Ciliate and phytoplankton species composition in the sea-surface microlayer in the outer Oslofjord

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Abstract

The sea-surface microlayer is the boundary layer between the water of the oceans and the atmosphere occupying approximately “1000 micrometer” uppermost part of the sea-surface microlayer (SML) (Liss and Duce, 1997). Nutrients and pollutants are known to be concentrated in the SML compared to the water column. The unique physical and chemical features of the SML may provide a habitat for a large number and diverse groups of neuston (microorganisms living in the SML) such as ciliates and phytoplankton. However, these microorganisms in the SML may be exposed to the higher concentrations of nutrients and pollutants, compared to the water column, probably affecting their diversities and species composition. The diversity and species composition of the ciliates and phytoplankton were assessed in the sea-surface microlayer (SML) and sub-surface layer during an experiment at three locations in the outer Oslofjord during July 2009. Verdens Ende was expected to have low amounts of nutrients and oil pollution. Ferjeodden (a harbor) and Bustangen (close to a farm) were expected to have elevated concentrations of oil and nutrients, respectively. The results showed that there were variations in the number of individuals of ciliates and phytoplankton between replicates collected from each habitat and location. Measures of diversity indicated that there were not any differences in ciliate and phytoplankton between the SML and the sub-surface layer and between the SML samples at the three locations. The ciliate and phytoplankton species compositions in the two layers were not significantly different but separations were observed between the two layers. Significant differences were found in ciliate and phytoplankton species compositions in the SML samples from the three locations. Oligotrich ciliates and diatoms were identified as the most responsible taxonomic groups contributing to separations between neuston and plankton and between the neuston at the three locations. Adaptation to eutrophication, oil pollution, and ultraviolet radiation (UVR), predator pressure reduction and food availability could be possible explanations for this observation. Finally, species composition could not be related to the environmental factors (temperature and salinity) because the environmental factors did not vary substantially at the three locations.

Content

Abbreviations

SML	The sea-surface microlayer
UVR	Ultraviolet radiation
PAHs	Polyaromatic hydrocarbons
NMDS	Non-metric multidimensional scaling
VES	Verdens Ende sea-surface layer samples
FjS	Ferjeodden sea-surface layer samples
BtS	Bustangen sea-surface layer samples

1. Introduction

The sea-surface microlayer is the boundary layer between the water of the oceans and the atmosphere. It occupies approximately “1000 micrometer” uppermost part of the sea-surface microlayer (SML) (Liss and Duce, 1997). The SML contains organic matters, primarily lipids (Carlson and Mayer, 1980), as well as inorganic compounds such as inorganic phosphorus (Williams, 1967) and nitrogen (Hardy, 1982; Gladyshev, 1986; Kuznetsova et al., 2004). A recent study has shown the enrichment of transparent exopolymer particles (TEP) being enriched in the SML in higher concentrations than in sub-surface layers (Wurl and Holmes, 2008). They are ubiquitous gels in the oceans, produced in surface waters from the coagulation of phytoplankton-derived dissolved polysaccharides (Alldredge et al., 1993; Verdugo et al., 2004). Because of their “sticky” features, TEP facilitate the aggregation of microorganisms (Alldredge et al., 1993; Verdugo et al., 2004) in the SML. The physical and chemical features of the SML including its high concentration of organic matters or nutrients, provides a habitat for a large number and diverse groups of neuston (organisms living in the sea-surface microlayer) such as bacterioneuston, phytoneuston (such as diatoms and dinoflagellates), ciliates (such as oligotrichs and tintinnids) and zooneuston accumulating in the SML (Hardy, 1982). Such organisms will commonly be found in a higher abundance in the SML compared to sub-surface layers (Liss and Duce, 1997). Environmental conditions are different between the SML and the sub-surface layers. For example, light intensity, temperature, salinity and the concentration of organic and inorganic substances are known to be higher in the SML than in the sub-surface layers. Neuston lives and thrives in the SML because they have adapted to this unique habitat (Zaitsev, 1971; Zaitsev and Liss, 1997). The neuston feeds, grows, and reproduces in the SML (Liss and Duce, 1997). This interface can also serve as both a sink and a source of anthropogenic compounds, including chlorinated hydrocarbons, organotin compounds, petroleum hydrocarbons and heavy metals due to its unique chemical composition, in particular its high content of lipids and protein (Southwood et al., 1999). The neuston community can be at greater risk of being exposed to higher concentrations of environmental contaminants in the SML compared to the sub-surface layer (Liss and Duce, 1997) for two reasons. First, because of the increasing amounts and types of anthropogenic substances deposited into the atmosphere, thus, into the sea surface (Liss and Duce, 1997); second, because both chemical contaminants and organisms tend to accumulate in the same phase (Hardy, 1997). Therefore, assessing neuston community relative to

pollution might be useful to indicate any alterations in marine environment due to excess nutrients and contamination (Gladyshev et al., 1997).

Studies in both marine and freshwater environment have found dissimilarities in the amounts of organic matters, nutrients and contaminants, and microorganism abundance between the SML and the sub-surface layer (Estep and Remsen, 1985). For example, Anikiev and Urbanovich (1989) collected samples from the SML and sub-surface layer in various oceans of the world and found higher mean concentration of total petroleum hydrocarbons in the SML compared with those in the sub-surface layer. The highest mean concentrations of peroxide, a product of the photo-oxidation of PAHs (polycyclic aromatic hydrocarbons), was measured in the SML in the Red Sea, where high UV intensities most probably resulted in the high concentration. High concentrations of PAHs in the SML have been found at sampling locations associated with anthropogenic coastal activities, particularly shipping harbors (Hardy et al., 1990). A comprehensive study by Parker and Hatcher (1974) on the distribution of dissolved and total nutrients in a freshwater lake revealed significant difference between surface and sub-surface layer concentration, 55.4% of the time.

One of the most important research areas relevant to the SML involves the species composition and health of neuston organisms. Phytoplankton are primary producers having an important role in the marine food web and energy flux (Huang et al., 2010), and ciliates, particularly tintinnids and oligotrichs, have been considered to be the major consumers of nano- (2-20 µm) and picoplankton (0.2-2 µm) (Fenchel, 1987), the dominant primary producers in aquatic ecosystems (Malone, 1980; Stockner, 1988). Thus, because of the importance of these two groups, the effect of pollution on these organisms should be considered. One approach to the assessment of community structure is the use of diversity indices such as species richness, diversity and evenness. A second way to assess the community is the use of multivariate statistical analysis such as non-metric multidimensional scaling (NMDS).

Neuston communities have been investigated by previous studies. Hardy (1973) found that phytoneuston communities in a temperate marine lagoon had lower species richness, diversity and evenness, and greater abundance and species dominance, and exhibited increased photosynthetic assimilation ratios when compared with sub-surface populations. He further suggested that greater environmental variations and amounts of nutrients in the SML compared with those in the sub-surface layer might be a possible reason. Parker and Hatcher

(1974) observed differences in algal community structures between the SML and sub-surface layer at freshwater ponds. They further found that availability of different organic and inorganic chemicals for the neuston and plankton have probably resulted in possessing different metabolic rates, microbial growth rates and community sizes in phytoneuston compared with those in phytoplankton communities. In an estuary, higher abundance in phytoneuston communities was observed that could be a result of accumulating and concentrating of rising bubbles which move materials from sub-surface layers to the SML. In addition, some algal species were missing in the SML leading to lower species diversity of phytoneuston compared with the phytoplankton communities that were probably caused by vertical mixing and total phytoplankton cell densities (Manzi et al., 1977). However, Estep and Remsen (1985) found that even though nutrient abundance was higher in the SML compared with those in the sub-surface layer, phytoplankton abundance was not consistently higher in the SML than in the sub-surface layers suggesting that the SML could be an area for microalgal accumulation. It is also a phase with high cell damage caused by ultraviolet radiation or algal consumption by protists. In addition, species richness, diversity and evenness of phytoneuston community were higher than those of phytoplankton community suggesting that the increased diversity was because of increased diversity of diatom group having a high affinity for the SML (Estep and Remsen, 1985). Using multivariate statistical analysis, significant differences were found in combined algal and cyanobacterial community structures between SML and sub-surface layer at two ponds suggesting that dominante algal species might be a possible reason for the difference between the two communities. However because of few number of studies on neuston communities, there was no clear conclusion of a possible explanation for the result (Butler et al., 2007). Only one molecular biological method determines that ciliate community in the surface microlayer is more abundant than the underlying layers (Cunliffe and Murrell, 2009).

There have been few studies on how pollutants and excess nutrients may affect neuston communities. However, there have been studies on the effects of pollutants and excess nutrients on plankton communities. Pollutants such as pesticides, heavy metals, or oil may affect few or all organisms. PAHs (polycyclic aromatic hydrocarbons) and petroleum hydrocarbons belong to persistent organic pollutants (POPs) group in the SML (Wurl and Obbard, 2004). In general, high concentrations of PAHs in the SML have been found at sampling locations associated with anthropogenic coastal activities, particularly shipping

harbors (Hardy et al., 1990). Petroleum hydrocarbon concentrations have been found in higher concentrations in the SML than in the sub-surface layer (Anikiev and Urbanovich, 1989).

Marine eutrophication is a process in which nutrient levels increase resulting in the production of particulate organic matter (POM)/ dissolved organic matter (DOM) production; this is mainly a result of an increase in the abundance and production of phytoplankton (Horrigan et al., 2002), bacteria and zooplankton in the water column (Chapman and Craigie, 1977). Finally, eutrophication leads to organic matter degradation resulting in decreased oxygen concentrations in the water column (Gray et al., 2002). Nixon and Pilsom (1983) suggested that agricultural fertilizers input and domestic sewege, via rivers, could be the major external sources of DOM/POM to coastal waters. Organic nutrients have been known to have higher concentrations in the SML than in the sub-surface layers (Estep and Remsen, 1985).

How oil pollution affects plankton ciliates and phytoplankton communities, have been studied extensively both in the laboratory and in the field. Oil slicks on the surface will limit gas exchange through the air sea interface and reduce light penetration into the water column affecting phytoplankton photosynthesis (González et al., 2009). Ciliates have been thought to ingest small globules of crude oil because these organisms have been seen to gather around the globules of crude oil (Spooner, 1968). Bloom of flagellates associated with oil particles has been observed (Smith, 1968). Phytoplankton abundance and species diversity increased in response to oil contamination in a microcosm experiment (Vargo et al., 1982). In an experiment of crude oil effects on plankton communities in an enclosed system, however, diatom and copepod abundance decreased and bacterial and tintinnid abundance increased (Dahl et al., 1983). Increased growth of marine ciliates and large droplets of oil in ciliate food vacuoles in an oil-contaminated seawater have also been observed (Andrews and Floodgate, 1974; Skjoldal et al., 1982). Studies of crude oil in enclosed ecosystems showed an initial toxic effect on marine protozoans and a decrease of heterotrophic ciliate abundance and a complete removal of these organisms below an oil slick within the enclosed system. However, the toxic effect decreased by adding nutrients to the experimental system (Dale, 1988). Blooms of tintinnids (loricated ciliates) (Dale, 1988) and flagellates (Dahl et al., 1983) were detected at later stages of these experiments. Oil-tolerant heterotrophic nanoflagellates have been identified to be important grazers of bacterial population in the marine oil biodegradation process (Dalby et al., 2008). It has been suggested that environmental factors such as ultraviolet radiation might influence the oil toxicity (Sargian et al., 2005; Belzile et al., 2006; Sargian et al., 2007).

Eutrophication or an increase in nutrient and dissolved organic matter (DOM) concentrations over natural levels (Smith, 1984) may involve an increase in phytoplankton (Hodgkiss and Lu, 2004; Liu, 2008) and ciliate abundance (Beaver and Crisman, 1982; Revelante et al., 1985; Beaver and Crisman, 1989; Pfister et al., 2002; Xu et al., 2005). One reason why eutrophication may lead to changes in phytoplankton and ciliate abundance may be their relationships in the microbial loop. The microbial loop describes a trophic pathway in the microbial food web where dissolved organic carbon (DOC) is returned to higher trophic levels: the organic carbon is consumed by bacteria which can be grazed by phytoplankton and microzooplankton (such as ciliates); these organisms can be ingested by mesozooplankton such as copepods (Fenchel, 2008). Increased phytoplankton may be a result of decreased predator pressure followed by decreased zooplankton abundance (Pfister et al., 2002). It is well known that certain phytoplankton and ciliate species are more common in nutrient-rich (eutrophic) than in oligotrophic waters (Revelante et al., 1985). For example, a community change toward the smaller size classes of phytoplankton (Gilmartin and Revelante, 1980) and plankton ciliates (Beaver and Crisman, 1982; Barría de Cao et al., 2003; Selifonova, 2009) were observed following eutrophication. In 1998, a diatom *Skeletonema costatum* bloom was observed in Jiaozhou Bay, China, after a large nutrient input from two days of heavy rain. During the bloom, species diversity and evenness decreased (Liu et al., 2005). Xu et al. (2005). found that eutrophication can cause a decrease in number of protozoan species (such as flagellates and ciliates) in lakes, and Tas et al. (2009) observed an increase in the species richness and a major change in phytoplankton community structure followed by decreased eutrophication. Other studies reported that the number of individuals and community diversity (number of species and Shannon-Weiner index) of ciliates increased with increasing eutrophication (Beaver and Crisman, 1989; Pfister et al., 2002). According to a study of phytoplankton community structure in two lakes differing in their trophic states, diatoms dominated the lake with high nutrients concentrations and dinoflagellates dominated the lake with low nutrient concentrations(Liu, 2008). Therefore, ciliates and phytoplankton could be used as indicators of eutrophication (Arndt et al., 1990; Devlin et al., 2007).

The outer Oslofjord is a large area that includes open seas, fjords and Norway's largest estuary (Hvaler). It is a very dynamic and open fjord system. The degree of nutrient input from the rivers into the outer Oslofjord can be influenced by the amount of precipitation. Agriculture is the main source of anthropogenic input of nutrients, Nitrogen (N) and Phosphorus (P), to the outer Oslofjord. A high nutrient concentration in late spring and

summer will be due to input from outside source or by runoff from land (Walday et al., 2009). Because the Oslofjord has had a history of coastal eutrophication (De Jong, 2006; Berge, 1990), eutrophication was expected to occur along some of the coasts of the Oslofjord, specially the coastal areas close to river mouths agricultural fields. In addition, because some of the coastal areas in the Oslofjord are used as harbors, oil pollution was expected to occur along the coasts of harbors in the Oslofjord.

Three inshore locations in the outer Oslofjord were chosen as sampling sites expecting to be different in their amounts and types of pollutants. Verdens Ende, located at the southernmost tip of the island of Tjøme in Vestfold, outer Oslofjord, was considered as a reference site probably having low amounts of pollution. Ferjeodden (Røeds), located in northernmost tip of the island Tjøme in Vestfold, is a small harbor probably having considerable amounts of oil pollution. Bustangen (Kråkere) is located in the north of the island Tjøme. It is close to a farm assuming that high amounts of organic pollution from the farm may find their way into the shallow sampling area.

Due to its unique properties, the sea-surface micro-layer might be an important habitat for growth and production of a large number of ciliates and phytoplankton communities. On the other hand, contaminants such as organic materials and anthropogenic chemicals tend to be enriched to higher concentrations in the SML than in sub-surface layer. Moreover, amounts of contaminants entering to the atmosphere and ocean tend to increase and could further add to their amounts in the SML. Therefore, neuston community (organisms living in the SML) might be exposed to higher amounts of nutrients and pollution compared to plankton community (organisms living in the sub-surface layers) affecting their species diversity and community structure.

There are three main objectives in this thesis:

Are there differences in phytoplankton and ciliates diversity between the SML and sub-surface water at different coastal locations?

Will hydrocarbon pollution affect the diversity of ciliates and phytoplankton?

Will eutrophication affect the diversity of ciliates and phytoplankton?

2. Materials and Methods

2.1. Locations

In July 2009, sea-surface microlayer (SML) and sub-surface water samples were collected from coastal areas of three different locations in the outer Oslofjord in the southeast part of Norway; Verdens ende, Ferjeodden (Røeds), and Bustangen (Kråkere) (Figure 2.1).

Verdens ende is located at the southernmost tip of the island of Tjøme in Vestfold, Norway. As a shallow and rocky bay, mostly used as an outdoor recreation area (The Climate and Pollution Agency, 2010), this location was expected to have low amounts of nutrients and water pollution. Hence, reference samples were taken from this location.

Ferjeodden (Røeds), located in northernmost tip of the island Tjøme in Vestfold, Norway, is a small harbor where ships and boats, and barges commute probably leading to oil contamination in the location. So, this location were expected to be influenced by hydrocarbon pollutants.

Bustangen (Kråkere) is located also in the north of the island Tjøme. This sampling site is located close to a farm; so, nutrients such as nitrogen and phosphorus might find their way into the coastal areas close to the sampling location probably leading to eutrophication.

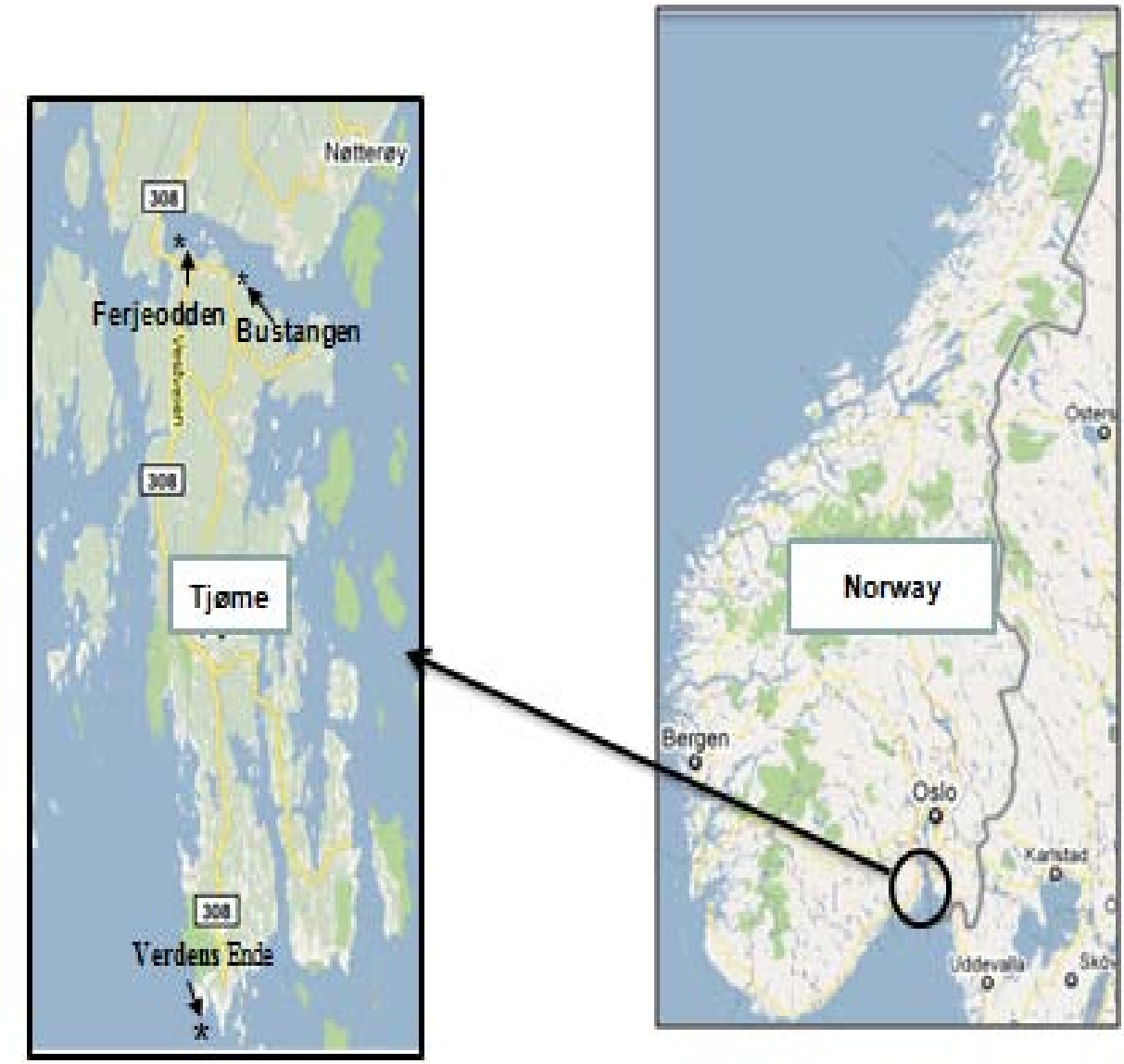


Figure 2.1. Map of the three sampling sites in the southeast of Norway: Ferjeodden, Bustangen and Verdens ende (http://maps.google.no/maps).

2.2. Sample collection

The SML and sub-surface water samples were taken at three different occasions from each location in the period July 17-26 in 2009 (see Table 3.1 for details).

Samples from the SML were taken by a remotely operated boat equipped with a Teflon-coated rotating drum (RD) (Figure 2.2) (Harvey, 1966; Hardy et al., 1988). The boat and the rotation of the drum were operated by a storage battery. The drum was rotated while the boat was driven forward pushing the drum ahead and a wiper attached on the drum collected the SML sample directing it into a 4L-glass bottle (collection jar) (Figure 2.3). At each time of

sample collection, 0.5 to 1 liter of SML sample was taken. To avoid contamination and vertical mixing, calm days without recent precipitation were chosen for sample collection. The drum and all the components of the collecting system were cleaned with 10% bleach solution and distilled fresh water before each use.

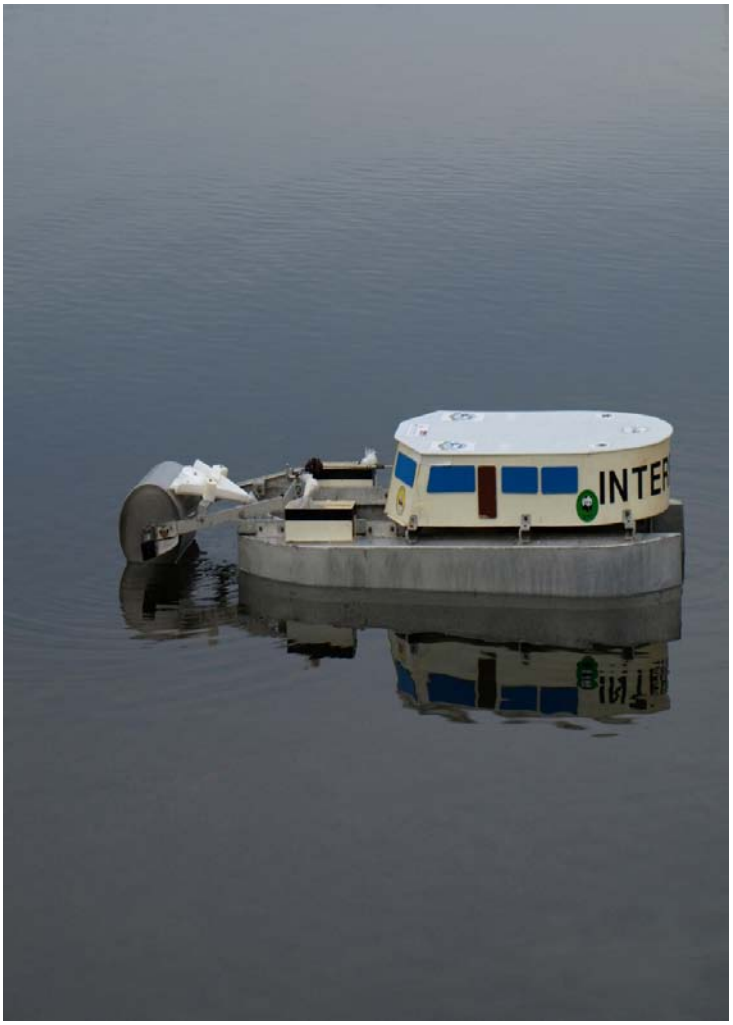


Figure 2.2. A picture of the SML sampler. Image credit: Hege Vestheim.

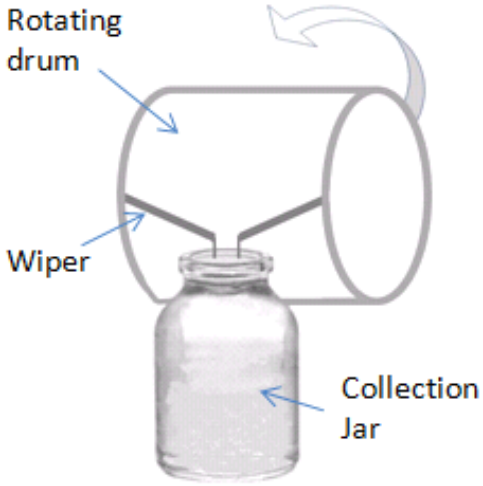


Figure 2.3. Schematic diagram of the SML sampler.

Sub-surface water samples were collected by submerging clean 300-ml dark bottles with closed lids into the water and opening the lids when the mouth of the bottles reached 15 cm below the surface.

Environmental factors including pH, salinity and temperature (°C) (Table 3.1) were measured at each sampling occasion. Salinity and temperature were measured by a S-C-T (salinity-conductivity-temperature) meter, YSL model 33, with a manual temperature compensation (accuracy: +/-0.7 ppt).

2.3. Sample treatment and counts

To preserve the samples, 250 ml of water samples were fixed with 1.5 ml pseudo-Lugol's solution. For pseudo-Lugol's solution (Thomas D., unpublished results), equal volume of solution A (30 g KI and 19.5 g I_2 dissolved in 500 ml of deionized water) and solution B (combine 135 ml of deionized water + 315 ml of absolute alcohol + 35 ml of 25% glutaraldehyde + 15 ml of glacial acetic acid) were mixed. Preserved samples were then stored in 4°C in the dark until they were analyzed in the laboratory by the inverted-microscope method (Hasle, 1978).

For ciliate and microalgae enumeration, the inverted-microscope method, or Utermohl method, was used (Hasle, 1978). After gentle shaking of the water samples for 120 times (for about 2 minutes), subsamples with different volumes (2, 2.5, 5, 10 or 50 ml), depending on

the cell density of the samples, were measured. Next, the subsamples were settled in combined plate chambers with different sizes of top cylinders (10 and 50 ml) according to the volume of the subsamples (Figure 2.4). Since the top cylinders with volumes lower than 10 ml were not available, 24-well micro plate (maximum volume of each well: 3 ml) was used to concentrate the subsamples with 2 and 2.5 ml volume. A settling time of 24 hours was used for all the subsamples. Where possible, cells were identified to genus and species levels according to Throndsen and Eikrem (2001), Strüder-Kypke et al (2003), Mathias (2006), and Throndsen et al. (2007). The number of individuals counted in relative volumes was calculated to the number of cells per ml. A Nikon Eclipse TE300 inverted microscope equipped with a Nikon D50 Digital Camera was used to observe and count ciliates and microalgae in the water samples.

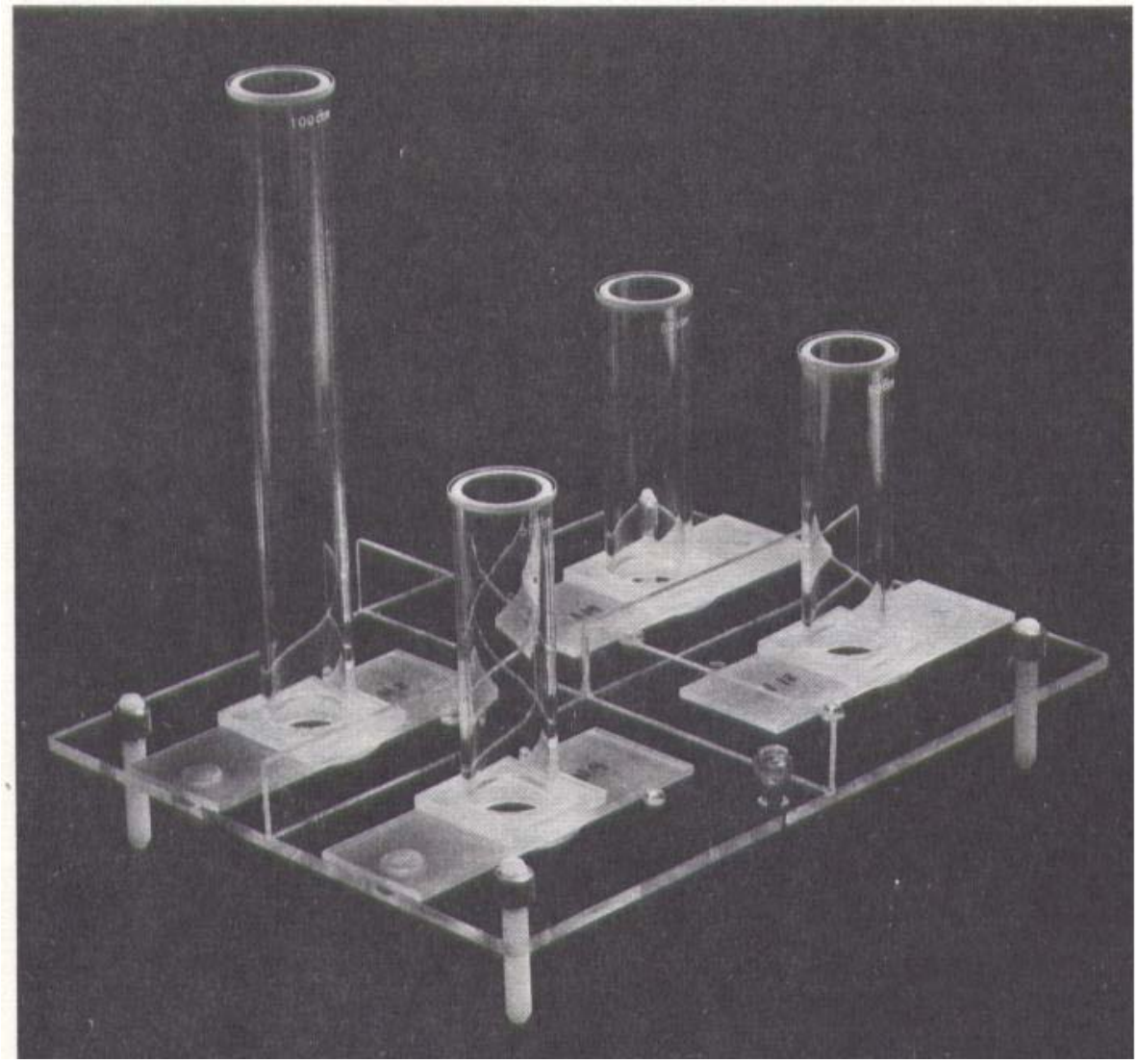


Figure 2.4. Sedimentation table with combined plate chamber set for sedimentation (Tangen, 1976).

2.4. Statistical analysis

Data were analyzed using the PAST (palaeontological statistics) software package (Hammer and Harper, 2001).

Four diversity measures were chosen; total number of species, Margalef's species richness (d), Shannon-Weiner index (H'), and Pielou's evenness (J'), in order to determine the diversity of different ciliate and phytoplankton communities in the SML and sub-surface layers at the three stations (Hammer and Harper, 2001).

Margalef's species richness takes S (the number of species) and N (the total number of individuals of all the species) into account in the following equation:

$D_{Mg} = (S-1)/\ln(n)$ (Clifford and Stephenson, 1975).

Shannon-Weiner index takes the number of individuals and number of taxa into account. It assumes that individuals are randomly sampled (Pielou, 1975) and all the species are present in the sample. It is calculated from the equation:

$H` = -sum ((ni/n)ln(ni/n))$

Where $H`$ is Shannon index, n_i is number of individuals of taxon i and n is number of individuals in all taxa. It varies from 0 for communities with one single species to high values for communities with many species having few individuals (Shannon, 1949).

Equitability (evenness) is another diversity measurement which belongs to the information theory indices. It measures how evenly species are distributed in a community. It is calculated from the equation:

$E = H`/H_{max} = H` /ln\ S$

Where $H`$is Shannon index, H_{max} is the maximum diversity which could possibly occur (which could be found in a situation where all species were equally abundant) and S is number of taxa (Pielou, 1969).

Two-tailed Mann-Whitney U test (Press et al., 2007) was applied to the diversity measurements of the communities to test if there were significant differences ($P<0.05$) in the species diversities between the SML and sub-surface layer at each location. In addition,

Kruskal-Wallis test, a non-parametric ANOVA (Zar, 1996), was performed to test if there were significant differences in the diversities between the SML and sub-surface layers at the three locations.

Multivariate community analyses were used to measure and display differences in the species composition of ciliates and phytoplankton communities between the three SML samples as well as between the SML and sub-surface layer samples at the three locations. Before further data analysis, log (x+1) transformation was done in order to down weight the effect of dominating species. Log (x+1) was preferred because the zero values produce log (0) = $-\infty$. Furthermore, in order to test for significant dissimilarities ($P<0.05$) in the ciliate and phytoplankton community structures between the SML and sub-surface layers as well as between the SML at the three locations, 1-way ANOSIM (analysis of similarities) (Clarke, 1993), a non-parametric test, based on Bray-Curtis similarity was performed. Bray-Curtis similarity is often a suitable coefficient for biological data on community structure (Clarke and Warwick, 2001). In order to display differences in the community structures of ciliates and phytoplanktons between the SML and sub-surface layer as well as between the SML at the three locations, NMDS (non-metric multidimensional scaling) was performed (Hill and Gauch, 1980). Experience with ecological data suggested that two-dimensional diagram might be more useful and accessible summary despite having a higher stress level compared to a three-dimensional diagram (Clarke and Warwick, 2001). Moreover, the species responsible for differences in the neuston (organisms living in the SML) and plankton (organisms living in the sub-surface layer) communities between the three locations were identified using SIMPER (the similarity percentage) (Hammer and Harper, 2001). The introduction of the original data (the number of individuals of phytoplankton and ciliate species) to SIMPER resulted mostly in identification of phytoplankton species contributing most to differences between the groups (this was because there was greater number of individuals of phytoplankton species than ciliate species in the samples). To avoid this problem, phytoplankton and ciliate groups were separated before introducing them to ANOSIM, NMDS, and SIMPER. Normally, SIMPER calculates the mean number of individuals of each group (when all the replicates are chosen as one group) before comparing the groups. Because there were only three replicates in each group, taking the mean should be avoided; therefore, before introducing the groups to SIMPER, the sum of the number of individuals of species were calculated out of the three replicates of each group.

3. Results

3.1. Environmental conditions

Environmental variables are shown in table.3.1. Samples were collected different times of the day. Temperature and salinity were not significantly different between the three locations using Kruskal-Wallis test. Temperature was always between 19˚C to 22˚C and salinity varied between 20 to 23 and increasing to 26 in 17th of July at Verdens Ende (Table 3.1).

Table 3.1. Environmental conditions (depth: cm, date: July, time: hour, temperature: ˚C) measured in the study period.

Location	Depth	Date	Replicate	Time	Temperature(˚C)	Salinity
Verdens Ende	Surface	July 17	1	22:00	20	26,5
	Surface	July 24	2	10:00	20	22
	Surface	July 26	3	10:00	19	21,2
	Sub-surface	July 17	1	22:00	20,08	26,5
	Sub-surface	July 24	2	10:00	20	22
	Sub-surface	July 26	3	10:00	19,5	21,2
Ferjeodden	Surface	July 19	1	22:00	19,7	21
	Surface	July 20	2	22:30	19,9	23
	Surface	July 22	3	08:25	21	22
	Sub-surface	July 19	1	22:00	19,7	21
	Sub-surface	July 20	2	22:30	19,9	23
	Sub-surface	July 22	3	08:25	21	22
Bustangen	Surface	July 19	1	20:30	20,3	20
	Surface	July 20	2	21:30	20,4	22,8
	Surface	July 22	3	10:30	22	22,2
	Sub-surface	July 19	1	20:30	20,3	20
	Sub-surface	July 20	2	21:30	20,4	22,8
	Sub-surface	July 22	3	10:30	22	22,2

3.2. Species abundance

The numbers of individuals of ciliate and phytoplankton groups, taken from the SML and sub-surface layer at the three stations, were presented in table 5.1. In addition, the total numbers of individuals of main ciliate and phytoplankton groups were shown in table 3.2. No statistical test was performed on the number of individuals of ciliates and phytoplankton.

Table 3.2. The number of individuals (cells/ml) of important ciliate and phytoplankton groups.

		Replicate	Oligotrichs	Tintinnids	other_ciliates	Ciliates	Dinoflagellates	Diatoms	Total Phytoplankton
Verdens Ende	Surface	1	0,40	44,00	4,40	92,40	85,60	360,80	446,40
		2	0,06	2,38	0,56	5,32	21,76	9,66	31,42
		3	4,58	0,00	2,40	2,40	166,50	224,40	390,90
	Sub-surface	1	1,50	8,10	5,40	21,60	7,30	16,30	23,60
		2	0,00	0,06	19,08	19,20	0,92	4,70	5,62
		3	0,00	1,60	0,40	3,60	12,40	24,00	36,40
Ferjeodden	Surface	1	4,00	38,80	6,80	84,40	406,00	61,20	467,20
		2	0,80	7,20	9,60	24,00	435,60	66,40	502,00
		3	10,00	0,40	9,20	10,00	122,40	39,20	161,60
	Sub-surface	1	2,50	3,40	5,50	12,30	51,60	5,30	56,90
		2	0,00	1,84	0,18	3,86	11,38	47,48	58,86
		3	1,02	2,98	1,68	7,64	37,34	5,12	42,46
Bustangen	Surface	1	1,00	2,25	1,50	6,00	16,50	119,50	136,00
		2	0,00	4,00	16,00	24,00	166,00	188,00	354,00
		3	0,40	5,20	1,60	12,00	43,60	690,00	733,60
	Sub-surface	1	0,40	7,00	0,00	14,00	1,00	24,10	25,10
		2	1,00	2,70	1,90	7,30	91,10	10,00	101,10
		3	0,04	1,66	0,02	3,34	0,50	24,84	25,34

3.3. Diversity

3.3.1. Neuston and plankton

The diversities of neuston and plankton communities (Table 3.3) were not significantly different at the three locations according to the Mann-Whitney U test ($p > 0.05$).

Table 3.3. Measurements of diversity indices of neuston and plankton communities: the number of species (no. of species), Shannon-Weiner (H´), Margalef's species richness, and Equitability (J´).

		Replicates	Number. of species	Shannon_Weiner (H´)	Margalef	Equitability (J´)
Verdens Ende	Surface	1	7	1,14	0,56	0,58
		2	5	1,32	0,49	0,82
		3	4	0,77	0,34	0,55
	Sub-surface	1	10	1,62	0,9	0,7
		2	5	0,05	0,41	0,03
		3	2	0,5	0,13	0,72
Ferjeodden	Surface	1	7	1,15	0,56	0,59
		2	6	1,48	0,51	0,83
		3	7	1,37	0,6	0,7
	Sub-surface	1	13	1,82	1,26	0,71
		2	8	1,17	0,92	0,56
		3	12	1,81	1,25	0,73
Bustangen	Surface	1	8	1,94	0,81	0,93
		2	7	1,22	0,61	0,63
		3	7	1,44	0,67	0,74
	Sub-surface	1	6	0,82	0,56	0,46
		2	9	1,57	0,91	0,71
		3	6	1,22	0,64	0,68

3.3.2. Neuston at the three locations

The diversities of the neuston communities were compared at the three locations (Table 3.3) and no significant difference were found between the diversities according to the Kruskal-Wallis test ($p > 0.05$).

3.4. Species composition

3.4.1. Neuston and plankton

The neuston refers to organisms living in the SML and the plankton refers to organisms living in the sub-surface layer.

3.4.1.1. Ciliates

There were no significant differences in species composition between the SML and sub-surface water layer at the three locations (Table 3.4).

Table 3.4. Differences in species composition between the SML and sub-surface layer (1-way ANOSIM analysis).

	Verdens Ende		**Ferjeodden**		**Bustangen**	
	R	p	R	p	R	p
Ciliates	-0.05	0.6	0.14	0.4	0.20	0.3

In addition, the neuston and plankton communities appeared to be separated at two locations using NMDS method (Figure 3.2 and 3.3) except Verdens Ende (Figure 3.1). The distance between the groups showed how much the groups were separated.

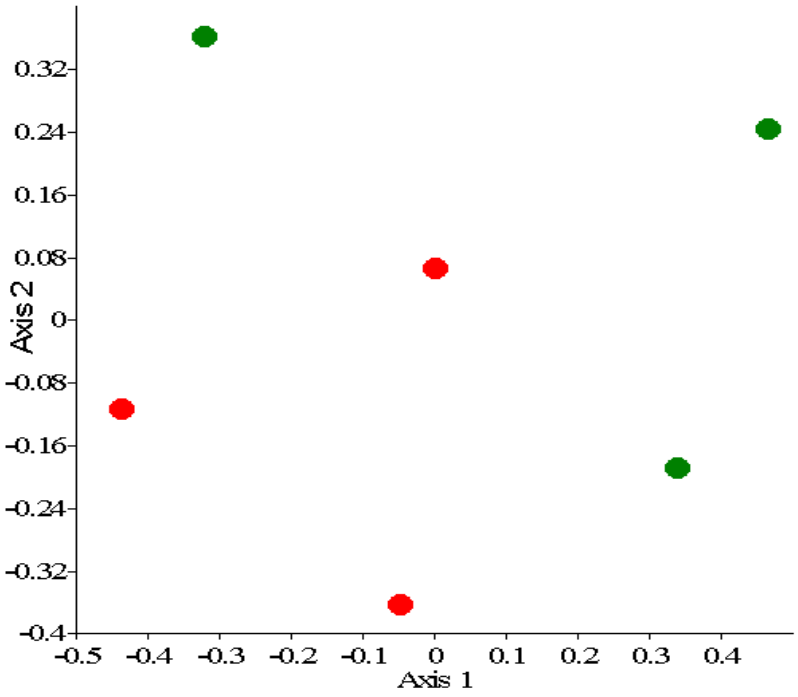


Figure 3.1. Non-metric multi-dimensional scaling ordination (2D) of the species composition of ciliates at Verdens Ende. Red circles represented the SML samples and green circles represented the sub-surface layer samples (Stress: 0.14).

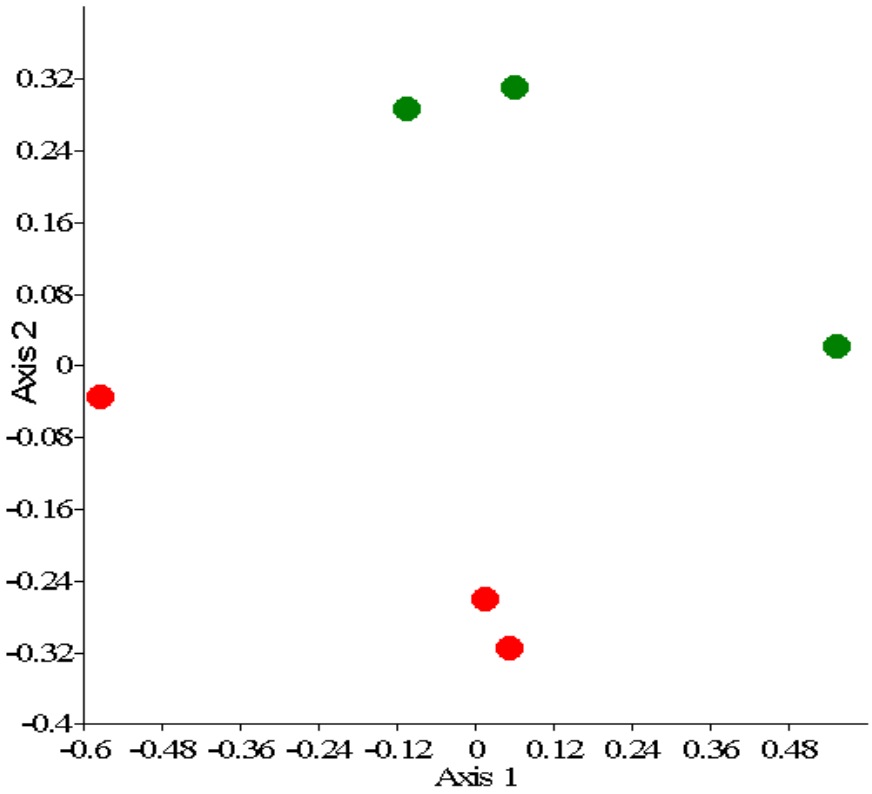


Figure 3.2. Non-metric multi-dimensional scaling ordination (2D) of the species composition of ciliates at Ferjeodden. Red circles represented the SML samples and green circles represented the sub-surface layer samples (Stress: 0.05).

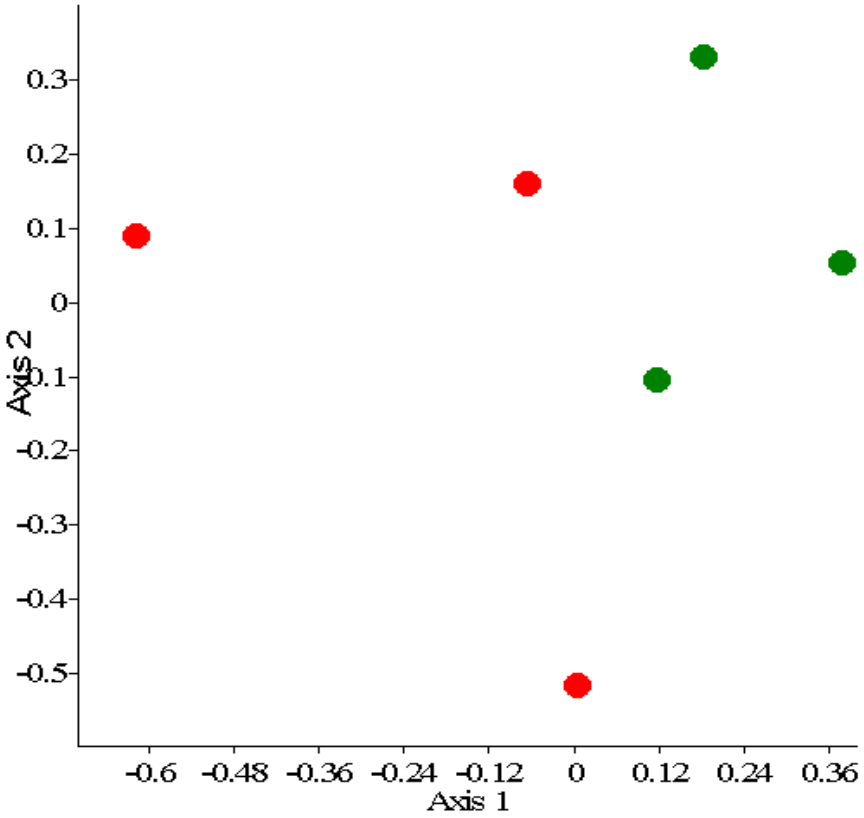


Figure 3.3. Non-metric multi-dimensional scaling ordination (2D) of the species composition of ciliates at Bustangen. Red circles represented the SML samples and green circles represented the sub-surface layer water samples (Stress: 0).

Moreover, the most responsible species for separations between the SML and sub-surface layer at the three locations were identified. At Verdens Ende, an oligotrich ciliate (*Strombidium* spp.2) was only found in the SML and tintinnids (although in different species) were found in both layers (Table 3.5). At Ferjeodden, in the SML, only two oligotrichs (*Tontonia* spp. and *Strombilidid* spp.) were present and tintinnids and other ciliate groups were only present in the sub-surface layer (Table 3.6). At Bustangen, tintinnids and oligotrichs (*Strombidium* spp.2) were only present in the SML (Table 3.7).

Table 3.5. SIMPER analysis of combined samples from Verdens Ende.

Taxon	The number of individuals of neuston	The number of individuals of plankton.
Helicostomella spp.	0,40	0,00
Eutintinnus spp.1	0,10	0,00
Strombidium spp.2	0,04	0,00
Favella spp.	0,00	0,02
Tintinnina spp.1	0,00	0,02

Table 3.6. SIMPER analysis of combined samples from Ferjeodden.

Taxon	The number of individuals of neuston	The number of individuals of plankton
Tintininna spp.3	0,00	0,10
Tintininna spp.1	0,00	0,04
Tiarina fusus	0,00	0,10
Hypotrichia spp.1	0,00	0,04
Strombidium spp.1	0,00	0,42
Tontonia spp.	0,40	0,00
Strombilidid spp.	0,80	0,00

Table 3.7. SIMPER analysis of combined samples from Bustangen.

Taxon	The number of individuals of neuston.	The number of individuals of plankton
Strombidium spp.2	0,70	0,00
Tintinnina spp.3.	0,50	0,00
Eutintinnus spp.2.	0,40	0,00
Salpingella spp.	0,40	0,00

3.4.1.2. Phytoneuston and phytoplankton

There were no significant differences in species composition between SML and sub-surface water layer at the three locations (Table 3.8).

Table 3.8. Differences in species composition between the SML and sub-surface layer (1-way ANOSIM analysis).

	Verdens Ende		**Ferjeodden**		**Bustangen**	
	R	p	R	p	R	p
phytoplankton community	-0.03	0.6	0.5	0.1	0.7	0.09

Neuston and plankton communities were separated at Ferjeodden and Bustangen (Figure 3.5and 3.6). However, at Verdens Ende, the two communities were not well separated and separations could be seen within the groups (between the replicates of each group) especially within the phytonplankton in the sub-surface layer (green circles) (Figure 3.4).

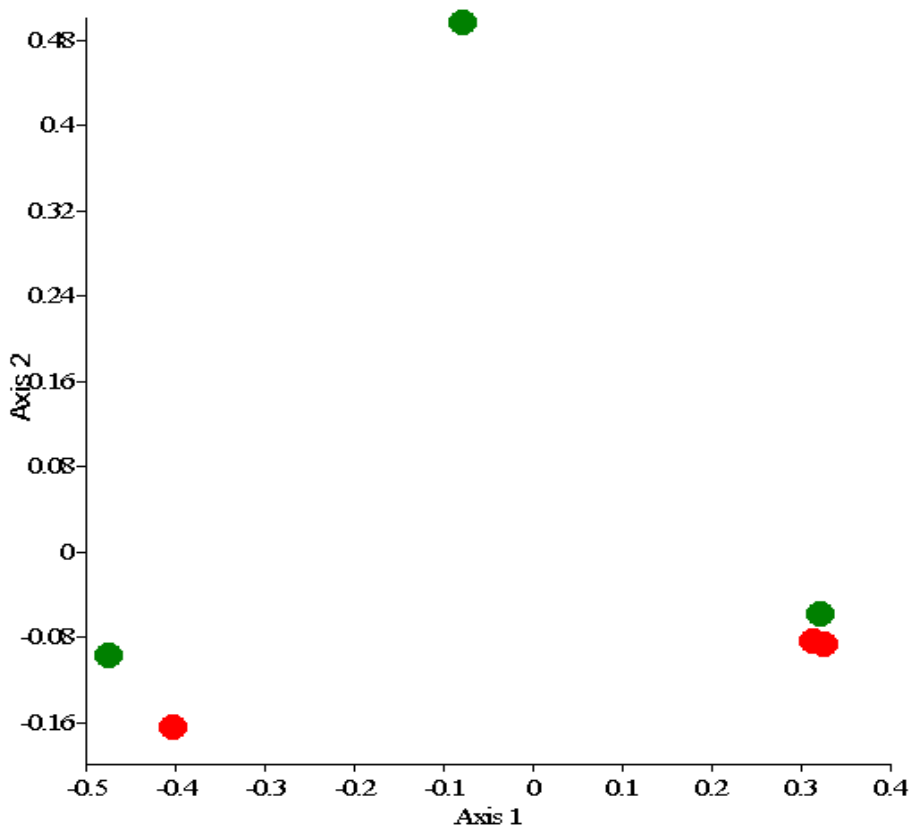


Figure 3.4. Non-metric multi-dimensional scaling ordination (2D) of the phytoneuston and phytoplankton at Verdens Ende. Red circles represented the SML samples and green circles represented the sub-surface samples (Stress: 0.12).

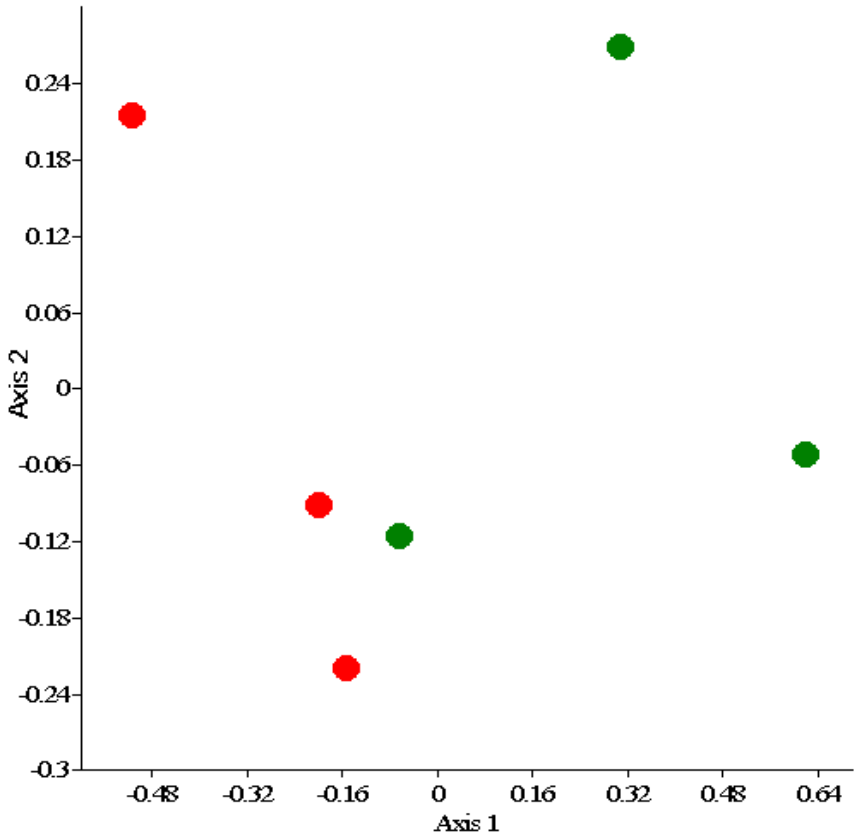


Figure 3.5. Non-metric multi-dimensional scaling ordination (2D) of the phytoneuston and phytoplankton at Ferjeodden . Red circles represented the SML samples and green circles represented the sub-surface samples (Stress: 0).

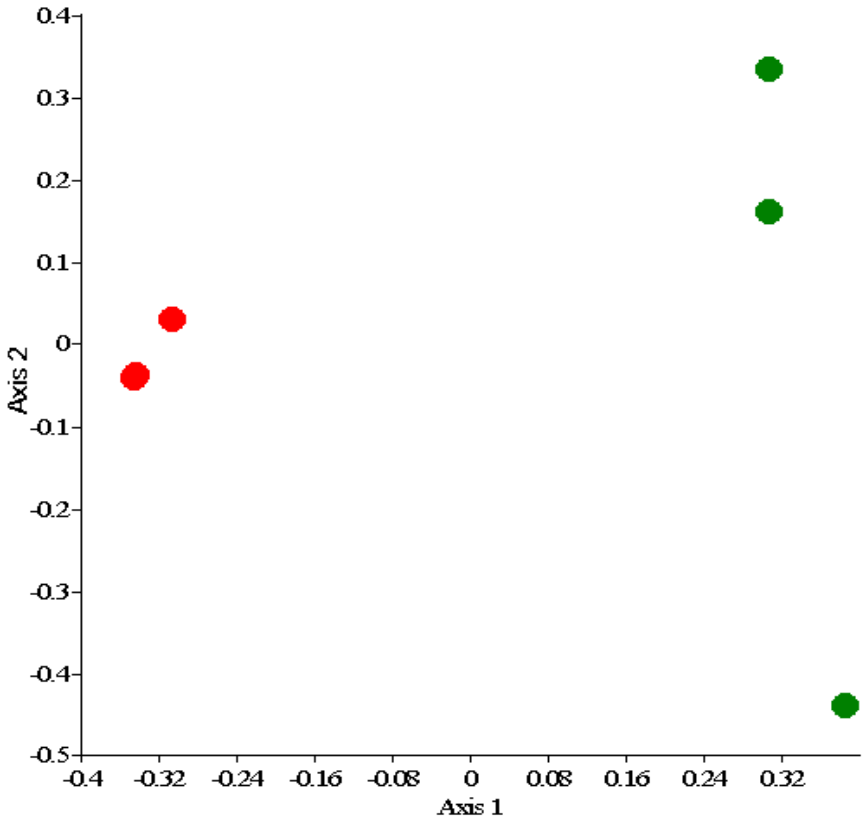


Figure 3.6. Non-metric multi-dimensional scaling ordination (2D) of the phytoneuston and phytoplankton at Bustangen. Red circles represented the SML samples and green circles represented the sub-surface samples (Stress: 0.05).

Species contributing most to separations between the SML and the sub-surface layer at the three locations were identified. At Verdens Ende, three dinoflagellates and a diatom *Paralia* spp. were only present in the SML and the diatoms *Melosira* spp. and another three dinoflagellates were present only in the sub-surface layer (Table 3.9). At Ferjeodden, the diatom *Leptocylindrus danicus* and three dinoflagellates were observed only in the sub-surface layer and the diatom *Chaetoceros* spp. and three dinoflagellate species were present only in the SML (Table 3.10). At Bustangen, the diatoms *Skeletonema* spp. and *Leptocylindrus danicus* and two dinoflagellates were seen only in the SML while the diatom *Pseudo-nitzshia* spp. and another four dinoflagellates were present in the sub-surface layer (Table 3.11).

Table 3.9. SIMPER analysis of combined samples from Verdens Ende.

Taxon	The number of individuals of neuston	The number of individuals of plankton
Heterocapsa spp.	0,40	0,00
Dinophysis spp.3	0,08	0,00
Paralia spp.	0,06	0,00
Ceratium longipes	10,02	0,00
Protoceratium spp.	0,00	0,10
Scrippsiella spp.	0,00	0,20
Melosira spp.	0,00	0,10
Gonyualax spp.	0,00	0,40

Table 3.10. SIMPER analysis of combined samples from Ferjeodden.

Taxon	The number of individuals of neuston	The number of individuals of plankton
Protoperidinium spp.	0,00	0,02
Leptocylindrus danicus	0,00	0,02
Lessardia spp.	0,00	0,16
Chaetoceros spp.	2,00	0,00
Ceratium macroceros	0,50	0,00
Scrippsiella spp.	1,20	0,00
Dinophysis acuminata	1,20	0,00

Table 3.11. SIMPER analysis of combined samples from Bustangen.

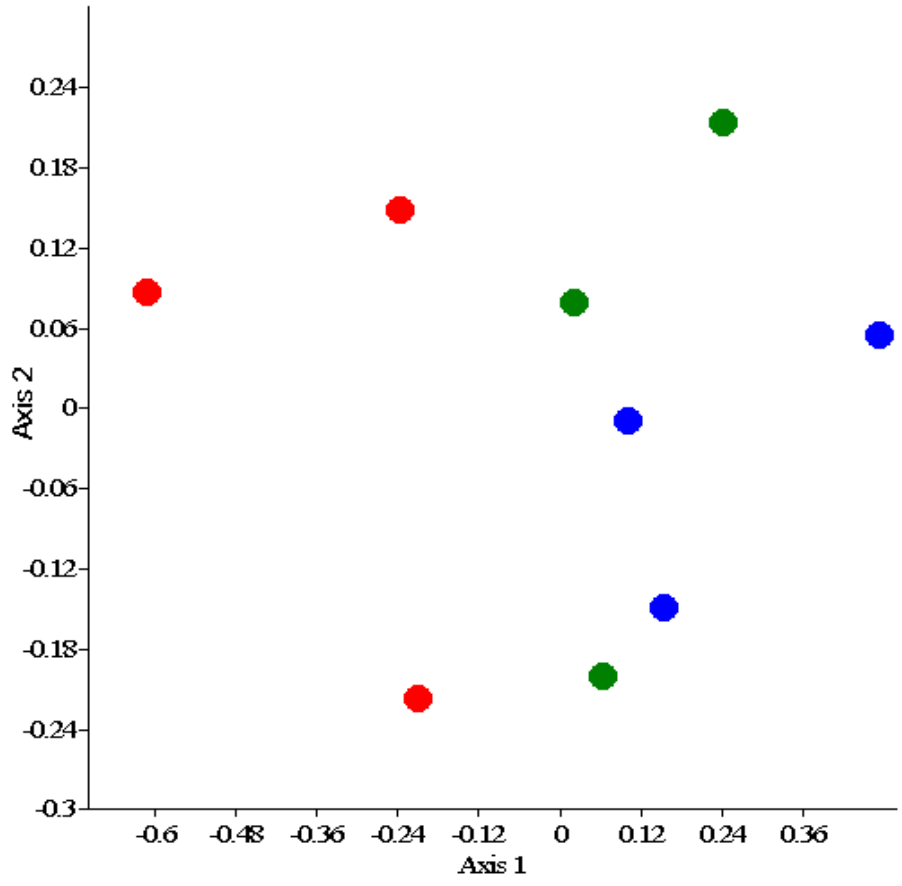
Taxon	The number of individuals of neuston	The number of Individuals of plankton
Ceratium spp.	0,50	0,00
Lessardia spp.	0,00	8,00
Skeletonema spp.	6,00	0,00
Dinophyceae spp.1	0,00	83,20
Ceratium macroceros	0,00	0,12
Leptocylindrus danicus	0,90	0,00
Dinophysis acuta	1,20	0,00
Pseudo-nitzshia spp.	0,00	0,04
Dinophysis norvegica	0,00	0,02

3.4.2. Neuston at the three locations

3.4.2.1 Ciliate communities

There was a significant difference in neuston communities between the three locations according to 1-way ANOSIM analysis (R: 0.27, p: 0.04).

Differences in the neuston communities between the three locations were apparent using NMDS (Figure 3.7). At Ferjeodden and Bustangen, the neuston communities appeared to be less different (the groups seem to be closer to each other) compared to the neuston community at Verdens Ende.



Figur 3.7. Non-metric multi-dimensional scaling ordination (2D) of the neuston ciliates from the three locations. Red, blue, and green circles represented Verdens Ende, Ferjeodden and Bustangen, respectively (Stress: 0.12).

Lohmanniella spp.1 and *Strombidium* spp.5 were identified as the most responsible species for the overall difference in the neuston communities between the three locations (Table 3.12).

Table 3.12. SIMPER analysis of combined neuston ciliate samples from the three locations.

Taxon	**The Number of individuals in Verdens Ende**	**The number of individuals in Ferjeodden**	**The number of individuals in Bustangen**
Lohmanniella spp.1	0,00	16,00	4,10
Strombidium spp.5	0,00	12,40	0,50
Helicostomella spp.	0,40	0,00	0,00
Strobilidiids spp.	0,00	0,80	0,00
Tontonia spp.	0,00	0,40	0,00
Tintinnina spp.2	0,00	0,40	0,00
Salpigella spp.	0,00	0,00	0,40
Strombidium spp.2	0,04	0,00	0,70

The numbers of individuals of *Lohmanniella* spp.1 and *Strombidium* spp.5 at the three locations were shown in figure 3.8 and 3.9, respectively. There was a significant difference in the number of individuals of *Lohmanniella* spp.1 between the three locations using Kruskal-Wallis test (H: 6.82, p: 0.03, n: 3). However, no significant difference was found in the number of individuals of *Strombidium* spp.5 between the three locations (H: 4.62, p: 0.09, n: 3).

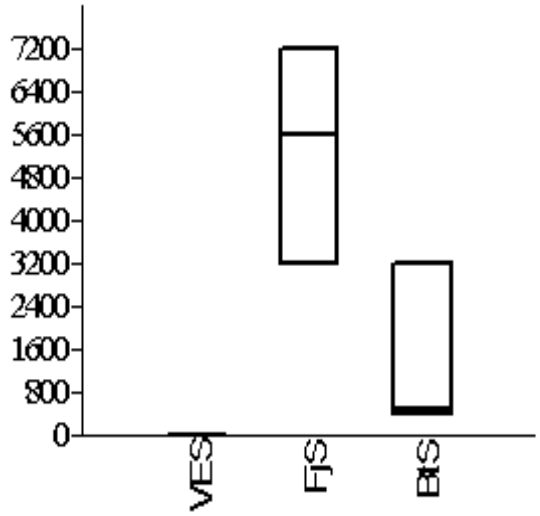


Figure 3.8. The number of individuals of *Lohmanniella* spp. in the SML at the three locations. VES: Verdens Ende, FjS: Ferjeodden, BtS: Bustangen.

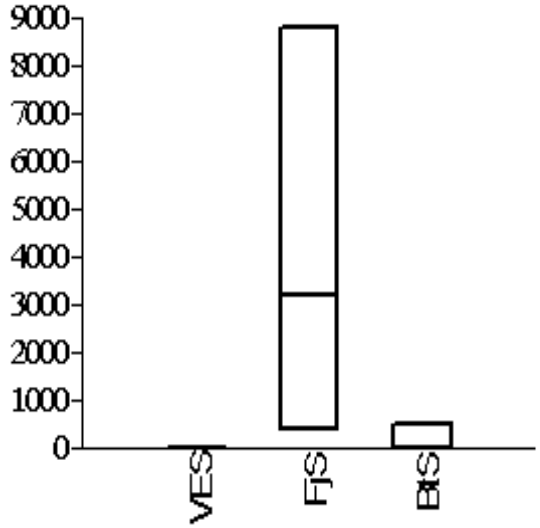


Figure 3.9. The number of individuals of *Strombidium* spp.5. in the SML at the three locations. VES: Verdens Ende, FjS: Ferjeodden, BtS: Bustangen.

3.4.2.2. Phytoneuston communities

A Significant difference was found in neuston communities between the three locations using 1-way ANOSIM (R: 0.48, p: 0.003).

The neuston communities at the three locations did not seem to be well separated using NMDS diagram. However, there seem to be a distance between the groups (Figure 3.10).

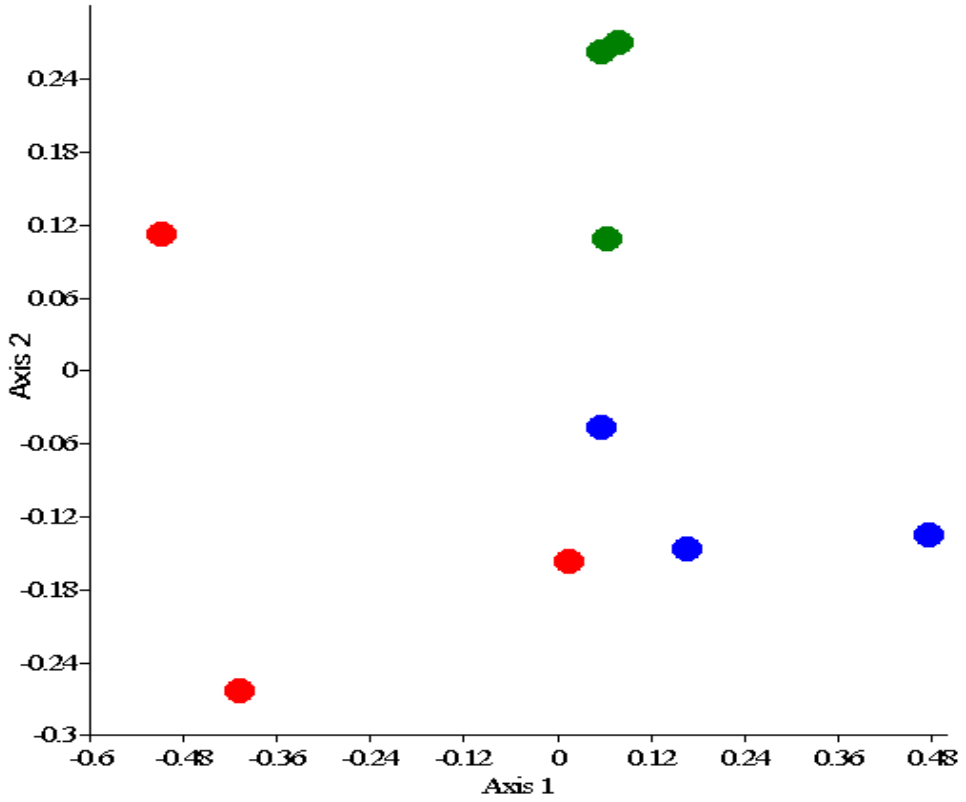


Figure 3.10. Non-metric multi-dimensional scaling ordination (2D) of the phytoneuston communities from the three locations. Red, blue, and green circles represented Verdens Ende, Ferjeodden and Bustangen, respectively (Stress: 0.09).

The species responsible for the difference in the neuston communities between the three locations were identified. Among these species, centric diatoms and dinoflagellates were the most important phytoplankton groups for the overall difference between the groups (Table.3.13).

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Table 3.13. SIMPER analysis of combined neuston ciliate from the three locations.

Taxon	The number of individuals at Verdens Ende	The number of individuals at Ferjeodden	The number of individuals at Bustangen
Centrales spp.	0,00	0,40	2,20
Melosira spp.	0,00	0,40	5,00
Ceratium lineatum	0,00	0,40	1,00
Ceratium spp.	0,00	1,20	0,50
Dinophysis acuta	0,00	0,04	1,20
Ceratium longipes	0,02	0,00	0,00
Dinophyceae spp.1	187,58	0,00	0,00
Heterocapsa spp.	0,40	0,00	0,00

The number of individuals of centric diatoms and dinoflagellates at the three locations were shown in figure 3.11 and 3.12, respectively. There was a significant difference in the number of individuals of centric diatoms between the three locations using the Kruskal-Wallis test (H: 6.2, p: 0.04, n: 3). However, there was not any significant difference in the number of individuals of dinoflagellates between the three locations (H: 2.6, p: 0.3, n: 3).

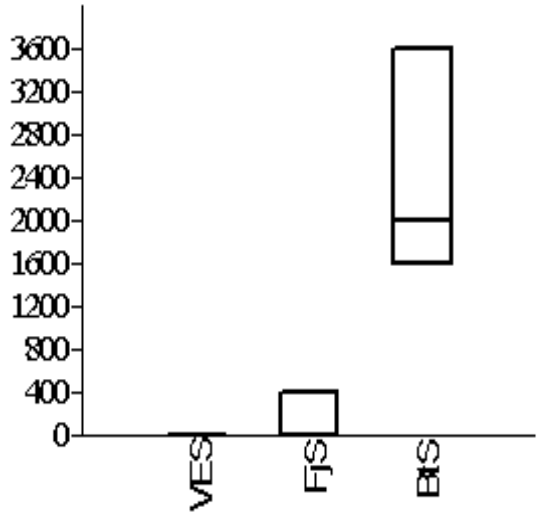

Figure 3.11. Box plots of the number of individuals of centric diatoms. at the three locations. VES: Verdens Ende, FjS: Ferjeodden, BtS: Bustangen.

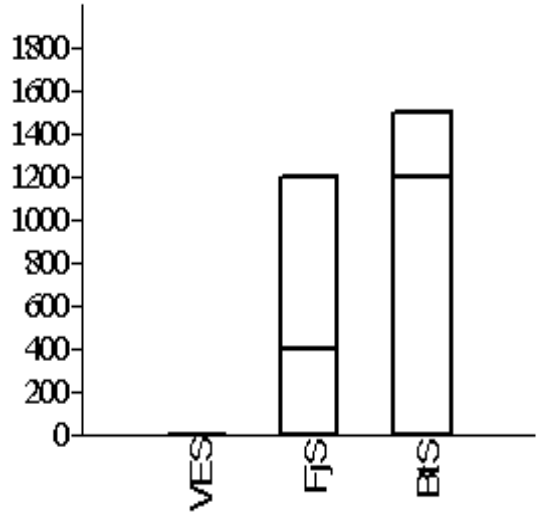

Figure 3.12. Box plots of the number of individuals of dinoflagellates at the three locations. VES: Verdens Ende, FjS: Ferjeodden, BtS: Bustangen.

4. Discussion

4.1. Environmental conditions

The environmental conditions (temperature and salinity) (Table 3.1) did not vary substantially at the three locations and in the different dates. Salinity was stable but there was an increase in 17th of July at Verdens Ende.

4.2. Diversity

No significant differences were determined in the ciliate and phytoplankton diversities between the SML and the sub-surface layer and between the SML samples at the three locations (sections 3.3.1. and 3.3.2.) applying statistical tests (Mann-Whitney U test and Kruskal-Wallis test) to the diversity indices (Table 3.3). One possible reason could be that the diversities of ciliates and phytoplankton groups were not influenced by expected eutrophication and oil pollution at Bustangen and Ferjeodden, respectively. This result was different from results obtained in previous studies that indicated differences between the diversity of neuston and plankton communities (Hardy, 1973; Parker and Hatcher, 1974; Manzi et al., 1977; Estep and Remsen, 1985). The differences between the result of the present study and previous studies could be because of the number of replicates (three) which might not be enough for any statistical test to detect possible "true" differences between the groups (Zar, 1996). Also, the diversity indices might not be suitable to correctly represent the species diversity of a community despite their popularity in the aquatic systems (Washington, 1984). Furthermore, there could be natural variations in the number of individuals of each species as well as the number of species in a microbial community (Cairns Jr et al., 1971; Cairns Jr et al., 1972). These natural variations within a community might be higher than possible differences between the compared communities. In order to find differences between the communities in locations differing in their amounts of nutrients and pollution, differences between the communities should be significantly higher than the natural differences within the communities (Cairns Jr et al., 1972).

4.3. Species composition

In the present study, no significant difference in species composition was found between neuston and plankton (Table 3.4 and 3.8). An explanation for this could be that the expected higher concentrations of nutrients (Hardy, 1982) and contaminants (Liss and Duce, 1997) in the SML than in the sub-surface layer did not affect the species composition of the neuston relative to the plankton. Another explanation could be that the 1-way ANOSIM test did not have enough power to determine differences or similarities between the groups. The number of individuals of species within the groups varied; this was evident from the test results where R= - 0.05 (for ciliates) (Table 3.4) and R= - 0.03 (for phytoplankton) (Table 3.8). In ANOSIM test, R< 0 indicates that dissimilarity between the groups are lower than the dissimilarity within the groups (Clarke, 1993), and ANOSIM test is sensitive to differences in dispersion (within-group variation) among groups. In addition, because there were only three replicates for each water layer and location in the present study, 1-way ANOSIM, like any other statistical tests, might have insufficient power to detect differences or similarities between the chosen groups (Clarke et al., 2006). In order to reliably detect small shifts in community composition, larger sample sizes (higher number of replicates) would be needed. Significant differences have been found between phytoneuston and phytoplankton communities by other studies (Hardy and Valett, 1981; Hardy et al., 1988) that was in contrast to the results obtained in the present study. One possible explanation of the difference between our result and the results of other studies could be using different statistical tests, with different amounts of power, by the other studies. Another explanation might be that in the previous studies, differences in the amounts of nutrients and pollutants between the SML and the sub-surface layer could have been greater than those in the present study. Therefore, the possible small differences in nutrient and pollution levels between the two water layers in the present study might have resulted in high similarity between neuston and plankton communities.

However, separations have been observed in ciliate communities in the SML and the sub-surface layer at Ferjeodden and Bustangen (Figure 3.2 and 3.3) and ciliate species responsible for the separations were identified (Table 3.5, 3.6 and 3.7). One of the most interesting outcomes, seen in species responsible for separations between neuston and plankton ciliates, was that only oligotrichs were found in the SML at Ferjeodden (Table 3.6). The existence of only oligotrichs in the SML at Ferjeodden, could be a result of their adaptation to oil-pollution or ultraviolet-B (UVB) radiation. According to Anikiev and Urbanovich (Anikiev and

Urbanovich, 1989), mean concentrations of total petroleum hydrocarbons in the SML in various oceans of the world, was higher compared with those in the sub-surface layers. Therefore, at Ferjeodden, a harbor, hydrocarbon pollutants would be expected to accumulate to higher concentrations in the SML than in the sub-surface layer. The effects of oil pollution on plankton ciliates was investigated in an experiment in an enclosed water column and it was revealed that the abundance of oligotrichs was higher in bags containing oil than those in control bags (Skjoldal et al., 1982). Also, UVB reaching the SML has higher intensity than those in the sub-surface layer (Liss and Duce, 1997); tolerating and adapting to high UV radiation is species-specific among ciliates (Marangoni et al., 2006) and there is little known about adaptations of specific ciliate groups to UV radiation. However, neuston community might be highly adaptive to environmental stressors (Zaitsev, 1971). Another possible explanation of the presence of only oligotrich ciliates in the SML at Ferjeodden might be decreased pressure from their predators, such as some copepod species, which might be sensitive to oil-pollution (Raffaelli and Mason, 1981) and UV radiation (Browman et al., 2000). Plankton ciliates particularly the oligotrichs that generally dominate in plankton systems are often strongly grazed by various copepod species (Browman et al., 2000; Calbet and Saiz, 2005). Consequently, if copepod abundance in the SML decrease as a result of sensitivity to oil pollution or UV radiation, predation pressure (Zöllner et al., 2009) on oligotrich ciliates may decrease resulting in increasing the ciliate abundance (Sakka Hlaili et al., 2008) in the SML. The presence of oligotrichs at Ferjeodden could be a result of an increased number of their prey, bacteria (Paranjape and Gold, 1982; Fenchel and Jonsson, 1988), being known to be highly abundant in oil-polluted areas (Dahl et al., 1983).

At Bustangen, where eutrophication was expected to occur, oligotrich and tintinnid ciliates contributed most to the separation between neuston and plankton communities. These ciliate groups were only present in the SML (Table 3.7). This result suggested that these ciliate groups might tolerate the expected higher amounts of nutrients in the SML than in the sub-surface layer. This result was similar to results obtained from other studies on pelagic systems. In eutrophic waters with high concentration of particulate organic matter and nutrients, oligotrichs such as *Strombidium* spp. were abundant (Beaver and Crisman, 1982; Barría de Cao et al., 2003; Kim et al., 2007; Selifonova, 2009) suggesting that this ciliate group might be tolerant to eutrophication (Barría de Cao et al., 2003; Selifonova, 2009). These ciliate groups might be present in eutophic waters because of availability of their favored prey, bacteria (Paranjape and Gold, 1982; Fenchel and Jonsson, 1988) and

nanoflagellates, found in high abundance in eutrophic waters (Beaver and Crisman, 1982; Azam et al., 1983; Gast, 1985; Pierce, 1989; Kim et al., 2007). Bacteria are known to be abundant in the most productive lakes (Fenchel, 1980) specially attached to surface film at the SML (Cunliffe and Murrell, 2009); also, nanoflagellates are known to be an important group of the microbial food web in the SML (Joux et al., 2006). In addition to oligotrichs, tintinnids were found to be abundant at eutrophic lakes (Beaver and Crisman, 1982); however, studies on tintinnids relative to eutrophication are rare (Barría de Cao et al., 2003) probably because tintinnids are known to be highly sensitive to eutrophication (Curds, 1982). In another study in eutrophic waters, the abundance of tintinnid ciliates was low (Selifonova, 2009). However, the presence of tintinnids only in the SML at Bustangen might be because of availability of their food, bacteria (Hollibaugh et al., 1980) that are known to be abundant in eutrophic areas (Fenchel, 1980) and particularly in the SML (Cunliffe and Murrell, 2009). It has been reported that bacteriovorous (such as tintinnids) ciliates might have the highest abundance of ciliates in some eutrophic estuaries and may be able to consume one third of annual bacterial production (Arndt et al., 1990).

Separations between phytoneuston and phytoplankton were also observed at Ferjeodden and Bustangen (Figure 3.4, 3.5 and 3.6) and species responsible for the separations were identified (Table 3.9, 3.10 and 3.11). An important finding was that genus *Chaetoceros* and three dinoflagellate species, being present only in the SML, were among the most responsible species for the separation between phytoneuston and phytoplankton at Ferjeodden with expected oil-pollution (Table 3.10). This finding suggested that genus *Chaetoceros* and the three dinoflagellate species might be tolerant to oil pollution at Ferjeodden. Vargo (1982) also found that the centric diatom *Chaetoceros,* dominated the phytoplankton community in oil tanks compared to controlled tanks; however, Vargo et al. (1982) did not explain clearly why he obtained such a result. In contrast, other studies reported diatoms as more sensitive to elevated oil concentrations than flagellates (Pulich et al., 1974; Hsiao et al., 1978). Davenport et al. (1982) reported that low hydrocarbon concentration may increase microflagellate abundance and decrease diatom abundance. Vargo et al. (1982) suggested that the difference between the results of the above reports may be due to the source, kind of oil and specific components of the oil. He added that the reason for *Chaetoceros* dominance could be the reduction of predator pressure because of changed feeding behavior or non-lethal oil concentrations in the water column. Decreased predation pressure could be as a result of a

remarkable negative effect that oil pollution have on some copepod species and other microalgal predators (Davenport et al., 1982).

The species responsible for the separation between the phytoneuston and phytoplankton at Bustangen, with expected eutrophication were identified (Table 3.11). The diatoms *Skeletonema* and *Leptocylindrus* and three dinoflagellates were only seen in the SML suggesting that these species might be tolerant to expected higher concentrations of organic nutrients in the SML than in the sub-surface layer. This finding was in agreement with Selifonova's (2009) findings which indicated that *Skeletonema, Leptocylindrus* and dinoflagellates could be tolerant to eutrophication. Another study also reported *Leptocylindrus,* a tolerant genus to industrial effluents and sewage discharges (Verlecar et al., 2006). There were two *Ceratium* species (dinoflagellates) among the responsible species for the differences between phytoneuston and phytoplankton at Bustangen (Table 3.11) suggesting that this genus (*Ceratium*) could be tolerant to eutrophication. This finding was similar to another study's finding in which *Ceratium* genus was assossiated with eutrophic coastal areas (Drira et al., 2010).

The neuston at the three locations, may also be influenced by or tolerate ultraviolet radiation. (UVR). We know UVR may affect growth and reproduction of plankton leading to changes in species composition of the community (Villafane et al., 1995). Intense radiation precludes microalgal species from the surface layers (Albright, 1980). For temperate areas, important shifts in marine plankton species composition were found after exposing the community to UVR (Santas et al., 1997; Helbling et al., 2005) leading to dominance of some species. A few studies suggest that phytoflagellates compared to diatoms (especially pennate diatoms), have more sensitivity towards UVR (Helbling et al., 1994; Villafane et al., 1995; Hernando and San Román, 1999). However, we found both diatoms and dinoflagellates in the SML that could be because the two groups were tolerant to UVR.

It is difficult to explain why significant differences were found in the species composition of neuston ciliates (p: 0.04, section 3.4.2.1.) and microalga (p: 0.003, section 3.4.2.2.) between the three locations because to our knowledge, there is no previous research on this subject. However, differences have been found in zooneuston (zooplankton living in the SML) (Holdway and Maddock, 1983) as well as in plankton (Vargo et al., 1982; Gillbricht, 1988; Liu et al., 2005; Devlin et al., 2007; Liu, 2008; Tas et al., 2009) between locations differing in their degrees of eutrophication and pollution. Zooneuston and plankton might have a close

relationship, with the neuston found in the present study, in the microbial food web and changes in their communities might lead to changes in predator (copepods) pressure and amounts of food (bacteria) availability. These changes might lead to changes in ciliate and phytoplankton communities (as mentioned above).

Separations between the neuston ciliates at the three locations were observed (Figure 3.7) and *Lohmanniella* spp. and *Strombidium* spp.5 were identified as the ciliate species contributing most to the separations in neuston between the three locations (Table 3.12). Because the two species were found only in the locations with expected eutrophication (Bustangen) and oil pollution (Ferjeodden), these two species could be tolerant to eutrophication and pollution. These two species are known to resist oil pollution (Skjoldal et al., 1982) and eutrophication (Barría de Cao et al., 2003; Selifonova, 2009). Another explanation regarding the presence of oligotrichs (*Strombidium spp.5.* and *Lohmanniella spp.*) in the SML at the two polluted locations could be that they are known to feed on bacteria (Paranjape and Gold, 1982; Fenchel and Jonsson, 1988), having a high number of individuals in eutrophic (Beaver and Crisman, 1982; Azam et al., 1983; Gast, 1985; Pierce, 1989; Kim et al., 2007) and oil polluted areas (Dahl et al., 1983). A possible reason why there was a significant difference in the number of individuals of *Lohmanniella* spp. between the three locations but not in the number of individuals of *Strombidium* spp.5. (section 3.4.2.1.), could be the statistical test used. The results of Kruskal-Wallis test like any other statistical test are less accurate with groups having less than 5 replicates (Zar, 1996).

Separations in phytoneuston community structures between the three locations (Figure 3.10) were explained by centric diatoms and dinoflagellates as the most responsible phytoneuston groups for the separations (section 3.4.2.2. and Table 3.13). Finding a significant difference only in the number of individuals of centric diatoms but not dinoflagellates may be explained by the statistical test, Kruskal-Wallis, that could be less accurate with groups having less than 5 replicates (Zar, 1996); however, centric diatoms are known to tolerate oil pollution (Vargo et al., 1982), eutrophication (Ruping, 1991; Ramaiah and Nair, 1998; Verlecar et al., 2006). Therefore, centric diatoms could be identified as indicators of high organic nutrients (Harrison et al., 1991; De et al., 1994) and oil pollution.

4.4. Links between diversity and environmental variables

There were small variations in the environmental variables between the three locations and sampling times, and any differences in neuston communities between the three locations could not be related to the environmental variables. In fact, neuston communities are known to adapt with their unique habitat (Zaitsev, 1971; Zaitsev and Liss, 1997), a stressful environment for microorganisms. Coastal and estuarine phytoplankton species seem to tolerate salinity changes in their environments. Estuarine phytoplankton species have a very wide range of salinity-tolerance being able to reproduce even in a salinity of 5-4 (Brand, 1984). Plankton ciliates can tolerate a salinity of 20-30 (Montagnes, 2001). Diatoms are able to grow within a temperature range of 5-25˚C with optimum growth at 15˚C (Ryther, 1954). Some dinoflagellates can grow within a temperature range of 10-25˚C or 10-30˚C (Pearce and Hallegraeff, 2004). Plankton ciliates may tolerate a temperature range of 7-22˚C (Montagnes, 2001). It was difficult and speculative to relate variation in communities to variation in recorded environmental factors that only represents small intervals well within the tolerance limits of the studied organisms.

Conclusions

There was no significant difference in the diversity of ciliates and phytoplankton between the SML and water column and between the SML samples at the three locations as opposed to other studies discussed in the previous chapter (Hardy, 1973; Parker and Hatcher, 1974; Manzi et al., 1977; Estep and Remsen, 1985). This result does not rule out possible “true” but unidentified differences because the representation of communities with diversity indices could be unsatisfactory. Also, insufficient number of replicates (n=3) contribute to much variation and large standard errors.

Ciliate and phytoplankton species compositions in the SML were compared with those in the sub-surface layer at the three locations and no significant differences were found between them. However, separations were observed in ciliate and phytoplankton between the two water layers at the three locations. Significant differences were found in ciliate and phytoplankton species compositions in the SML samples from the three locations. Oligotrich ciliates and diatoms were identified as the taxonomic groups contributing most to separations between neuston and plankton and between the neuston at the three locations. Adaptation to eutrophication, oil pollution, and UVR (ultraviolet radiation), predator pressure reduction and food availability could be possible reasons for this result.

The results obtained in the present study could not be related to the environmental factors (temperature and salinity) because the environmental factors did not vary substantially at the three locations.

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5. Appendix

Table 5.1: The number of individuals (cells/ml) of ciliate (oligotrichs, tintinnids, and other ciliates) and phytoplankton (dinoflagellates and diatoms) species in Verdens Ende, Ferjeodden, and Bustangen. (untronsformed data)

	Verdens Ende						Ferjeodden						Bustangen					
	Surface			Sub-surface			Surface			Sub-surface			Surface			Sub-surface		
Replicate	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Oligotrichs																		
Strombidium spp.1	0,40	0,00	0,14	0,40	0,00	0,00	0,00	0,00	0,00	0,40	0,00	0,02	0,50	0,00	0,00	0,10	0,10	0,00
Strombidium spp.2	0,00	0,00	0,04	0,00	0,00	0,00	0,00	0,00	0,00	0,80	0,00	0,10	0,00	0,70	0,00	0,00	0,00	0,00
Strombidium spp.3	0,00	0,06	4,4	0,80	0,00	0,00	0,00	0,40	0,00	0,90	0,00	0,82	0,00	0,00	0,40	0,10	0,10	0,04
Strombidium spp.4	0,00	0,00	0,00	0,30	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Strombidium spp.5	0,00	0,00	0,00	0,00	0,00	0,00	3,20	0,40	8,80	0,40	0,00	0,08	0,50	0,00	0,00	0,20	0,10	0,00
Strobilidiina spp.1	0,00	0,00	0,00	0,00	0,00	0,00	0,80	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Lohmanniella spp.1	0,00	0,00	0,00	4,50	0,00	0,40	3,20	5,60	7,20	5,10	0,02	0,00	0,50	3,20	0,40	0,00	1,80	0,00
Tontonia spp.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,40	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Total number of Oligotrichs	0,40	0,06	4,58	1,50	0,00	0,00	4,00	0,80	10,00	2,50	0,00	1,02	1,00	0,00	0,40	0,40	1,00	0,04
Tintinnids																		
Tintinnopsis spp.	23,20	1,04	0,00	1,00	0,02	0,00	1,20	2,00	0,00	0,20	0,38	0,82	1,75	1,20	0,40	1,50	0,10	0,98
Tintinnidium spp.	19,20	1,34	0,00	7,00	0,00	1,60	34,0	5,20	0,00	3,00	1,34	2,08	0,00	2,00	4,40	5,50	2,60	0,66
Tintinnina spp.1	0,00	0,00	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,02	0,02	0,00	0,00	0,40	0,00	0,00	0,02
Tintinnina spp.2	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,40	0,10	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,00
Tintinnina spp.3	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,10	0,00	0,00	0,50	0,00	0,00	0,00	0,00	0,00
Favella spp.	0,00	0,00	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Helicostomella spp.	0,40	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Eutintinnus spp.1	0,10	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

Eutintinnus spp.2	1,20	0,00	0,00	0,00	0,00	0,00	3,60	0,00	0,00	0,00	0,10	0,04	0,00	0,40	0,00	0,00	0,00	0,00
Salpingella spp.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,40	0,00	0,00	0,00	0,00
Total number of tintinnids	44,00	2,38	0,00	8,10	0,06	1,60	38,8	7,20	0,40	3,40	1,84	2,98	2,25	4,00	5,20	7,00	2,70	1,66
Other ciliates																		
Hypotrichia spp.1	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,04	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Steenstrupiella spp.	0,40	0,00	0,00	0,20	0,00	0,00	3,60	4,00	2,00	0,10	0,12	0,22	0,50	12,40	1,20	0,00	0,10	0,02
Tiarina spp.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,10	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Paramecium spp.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,50	0,00	0,00	0,00	0,00	0,00
Total number of other ciliates	4,40	0,56	2,40	5,40	19,1	0,40	6,80	9,60	9,20	5,50	0,18	1,68	1,50	16,00	1,60	0,00	1,90	0,02
Total number of ciliates	92,40	5,32	2,4	21,60	19,2	3,60	84,4	24,00	10,00	12,30	3,86	7,64	6,00	24,00	12,00	14,00	7,30	3,34
Dinoflagellates																		
Ceratium fusus	0,00	0,00	0,00	0,00	0,00	0,00	0,80	0,00	0,00	0,00	0,02	0,02	0,50	0,40	0,00	0,00	0,10	0,00
Ceratium longipes	0,00	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Ceratium lineatum	0,00	0,00	0,00	0,00	0,00	0,00	0,40	0,00	0,00	0,00	0,00	0,00	1,00	0,00	0,00	0,00	0,00	0,02
Ceratium macroceros	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,04	0,46	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,10	0,02
Ceratium spp.	0,00	0,00	0,00	0,00	0,00	0,00	0,80	0,00	0,40	0,00	0,00	0,02	0,50	0,00	0,00	0,00	0,00	0,00
Dinophysis acuta	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,04	0,00	0,00	0,00	0,00	0,00	1,20	0,00	0,00	0,00	0,00
Dinophysis acuminata	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,20	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Dinophysis norvegica	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,20	0,00
Dinophysis spp.4	0,00	0,08	0,08	0,30	0,02	0,00	11,6	0,00	0,80	0,00	0,04	0,04	0,00	4,80	0,40	0,00	0,00	0,00
Gymnodinium spp.	34,80	0,00	0,00	3,10	0,8	0,00	137,	15,60	81,20	0,00	0,94	0,00	0,00	0,00	14,00	0,00	0,00	0,28
Protocentrum micans	18,40	0,06	0,00	0,50	0,00	0,00	70,4	19,20	7,60	0,20	0,06	0,40	3,75	77,60	0,40	0,00	0,20	0,02
Protoperidinium spp.	0,40	0,00	0,00	0,10	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,50	0,00	0,00	0,00	0,00	0,00
Scrippsiella spp.	0,00	0,00	0,00	0,20	0,00	0,00	0,00	1,20	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Diplopsalis spp.	1,20	0,04	0,00	1,30	0,00	0,00	6,80	0,40	7,60	1,00	0,16	0,86	1,00	6,40	0,40	0,10	0,20	0,00
Dinophyceae spp.1	0,00	21,58	166,	0,00	0,00	12,0	0,00	0,00	0,00	50,40	0,00	35,3	0,00	0,00	0,00	0,90	82,3	0,00
Dinophyceae spp.2	30,40	0,00	0,00	1,70	0,10	0,00	177,	399,2	24,80	0,00	10,1	0,00	9,25	75,60	28,40	0,00	0,00	0,16
Lessardia spp.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,16	0,00	0,00	0,00	0,00	8,00	0,00

Heterocapsa spp.	0,40	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Protoceratium spp.	0,00	0,00	0,00	0,10	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Gonyaulax spp.	0,00	0,00	0,00	0,00	0,00	0,40	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Total number of dinoflagellates	85,60	21,76	166,	7,30	0,92	12,4	406,	435,6	122,4	51,60	11,3	37,3	16,50	166,0	43,60	1,00	91,1	0,50
Diatoms																		
Pennales spp.1	69,20	5,82	46,4	0,40	1,96	18,4	40,4	25,60	18,00	1,00	34,8	1,24	81,50	90,80	517,6	16,40	4,90	13,62
Centrales spp.1	0,00	0,00	0,00	0,00	0	0,00	0,00	0,00	0,40	0,00	0,06	0,00	1,00	0,80	0,40	0,30	0,00	0,00
Diatom spp.1	172,0	2,3	136,	2,50	2,12	3,60	1,60	1,20	0,00	0,00	1,18	0,10	1,00	0,00	0,40	0,10	0,00	0,02
Skeletonema spp.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	6,00	0,00	0,00	0,00	0,00	0,00
Striatella spp.	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,80	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Leptocylindrus danicus	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,02	0,00	0,50	0,40	0,00	0,00	0,00	0,00
Thalasiosira spp.	0,00	0,00	0,00	0,00	0,00	0,00	0,80	0,80	3,20	0,20	0,16	0,08	0,00	0,00	0,00	0,00	0,00	0,00
Paralia spp.	0,00	0,06	0,00	0,00	0,00	0,00	0,00	0,80	0,00	0,00	0,04	0,00	1,00	0,80	1,60	1,80	0,80	0,36
Dactyliosolen spp.	6,80	0,04	8,4	0,90	0,36	0,40	2,80	2,40	0,00	0,20	1,24	0,96	1,00	4,00	0,00	0,40	0,00	0,16
Pleurosigma normanii	0,40	0,02	0,8	0,00	0,06	0,40	1,60	1,60	0,00	0,00	0,24	0,06	3,75	4,40	4,40	2,90	0,40	1,76
Pseudo-nitzshia spp.	0,00	0,04	0,00	0,00	0,02	0,00	0,00	0,00	1,60	0,00	0,42	0,68	0,00	0,00	0,00	0,00	0,00	0,04
Cylindrotheca closterium	18,00	1,22	32	0,00	0,18	0,80	10,4	16,40	15,20	0,40	5,52	0,58	21,25	67,60	149,2	1,30	0,60	8,48
Proboscia spp.	93,60	0,16	0,4	12,40	0,00	0,00	3,60	15,20	0,00	3,50	3,70	1,42	1,50	18,40	13,20	0,80	3,20	0,14
Melosira spp.	0,00	0,00	0,00	0,10	0,00	0,00	0,00	0,40	0,00	0,00	0,04	0,00	1,00	0,80	3,20	0,10	0,10	0,26
Chaetoceros spp.	0,80	0,00	0,00	0,00	0,00	0,40	0,00	2,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Total number of diatoms	360,	9,66	224,	16,30	4,7	24,00	61,2	66,40	39,20	5,30	47,4	5,12	119,5	188,	690,0	24,10	10,0	24,84
Prasinophyceae	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Pterosperma spp.	0,40	0,00	0,00	0,00	0,00	0,40	0,80	1,20	0,00	0,00	0,00	0,00	0,00	0,00	1,20	0,00	0,00	0,00
Euglenophyceae	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
Euglenophyceae spp.	20,80	0,64	8,40	0,40	0,30	1,20	34,00	10,00	17,60	0,00	1,12	0,22	1,50	9,60	8,80	0,20	0,10	0,08
Total number of Phytoplankton	446,40	31,42	390,90	23,60	5,62	36,40	467,20	502,00	161,60	56,90	58,86	42,46	136,00	354,00	733,60	25,10	101,10	25,34