The effect of marinas on infaunal communities in Zostera marina meadows and unvegetated sediments

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Abstract

The aim of this study was to investigate the effects from marinas on infaunal communities in Zostera marina meadows and in adjacent unvegetated sediments. The results from the study showed that Z. marina meadows supported a lower infaunal diversity than unvegetated sediments. However, no direct effects from marinas could be detected. Low infaunal diversity in meadows may be explained by high organic loadings, hypoxic and sulfidic sediments, and the chemical and physical disturbances commonly associated with the activities at marinas.

Sediment samples for fauna, grain size and chemistry from two locations close to marinas and two locations remote from marinas in the Oslofjord, Norway were analysed. A total of 1535 individuals were found, representing 33 taxa and comprising mainly the groups Insecta, Polychaeta, Oligochaeta and Gastropoda. The gastropods represented 53 % of all individuals encountered, followed by insects (22%) and oligochaetes (18%). The polychaetes were the most taxa rich group (10 taxa), followed by crustaceans (6 taxa) and gastropods and bivalves (both, 5 taxa).

There could not be detected a direct negative effect from marinas since locations remote from marinas had higher contaminant loadings than locations close to marinas. The most contaminated stations were dominated by taxa typically for polluted areas. Investigations of the differences in infaunal composition and contamination patterns between Z. marina meadows and unvegetated sediments, indicates that infauna in Z. marina meadows were more affected by contaminants than infauna in unvegetated sediments. Considering their importance, more emphasis should be put on the seagrass ecosystem in coastal management. Relative simple and low cost installments, such as sediment catch basins may be effective in reducing contaminant loads to seagrass meadows.
Preface

First, I want to thank my supervisors Kjell Magnus Norderhaug, Torgeir Bakke and Eli Rinde for advices, excellent feedback, and for introducing me to the fascinating field of sediment ecology.

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1 Introduction

The coastal waters of Norway harbor a high diversity of species and habitats. Seagrass meadows are one such species rich habitat which is considerably more widespread than previously estimated and exist along the entire Norwegian coast (Bekkby et al. 2011). However, in recent years, there has been an increased use of coastal waters for recreational purposes, increasing the demand for marinas. This demand has led to a growing concern about their potential effects on the seagrass ecosystem.

1.1 The seagrass ecosystem

The most common seagrass species in Norway is Zostera marina (Linnaeus, 1753), which forms dense meadows along the entire Norwegian coast. It is native to Eurasia and North America and is the most widespread seagrass species in the northern hemisphere. Z. marina grows in sand and mud in sheltered bays, estuaries, and beaches to a depth of approximately 10 meters (Green and Short 2003).

Seagrass meadows are one of the ocean’s most productive ecosystems, yielding numerous goods and services. For example, primary production from seagrass ecosystems may exceed that of cultivated terrestrial ecosystems (Duarte and Chiscano 1999). Additionally, seagrasses produce enormous amounts of carbon, which enters the detrital pool, where much is buried within the sediment, forming hotspots for carbon sequestering (Duarte et al. 2005; Kennedy et al. 2010). Seagrasses also act as nursing grounds for many commercially important species of fish and shellfish (Nelson 1980; Gotceitas 1997; Beck et al. 2001; Heck Jr et al. 2003). Therefore, seagrass meadows are extremely important marine habitats (Costanza 1997).

Seagrasses harbor diverse flora and fauna. The seagrass canopy alter water flow, enhance sedimentation and traps nutrients (Ginsburg and Lowenstam 1958; Sternberg 1968; Fonseca et al. 1982; Thomas and Cornelis 2003; Peterson et al. 2004). Further, the seagrass canopy provides substrates and habitats for animals (Neckles 1993; Fredriksen
et al. 2005) and algae (Fredriksen and Christie 2003), whereas the root system provides sediment stability for fauna living in the sediment (Fonseca 1983; Boström et al. 2006).

A main component in the seagrass ecosystem is the infaunal community. Infauna move through the sediment, ingest and egest particles, and draw oxygen-rich water down from the sediment surface to its depths (bioturbation) (Rhoads 1974). This sediment oxygenation creates a more favorable living environment for other species, including the seagrass itself. Bioturbation also alters nutrient fluxes between the water column and the sediment. In this manner, infauna may have an impact on the overall seagrass ecosystem (Kaiser et al. 2005; Norkko and Shumway 2011). Bioturbating infauna also alter the uptake rate and the distribution of contaminants in the sediment, making the contaminants more available to other infaunal species (Rasmussen et al. 1998).

Seagrass meadows are expected to support a greater infaunal abundance and diversity than unvegetated sediments (Stoner 1980; Edgar et al. 1994; Boström and Bonsdorff 1997; Webster 1998; Fredriksen et al. 2010). In turn, infaunal abundance and species richness is related to the physical and chemical characteristics of the seagrass meadow. Through their leaves, plants transport oxygen to their roots and rhizomes (Pedersen et al. 1998), thus avoiding anoxia and sulfuric sediments (Holmer and Nielsen 1997; Mateo et al. 2006). Further, the roots and rhizomes form a complex, interlocking matrix that offers shelter from predation (Patriquin 1975; Fonseca 1983; Koch 2001; Boström et al. 2006).

The seagrass shoots may also be important in structuring the infaunal community (Webster 1998). With increasing shoot density, the below ground biomass may increase and make the habitat more complex and provide more substrate for fauna. Also, the physical characteristics of the seagrass canopy enhance the ability to trap drifting algae. The algae further decompose, and the resulting organic matter may either enhance infaunal production through an increased food supply (Moksnes et al. 2008) or make the sediment anoxic and cause infaunal death (Norkko and Bonsdorff 1996a; Burkholder et al. 2007).
1.2 Marinas

On a global scale, anthropogenic activities have altered the coastal zone, causing a significant loss of seagrass habitats. The consequences include eutrophication (Burkholder et al. 2007), climate change (Short and Neckles 1999), and introduced species (Williams 2007), all of which have attracted wide attention. Another more local source of disturbance may be the activities associated with the construction and use of recreational marinas. Marinas and docks cause shadowing and alter water circulation. Boating cause contamination, dredging cause smothering while anchoring may tear up the seagrass. These disturbances have caused substantial loss of seagrass meadows (Loflin 1995; Burdick and Short 1999; Francour et al. 1999; Burkholder et al. 2007). The coastal zones may change too quickly for seagrasses and associated species to adapt.

Approximately 60% of Norway’s inhabitants are concentrated around the Oslofjord area, and the fjord experiences heavy traffic from shipping and recreational boats. The area has undergone high levels of development in recent decades, and increased recreational use of boats has intensified the construction of docks and marinas (Rinde et al. 2011). Construction of recreational marinas occurs in a “piece by piece” fashion, often without a regulation plan. Furthermore, an unknown number of smaller, private docks are constructed illegally (Bristøl 2008; Kalvsjøhagen 2010; Rennestraum 2011). Thus, there is an unknown, increasing number of marinas and harbors in Norwegian coastal waters.

Marinas are often placed in sheltered bays, which are areas often vegetated by seagrasses. Large volumes of sediment accumulate in sheltered areas due to resuspension and deposition. Fine particulate sediment adsorbs dissolved metals from the water column and binds the metals into the sediment. Additionally organic compounds, which do not dissolve in water, tend to accumulate in sediment by adsorption to organic matter. Therefore, sheltered bays and polls are often contaminant sinks.

Marina-related activities, such as fuel combustion in boat engines, storm water run-off from impermeable surfaces, waste from boat maintenance, and overboard sewage discharge, can introduce a variety of chemical contaminants into the marine environment (Tjärnlund et al. 1996; Durand 2004; Lahti et al. 2010). Many of these compounds,
including metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and byproducts of antifouling paints, adsorb onto particulate matter and accumulate in marina sediments (Voudrias and Smith 1986; Marcus et al. 1988; Unger et al. 1988; Schiff et al. 2007).

The most important source of contamination in Norwegian marinas is related to the general maintenance of boat hulls (KLIF 2010). The washing and preparation of hulls creates contaminated waste that may be directly disposed in nearby seawater. In some marinas, this waste is drained and filtered through a sediment catch basin\(^1\). When the waste enters the seawater, it is diluted and is further transported to nearby sediments and water. Accordingly, the concentrations of contaminants in marinas usually decline in a gradient from the sediment catch basin to nearby sediments within the marina (KLIF 2010). The most common contaminants found in Norwegian marinas are listed below.

Tributyl tin (TBT) is in Norwegian marinas often found at concentrations classified as extreme (Næs et al. 2000; Næs et al. 2002; KLIF 2010). Concentrations may, however, vary within each marina. TBT has various effects on benthic organisms, including impossex and reduced reproduction in gastropods (Bryan et al. 1989), shell thickening (Chagot et al. 1990) and reduced growth in bivalves (Ruiz et al. 1995).

Polycyclic aromatic hydrocarbons (PAH) are often found in concentrations considered to be moderate and extreme in the marinas of south Norway (Næs et al. 2000; Næs et al. 2002; KLIF 2010). PAH are naturally occurring and ubiquitous in the environment. They consist of a range of different congeners, some being carcinogenic. PAH are lipophilic and may accumulate in benthic organisms (State of the Environment Norway 2011).

Similar to PAH, polychlorinated biphenyls (PCB) may be found at high concentrations in Norwegian marinas (Næs et al. 2000; Næs et al. 2002; KLIF 2010). PCB consists of several congeners with different toxicities. They are highly lipophilic, biomagnify in the food chain, and are extremely persistent in the environment. PCB have acute toxicity and are carcinogenic (State of the Environment Norway 2011).

\(^1\) Underground retention system designed to remove debris and contaminants from stormwater run-off.
Metals are generally found in moderate concentrations in Norwegian marinas (Næs et al. 2000; Næs et al. 2002). However, high concentrations of cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg), and zinc (Zn) have been found in some marinas. Some metals are carcinogenic and may reduce the reproduction and survival of organisms (State of the Environment Norway 2011).

The contamination from marinas may impact the infaunal community, because many species are sedentary and cannot avoid disturbances. Contamination often leads to structural changes in benthic communities (Pearson and Rosenberg 1978). A small number of opportunistic species will increase in abundance, whereas less tolerant species will become rare or disappear. Reduced biodiversity may alter the function of infauna on structuring the sediment (Solan et al. 2004) and, thus, the seagrass ecosystem.

Little is known about the effects of marinas on the infaunal community in seagrass ecosystems. In particular, it is unknown whether infaunal communities in seagrass meadows are affected differently to infaunal communities in unvegetated sediments. Therefore, it is important to assess the effect of marinas on this component of marine biodiversity.

### 1.3 Aims

The aims of this study were as follows:

1. Examine if infaunal communities in *Z. marina* meadows differ from those in unvegetated sediments.

2. Examine if infaunal communities in *Z. marina* meadows close to marinas differ from those in *Z. marina* meadows remote from marinas.

3. Examine if infaunal communities in unvegetated sediments close to marinas differ from those in unvegetated sediments remote from marinas.

4. Examine which factors explaining the observed pattern in infaunal communities.
2 Materials and methods

2.1 Description and choice of study locations

Sediment samples were collected from *Z. marina* meadows (hereafter called meadows) and unvegetated sediments (hereafter called sand) in Sandspollen and Sætrepollen of inner Oslofjord and in Horneskilen and Skjebergkilen of outer Oslofjord (8 stations in total) (Figure 1) during field work carried out in May 2010. Sandspollen and Horneskilen are situated remote from marinas and functioned as control locations, whereas Sætrepollen and Skjebergkilen are situated close to marinas and functioned as disturbed locations. All four locations support relatively large meadows with nearby sand. The locations were chosen for their similar physical conditions, such as degree of wave exposure and sediment type.

![Figure 1: Map of the four study locations in the Oslofjord. The control location Sandspollen (Sa) and the disturbed location Sætrepollen (Sæ) are located in the inner Oslofjord, while the control location Horneskilen (Ho) and the disturbed location Skjebergkilen (Sk) are located in the outer Oslofjord. Green areas indicate earlier recordings of *Z. marina* meadows performed by NIVA and IMR. The maps are from The Norwegian Coastal Administration.](image-url)
2.1.1 Sandspollen

Sandspollen is a poll located on the west side of the Oslofjord, situated just inside a shallow sill separating the Oslofjord into an inner and outer region. The land surrounding the poll is forested, and the topography is rather hilly. The poll is oriented in a northwest-southeast direction with the inlet situated on the northeast side of the poll. There are four smaller bays along the longitudinal axis of the poll (Figure 2): Tangenbukta and Verpenbukta in the northwest and Kapellkilen and Lagbukta in the southeast.

Sandspollen is approximately 1,200 m long and 500 m wide, with a total area of approximately 370,000 m². The inlet is approximately 100 m wide. Sandspollen has two basins situated near the middle of the poll with maximum depths of 14.2 m and 12.6 m. The four bays are rather shallow (<6 m). There is groundwater seepage in Lagbukta, while the other bays receive freshwater only periodically (Wistrøm 1978). The sediment consists of mud.

Three of the bays (Tangenbukta, Verpenbukta, and Kapellkilen) in Sandspollen support meadows varying in size from approximately 1,000 to 31,000 m² (Figure 2). All three

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2 An enclosed bay with a sill positioned shallower than the halocline.
meadows are classified as regionally important marine habitats by the DN criteria (2007). The field samples were taken from the meadow and the sand in Kapellkilen (Figure 2).

**Pollution in Sandspollen**

Although there is no large marina in Sandspollen, it is a popular recreational area frequently used by boaters, similar to many other polls in the Oslofjord.

### 2.1.2 Sætrepollen

Sætrepollen is located north of Sandspollen and is very similar. The poll is surrounded by agricultural land and forested areas. As with Sandspollen, the poll is oriented in a northwest-southeast direction. It has two smaller bays in the south, one in the southwest and one in the southeast. There are two inlets to the poll, one in the north between the land in the west and the island of Torvøya in the east and one on the east side of the poll between Torvøya and the mainland in the south (Figure 3).

Sætrepollen is approximately 1,900 m long and 500 m wide, with a total area of approximately 600,000 m². The inlets in the north and east are approximately 65 m and 300 m, respectively. The deepest part of the poll is situated near the inlet in the east, with a depth of 16 m. The inlet in the north has depths of between 0.1 and 10 m. The two bays are quite shallow with depths <4–5 m. Five small rivers have outlets into the poll: one in the northwest, one in the southwest, two in the southwest bay, and one in the southeast bay. The sediment in the poll consists of mud.

The north side of the poll and both bays in the south support several large meadows varying in size from approximately 20,000 to 46,000 m² (Figure 3). All meadows are classified as very regionally important marine habitats by DN. The field samples were taken from the meadow and the sand located in the southwest bay (Figure 3).
Pollution in Sætrepollen

There are several marinas within the poll, the largest located in close proximity to the study site (Figure 3). Together, these marinas support approximately 390 boats. There are approximately 200 spots used for the storage of boats during winter.

There is no sediment catch basin in the marina in Sætrepollen. Therefore, waste produced by the maintenance of boats directly enters the sea. Additionally, accidental spillage of fuel, antifreeze agents, and other chemicals occurs sporadically (Daniel Tørring Ingebretsen (02.12.2011), Sætre båtforening, written communication). Dyno Nobel ASA produces explosives on the small peninsula of Engene (Figure 3). In a report from 1999, the Norwegian Society for the Conservation of Nature regarded these industries as the most important sources of PCB in the Oslofjord, mainly originating from run-off from contaminated land (NNV 1999).

Figure 3: Detailed map of Sætrepollen, the disturbed location in inner Oslofjord. Green areas indicate earlier recordings of Z. marina beds performed by NIVA and IMR. Red dots indicate sampling area. The names represent the four bays inside the poll. The map is from The Norwegian Coastal Administration.
2.1.3 Horneskilen

Horneskilen is a kile\(^3\) oriented in a northwest-southeast direction northwest in the bay of Skjebergkilen and is surrounded by agricultural land and some forested areas. The kile is sheltered, as it is positioned in the inner part of Skjebergkilen.

The kile is approximately 1.5 km long and 200–500 m wide, with a total area of 450,000 m\(^2\). The inlet is approximately 200 m wide and constitutes the deepest part (16 m). The inner part of the kile is the shallowest, with a depth of <2 m. Two small rivers have their outlet north in Horneskilen. The sediment consists mainly of clay.

Horneskilen has one large meadow with a size of 128,000 m\(^2\) (Figure 4). This meadow is classified as a very important marine habitat based on the criteria set by DN. The field samples were taken from the meadow and the adjacent sand.

Pollution in Horneskilen

The agricultural land surrounding Horneskilen drains into the kile. There is a small craft harbor sustaining approximately 70 boats on the west side of the kile (Figure 4).

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\(^3\) A wedge-shaped bay.

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**Figure 4:** Detailed map of Horneskilen, the control location in outer Oslofjord. Green areas indicate earlier recordings of *Z. marina* beds performed by NIVA and IMR. Red dots indicate sampling area. The circle indicates a small craft harbour. The map is from The Norwegian Coastal Administration.
2.1.4 Skjebergkilen

Skjebergkilen is a kile located on the east side of the outer Oslofjord. The kile is surrounded by industrial areas and forested land. The kile is positioned in a north-south direction and is sheltered between islands and points. There are two bays in the north: Horneskilen in the northwest and another in the northeast. The areas are given as the total for Skjebergkilen in this section.

The kile is approximately 4,600 m long and 1,900 m wide, with a total area of approximately 4.7 km$^2$. The inlet is situated in the south, is approximately 500 m wide, and constitutes the deepest part (52 m). In the middle part of the kile, the depth is 30–40 m. The shallowest part is situated in the two bays in the north, with a depth of <2–8 m. There are five smaller rivers with outlets to Skjebergkilen, but none in the immediate vicinity of the studied area. The sediment in the kile consists mostly of clay and mud.

Skjebergkilen supports several meadows of different sizes (Figure 5), ranging from 5,000 to 34,000 m$^2$. There is one large marina sustaining approximately 1,000 boats situated in

Figure 5: Detailed map of Skjebergkilen, the disturbed location in outer Oslofjord. Green areas indicate earlier recordings of Z. marina beds performed by NIVA and IMR. Red dots indicate sampling area. The map is from The Norwegian Coastal Administration.
the west side of the kile near the largest meadow (Figure 15). This meadow is classified as an important marine habitat by DN. In addition, 550 boats are stored on the land during the winter. The field samples were taken in the meadow and sand immediately associated with the marina (Figure 5).

**Pollution in Skjebergkilen**

Washing and maintenance of boats is performed on land, and wastewater is collected in a sediment catch basin.

The Norwegian Climate and Pollution Agency (KLIF) (KLIF 2010) has classified the sediment in Skjebergkilen marina as relatively acceptable regarding environmental contaminants. The concentrations of metals, hydrocarbons, PCB\textsubscript{7}, and TBT were classified as being non-toxic. However, toxic concentrations of TBT and Cu were found.

### 2.2 Sampling

For fauna analysis, five core samples (10 cm in diameter and 20 cm in depth) were randomly collected in the sand and in the meadow at the four study locations, for a total of 40 samples. This allowed for fauna of the sand and meadows to be compared at two levels: (1) within locations and (2) between locations.

The samples were collected by SCUBA diving between May 18 and 21, 2010. When sampling the fauna, the corer was pushed down into the sediment to a depth of approximately 10 cm, yielding a sample volume of \( \sim 785 \text{ cm}^3 \). This volume is not exact, as there were soft and fluffy sediment at some stations, making accurate sampling difficult. The sample was transferred to a plastic zip-top bag pre-labeled with the location name, substrate type (sand or meadow), and core number. Onshore, the samples were immediately washed through a 500-\(\mu\)m mesh sieve. This mesh size was chosen in order to maintain macrofauna and eliminate meiofauna (e.g., nematodes and copepods). Larger objects that could damage the fauna were removed. The fauna was then carefully transferred to plastic bottles and fixed in 96% ethanol to ensure a high alcohol level after sieving with water. A waterproof label was placed inside each bottle.
For grain size analysis, one core sample (10 cm in diameter and 10 cm in depth) was taken in the sand and in the meadow at each location, for a total of eight samples. The sediment in the corers was transferred to plastic zip-top bags and stored at -18°C in the laboratory until further analysis.

Three core samples were taken in the sand and in the meadow at each location (24 samples in total) for determination of the redox potential discontinuity depth (RPD) and sediment sulfur content. For chemical analysis, three core samples were taken in the meadow at each location, for a total of 12 samples. For estimation of plant biometric variables for each meadow, all above-ground *Z. marina* plants within five 20 × 20 cm frames were collected (biomass). The plant shoots were then counted, and the canopy height and percentage coverage of *Z. marina* in five additional randomly dropped frames (50 × 50 cm) were estimated.

### 2.3 Laboratory work

#### 2.3.1 Faunal samples

In the laboratory, the alcohol was replaced by 70% alcohol to assure a high alcohol content. The faunal samples were then stained with rose bengal to facilitate sorting of the fauna. The samples were further washed with water through a 500-μm mesh sieve. The fauna were sorted under a magnifying lamp and a stereo microscope into six groups: Crustacea, Echinodermata, Mollusca, Oligochaeta, Polychaeta, and Insecta. The fauna were then identified to the lowest taxonomical level possible under a stereo microscope and a microscope. Meiofauna remaining in the samples were not included in the analysis.

#### 2.3.2 Quality control of identified animals

Bivalves and gastropods were identified using the Marine Species Identification Portal (MSIP) and literature by Tebble (1966), and with guidance and quality control by Hartvig Christie at NIVA and Professor Jon-Arne Sneli. Polychaetes were identified using MSIP and literature by Kirkegaard (1992a, 1992b) with guidance from Fredrik Melsom.
Amphipods were identified using MSIP and literature by Hayward and Ryland (1995) together with masters student Marc Silberberger. Echinoderms were identified using MSIP. Chironomids were identified to the genus level by Dr. Elisabeth Stur at the Norwegian University of Science and Technology. Oligochaetes were identified with guidance from Professor Christer Erseus at the University of Gothenburg.

2.3.3 Environmental variables

Samples for grain size were analyzed and resulting grain size parameters were used to describe the stations. The samples were wet-sieved through a series of sieves with mesh sizes of 2,000, 1,000, 500, 250, 125, and 63 μm following the Udden/Wentworth scale (Wentworth 1922). Each fraction was then dried at 90°C until all water had evaporated. Each sample was then weighed and calculated as a proportion of the total sediment dry weight (Bale and Kenny 2007). The parameters included in this study were the mean grain size diameter, sorting, skewness and kurtosis. The grain size parameters were based on the Folk and Ward method (1957). Formulas and descriptions of the parameters are given in Appendix F.

Due to the small sample size (one replicate per station), the grain size parameters did not satisfy the assumptions underlying a rigorous multivariate analysis. Therefore, they were excluded from the numerical analysis and only interpreted relative to the faunal observations.

Analysis of plant biometric parameters and sediment chemistry measurements was performed by NIVA and was made available for the study. For each sample, the following methods were used:

*Plant biometrics:* plant biomass (Z. marina wet weight per m$^2$), coverage (percentage of the area covered by Z. marina within each frame), canopy height (the average height of Z. marina in each frame), and shoot density (number of Z. marina shoots per m$^2$).
Mercury was determined by mixing ionic mercury with a reducing agent (SnCl$_2$) to convert mercury species to elemental mercury (Hg). The mercury was then quantified by transferring it into a cold-water spectrometer by the use of an inert gas (argon).

Remaining metals (Pb, Cd, Cu, Hg, and Zn) were determined by adding nitric acid to a known volume of sediment and autoclaving at 120°C. Determination of each metal species was performed in the liquid phase by inductively coupled plasma atomic emission spectroscopy or inductively coupled plasma mass spectrometry.

Total amount of organic carbon (TOC) and nitrogen (TN): Prior to analysis, samples were acidified to remove inorganic carbon. TOC and TNT were then determined by combustion of dried and weighed sediment samples in oxygen-saturated helium gas at 1,800°C to convert organic carbon to CO$_2$ and nitrogen compounds to nitrogen oxides. Complete combustion was ensured by the use of catalysts. Excess oxygen was removed over copper at 650°C, which also reduced the nitrogen oxides to N$_2$ gas. The combustion gases were shunted through a chromatographic column, and the N$_2$ and CO$_2$ gases were detected by a hotwire detector.

PAH was determined by adding internal standards to samples and Soxhlet-extracting with dichloromethane. The extract was then rinsed to remove interfering substances. The extract was then analyzed with gas chromatography/mass selective detector (GC/MSD). PAH components were identified with MSD by their retention times and the components molecular ions. PAH was measured as benzo[a]pyrene (b[alp]) and PAH$_{16}$.

Dichlorodiphenyltrichloroethane (DDT) and PCB were determined by adding internal standards to samples and extracting with organic solvents. The extracts were then rinsed to remove interfering substances and then analyzed using a gas chromatograph-electron capture detector. The organochloride compounds were identified by their respective retention times. DDT is the sum of DDT and the degradation products DDE and DDE. PCB was measured as PCB$_7$.

TBT was determined by adding internal standards to samples and adding an alcohol base. After pH calibration and derivatization, tin organic substances were extracted with
organic solutions, and samples were rinsed using gel permeation chromatography. Samples were then analyzed by GC coupled with atomic emission detection. Methyl- and phenyl compounds were identified by their retention times, and quantification was performed using the internal standard.

The redox potential \((E_h)\) was measured with a radiometer 201 platinum electrode, and sulfide \((pS)\) was measured with a radiometer F1212S sulfide-ion \((S^2-\) \) selective electrode. Electrodes were coupled to a switch against a silver-silver chloride electrode. For each sample, the half-cell potential of the reference electrodes was added to the observed potentials to estimate \(E_h\). \(pS\) was measured from \(E_o\) as the concentration of \(H_2S\) \((H_2S+HS^-+S)\) expressed as \(-\log [H_2S]\) as described in Schaanning et al. (1996). The pH was set to 7.2 in all cases.

For each sample, RPD was defined as the depth where the sediment changed from oxic to anoxic. The sediment sulfide content was defined as the \(pS\) value at a depth of 5 cm.

### 2.4 Numerical analysis

#### 2.4.1 Analysis of the environmental data set

The environmental variables measured within meadows and subjected to numerical analysis included the amount TN, TOC, Pb, Cd, Cu, Hg, Zn, b[a]p, PAH\(_{16}\), PCB\(_7\), DDT, TBT, sediment sulfide content, RPD, plant biomass, coverage, canopy height, and shoot density.

To investigate differences in organic loading and contamination between meadows, TOC and contaminants were graphed using 95 % confidence intervals where a lack of overlap indicated a significant difference. \(\alpha\) was set to 0.05 in all cases. Sediment redox and sulfide profiles where graphed with their means and standard deviations. The sediment oxygen zonation as proposed by Wildish et al. (2001) where used. Positive \(E_h\) values were indicative of oxic sediments, \(E_h\) values from 0 to -100 indicated hypoxic sediments and \(E_h\) values <-100 indicated anoxic sediments.
One-way ANOVAs were used to investigate differences in plant biometrics between the meadows. The parameters were checked for normality using the Anderson Darling test (Anderson and Darling 1952) and for equal variances using the Levene’s test (Levene 1960) (descriptive statistics are given in Appendix H). Plant canopy height, coverage, and shoot density were then analyzed using a standard one-way ANOVA, while a Kruskal-Wallis one-way test on ranks (Kruskal 1964) was used to analyze plant biomass.

An assessment of the ecological quality of the sediments was performed by classifying the metals, PAH$_{16}$, PCB$_7$, and TBT into ecological quality classes according to The Norwegian Climate and Pollution Agency’s (KLIF) classification system for metals and organic contaminants in seawater and sediments (KLIF 2007) (Table 1). Each ecological class is defined based on the substance’s toxicity and its effect on organisms. TOC concentrations were classified according to KLIF’s classification of environmental quality in fjords and coastal waters (Molvær et al. 1997).

The environmental variables$^4$ were log transformed ($\log{(x+1)}$) to avoid skewness and to obtain normality (Clarke and Gorley 2006). Pearson’s correlations were applied to the variables (Appendix I), and variables with correlation coefficients $> 0.95$ and $< -0.95$ where treated as a single variable. TOC, TN, and Cd were correlated, and the values for TOC were used. Pb and Hg were correlated, and the values for Pb were used. B[a]p and PAH$_{16}$ were correlated, and the values for b[a]p were used. Hereafter, these are referred to and treated as single variables. To account for different scales in the environmental measurements, the resulting data set was normalized by subtracting the mean from each variable and dividing by the standard deviation.

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$^4$ To match the number of chemical samples, two random samples from the plant biometric parameters were excluded from further analysis. The excluded replicates are indicated by an asterisk (*) in Appendix D.
Table 1: Classification of metals and organic contaminants in sediments. Roman numerals (I-V) indicate the upper limit for a given contaminant, and color code represents the different sediment quality classes. Based on KLIF’s classification guidelines (Molvær et al. 1997; KLIF 2007).

<table>
<thead>
<tr>
<th>Substance</th>
<th>Ecological classes based on substance concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I Very good</td>
</tr>
<tr>
<td></td>
<td>Background levels</td>
</tr>
<tr>
<td>Metals</td>
<td>mg kg⁻¹</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>30</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.25</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>35</td>
</tr>
<tr>
<td>Mercury (Hg)</td>
<td>0.15</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>150</td>
</tr>
<tr>
<td>PAH’s</td>
<td>µg kg⁻¹</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>6</td>
</tr>
<tr>
<td>PAH₁₆</td>
<td>300</td>
</tr>
<tr>
<td>Other organic</td>
<td>µg kg⁻¹</td>
</tr>
<tr>
<td>PCB₇</td>
<td>5</td>
</tr>
<tr>
<td>ΣDDT</td>
<td>20</td>
</tr>
<tr>
<td>TBT</td>
<td>1</td>
</tr>
<tr>
<td>TOC</td>
<td>&lt;20</td>
</tr>
</tbody>
</table>

The environmental variables were analyzed using a principal component analysis (PCA) to assess the environmental characteristics in control meadows and disturbed meadows. The environmental data set is represented in two independent axes, where axis one (PC1) is a linear combination of the original variables that minimizes the sum of the squared deviations of points on the new axis (i.e., in the direction of maximum variation in the data). PC1 gives the main trend in the data, while axis two (PC2) gives the second most important trend (Clarke and Warwick 2001). Vectors lying close to each other are
positively correlated. Vectors at a 90° angle to one another are uncorrelated, while vectors pointing in opposite directions are negatively correlated. The length of the vectors represents the extent of contribution in the two-dimensional plot; the longer the vector, the stronger the contribution. This analysis was performed on the normalized environmental variables.

2.4.2 Analysis of the faunal data set

To avoid over estimation of the recorded number of taxa, juveniles and other individuals that could not be separated into individual taxonomic groups (e.g., species or genus) were excluded from the numerical biodiversity analysis.

A set of diversity indices was calculated to measure the macrofaunal diversity between the stations. This study included the estimated number of individuals \( \cdot m^2 \), the number of taxa, Shannon diversity \( \exp(H') \) (Shannon and Weaver 1963), and Hurlbert’s diversity index \( \text{ES}_{100} \) (Hurlbert 1971). Descriptions of Shannon diversity and Hurlbert’s rarefaction are given in Appendix E.

The diversity measures were then graphed. \( N, S, \) and \( H' \) were plotted with 95% confidence intervals to test the null hypothesis of no difference in faunal diversity (1) between sand and meadows and (2) between control stations and disturbed stations. A lack of overlap indicated a significant difference. \( \alpha \) was set to 0.05 in all cases.

\( H' \) and \( \text{ES}_{100} \) were used to determine the ecological status (EQS) of each station according to the Norwegian classification guide for ecological quality of coastal waters (Vannportalen 2009) (Table 2). The classification is based on 1000 cm\(^2\) grab samples (see Chapter 4.4.1).

<table>
<thead>
<tr>
<th>Index</th>
<th>Ecological status based on the observed index value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( H' )</td>
<td>( \geq 3.8 )</td>
</tr>
<tr>
<td>( \text{ES}_{100} )</td>
<td>( \geq 25 )</td>
</tr>
</tbody>
</table>
Non-metric multi-dimensional scaling (nMDS) (Shepard 1962; Kruskal 1964) was applied to the faunal data to visualize the difference in faunal composition (1) between sand and meadows and (2) between control stations and disturbed stations. The faunal data set was square-root transformed prior to the analysis to reduce the importance of highly abundant species and to reduce stress (Clarke and Warwick 2001). The procedure was based on a Bray-Curtis similarity matrix (Bray and Curtis 1957) which was calculated using the transformed faunal data set. The Bray-Curtis formula is given in Appendix G.

NMDS ordination seeks the main trends in a data set and reduces high-dimensional data into a two-dimensional space. Here, the distance between two samples reflects their relative similarity in faunal composition based on the rank order of the data. It then constructs a plot showing the similarities. Samples situated closer together have more similar faunal compositions than samples that are farther apart. The degree to which the map matches the data, or “the goodness-of-fit,” is reported as Kruskal’s stress formula 1 (Kruskal 1964). (Kruskal’s stress formula 1 is given in Appendix G). A stress value <0.05 indicates that the map is an excellent representation of the data with no prospect of misinterpretation. Values <0.10 indicate a good relationship with the data with little prospect of misinterpretation. Values <0.20 indicate that useful information can be extracted, but one should not rely heavily on the details of the plot. Values >0.30 indicate a poor relationship with the data and that points are close to being randomly placed in the ordination space. The analysis was run with 100 iterations to ensure that an optimal solution had been found. The Bray-Curtis similarities were superimposed onto the nMDS plot to visualize similarities in faunal composition between stations.

Analysis of similarities (ANOSIM) was conducted to determine (1) whether the faunal compositions inside the meadows were significantly different from those of the sand and (2) whether the faunal compositions in the control stations were significantly different from those of the disturbed stations. ANOSIM is a distribution-free, multivariate analogue of ANOVA that tests for differences between groups defined a priori (Clarke 1993). The analysis was performed two times; first using sand/meadows, second time using control/disturbed stations as factor B. Inner/outer Oslofjord was in both cases used
as factor A. The reported R statistic measures the degree of separation among the groups. It is calculated for each station and averaged to give the global R. The R statistics are always between 0 and 1. If R = 1, all samples within a group are more similar to each other than any samples from other groups. If R = 0, the similarities between and within groups are the same (Clarke 1993). The significance level for group differences is given by a permutation test (999 permutations) generating a permutation distribution with the observed global R.

Similarity percentages (SIMPER) analysis was performed to examine the contribution of individual species to the differences (1) between sand and meadows and (2) between control stations and disturbed stations. The method computes the contribution from each species to any difference between groups of samples. Each species’ contribution is then defined as the average dissimilarity between groups. Species contributions are presented as percentage of dissimilarity. A measure of how well a species contributes to the observed dissimilarity is given by the dissimilarity of each species divided by the standard deviation (Clarke 1993). The analysis was performed on the Bray-Curtis similarities. Due to the lack of a three-way design option in PRIMER, the data set was divided into two datasets: fauna from inner Oslofjord and fauna from outer Oslofjord. Control/disturbed stations were used as factor A and sand/meadows were used as factor B.

2.4.3 Linking environmental variables to faunal data

The BIOENV function in PRIMER was applied to identify the environmental variables that best explained the pattern in faunal composition (1) between sand and meadows and (2) between control stations and disturbed stations. The method measures how closely related the faunal and environmental variables are by calculating a rank correlation coefficient (Spearman’s ρ) between all elements of the two data sets. The result is the environmental variable or the set of environmental variables that best explains the observed pattern in faunal composition. The method was applied on the Bray-Curtis similarity matrix based on transformed faunal data and a Euclidian distance matrix based on log-transformed and normalized environmental variables. Two replicate fauna samples
from each station were randomly excluded (these samples are marked with an asterisk, *, in Appendix A) prior to the analysis to match the number of environmental variables. The BEST function in PRIMER was applied to test the null hypothesis of $\rho = 0$, indicating that the set of environmental variables is not better than any random combination of variables.

Confidence intervals were computed using SigmaPlot version 11.0. All other univariate statistical analyses were performed using Minitab version 15. All multivariate analyses were computed using PRIMER (Plymouth Routines In Multivariate Ecological Research) version 6 (Clarke 1993; Clarke and Warwick 2001; Clarke and Gorley 2006). The grain size analysis was performed using the Microsoft Excel spreadsheet Gradistat (Blott 2001).
3 Results

3.1 Environmental variables

The raw data for faunal abundances and environmental variables are given in the Appendices A, B, C, and D.

3.1.1 Physical variables

The sediment from most of the stations had a high proportion of dead plant material (Table 3). A layer of ephemeral algae was observed in the meadows in Horneskilen and Skjebergkilen.

The sand in Sætrepollen consisted of dark sediments with many small coal fragments. A sulfidic odor characterized the sediments from both stations in Sandspollen.

The sediment in the meadows generally consisted of poorly sorted, very fine sand or very coarse silt. There were no clear patterns distinguishing the sediment parameters between the stations, except that the sand had a larger proportion of gravel and a lower grain size kurtosis than meadows. Meadows in Sandspollen and Sætrepollen had higher proportions of mud and a higher mean grain size than the sand. This pattern was reversed in Horneskilen and Skjebergkilen, where meadows had smaller proportions of mud and a lower mean grain size than the sand. The meadows in Sandspollen and Sætrepollen were characterized by finer particulate sediment than nearby sand. Disturbed meadows had higher mud contents than control meadows in both areas.

Overall, the stations had poor or very poor sediment sorting. Two stations were coarse skewed implying sediments skewed towards coarse material. Four stations were fine skewed implying sediments skewed towards finer material. The sand in Horneskilen and the meadow in Skjebergkilen had symmetrical skewness. There was no pattern between stations in the degree of grain size distribution “peakedness”. Stations were leptokurtic, platykurtic or mesokurtic.
Table 3: Physical characteristics for each station. The table lists the sample coordinates (latitude and longitude), sampling depth, the relative amounts of gravel, sand and mud, the sediment parameters mean grain size, grain size sorting, grain size skewness and grain size kurtosis, and a description of the sediment. Other remarks are also given. Sediment parameters are based on the Folk and Ward method (1957) and are given in phi units.

<table>
<thead>
<tr>
<th></th>
<th>Sandspollen</th>
<th>Sætrepollen</th>
<th>Horneskilen</th>
<th>Skjebergkilen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sand</td>
<td>Meadow</td>
<td>Sand</td>
<td>Meadow</td>
</tr>
<tr>
<td>Longitude</td>
<td>10 35.007</td>
<td>10 35.054</td>
<td>10 31.995</td>
<td>10 32.052</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Gravel (%)</td>
<td>10.9</td>
<td>1.7</td>
<td>23.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>83.1</td>
<td>70.0</td>
<td>49.1</td>
<td>57.4</td>
</tr>
<tr>
<td>Mud (%)</td>
<td>5.9</td>
<td>28.3</td>
<td>27.3</td>
<td>41.8</td>
</tr>
<tr>
<td>Mean (φ)</td>
<td>1.83</td>
<td>3.74</td>
<td>2.38</td>
<td>4.40</td>
</tr>
<tr>
<td>Sorting φ)</td>
<td>1.88</td>
<td>2.24</td>
<td>3.20</td>
<td>2.33</td>
</tr>
<tr>
<td>Skewness φ)</td>
<td>0.70</td>
<td>0.35</td>
<td>0.38</td>
<td>-0.03</td>
</tr>
<tr>
<td>Kurtosis φ)</td>
<td>4.37</td>
<td>2.09</td>
<td>1.65</td>
<td>1.55</td>
</tr>
<tr>
<td>Description</td>
<td>Medium sand, poorly sorted, coarse skewed, leptokurtic</td>
<td>Very fine sand, very poorly sorted, fine skewed, platykurtic</td>
<td>Fine sand, very poorly sorted, fine skewed, platykurtic</td>
<td>Very coarse silt, very poorly sorted, fine skewed, platykurtic</td>
</tr>
<tr>
<td>Other remarks</td>
<td>Sulfidic odor, Much dead plant material</td>
<td>Sulfidic odor, Much dead plant material</td>
<td>Dark sediment, Much dead plant material</td>
<td>Much dead plant material</td>
</tr>
</tbody>
</table>
There were marked differences in plant structure between the meadows (Table 4). Disturbed meadows had significantly higher canopies than control meadows. The higher plant canopy in disturbed meadows corresponds to the high mud content of the sediment in these meadows (Table 3). Sandspollen had the highest plant biomass (1,600 g·m⁻²), which was twice the amount in the meadow in Sætrepollen (800 g·m⁻²). Meadows in the outer Oslofjord were characterized by lower biomass (200 g·m⁻²) than meadows in the inner Oslofjord. Sandspollen also had the largest average plant coverage (75%) and the highest shoot density (113.6 shoots·m⁻²), although they were not significantly higher than those in the meadows of Sætrepollen and Skjebergkilen. Although non-significant, plant cover and shoot density were higher in inner Oslofjord compared to outer Oslofjord. With the exception of shoot density, the meadow in Horneskilen had the lowest plant biometric values.

Table 4: Plant structure parameters given as biomass·m⁻² (wet weight), plant cover (%), canopy height (cm), and the number of shoots·m⁻² from the four stations. The parameters at each station are given as the average of five samples ± their standard deviations. Similar letters indicate no significant difference (p > 0.05).

<table>
<thead>
<tr>
<th></th>
<th>Sandspollen</th>
<th>Sætrepollen</th>
<th>Horneskilen</th>
<th>Skjebergkilen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass m⁻² (g WW)</td>
<td>1600 ± 400ᵃ</td>
<td>800 ± 50ᵇ</td>
<td>200 ± 30ᶜ</td>
<td>200 ± 70ᶜ</td>
</tr>
<tr>
<td>Cover %</td>
<td>75 ± 25ᵃ</td>
<td>45 ± 21ᵇ</td>
<td>16 ± 8ᶜ</td>
<td>34 ± 20ᵇᶜ</td>
</tr>
<tr>
<td>Canopy height (cm)</td>
<td>43.6 ± 5.0ᵃ</td>
<td>60.0 ± 6.1ᵇ</td>
<td>19.0 ± 2.2ᶜ</td>
<td>59.0 ± 8.9ᵇ</td>
</tr>
<tr>
<td>No. of shoots·m⁻²</td>
<td>113.6 ± 33.5ᵃᵇ</td>
<td>93.6 ± 29.7ᵇᶜ</td>
<td>75.2 ± 15.3ᶜᵈ</td>
<td>72.8 ± 34.9ᵃᵇᵈ</td>
</tr>
</tbody>
</table>

3.1.2 Chemical variables

Organic loading

All sediment samples for determination of organic and contaminant loading were taken in meadows. Sediment TOC was highest in the control meadow in Sandspollen (123.3 ± 2.3 µg·mg⁻¹) while the disturbed meadow in Sætrepollen had the second highest TOC concentrations (74.0 ± 2.3 µg·mg⁻¹, Figure 6). The control meadow in Horneskilen and the disturbed meadow in Skjebergkilen had very similar concentrations (~48.0 ± 4.0 µg·mg⁻¹). TOC was positively correlated with all chemical parameters, except for DDT.
(Appendix I). All stations constitute very bad sediment quality according to the observed TOC values (Figure 6).

**Figure 6:** The average amount and standard deviation of total organic carbon (TOC) from three sediment samples taken in Zostera meadows in the Oslofjord in May 2010. TOC is classified according to KLIF’s classification guide (Molvær et al. 1997). Roman numerals indicate the upper level for the given contaminant, and color code represents the different sediment quality classes. See Table 1 for contamination limits for ecological classes. Station abbreviations: Sa = Sandspollen, Sæ = Sætrepollen, Ho = Horneskilen, Sk = Skjebekgiken, Z indicates Zostera.

**Contaminants**

In general, the sediment in disturbed meadows was less contaminated than the nearby control meadows (Figure 7a-e). The meadow in Sandspollen had the greatest contamination loadings, while the meadow in Skjebekgiken had the lowest concentrations. The high concentrations of Pb (100.6 ± 8.5 mg·kg⁻¹, Figure 7b) and Cu (109.3 ± 4.9 mg·kg⁻¹, Figure 7c) in the meadow in Sandspollen implies bad sediment quality. According to the concentrations of the other metals, the meadows were classified to either very good or good conditions.
Figure 7: The average amount and standard deviation of a) mercury (Hg), b) lead (Pb), c) copper (Cu), d) zinc (Zn), and e) cadmium (Cd) from three sediment samples taken in Zostera meadows in the Oslofjord in May 2010. Each metal is classified according to KLIF’s classification guide (KLIF 2007). Roman numerals (I-V) indicate the upper level for the given contaminant, and the color code represents the different sediment quality classes. See Table 1 for contamination limits for ecological classes. Station abbreviations: Sa = Sandspollen, Sæ = Sætrepollen, Ho = Horneskilen, Sk = Skjebergkilen, Z = Zostera.
The sediment in the meadow in Sandspollen also had the highest concentrations of organic contaminants (Figure 8a-e). The contamination pattern generally reflected that of the metals and was higher in inner Oslofjord than in outer Oslofjord. Both disturbed meadows had higher TBT concentrations than their nearby control meadows (Figure 8d). The highest TBT concentration was found in Sætrepollen \( (70.7 \pm 16.6 \, \mu g \cdot kg^{-1}) \), while the lowest concentration was found in Horneskilen \( (9.4 \pm 1.8 \, \mu g \cdot kg^{-1}) \). The concentrations of DDT showed an opposite pattern; the highest concentration was found in the meadow in Skjebergkilen \( (3.8 \pm 0.8 \, \mu g \cdot kg^{-1}) \), while the lowest concentration was found in the meadow in Sandspollen \( (1.2 \pm 0.2 \, \mu g \cdot kg^{-1}) \). TBT was found in concentrations classified as very bad sediment quality in all meadows (Figure 8d). All other contaminants comprised very good or good conditions in all meadows.
Figure 8: The average amount and standard deviation of a) benzo[a]pyrene (b[a]p), b) polycyclic aromatic hydrocarbon 16 (PAH$_{16}$), c) polychlorinated biphenyl 7 (PCB$_{7}$), d) tributyl tin (TBT), and e) dichlorodiphenyltrichloroethane (DDT) from three sediment samples taken in Zostera meadows in the Oslofjord in May 2010. Each substance is classified according to KLIF’s classification guide (KLIF 2007). Roman numerals (I-V) indicate the upper limit for the given contaminant, and the color code represents the different sediment quality classes. See Table 1 for contamination limits for ecological classes. Station abbreviations: Sa = Sandspollen, Sæ = Sætrepollen, Ho = Horneskilen, Sk = Skjebergkilen, Z = Zostera.
Based on the redox potentials, the meadows in Sandspollen and Sætrepollen were oxic to a greater depth than the sand stations, while the opposite was true in Skjebergkilen (Figure 9, upper panel). The redox potentials were similar in the sand and the meadow in Horneskilen. There were no visual differences between the control stations and the disturbed stations. All stations had oxic sediments to a depth of approximately 3 cm. The highest redox potentials were observed in the meadow in Sandspollen and in the sand in Skjebergkilen, where the sediment was oxic to a depth of 9 cm.

The meadows in Sandspollen, Sætrepollen and Skjebergkilen had higher sulfur concentrations than sand (Figure 9, lower panel). High sulfur concentrations (pS = ~ 5) were observed at approximately five cm and downwards in the meadow in Sandspollen, and in both substrates in Sætrepollen. Sulfur concentrations in Horneskilen and Skjebergkilen were non-toxic in both substrates. All meadows had low sulfur concentrations in the top sediment layer (above 5 cm).
Figure 9: Comparisons of sediment redox profiles and sulfur profiles in sand and in Zostera meadows at four locations in the Oslofjord in Mai 2010. Upper panel: sediment redox profiles ($E_h$) with reference lines dividing oxic from anoxic sediments. Lower panel: sediment sulfur profiles where pS is the estimated concentration of $H_2S$ ($H_2S + HS^- + S$) expressed as $-\log [H_2S]$. Values are the means and standard deviations from three sediment samples taken in sand (open circles) and Zostera meadows (black circles) in Mai 2010. Station abbreviations: Sa = Sandspollen, Sær = Sætrepollen, Ho = Horneskilen, Sk = Skjebergkilen.

The PCA plot of the environmental variables shows that there are two main gradients among meadows (Figure 10). The combination of TOC, TN, metals, PCBs, and plant biomass is an important variable in structuring PC1 in the positive direction. DDT contributes in structuring PC1 in the negative direction. RPD and sediment sulfide content strongly contribute in structuring PC2 in the negative direction, while plant canopy and, to some degree, TBT
structure PC2 in the positive direction. The PCA analysis shows that Sandspollen and Sætrepollen had the highest contaminant loadings. The samples from each meadow were grouped together in the plot, and the plot reflects the contamination pattern observed in the graphs above. Meadows in Sandspollen and Sætrepollen are on the right side of the plot. The control meadow in Sandspollen had higher concentrations of metals, hydrocarbons, TOC, and nitrogen as well as the highest plant biomass and coverage. The disturbed meadow in Sætrepollen had the highest TBT concentrations. High sediment sulfide content is indicated by low S values. Thus, the control meadow Horneskilen had the lowest sediment sulfide concentrations, while the meadow in Sætrepollen had the highest concentrations. The disturbed meadow in Skjebergkilen had the highest DDT concentrations. Plant canopy was highest in Sætrepollen and, to some degree, in Skjebergkilen. In the plot, PC1 accounted for 63.6% of the variation in the environmental data set from the meadows, and PC2 accounted for 17.7%. Together, PC1 and PC2 accounted for 81.4% of the variance among stations.

**Figure 10:** PCA of 17 environmental variables for 12 core samples (sediment chemistry variables randomly selected from a total of 20 samples) collected in *Zostera* meadows at four locations in the Oslofjord in Mai 2010. More than 80% of the variance between stations in environmental characteristics is explained by the first two PC axes. Pb, Hg = lead and mercury, Cu = copper, Zn = zinc, TOC, TN, Cd = total organic carbon, total nitrogen and cadmium, TBT = tributyl tin, hyd = PAHs, PCB7 = polychlorinated biphenyl 7, DDE = dichlorodiphenyltrichloroethane, RPD = redox discontinuity depth, S = sediment sulfur content, bio = plant biomass, cov = plant cover, can = canopy height, sho = shoot density. Station abbreviations: Sa = Sandspollen, Sæ = Sætrepollen, Ho = Horneskilen, Sk = Skjebergkilen, Z = *Zostera*. 

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3.2 Faunal data

A total of 1,535 individuals >500 µm from 33 taxa (excluding juveniles and unidentifiable individuals) were registered from the 40 core samples (Table 5). Gastropods dominated the fauna at 52.7% of the total number of individuals. Polychaetes were the most taxa-rich group, representing 30.3% of the total number of taxa recorded.

Table 5: The total number of individuals and taxa within the major taxonomic groups and the percentage of individuals and taxa within each group. The number of taxa is based on the reduced species matrix.

<table>
<thead>
<tr>
<th>Taxonomic Group</th>
<th>Individuals</th>
<th>Taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>Echinodermata</td>
<td>2</td>
<td>0.13</td>
</tr>
<tr>
<td>Crustacea</td>
<td>8</td>
<td>0.52</td>
</tr>
<tr>
<td>Insecta</td>
<td>331</td>
<td>21.56</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>809</td>
<td>52.70</td>
</tr>
<tr>
<td>Bivalvia</td>
<td>31</td>
<td>2.02</td>
</tr>
<tr>
<td>Oligochaeta</td>
<td>276</td>
<td>17.98</td>
</tr>
<tr>
<td>Polychaeta</td>
<td>78</td>
<td>5.08</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1535</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 6 shows that the numerically most abundant taxa were *Hydrobia ulvae* with 731 individuals comprising 48.9% of all individuals, marine insects (*Chironomus* sp.) with 331 individuals comprising 22.2% of all individuals, and *Tubificoides benedii* with 236 individuals comprising 15.8% of all individuals. Together, they accounted for 86.9% of the total number of individuals. Of all identified taxa, 13 taxa were represented by only a single individual in the samples.
The average abundance, number of taxa, Shannon diversity ($H'$), Hurlbert rarefaction ($ES(100)$) and the estimated number of individuals-m$^{-2}$ within each station are shown in Figure 11a-e. The figure also gives a classification of stations into EQS based on $H'$ and $ES(100)$.

The highest number of individuals was estimated to be in the sand in Sandspollen and the lowest in the meadow in Sandspollen (Figure 11a). There were, however, no significant differences in infaunal abundances between sand and meadows within or between locations. The confidence intervals were relatively large, particularly for samples taken in sand. This indicates a large variance in the number of individuals between core samples. The highest abundances were found in the sand in Sandspollen, with an average of 62 individuals per sample. The meadow in Sandspollen had the lowest abundances, with an average of 16.4 individuals per sample. On average, all meadows supported a lower number of taxa than did sand. Only 2.6 taxa were found in the meadows in Sandspollen and Horneskilen, while the sand in Sandspollen supported an average of seven taxa. This pattern was not significant, as the confidence intervals overlapped. However, Shannon diversity was significantly higher in the sand than in the meadows at all locations except Skjebergkilen. Sand in Sandspollen had the highest Shannon diversity (2.2) while the meadows in Horneskilen had the lowest (0.4).
Hurlbert’s diversity was near identical with the recorded number of taxa in samples (see Chapter 4.4.2). All meadows had a non-significant lower Hurlbert’s rarefaction than sand (Figure 11e). Hurlbert’s rarefaction was highest in the sand in Sandspollen (7.0) and lowest in the meadow in Sandspollen and Horneskilen (2.6).

The average faunal abundance varied between control and disturbed stations, and no overall pattern could be detected (Figure 11b). The number of taxa varied little between control and disturbed stations and no difference could be observed (Figure 11c). Disturbed meadows had a significantly higher Shannon diversity than control meadows (Figure 11d). Hurlbert’s diversity was very similar with respect to the number of taxa and showed no difference between control stations and disturbed stations (Figure 11e).

Most stations had bad or very bad EQS according to the classification criteria (Figure 11d-e). Based on Shannon diversity, the sand in Sandspollen had the best EQS and was classified as moderate. Classification by the use of Hurlbert’s rarefaction showed that three of the four sand stations had bad EQS, while all meadows were classified as very bad. Disturbed meadows were classified as bad according to the Shannon diversity index, while control meadows were classified as very bad.
<table>
<thead>
<tr>
<th>Station</th>
<th>Total abundance (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SaS</td>
<td>0</td>
</tr>
<tr>
<td>SaZ</td>
<td>20</td>
</tr>
<tr>
<td>SæS</td>
<td>40</td>
</tr>
<tr>
<td>SæZ</td>
<td>60</td>
</tr>
<tr>
<td>HoS</td>
<td>80</td>
</tr>
<tr>
<td>HoZ</td>
<td>100</td>
</tr>
<tr>
<td>SkS</td>
<td>120</td>
</tr>
<tr>
<td>SkZ</td>
<td>140</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Station</th>
<th>Total number of taxa (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SaS</td>
<td>0</td>
</tr>
<tr>
<td>SaZ</td>
<td>2</td>
</tr>
<tr>
<td>SæS</td>
<td>4</td>
</tr>
<tr>
<td>SæZ</td>
<td>6</td>
</tr>
<tr>
<td>HoS</td>
<td>8</td>
</tr>
<tr>
<td>HoZ</td>
<td>10</td>
</tr>
<tr>
<td>SkS</td>
<td>12</td>
</tr>
<tr>
<td>SkZ</td>
<td>14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Station</th>
<th>Hurlbert’s rarefaction (ES(100))</th>
</tr>
</thead>
<tbody>
<tr>
<td>SaS</td>
<td>0</td>
</tr>
<tr>
<td>SaZ</td>
<td>2</td>
</tr>
<tr>
<td>SæS</td>
<td>4</td>
</tr>
<tr>
<td>SæZ</td>
<td>6</td>
</tr>
<tr>
<td>HoS</td>
<td>8</td>
</tr>
<tr>
<td>HoZ</td>
<td>10</td>
</tr>
<tr>
<td>SkS</td>
<td>12</td>
</tr>
<tr>
<td>SkZ</td>
<td>14</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Station</th>
<th>Shannon diversity (H')</th>
</tr>
</thead>
<tbody>
<tr>
<td>SaS</td>
<td>0.0</td>
</tr>
<tr>
<td>SaZ</td>
<td>0.5</td>
</tr>
<tr>
<td>SæS</td>
<td>1.0</td>
</tr>
<tr>
<td>SæZ</td>
<td>1.5</td>
</tr>
<tr>
<td>HoS</td>
<td>2.0</td>
</tr>
<tr>
<td>HoZ</td>
<td>2.5</td>
</tr>
<tr>
<td>SkS</td>
<td>3.0</td>
</tr>
<tr>
<td>SkZ</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Figure 11: Bar charts of a) the estimated number of individuals m^-2 and the mean and 95% confidence intervals for b) infaunal abundance (N), c) number of taxa, d) Shannon’s diversity (H’) and e) Hurlbert’s rarefaction (ES100). Based on core samples collected in sand and in Zostera meadows in the Oslofjord in Mai 2010. The diversity indices is classified into ecological classes according to the Norwegian classification guide for ecological quality (Vannportalen 2009). Color codes for the different quality classes in the legend, see Table 2 for EQS limits. Station abbreviations: Sa = Sandspollen, Sæ = Sætrepollen, Ho = Horneskilen, Sk = Skjebergkilen, Z = Zostera, S = sand.
The relative abundance of infauna in sand was fairly consistent between locations and no clear differences between stations could be detected (Figure 12). Dominating taxa were marine insects (*Chironomus* sp.), gastropods and oligochaetes. Gastropods, oligochaetes, polychaetes and insects dominated sand samples while insects, gastropods and oligochaetes dominated samples from meadows. From the 33 taxa identified, 16 taxa, consisting mainly of crustaceans and polychaetes, were only found in the sand. Six taxa, consisting mainly of gastropods and polychaetes, were found exclusively in meadows. No bivalves were found in the meadows. Taxa found in both substrates included *Nassarius reticulatus* and *Arenicola marina*.

Oligochaetes made up 30%–40% of the recorded fauna in the disturbed meadows but were virtually absent from control meadows (Figure 12). In the inner Oslofjord, the control meadow had higher proportions of insects than did the disturbed meadow. In the outer Oslofjord, the fauna in the control meadow comprised almost completely gastropods. Here, the disturbed meadow was dominated by both gastropods and oligochaetes. Of the 33 taxa, eight taxa, consisting mainly of crustaceans, were found exclusively in control sand. Six taxa, consisting mainly of polychaetes, were found only in disturbed sand. The gastropod *Rissoa parva* was only found in control meadows, while four taxa, the amphipod *Microdeutopus gryllotalpa*, the gastropod *R. membranaea*, the oligochaete *Heterochaeta costata*, and the polychaete *Eteone* cf. *longa*, were found only in disturbed meadows.

![Figure 12: Relative abundances of echinoderms, crustaceans, insects, gastropods, bivalves, oligochaetes, and polychaetes in core samples collected in sand (left) and in *Zostera* meadows (right) in the Oslofjord in Mai 2010. Color codes for the taxonomic groups are defined in the legend. The figure shows compiled data from all stations. Sa = Sandspollen, Sæ = Sætrepollen, Ho = Horneskilen, Sk = Skjebergkilen, S = Sand, Z = *Zostera.*](image-url)
The low stress value of 0.4 indicated that the nMDS plot was an excellent representation of the faunal composition (Figure 6) (Clarke and Warwick 2001). Stations in the inner Oslofjord are situated on the left side of the plot, while stations from the outer Oslofjord are situated on the right side. Thus, the faunal composition in the outer Oslofjord differs from that in the inner Oslofjord.

The nMDS ordination differentiates between sand and meadows (Figure 13), meaning that the faunal composition differed between these substrates. This difference was verified by the ANOSIM, which found that the faunal composition in sand was significantly different from that in the meadows \((p = 0.01, \text{ global } R = 0.32)\). The black similarity contours on the nMDS plot indicate that the faunal composition in sand and meadows in Sandspollen and Sætrepollen was less than 40% similar. However, the red similarity contours indicates that the difference between meadows and sand was not obvious in outer Oslofjord; the faunal composition in sand in Horneskilen was at least 60% similar to that in sand and meadows in Skjebergkilen.

The nMDS ordination shows a larger separation between control meadows and disturbed meadows than between control sand and disturbed sand (Figure 13). Differences between stations are shown by a differentiation between all stations within the plot. Disturbed stations are generally situated above control stations. However, differences between control sand and disturbed sand are not readily detected; stations in Horneskilen and Skjebergkilen group together in the lower right corner of the plot and are at least 60% similar in faunal composition.

The two-way ANOSIM showed that the faunal composition in control meadows was significantly different from that in disturbed meadows \((p = 0.03, \text{ global } R = 0.54)\). The test showed, however, that there was no significant difference in faunal composition between control sand and disturbed sand \((p = 0.15, \text{ global } R = 0.11)\). The low R value (0.11) is in agreement with the nMDS plot and suggests overlap in faunal composition between control sand and disturbed sand.
Figure 13: Two-dimensional nMDS plot of infauna from core samples collected in sand and in meadows in the Oslofjord in May 2010. Positions are based on Bray-Curtis similarities of square root transformed data (stress = 0.04). The distance between samples is proportional to their relative similarity. The axes have no units. The Bray-Curtis similarities are superimposed as black (40% similarity) and red (60% similarity) contours. Station abbreviations: Sa = Sandspollen, Sæ = Sætrepollen, Ho = Horneskilen, Sk = Skjebergkilen. Z = Zostera, S = sand.

Table 7a-c shows that two to four species contributed to more or less 50 percent of the dissimilarity between all groups. Having its greatest abundance in sand, the oligochaete *T. benedii* contributed, in both regions, approximately 20 % to the dissimilarity between sand and meadows (Table 7a). In inner Oslofjord also *H. ulvae* was a good discriminator having its highest abundance in sand and contributing approximately 19 % to the difference between substrates (Table 7a).

*T. benedii* was more abundant in disturbed meadows than in control meadows and contributed approximately 20 % to the dissimilarity between stations (Table 7b). In inner Oslofjord, *H. ulvae* had higher abundance in the control sand than in the disturbed sand, contributing 19.1 % to the dissimilarity between stations (Table 7c). The oligochaete *T. pseudogaster* was the best discriminator between control sand and disturbed sand in the outer Oslofjord (Table 7c) and contributed 11.9 % to the dissimilarity between stations.
Table 7: List of taxa contributing to the differences in faunal composition between (A) sand and meadows in inner and outer Oslofjord, (B) control meadows and disturbed meadows in inner and outer Oslofjord, and (C) control sand and disturbed sand in inner and outer Oslofjord determined by the SIMPER analysis in PRIMER. Columns one and two give the average abundance of each species; column three is the species contribution to the between-group dissimilarity; column four gives the ratio of the average contribution (column three) divided by the standard deviation (SD) of those contributions across all pairs of samples making up this average and is a measure of how consistently the species contribute to the group differences. Column five is the contribution of each species to the total dissimilarity, and column six is the cumulative contribution of the identified influential taxa (50% cut-off point).

Table 7a

<table>
<thead>
<tr>
<th>Species</th>
<th>Av. ab. sand</th>
<th>Av. ab. Meadow</th>
<th>Av. dissim.</th>
<th>Av. dissim. /SD</th>
<th>Contribution %</th>
<th>Cum. cont. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrobia ulvae</td>
<td>2.5</td>
<td>0.7</td>
<td>11.6</td>
<td>1.4</td>
<td>19.4</td>
<td>19.4</td>
</tr>
<tr>
<td>Tubificoides benedii</td>
<td>2.7</td>
<td>1.6</td>
<td>10.4</td>
<td>1.8</td>
<td>17.4</td>
<td>36.9</td>
</tr>
<tr>
<td>Chironomus sp.</td>
<td>3.0</td>
<td>3.4</td>
<td>8.22</td>
<td>1.3</td>
<td>13.8</td>
<td>50.6</td>
</tr>
</tbody>
</table>

Species contributing to the differences in faunal composition between sand and meadows in outer Oslofjord, total dissimilarity = 53.12

<table>
<thead>
<tr>
<th>Species</th>
<th>Av. ab. sand</th>
<th>Av. ab. Meadow</th>
<th>Av. dissim.</th>
<th>Av. dissim. /SD</th>
<th>Contribution %</th>
<th>Cum. cont. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrobia ulvae</td>
<td>4.7</td>
<td>4.8</td>
<td>16.6</td>
<td>1.7</td>
<td>31.3</td>
<td>31.3</td>
</tr>
<tr>
<td>Tubificoides benedii</td>
<td>2.3</td>
<td>0.9</td>
<td>10.8</td>
<td>1.6</td>
<td>20.3</td>
<td>51.7</td>
</tr>
</tbody>
</table>
Species contributing to the differences in faunal composition between the control meadow and the disturbed meadow in the inner Oslofjord, total dissimilarity = 44.72

<table>
<thead>
<tr>
<th>Species</th>
<th>Av. ab. control</th>
<th>Av. ab. disturbed</th>
<th>Av. dissim.</th>
<th>Av. dissim. /SD</th>
<th>Contribution %</th>
<th>Cum. cont. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubificoides benedii</td>
<td>0.2</td>
<td>2.7</td>
<td>17.3</td>
<td>1.8</td>
<td>38.6</td>
<td>38.6</td>
</tr>
<tr>
<td>Chironomus sp.</td>
<td>3.8</td>
<td>4.3</td>
<td>8.4</td>
<td>1.6</td>
<td>18.7</td>
<td>57.4</td>
</tr>
</tbody>
</table>

Species contributing to the differences in faunal composition between the control meadow and the disturbed meadow in the outer Oslofjord, total dissimilarity = 60.25

<table>
<thead>
<tr>
<th>Species</th>
<th>Av. ab. control</th>
<th>Av. ab. disturbed</th>
<th>Av. dissim.</th>
<th>Av. dissim. /SD</th>
<th>Contribution %</th>
<th>Cum. cont. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrobia ulvae</td>
<td>6.8</td>
<td>2.8</td>
<td>23.1</td>
<td>2.1</td>
<td>38.4</td>
<td>38.4</td>
</tr>
<tr>
<td>Tubificoides benedii</td>
<td>0.0</td>
<td>1.9</td>
<td>10.8</td>
<td>1.7</td>
<td>18.0</td>
<td>56.4</td>
</tr>
</tbody>
</table>
Table 7c

<table>
<thead>
<tr>
<th>Species</th>
<th>Av. ab. control</th>
<th>Av. ab. disturbed</th>
<th>Av. dissim.</th>
<th>Av. dissim. /SD</th>
<th>Contribution %</th>
<th>Cum. cont. %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hydrobia ulvae</em></td>
<td>3.2</td>
<td>1.9</td>
<td>9.2</td>
<td>1.47</td>
<td>19.1</td>
<td>19.1</td>
</tr>
<tr>
<td><em>Bittium reticulatum</em></td>
<td>1.9</td>
<td>1.4</td>
<td>8.2</td>
<td>1.53</td>
<td>17.0</td>
<td>36.1</td>
</tr>
<tr>
<td><em>Tubificoides benedii</em></td>
<td>3.4</td>
<td>1.9</td>
<td>5.5</td>
<td>1.46</td>
<td>11.4</td>
<td>47.5</td>
</tr>
<tr>
<td><em>Scoloplos armiger</em></td>
<td>1.8</td>
<td>0.6</td>
<td>5.3</td>
<td>1.51</td>
<td>11.0</td>
<td>58.5</td>
</tr>
</tbody>
</table>

Species contributing to the differences in faunal composition between the control sand and the disturbed sand in the outer Oslofjord, total dissimilarity = 46.62

<table>
<thead>
<tr>
<th>Species</th>
<th>Av. ab. control</th>
<th>Av. ab. disturbed</th>
<th>Av. dissim.</th>
<th>Av. dissim. /SD</th>
<th>Contribution %</th>
<th>Cum. cont. %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hydrobia ulvae</em></td>
<td>4.6</td>
<td>4.8</td>
<td>15.2</td>
<td>1.9</td>
<td>32.7</td>
<td>32.7</td>
</tr>
<tr>
<td><em>Tubificoides benedii</em></td>
<td>3.0</td>
<td>1.6</td>
<td>8.1</td>
<td>1.7</td>
<td>17.3</td>
<td>50.0</td>
</tr>
<tr>
<td><em>Tubificoides pseudogaster</em></td>
<td>1.4</td>
<td>0.0</td>
<td>5.6</td>
<td>1.4</td>
<td>11.9</td>
<td>61.9</td>
</tr>
</tbody>
</table>
There was little similarity between grain size parameters and faunal diversity. However, a higher abundance and taxonomic richness in the sand station in Sandspollen was reflected in the sediment here having a higher sand content compared to the nearby meadow.

Of the two environmental variables subjected for analysis, RPD was the variable that best explained the difference in faunal composition between meadows and sand (\( \rho = 0.153 \), Table 8). The sediment sulfide content had a slight negative correlation with the community pattern (\( \rho = -0.053 \)). Both variables were, however, insignificant in structuring the faunal pattern (\( P > 0.05 \)).

Due to the high inter-correlation between the environmental variables within the meadows, many showed significant, high correlations with the observed faunal pattern (Table 8).

The BIOENV procedure revealed that TOC, TBT, and plant canopy made up the best set of environmental variables for explaining the pattern of infaunal community between meadows (\( \rho = 0.765 \), Table 8). The BEST analysis was highly significant (\( P = 0.01 \)). However, Zn, PAH\(_{16}\) and b[a]p, and plant biomass also had significant correlation coefficients (\( P < 0.05 \)). Thus, the faunal

3.3 Linking environmental variables to faunal data

<table>
<thead>
<tr>
<th>Most important single variable between substrates</th>
<th>( \rho )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPD</td>
<td>0.153</td>
<td>0.24</td>
</tr>
<tr>
<td>Sulfur</td>
<td>-0.053</td>
<td>0.68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Most important single variable in meadows</th>
<th>( \rho )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC, TN, Cd</td>
<td>0.488</td>
<td>0.04</td>
</tr>
<tr>
<td>Cu</td>
<td>0.373</td>
<td>0.12</td>
</tr>
<tr>
<td>Pb, Hg</td>
<td>0.304</td>
<td>0.21</td>
</tr>
<tr>
<td>Zn</td>
<td>0.520</td>
<td>0.01</td>
</tr>
<tr>
<td>PCB(_7)</td>
<td>0.467</td>
<td>0.07</td>
</tr>
<tr>
<td>DDT</td>
<td>0.236</td>
<td>0.24</td>
</tr>
<tr>
<td>B[a]p, PAH(_{16})</td>
<td>0.488</td>
<td>0.05</td>
</tr>
<tr>
<td>TBT</td>
<td>0.559</td>
<td>0.04</td>
</tr>
<tr>
<td>RPD</td>
<td>0.067</td>
<td>0.23</td>
</tr>
<tr>
<td>Sulfur</td>
<td>-0.084</td>
<td>0.72</td>
</tr>
<tr>
<td>Biomass</td>
<td>0.461</td>
<td>0.05</td>
</tr>
<tr>
<td>Cover</td>
<td>0.212</td>
<td>0.81</td>
</tr>
<tr>
<td>Canopy</td>
<td>0.527</td>
<td>0.03</td>
</tr>
<tr>
<td>Shoots</td>
<td>0.040</td>
<td>0.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The BEST* set of variables in meadows</th>
<th>( \rho )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOC, TBT, Zostera canopy</td>
<td>0.765</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 8: Relationship between environmental variables and faunal pattern, given as Spearman’s rank correlation \( \rho \), between each environmental variable measured (1) between substrates, (2) inside meadows, and the Bray Curtis similarities of the normalized faunal data, using BIOENV in PRIMER. * shows the best set of explanatory variables from the BEST analysis. \( \rho \) is given for each environmental variable. All variables were log-transformed prior to the analysis. Variables with correlation coefficients >0.95 are treated as a single variable. \( P \) is the test statistics given by the BEST analysis. Significant values are in bold (\( \alpha = 0.05 \)).
composition was structured by a range of variables.
4 Discussion

The results from this study showed that meadows supported a lower number of taxa and a lower faunal diversity than sand. The low taxonomic diversity in meadows may result from high organic loading and sediment hypoxia. The seagrass canopy alters water flow and the deposition of organic matter and contaminants is probably more enhanced in meadows compared to sand.

The infaunal communities in meadows close to marinas were significantly different from those remote from marinas. In inner Oslofjord, the contaminated control meadow was completely dominated by *Chironomus* sp. In outer Oslofjord, *H. ulvae* dominated the control meadow while the disturbed meadow was more species rich. There could, however, not be detected a direct negative effect from marinas since high contamination was also recorded in the control locations. Nonetheless, the infaunal communities in the most contaminated meadows were dominated by opportunistic taxa typically of disturbed areas.

The infaunal communities in sand close to marinas were not different from those remote from marinas. Compared to meadows, accumulation of contaminants may be lower in sand.

Multiple factors may have caused the observed patterns in infaunal communities. The low taxonomic diversity recorded in the inner Oslofjord may be explained by high concentrations of TBT, Cu, nitrogen and TOC. Eutrophication may explain the high abundance of gastropods in outer Oslofjord. In general, meadows in sheltered bays and polls seem to support low species diversity.

4.1 General patterns in faunal diversity

The low fauna diversity in meadows compared to sand are in contrast to the general assumption that meadows support richer infaunal communities than sand. This indicates that the structuring mechanisms exerted by the seagrass ecosystem may have been overridden by other factors.

The taxonomic diversity in meadows in the Oslofjord is lower than those reported from other meadows on the Norwegian coast. Fredriksen et al. (2010) found an average of 6–14 species per
sample among four meadows on the south and west coasts of Norway. This is approximately twice the number of taxa recorded in the present study. The taxonomic numbers recorded in this study is more similar to those of Boström and Bonsdorff (1997), who studied meadows in the Baltic Sea and found an average of 5.9–8.8 species per sample.

Meadows situated in fjords and sheltered waters, characterized by periodic hydrodynamic fluctuations, may support fewer species than more wave exposed meadows (Degerman and Pihl 1985). Similar to the inner Oslofjord, the Baltic Sea experience fluctuating salinity which may naturally affect faunal distribution (Kaiser et al. 2005). Also, both areas receives nutrient run-off from land which may cause severe eutrophication outbreaks with detrimental effects on biota (Mirza and Gray 1981; Cederwall and Elmgren 1990). These similarities may partly explain the similarity in species number between the two areas. The areas differ, however, in being geographical remote from each other. In addition, the Baltic Sea has a young geological age which further may explain the low species diversity there (Schiewer 2008). Importantly, the high species diversity recorded by Fredriksen et al. (2010) did not vary significantly among meadows on the southern and western coast of Norway. These meadows are more exposed to waves and the waters are characterized of being oceanic, having a more stable salinity than waters in enclosed bays in the Oslofjord. According to the intermediate disturbance hypothesis (IDH), species diversity peaks at intermediate levels of disturbance and declines at lower and higher levels of disturbance (Connell 1978). In comparison, the higher species diversity on the southern and western coast of Norway suggests that meadows in the Oslofjord may have been subjected to high or low levels of disturbance. Such disturbance may originate from e.g. fluctuating salinity or contamination.

### 4.2 Causes for differences between sand and meadow infauna

The number of species recorded in sand (4.8–7.0) was similar to previous recordings suggesting that sand was less influenced by external factors than meadows. In sand, Fredriksen et al. (2010) recorded 6–12 species per sample, while Boström (1997) reported 4.6–5.5 species per sample. However, the ANOSIM analysis yielded an R value of 0.32, indicating that the fauna overlapped between the two substrates (Clarke and Gorley 2006). The factors influencing the distribution of
animals include habitat choice and degree of shelter from predation and other disturbances (Woodin 1978; Lewis 1984; Summerson and Peterson 1984; Gotceitas and Colgan 1989). Considering the difference in habitat structure between meadows and sand, the result from this study is surprising. Of the 33 taxa, six were exclusively found in meadows. From these, only three are commonly associated with macrophytes (*M. gryllotalpa* and *R. membranacea* and *R. parva*) (Fredriksen et al. 2005; Nilsen 2007). The 11 taxa that were found in both substrates included more omnipresent taxa such as *Chironomus* sp, *N. reticulatus*, *H. ulvae*, *T. benedii* and *A. marina*. The high overlap between sand and meadows may also be due to the “edge effect”. Species in the sand stations may have been interacting with the edges of the meadows where shoots are less dense.

The dominance of *Chironomus* sp. larvae in meadows in Sandspollen may be explained by the density and amount of plant biomass. *Z. marina* have high turnover rates, and a new leaf may be produced every two weeks in Scandinavian waters (Sand-Jensen 1975; Pinnerup 1980). The litter is then incorporated in the sediment and adds to the carbon pool. This may explain the high correlation between TOC and plant biomass. Both TOC and plant biomass were highest in Sandspollen, where the larvae of *Chironomus* sp. dominated most of the fauna. These larvae are often found attached to epibenthic detritus (e.g., seagrass litter), which provides shelter, a location for building their tubes, and a source of food and oxygen (Neumann 1976). Substrate availability would then increase with increasing plant biomass. In comparison, unvegetated sand is a two-dimensional substrate for these non-burrowing epibenthic insects, and space could be a limiting factor. The positive correlation between macrophyte biomass and abundance of *Chironomus* sp. has also been previously reported (Drake and Arias 1995). The sand in Sandspollen was more diverse and dominated by more infaunal taxa (e.g., polychaetes, gastropods) than the meadows.

The importance of grain size in structuring faunal composition in meadows is not clear. The higher abundance and species richness recorded in the sand in Sandspollen was reflected in the sediment grain size parameters. The mud content was lower, and the sand content was higher at this station compared to the nearby meadow (Table 3). Sediment grain size may be an important factor in structuring infaunal communities in seagrass meadows (Boström et al. 2006). However, there was no relationship between grain size parameters and faunal abundance or the number of
taxa in any of the other stations in this study. This is similar to the findings of Fredriksen et al. (2010), who did not find any relationship between grain size and taxa numbers in meadows on the southern and western coasts of Norway.

Differences in predation pressure are unlikely to have caused the differences in faunal diversity between the two substrates. The higher faunal diversity in sand may indicate that predation pressure was higher in meadows. However, this is unlikely, because the seagrass root system is believed to make predation less efficient in meadows than in sand (Orth et al. 1984 and references therein). Further, Webster (1998) found that faunal diversity increased with increasing shoot density. This was explained by the increase in biomass below ground with increasing shoot density, which provides more shelter for infauna. In the present study, the meadow with the highest shoot density (Sandspollen, Table 4) had the lowest number of taxa, Shannon diversity, and expected number of species per sample (Figure 11). Shoot density was within the range reported in other studies (Boström and Bonsdorff 1997; Webster 1998; Fredriksen et al. 2010), although they reported higher abundance and taxonomic richness in meadows than in sand. However, Webster’s findings (1998) somewhat contradicted the findings of Heck Jr. and Thoman (1981), who found that only dense seagrass meadows (674 shoots·m⁻²) significantly affected predation efficiency and that less dense meadows provided no more shelter than unvegetated sediments. Thus, the role of shoot density in the present study, which ranged from approximately 73 to 114 shots·m⁻², is not readily interpreted. Nevertheless, it is highly unlikely that the lower taxonomic diversity in meadows than in the sand biotope resulted from a higher predation pressure.

*H. ulvae* was probably attracted to a layer of ephemeral algae on top of the sediment in the meadow in Horneskilen. *H. ulvae* are known to colonize and commonly inhabit algal mats where it may appear in dense patches (Soulsby et al. 1982; Hull 1987; Norkko and Bonsdorff 1996b; Norkko et al. 2000). The algae may also decompose and provide a food supply for the snails which may then increase in abundance (Levinton and Bianchi 1981). Ephemeral algae were also present at other stations and *H. ulvae* could probably have influenced abundance numbers there as well. Nutrient run-off from agricultural land favors the growth of such algae.
The ability of the seagrass canopy to lend structure could have made the sediment more prone to hypoxia. The plant canopy alters water flow and traps fine particulate sediment and floating algae; the seagrass ecosystem produces more carbon than the community needs (Ginsburg and Lowenstam 1958; Sternberg 1968; Fonseca et al. 1982; Thomas and Cornelisen 2003; Peterson et al. 2004; Duarte et al. 2005). Thus, together with the high production of plant biomass, these factors may have continuously added fine particulate nutrients and sediment, plant litter, and algae to the ecosystem. These mechanisms are believed to be key factors underlying the high faunal diversity in seagrass meadows compared to sand. However, continuous deposition of particles causes accumulation of organic matter and consumes oxygen. In enclosed areas, the result is often anoxic sediments, which stimulate sulfate reduction (Holmer and Kristensen 1996).

Thus, a difference in the faunal composition between meadows and sand could be explained by meadows being more prone to hypoxic outbreaks. This theory is supported by the distribution pattern of H. ulvae. Since it is highly sensitive to low levels of sediment oxygen (Norkko and Bonsdorff 1996a), it was absent from meadows but present in sand in Sandspollen and Sætrepollen. If TOC concentrations are used as a proxy for the frequency of hypoxic events, then the meadows in inner Oslofjord can be considered more prone to hypoxia.

Through their leaves, plants transport oxygen to their roots and rhizomes (Pedersen et al. 1998) and may thereby avoid anoxia and sulfidic sediments (Holmer and Nielsen 1997; Mateo et al. 2006). This was also seen in the present study (Figure 9, upper panel). In agreement with the findings by Holmer and Nielsen (1997) and Lee and Dunton (2000), sediment sulfur content was higher in meadows than in sand (Figure 9, lower panel). The difference between substrates was explained by that the continuous input of plant biomass in meadows favor microbial breakdown and, hence, sulfate reduction. In the present study, this was reflected in the TOC concentrations which were higher in meadows with sulfidic sediments. The meadows in outer Oslofjord had lower TOC concentrations and did not have sulfidic sediments (Figure 9, lower panel). However, the top sediment was not anoxic at any of the stations. Animals living in this part of the sediment may therefore have avoided low oxygen levels and toxic sulfur concentrations. This can explain why the BIOENV analysis did not find any relationship between the fauna and the RPD and sediment sulfide content.
The low taxonomic diversity could be a result of periodic sediment hypoxia. Although the top sediment was oxic in all stations, microbial degradation and sulfate reduction vary by season (Holmer and Kristensen 1996; Perez-Dominguez et al. 2006). When investigating microbial degradation in sediments under a fish farm, Holmer and Kristensen (1996) found that sulfate reduction was highest in the summer months and declined when water temperatures decreased. The field work in the present study was performed in the spring, when the water temperature was still relatively low. Thus, the study did not capture the environmental conditions at the worst with respect to hypoxic events. Albeit not harmful for the plant, Z. marina roots and rhizomes also experience diurnal fluctuations, with changes from oxic to anoxic conditions within hours (Lee and Dunton 2000). Since these processes depend on photosynthesis, sediments tend to be anoxic at night. Thus, the sediment could have been affected by episodic oxygen depletion and sulfate reduction, although not indicated in all sediment sulfur profiles. If occurring with periods of recruitment, such event may significantly effect infaunal populations (Breitburg 1992). Therefore, the high presence of opportunistic species in meadows may also reflect episodic hypoxia.

4.3 The effect from marinas

In addition to high organic loading and episodic hypoxia, the low species diversity in meadows could also be a result of chemical and physical disturbances from marinas. Cu and TBT, being actively introduced into the marine environment for their toxic properties, could be responsible for the observed faunal pattern. These contaminants may accumulate in areas even more remote from marinas.

4.3.1 Sediment contamination

The classification of contaminants showed that the faunal communities in meadows could have been affected by contamination. In the Norwegian classification of sediment quality (KLIF 2007), the upper limit of class II (good) corresponds to a Predicted No Effects Concentration (PNEC) at chronic exposure. Long-term exposure to concentrations above this limit may damage faunal communities. Most contaminants were classified as good environmental quality (Figure 7-8), meaning that concentrations of these contaminants were not expected to have toxic effects on
organisms. However, the concentrations of Pb and Cu were in class IV (bad) in Sandspollen (Figure 7 b and c), meaning that short-term exposure to these concentrations may damage faunal communities. Concentrations of TBT were in class V (very bad) in all stations (Figure 8), meaning that short-term exposure to these concentrations may severely damage faunal communities (KLIF 2007).

The correlation between Cd and the faunal pattern was not likely a direct relationship. The Cd concentrations were orders of magnitudes lower than those found to affect soft bottom communities (Bryan and Langston 1992 and references therein). In addition, Cd concentrations were classified as representing good sediment qualities in all meadows (Figure 7e). Although the BIOENV analysis identified Cd as significant in explaining the faunal pattern, it was also highly correlated with TOC (Appendix I). Thus, Cd probably functioned as a proxy for TOC and its correlation with the fauna probably reflects this association. With the exception of Cu, few metals have been shown to affect infaunal communities. In cases where a direct fauna-metal relationship has been found (Bryan and Langston 1992 and references therein), concentrations are usually orders of magnitudes above those in the present study. Tranum et al. (2004) investigated the effect of metal contamination on invertebrate colonization of soft bottoms in the Oslofjord. Further, they tried to separate the effects of contamination from the effects of low oxygen levels. Only Cu had significant negative effects. The authors concluded that the effects of low sediment oxygen levels were more critical to the colonization of benthic fauna than the effects of high metal concentrations. Therefore, further discussion will focus on the potential effects of Cu and TBT.

Cu and TBT levels may explain the low taxonomic diversity in Sandspollen and Sætrepollen. Cu is one of the most toxic metals to marine organisms (Abel 1989) and has been found to greatly reduce the number of infaunal species in Norwegian coastal waters (Rygg 1985). Here, its effects overrode the effects of other metals (Pb and Zn) as well as natural factors, such as grain size and sediment organic content. These findings are relevant to the present study, as the contamination in Sandspollen may have overridden the structuring mechanisms exerted by the seagrass. As Cu was found to represent bad conditions only in the meadow in Sandspollen, only the fauna there should be affected. This explains why the BIOENV procedure identified no significant relationship between Cu and the faunal composition within meadows. The TBT concentrations
recorded in Sandspollen and Sætrepollen were within the same range as those found to affect mussels in other Norwegian marinas (Berge et al. 1997). TBT is toxic to various marine organisms, including mollusks (Laughlin et al. 1986; Spooner et al. 1991; Oehlmann et al. 1996; Horiguchi et al. 1997), crustaceans (Evans and Laughlin Jr 1984), and polychaetes (Meador and Rice 2001; Lau et al. 2007). In all cases, TBT has been shown to affect survival, reproduction, growth, and, hence, whole populations. Thus, it seems plausible that longer exposure to Cu and TBT may explain the low taxonomic diversity in meadows in inner Oslofjord.

Although not investigated in this study, marinas may also exert physical disturbance to seagrasses and associated fauna. Disturbances such as mechanical damage caused by anchoring and moorings (Walker et al. 1989; Hastings et al. 1995; Lloret et al. 2008), shadowing due to the presence of docks (Loflin 1995; Burdick and Short 1999), smothering caused by dredging, or indirect effects due to altered water turbidity (Onuf 1994).

### 4.3.2 Effects on the infaunal communities

Although no direct negative effects could be detected from marinas, the infaunal communities in the most contaminated meadows, were typical of impacted communities. For example, the oligochaete *T. benedii* is an indicator species of hypoxic and polluted sediments (Giere et al. 1999). It is a typical opportunistic species, being short lived and having a small body size (Pearson and Rosenberg 1978). *Chironomus* sp. larvae dominated the faunal community in meadows in Sandspollen and Sætrepollen. These insects tolerate high organic loadings and pollution (Waterhouse and Farrel 1985) and are used in the Ecological Quality (EcoQ) index (Simboura and Zenetos 2002) as indicator organisms. The occurrence of opportunistic species in these meadows corresponds with the high concentrations of chemicals, indicating that contaminants are responsible for the observed faunal composition. This was also reflected in the faunal diversity indices, which classified these meadows as bad and very bad EQS (Figure 11 d and e). Although less dominant, *T. benedii* was also found in the meadows in outer Oslofjord (Figure 12). However, oligochaetes are common in seagrass meadows (e.g., Boström and Bonsdorff 1997; Webster 1998; Bowden et al. 2001; Fredriksen et al. 2010), and, as opportunistic taxa, their distributions may not solely reflect the contamination pattern.
If the locations in inner Oslofjord are considered disturbed locations, and the locations in outer Oslofjord are considered control locations, then the faunal distribution pattern clearly reflects the contamination pattern. The dominance of pollutant-tolerant species in disturbed meadows coincides with the high concentrations of contaminants recorded there. Although the faunal communities in meadows in the outer Oslofjord were classified as bad and very bad EQS, they were not dominated by pollution-tolerant taxa. As discussed earlier, the dominance of *H. ulvae* in these meadows probably reflected eutrophication, a disturbance not directly related to marina activities. However, it is important to stress the large spatial heterogeneity that is naturally observed in benthic communities. In fact, faunal communities are patchily distributed on a range of spatial scales (Preston 1962; Morrisey et al. 1992; Archambault and Bourget 1996). Such heterogeneity becomes apparent when investigating the differences in dominating fauna between meadows (Figure 12). Thus, the difference in faunal composition may also reflected the heterogeneity characteristic of all natural environments (Levin 1992).

The univariate (Figure 11b-e) and multivariate methods did not identify any significant differences in infaunal composition between control sand and disturbed sand. As discussed earlier, the number of recorded taxa was similar to other studies in Scandinavia investigating unvegetated sand close to *Z. marina* meadows (Boström and Bonsdorff 1997; Fredriksen et al. 2010). However, those authors did not discuss whether the study locations were disturbed in any way. In the case of Fredriksen et al. (2010), one study location was situated on the west coast of Norway. Waters are rapidly replenished in this area, and benthic biodiversity is high. Even in this location, the average number of recorded taxa in sand (~10) did not significantly differ from those in the present study. However, the faunal composition in this study was dominated more by opportunistic species than on the west coast of Norway (Fredriksen et al. 2010).

The number of taxa and the estimated number of individuals per m² in sand was comparable between this study and a study from Sweden investigating the effects of marinas on soft bottom communities (Degerman and Pihl 1985). The authors proposed that poor sediment fauna was related to limited water circulation in marinas in enclosed bays; they emphasized the effects of contamination from boat maintenance and other effluents from marinas. Similarly, opportunistic taxa comprised most of the recorded fauna there.
Was the faunal community in meadows more affected by contamination than the sand communities?

The fauna in meadows may have experienced higher contamination loadings than those in the sand. The plant biomass may accumulate contaminants to concentrations higher than those found in the sediment (Pulich et al. 1976; Francois et al. 1989). Accordingly, concentrations in the plant may be orders of magnitudes greater than those in nearby ambient and interstitial waters (Lyngby et al. 1982). Decomposed seagrass enters the sediment and the detrital food web, and contaminants bound to the plant may become available to higher trophic levels (Zieman et al. 1984). Therefore, the rapid turnover rate of seagrass leaves continuously adds contaminants to the sediment, where it then accumulates (Kelly et al. 1990). As with organic matter, contaminant loading may be greater in meadows than in sand and override the positive effects exerted by the seagrass.

The distributional pattern of *H. ulvae* in the inner Oslofjord may indicate that the sand was less affected by contamination. This gastropod was almost absent from the meadows in inner Oslofjord; in the sand, it dominated 30%–40% of the fauna and contributed approximately 19% to the difference between the two substrates (Table 7a). This suggests that the sand offered a better living environment than meadows. The gastropod has also been found to be an effective indicator species of contaminated sediments (Araújo et al. 2011); when contamination is severe, it migrates through the sediment to attempt avoidance.

4.3.3 Possible causes for the observed contamination pattern

In the present study, the concentrations of contaminants were not elevated compared to Norwegian marinas (Næs et al. 2000; Næs et al. 2002; KLIF 2010). The PCA plot (Figure 10) performed on environmental variables showed a clear distinction between locations, confirming differences in sediment contamination. The control meadow in Sandspollen had the highest concentrations of most contaminants. The contaminants here were likely a result of high levels of boating. As a popular anchorage site, several hundred boats may visit Sandspollen during the summer months (Daniel Tørring Ingebretsen (02.12.2011), Sætre båtforening, written communication). Wear and tear of antifouling paints used on recreational boats can cause increased sediment contamination in popular anchorage areas (Warnken et al. 2004). However,
the sediment in disturbed locations had higher TBT concentrations than the nearby control locations. The obvious source of TBT and Cu is the maintenance of and leaching from boat hulls (Schiff et al. 2004), while high Pb concentrations may reflect leaded fuel spills. TBT was persistent in the environment even two decades after it was banned as an antifouling agent. The high TBT concentrations in the present study (Figure 8d) indicate that this is also true for marinas in the Oslofjord.

Being situated in the inner Oslofjord, the city of Oslo may partly explain the difference in contamination between inner and outer Oslofjord. The high organic loading in inner Oslofjord may partly originate from municipal waste water and run-off from land. Historically, the city of Oslo has been the main source of pollution in the inner Oslofjord (Arnesen 2001). Much of the contaminants are today removed in wastewater treatments plants, but contaminants may still be transported to the sea through run-off from land. Due to the Drøbak sill (Gade 1968), exchange of waters and removal of sediment is limited in inner Oslofjord. The presence of sills in polls would further limit water circulation and enhance sedimentation of contaminants in enclosed areas. The waters in the outer Oslofjord are not influenced by the Drøbak sill and are more often replenished.

Apart from being geographical remote from each other, differences in concentrations of contaminants between the two marinas may reflect a difference in waste management. The most obvious difference is that wastewater from the maintenance of boat hulls drains through a sediment catch basin in Skjebergkilen, while in Sætrepollen, it directly enters the sea. The efficacy of such installments is also suggested by the differences in contaminant concentrations and the size of the two marinas. The marina in Sætrepollen harbors 390 boats, while the marina in Skjebergkilen harbors approximately 1,000 boats. Thus, sediment catch basins seem efficiently to reduce contamination to nearby sediments.
4.4 Study evaluation

4.4.1 Sampling

That the recorded number of taxa in sand was similar to previous Scandinavian studies (Boström and Bonsdorff 1997, Fredriksen et al. 2010) indicates that there were little differences in sampling methodology. The sample design used in this study differs from that of earlier studies; therefore, comparisons should be made with caution. Fredriksen et al. (2010) used corers of 5 cm in diameter and approximately 10 cm in depth and included infauna >250 µm. Boström and Bonsdorff (1997) used corers of 4 cm in diameter and 10 cm in depth but included only infauna >500 µm. Thus, even smaller sample sizes collected the same or more species than in the present study. This further supports the conclusion that meadows in the Oslofjord support fewer infaunal species. The high species numbers recorded by Fredriksen et al. (2010) may be a direct result from the smaller mesh size and hence, a higher number of individuals retained in the sieve. However, infauna of 250–500 µm would consist of organisms that are difficult to identify (e.g., nematodes and copepods). In the case of Fredriksen et al. (2010), such small animals comprised only a small part of the total number of species. Therefore, it seemed reasonable to compare the number of species between these studies.

The number of species retrieved in a sample may be correlated with the size of the sampling device (Gray 2002). According to the guide (Vannportalen 2009), the classification of EQS is based on 1000 cm\(^2\) grabs. Therefore, based on the core size used in this study (78.5 cm\(^2\)) one cannot be certain that classification gave a satisfying representation of the station’s EQS.

Measurements of sediment redox potentials often suffer from low reproducibility. Metal electrodes may respond slowly and the recorded potentials often depend on previous samples. Furthermore, successful determination of redox potentials is highly dependent upon the individual practice of the field worker (Schaanning and Hansen 2005).
4.4.2 Numerical analyses

In the BIOENV analysis, the probability of detecting an environmental variable that matches the faunal pattern increases with an increasing number of variables and decreasing number of species. Ecological studies often suffer from having measured too few environmental variables. As such, if little relationship is found between environmental variables and fauna, the latent environmental gradient will not be identified. Therefore, the interpretation is often based on speculation. The opposite was the case in the present study. To avoid overfitting the data, highly correlated variables were removed. Even then, the number of variables was similar to the number of species included in the analysis. With a high number of variables and a small number of recorded species, the chances of a random fit are high. Further, an important explanatory variable could also be a proxy for an unmeasured latent variable. Therefore, the results should be interpreted with caution. Nevertheless, the procedure provided valuable information on the kind of environmental variables that structured the observed faunal pattern. These were variables found to be important in structuring infaunal communities also in other studies (see discussion above).

Importantly, the faunal data did not succeed in estimating Hurlbert’s rarefaction. To estimate the number of expected species in a sample with 100 individuals, one needs more than 100 individuals in a sample. In this study, several samples contained less than 100 individuals. This resulted in the estimated number of species being near identical with the actual recorded number of taxa. The number (100) was chosen in order to classify stations into EQS as described in the Norwegian classification guide (Vannportalen 2009).

The use of univariate indices gave most likely a satisfying representation of EQS. The classifications of the stations into EQS were consistent between Shannon diversity and Hurlbert rarefaction. However, the Norwegian classification guide (Vannportalen 2009) recommends a combination of univariate and multivariate indices when classifying into EQS. The degree to which single metric indices successfully detect environmental disturbance is under debate. Borja et al. (2011) argued that multimetric indices may be more applicable when detecting disturbance (e.g., in marinas where several stressors are present). The multivariate indices used in the Norwegian classification guide assume that a particular number of indicator species is present in a sample. However, the faunal samples in the present study contained too few indicator species.
for these indices to be used. It may further be argued that multivariate indices are not designed to describe seagrass fauna (Dr. Karl Norling (24.10.2011), NIVA, written communication). However, multivariate and univariate indices has been shown to produce very similar classification statuses in earlier investigations of sediment fauna in the Oslofjord (Berge et al. 2011).

Sediment samples for analysis of contaminants were only taken in meadows. Thus, it is impossible to identify factors underlying structuring differences in faunal composition between the substrates. In the BIOENV analysis, the number of faunal samples had to be reduced to match the number of sediment chemistry samples. Thus, this loss of information made the analysis less rigorous. However, the variables found to explain the infaunal pattern included variables that have been found to structure faunal composition also in other areas.

The confidence intervals for infaunal abundances were large at each station (Figure 11b). This is not surprising, as most marine invertebrates have pelagic recruitment and are patchily distributed with a high variance over small geographical scales (Preston 1962; Morrisey et al. 1992; Archambault and Bourget 1996). The wide intervals made it difficult to investigate whether there were significant differences in faunal abundance between stations.
5 Conclusions and further perspectives

This study showed that meadows supported a lower infaunal diversity than sand. These findings can be explained by the high TOC concentrations recorded in meadows. Although being important food for infauna, organic matter also stimulates sulfate reduction. As with organic matter, concentrations of contaminants may be higher in meadows than in sand. The negative effects associated with high organic loading and contamination by TBT and Cu most likely overrode the positive structuring mechanisms exerted by the meadows. This may explain the low species diversity recorded in meadows compared to sand. Infaunal communities in the most contaminated meadows were typically of impacted areas. Based on this study, a simple and precautionary mitigation measure would be to install sediment catch basins in marinas, as they may efficiently reduce outputs of contaminated waste from marinas.

It is of key interest to understand the factors that affect faunal communities in seagrass meadows. In general, the cumulative effects of multiple stressors are frequently unknown. How stressors interact and whether their effects on the seagrass ecosystem are additive or synergistic has implications for management strategies. For additive effects, a reduction in the magnitude of one stressor would predictably have a positive effect to the response of interest. In contrast, a reduction in a stressor involved in synergistic interactions would have greater than predicted effects. Thus, for effective management of these very important ecosystems, more knowledge about their threats and how they interact is needed.

In this study, control locations were heavily used by recreational boaters. This explains why no direct negative effect from marinas could be detected. Further studies should aim at doing preliminary sampling of sediment chemistry. This would ensure a suitable choice of study locations and make the sampling design more rigorous. Study locations could then be chosen based on their contaminant loadings. Further studies should also focus on analyzing chemical variables in both sand and meadows. This could determine the fate and effects of contaminants in the two substrates.
Identification literature

Marine Species Identification Portal: http://species-identification.org/
References


Vannportalen 2009. Klassifisering av miljøtilstand i vann. Økologisk og kjemisk klassifiseringssystem for kystvann, innsjøer og elver i henhold til vannforskriften (in Norwegian).


Appendices

Appendix A: Faunal abundance data

Compiled list of taxa identified in this study. Numbers are the number of individuals identified from each core sample at each station. Station abbreviations: Sa = Sandspollen, Sæ = Sætrepollen, Ho = Horneskilen, Sk = Skjebergkilen. Z = Zostera, S = sand. The numbers are the sample number (1-5). * denote the samples excluded from the BIOENV and BEST analysis.

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<th>SaS2</th>
<th>SaS3</th>
<th>SaS4</th>
<th>SaS5</th>
<th>SaZ1</th>
<th>SaZ2</th>
<th>SaZ3*</th>
<th>SaZ4</th>
<th>SaZ5*</th>
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**Appendix B: Chemical variables**

Sediment chemistry variables for sediment samples from meadows: total nitrogen (TN), total organic carbon (TOC), cadmium (Cd), copper (Cu), mercury (Hg), lead (Pb), zinc (Zn), polychlorinated biphenyl 7 (PCB7), dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbon 16 (PAH16), benzo[a]pyrene (b[a]p), and tributyl tin (TBT). Station abbreviations: Sa = Sandspollen, Sæ = Sætrepollen, Ho = Horneskilen, Sk = Skjebergkilen. Z = *Zostera*. The numbers are the sample number (1-3).

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## Appendix C: Redox and sulfur measurements

Electrode measurements and computed redox potentials and concentrations of H$_2$S for sediment samples from each station. Station abbreviations: Sa = Sandspollen, Sæ = Sætrepollen, Ho = Horneskilen, Sk = Skjebergkilen. Z = Zostera, S = sand. The numbers are the sample number (1-3).

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**Appendix D: Plant biometric variables**

Plant biometric variables from each meadow. Station abbreviations: Sa = Sandspollen, Sæ = Sætrepollen, Ho = Homeskilen, Sk = Skjebergkilen. Z = Zostera. The numbers are the sample number (1-3). "*" denote samples excluded from the PCA analysis.

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Appendix E: Faunal diversity indices

The number of individuals per square meter was calculated using the following formula:

\[
\text{No. individuals m}^{-2} = \frac{10,000 \text{ cm}^{-2}}{\pi r^2} \times \frac{\text{total number of individuals}}{\text{number of cores per station}}
\]

The Shannon Wiener diversity combines species richness and their relative abundance. The minimal value for \(H'\) is 0 and is obtained when only one species is in the sample. \(H'\) is undefined when there are no species in a sample. The index is computed as:

\[
H' = -\sum_{i=1}^{S} p_i \log_2 p_i
\]

Where \(s\) is the number of species, \(p_i\) is the proportion of individuals found in the \(i\)th species \((p_i = n_i / N)\), \(n_i\) is the number of individuals of species \(i\) in the sample, and \(N\) is the total number of individuals sampled.

Hurlbert’s rarefaction has the assumption that individuals arrive in the sample independently of each other, can be used to project back from the counts of total species \((S)\) and individuals \((N)\), how many species \((ES_n)\) would have been “expected” had we observed a smaller number \((n)\) of individuals. The index is given as

\[
ES_n = \sum_{i=1}^{S} \left[ 1 - \frac{(N - N_i)! (N - n)!}{(N - N_i - n)! N!} \right]
\]

The formula then generates an absolute measure of species richness (in this study, the number of expected species in a sample of 100 individuals) which can be compared across samples.
Appendix F: Grain size parameters

Formulas used for determination of grain size parameters. For all formulas: $\phi_x$ is the grain diameters in phi units ($\phi = -\log_2(x)$) at the cumulative percentile value of $x$.

 Mean grain size diameter is an estimate of the average grain size in a sample and is given as

$$M_z = \frac{\phi_{16} + \phi_{50} + \phi_{84}}{3}$$

 Sorting is a measure of the distributions of grain size around the median grain size. Poorly sorted (heterogeneous) sediment contains grains of mixed sizes (large variance), whereas well-sorted (homogenous) sediment mainly consists of grains of similar sizes (low variance). Sorting is given as

$$\sigma_I = \frac{\phi_{84} - \phi_{16}}{4} + \frac{\phi_{95} - \phi_{5}}{6.6}$$

 Skewness is a measure of the predominance of particular sediment fractions. It describes the symmetry in grain-size distribution in relation to the median grain size. A positive skewness value indicates that the grain size distribution is skewed toward fine particles; a value near zero indicates that the grain size distribution is symmetrical, and a negative value indicates that the grain size distribution is skewed toward coarse particles. Skewness is given as

$$Sk_I = \frac{\phi_{16} + \phi_{84} - 2\phi_{50}}{2(\phi_{84} - \phi_{16})} + \frac{\phi_{5} + \phi_{95} - 2\phi_{50}}{2(\phi_{50} - \phi_{5})}$$

 Kurtosis compares sorting in the central portion of the sample and assesses the shape of the grain-size distribution relative to a normal distribution. An excessively peaked distribution (leptokurtic) with narrow tails results in high kurtosis values, whereas a flattened distribution (platykurtic) with wider tails gives low kurtosis values. $K_G = 1$ gives a normal distribution. Kurtosis is given as

$$K_G = \frac{\phi_{95} - \phi_{5}}{2.44(\phi_{75} - \phi_{25})}$$
Appendix G: Formulas used in multivariate analysis

The Bray-Curtis similarity between samples 1 and 2 is defined as

\[ S_{jk} = 100 \left( 1 - \frac{\sum_i |y_{i1} - y_{i2}|}{\sum_i y_{i1} + \sum_i y_{i2}} \right) \]

Where \( y_{il} \) is the count for the \( i \)th (of \( p \)) species from sample 1, and \( \sum_i (...) \) denotes summation over those species.

Kruskal’s stress formula 1.

\[ Stress = \sqrt{\frac{\sum_j \sum_k (d_{jk} - D_{jk})^2}{\sum_j \sum_k d_{jk}^2}} \]

Where \( d_{jk} \) is the distance between objects \( j \) and \( k \) in the nMDS space, and \( D_{jk} \) is the distance predicted by the fitted regression line best representing \( d_{jk} \).
Appendix H: Descriptive statistics

Investigation of ANOVA assumptions. Top panel: QQ plots, means, standard deviations (StDev), total observations (N) and test statistics for the Anderson Darling test for plant biomass, cover, canopy and shoot density. Middle (left and right) and bottom (left and right) panels: 95% Bonferroni confidence intervals for the standard deviations and test statistics for the Levene’s test for plant biomass, cover, canopy and shoot density.
**Appendix I: Pearson’s correlations**

Pearson’s correlations between environmental variables. Grey cells indicate highly correlating variables ($\rho > 0.95$).

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