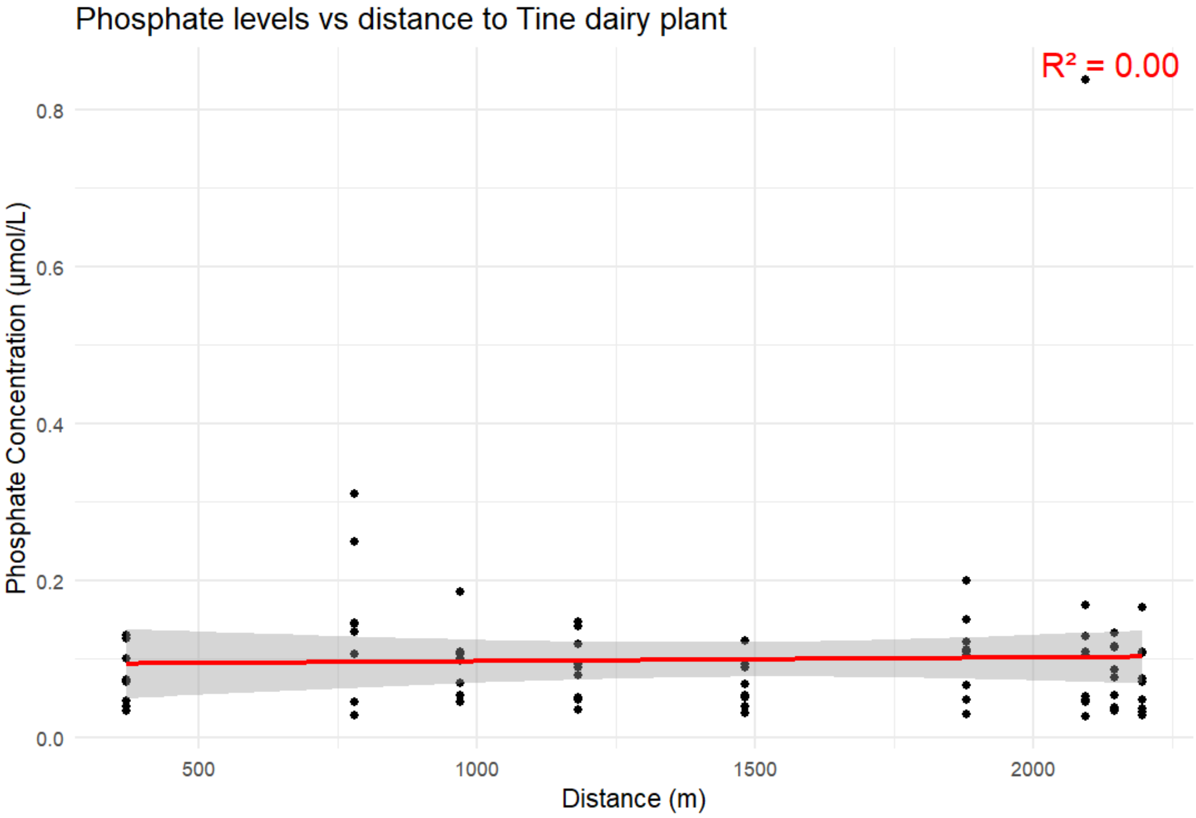

Figure 37: Phosphate concentration at different distances from Tine dairy plant discharge point (= distance 0m).

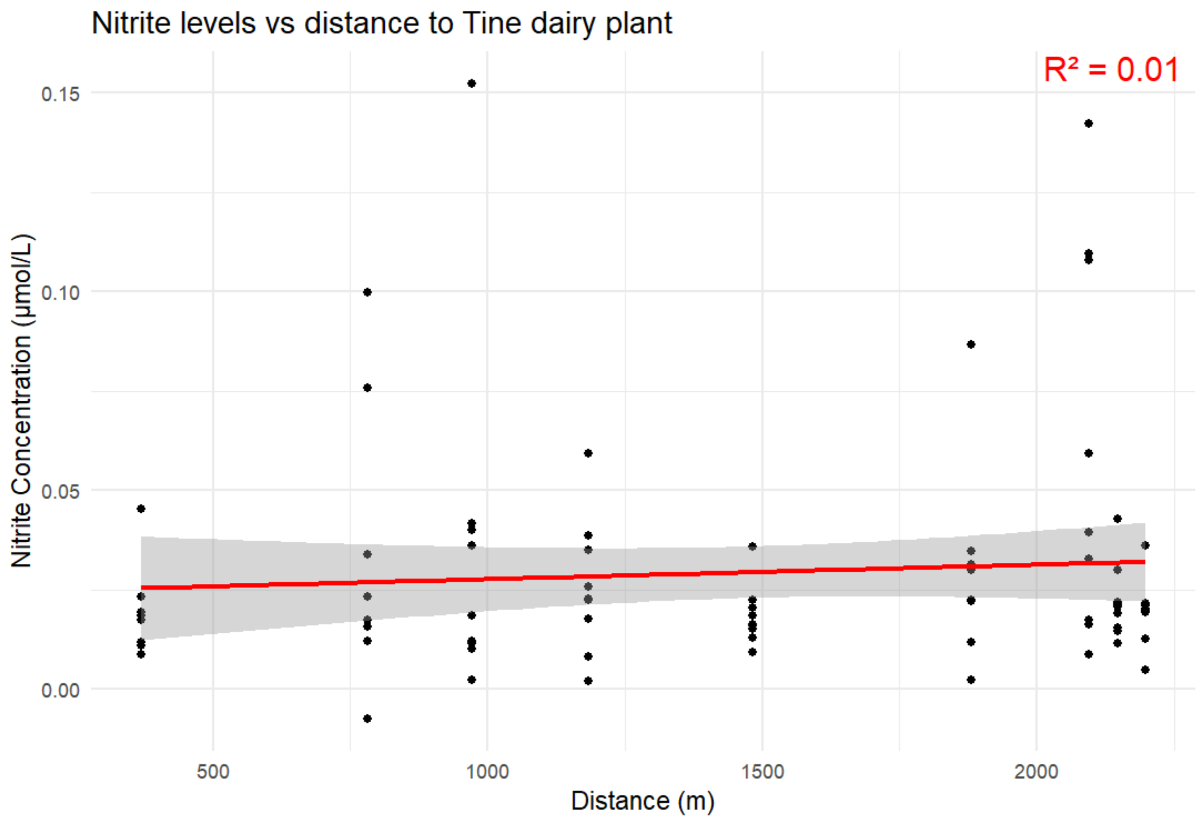

Figure 38: Nitrate concentration at different distances from Tine dairy plant discharge point (= distance 0m).