# Seasonal dynamics and diversity of microalgae and flagellate protists of two sandy shores in Oslofjorden

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# **Abstract**

The objective of this thesis was to investigate the diversity of microalgae (including cyanobacteria) and protist flagellates and their seasonality in two sandy shore locations of Oslofjorden; Huk in Bygdøy Peninsula and Nesset in Bunnefjorden. The samples were collected in the littoral zone from August 2014 to June 2015. Huk was sampled nine times and Nesset only seven due to ice freezing. Temperature and salinity were also recorded. After transferring the samples to the laboratory at the department of Bioscience at the University of Oslo, they were investigated by means of light and scanning electron microscopy.

In total, 71 different taxa were recorded in these two locations, 63 in Nesset and 44 at Huk, showing higher species richness in Nesset compared to Huk. The main protist groups recorded were dinoflagellates, haptophytes, cryptomonads, chlorophytes, euglenoids, heterokonts, diatoms, cercozoans, apusozoans and choanoflagellates. In addition a large number of cyanobacteria were detected, especially at Nesset.

The samples from Nesset generally contained both a higher abundance of organisms and species number. Environmental conditions such as differences in sediment grain size, wave exposure and salinity may explain the differences in species richness.

A new statistical method was used to test the significance of the difference in species richness between Huk and Nesset. This test estimated that the average probability of detecting species is larger than 50%. In this range, the performed probabilistic assessment showed that the difference in species richness between Huk and Nesset to be significant.

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#### 1 INTRODUCTION

#### 1.1 Aim of this research

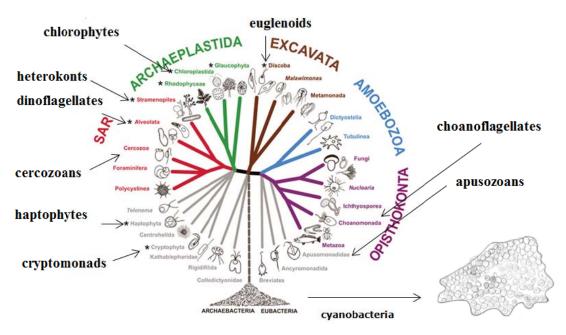
The main objective of this thesis was to investigate the diversity of flagellate protists and microalgae including cyanobacteria and their seasonal development at two sandy shores locations in Oslofjorden; Huk in Bygdøy Peninsula and Nesset in Bunnefjorden. The samples were collected from August 2014 to June 2015. Identification was done in the LM and SEM to the lowest possible taxon.

## 1.2 Protists and microalgae

In biological taxonomy schemes, protists are described as a large group of diverse eukaryotic microorganisms, mainly unicellular animals and plants, that do not form tissues (wikipedia.org-dated 10.09.15) and include the algae. The cyanobacteria are prokaryotic, but may for practical reasons be included with the microalgae (they are unicellular phototrophs). A schematic view of the tree of life with the positions of the groups investigated in this thesis is presented in Figure 1-1. In the present study, the main focus was on the flagellate protists including the dinoflagellates, haptohytes, cryptomonads, chlorophytes, euglenoids, heterokonts, diatoms, cercozoans, apusozoans and choanoflagellates. The cyanobacteria were also included. Ciliates were abundant in these habitats, but were outside the scope of this study. A few pictures of encountered ciliates are presented in appendix D.

Photosynthetic cyanobacteria, diatoms, haptophytes, cryptomonads and euglenoids living in the surface of the sand are the primary producers of the sandy shore ecosystem and are being preyed upon by heterotrophic and mixotrophic apusozoans, cercozoans, euglenoids, cryptomonads and ciliates within the microbial loop. The role of heterotrophic flagellates is important in the microbial loop according to Fenchel and Andersen (1985) as they act as predator on bacteria and diatoms.

In the last decades, a few investigations have been done to identify species diversity and the ecological role of protists including their abundance and importance in the 'microbial loop' of sandy sediments (Fenchel 1967, 1969; Lee and Patterson 2002; Anderson et al. 2003 and Flø Jørgensen et al. 2004).



**Figure 1-1** The tree of life with the position of the investigated groups marked with arrows, modified from Adl et al. (2012).

# 1.3 Sandy shores

Beaches are very dynamic environments due to influence from waves, tides and wind and are the places where the sea meets land. The basis of the shores could be rock, gravel, sand, silt or clay. The main difference between these materials is the size of the particles which affects their properties such as porosity and permeability. Sand (0.1 mm –1 mm) has grain size between clay and gravel. The main characteristics are listed below (McLachlan and Brown 2006; Das 2012):

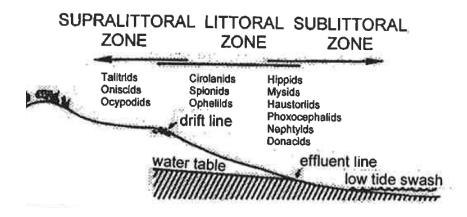
- Grain size: The majority of particles are in the range of 0.1 to 1.0mm. The finer particles sizes usually follow the water movements.
- Mineralogy: There are two major categories of quartz fragments and calcium carbonate.

  Norwegian sandy beaches belong to the quartz category.
- Grain shape: The sand particles could be a mixture of different shapes of round, sharp, angular, spherical etc.
- Porosity: The ratio between the total void volumes to the total sediment volume is titled porosity. This range is between 10 to 50% and depends on the sand density.
- Permeability: Permeability is the discharge rate of the water through the sand layer and depends on relative density of the sand which could be between 0.001 to 0.000001 m/s.
- Moisture content: The percentage of water in the sediment volume determines the water content in the layer. Finer soil has usually higher water content.

Supply of oxygen into the sediment is one of the main factors which heavily influences the biological communities. Low oxygen communities have a totally different species composition (Little 2000). The soil porosity and permeability is directly dependent on the particle size. Porosity has an important role regarding the biota type while the permeability is of great significance for the organism diversity in sand (Larsen 1985). Compared to the clay and muddy soils, sand has higher porosity and permeability, resulting in higher drainage, suspension and oxygen level (Patterson et al. 1989 & 1993). On the other hand, due to very limited water movement, the clay shores are nutrient rich compared to sandy shores.

The shore may be divided into three zones as illustrated in Figure 1-2. McLachlan and Jaramillo (1995) reviewed different criteria for the zonation. Among all available criteria, they put more weight of evidence for supporting three zones criterion. According to McLachlan and Brown (2006), these three zones are:

- Supralittoral zone: A top zone above the drift line. The main animal taxa for this zone are: talitrid amphidos, oniscid isopods and ocypodid crabs (see Figure 1-2).
- Littoral zone: This zone is below the supralittoral (middle zone of Dahls' theory, 1952 and Salvat 1964). It is a semi-saturated zone where the water line could be observed due to tide in this zone and the water movements are dampening the sand. Cirolanid ispodod, other ispodos such as Euzonus, haustoriid, amphipdos and sponoid polychaetes are examples of animal in this zone (see Figure 1-2).
- Sublittoral zone: The sublittoral zone is located below the semi-saturated littoral zone. The saturation percentage is 100%. Hippid crabs, mysids, donaci bivalves, nyphtyid and glyceride polychaetes, oedicerotid and haustoriid amphipdos are examples of animal in this zone (see Figure 1-2).



**Figure 1-2** The three zones of McLachlan and Brown (2006)

Under conditions with calm water (sheltered area), the benthic microflora and fauna is usually abundant (McLachlan and Brown 2006; Defo and McLachlan 2005), whereas on the exposed beaches, surf-zone diatoms may be far more important. In this study, the focus was on the species collected from the aerated top sediment layers of the littoral zone.

While some work has been conducted, (e.g. Kaas et al. 1985; Larsen 1987; Vørs 1992; Grimsrud 2001 and Zubizarreta 2005), the number of studies that have been carried out in this field is far behind that for rocky shores and other coastal communities. This dearth is especially seen in the Scandinavian region, and this was the motivation behind the present study.

#### 2 AN INTRODUCTION TO THE RECORDED GROUPS

Below follows a short description of the different groups observed.

## 2.1 Dinoflagellates

The species are photosynthetic or heterotrophic unicellular organism with two different flagella according to Graham et al. (2009) and Lebour (1925). The transverse flagellum provides forward motion while the other one trails behind providing little propulsive force. They have a complex cell covering called amphisma which is composed of flattened vesicles called alveoli (Zubizarreta 2005). Most of these species cells have two parts, an anterior epicone and posterior hypocone (Graham et al. 2009, see Figure 2-1). The two parts are separated by a groove that encircles the cell, known as cingulum where the smaller grove is known as sulcus. At the intersection of the cingulum and sulcus is a pore shape which the two flagella emerge from (Graham et al. 2009).

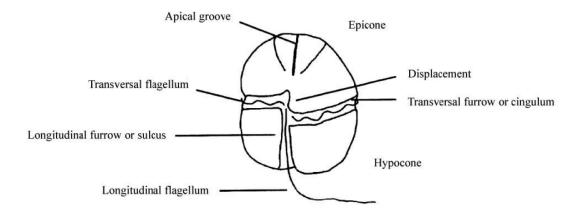


Figure 2-1 Example of dinoflagellate cell, Zubizarreta (2005)

# 2.2 Haptophytes

They are autotrophic and most of them are unicellular with two chloroplasts and two flagella as seen in Figure 2-2 (Zubizarreta 2005). These flagella could be equal or unequal. Haptonema is characteristic of many species, including the earliest-diverging forms. They play an important role in the food webs of both natural and aquaculture systems (Graham et al. 2009). Due to having small size, observation of these species in the light microscope (LM) is challenging and requires electron microscope (EM) or genetic methods.

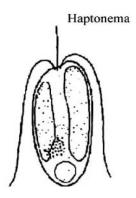


Figure 2-2 Example of haptophyte cell, Zubizarreta (2005)

# 2.3 Cryptomonads

They are unicellular flagellates with two flagella emerging from an anterior pocket, vestibulum (Graham et al. 2009). The main specification of cryptomonads is a presence of characteristic extrusomes called ejectisome (Graham et al. 2009). Ejectisome consist of two connected spiral ribbons held under tension (see Figure 2-3). If the cells are stressed they push the cell in a zigzag course away from the disturbance (Graham et al. 2009). Large ejectisomes, visible under the light microscope, are associated with the vestibulum; smaller ones occur underneath the periplast (Morall and Greenwood 1980; Grim and Staehein 1984). Most of cryptomonads have one or two chloroplasts. In some cases, there are no chloroplasts.

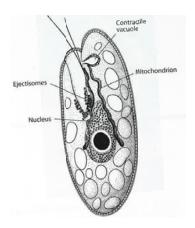


Figure 2-3 Example of cryptomonad cell, Graham et al. (2009)

# 2.4 Chlorophytes

Chlorophytes are predominately photosynthetic organisms with green-coloured cells. The reason of this green colour is that abundant chlorophylls *a* and *b* are not covered by large amounts of differently coloured accessory pigments (Graham et al. 2009). The various species of

chlorophytes can be unicellular, multicellular, or colonial. This group of green algae has one (sometimes two) or more flagella. Flagellate cells of chlorophytes have more or less symmetric morphology as shown in Figure 2-4. Eyespots are usually observed in their chloroplasts (Zubizarreta, 2005).

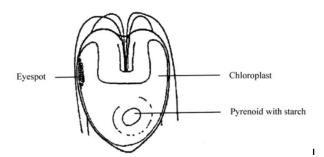


Figure 2-4 Example of chlorophyte cell, Zubizarreta (2005)

# 2.5 Euglenoids

Euglenoids are unicellular flagellates. Many of them are heterotrophic, but some are phototrophic. Two anterior, unequal flagella, rooted within a canal (See Figure 2-5). The cell surface is usually striped, composed of pellicular strips that make up the pellicular according to Leedale (1967). Schematic view of their euglenoids movements is illustrated in Figure 2-6.

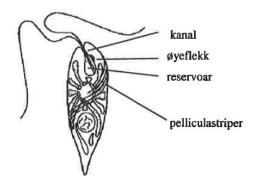


Figure 2-5 Example of euglenoid cell, Grimsrud (2001)

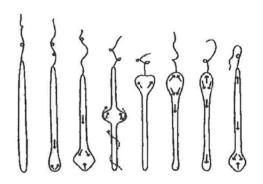


Figure 2-6 Shecmatic view of the euglenoid movements, Grimsrud (2001)

## 2.6 Choanoflagellates

The choanoflagellates are a group of free-living unicellular and colonial flagellate eukaryotes (wikipedia.org- dated 10.09.2015). They are small single-celled protists, found in both fresh waters and the oceans. They have single flagellum surrounded by a collar of 30–40 microvilli (from wikipedia.org) by which choanoflagellatea both move and take in food, See Figure 2-7.

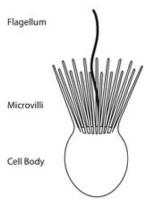
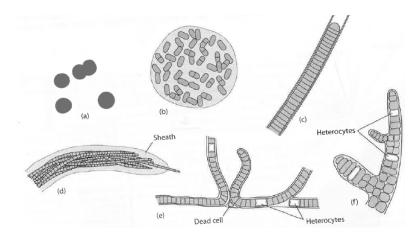


Figure 2-7 Example of choanoflagellatea cell, from tolweb.org

## 2.7 Cyanobacteria

Cyanobacteria or blue green algae occur in a diversity of body forms, single cells to more complexes from, usually unicellular, Graham et al. (2009). Also they are photosynthetic and contain chlorophyll *a*, the same photosynthetic pigment that plants use. Their morphology is shown in Figure 2-8. Cells attached end-to end in a row take the forms of filament, also known as trichome (Graham et al. 2009).



**Figure 2-8** Different types of cyanobacteria a) unicells such as *Synechocystis*, b) colonies of individual cells such as *Aphanothece*, c) unbranched filaments including Lyngbya, d) aggregations of multi trichomes in common sheath such as *Microcoleus*, e) **f**alse-branched forms including *Scytonema*, f) true-branched forms sych as *Stigonema*, Graham et al. (2009)

#### 2.8 Heterokonts

In this study, five groups of heterokonts, both photosynthetic and heterotrophic species were observed. Of these five, the diatoms were most abundant. Diatoms are algae with unique cell wall which mainly made of dioxide silicon. This wall is also known as frustule and consists of two valves, Graham et al. (2009). The presence of two different flagella is a characteristic of the heterokonts. One flagellum is usually smooth and short and the other one is longer and directed into the direction of swimming (Zubizarreta 2005; Van den Hoek et al. 1995). Figure 2-9 and Figure 2-10 show examples of this group.



Figure 2-9 Example of diatom cell

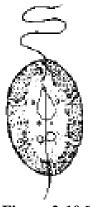


Figure 2-10 Example of *Olisthodiscus* cell, Hallegraeff and Hara(1995)

#### 2.9 Cercozoans

They are unicellular and have a variety of forms; therefore it is difficult to identify them with respect to the structural characteristics. *Metromonas* is example of this group observed in sand (Throndsen and Eikrem 2010). An example form of *Metromonas* is illustrated in Figure 2-11.

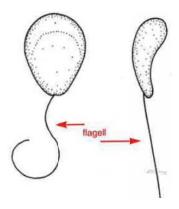


Figure 2-11 Example of cercozoan cell, modified from Throndsen and Eikrem (2010)

# 2.10 Apusozoans

Amastigomonas is the main species from this group. Their cells are the rice-grain shaped with a short snout including the base of the short flagellum illustrated in Figure 2-12.

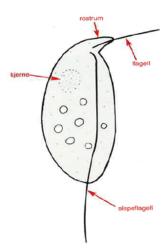


Figure 2-12 Example of apusozoan cell, Throndsen and Eikrem (2010)

#### 3 MATERIALS AND METHODS

## 3.1 Description of the selected locations

Two locations in Oslofjorden with differing properties were selected as sampling sites. The first location was Huk in Bygdøy Peninsula and the second one was Nesset in Bunnefjorden. The locations are shown in Figure 3-1.

Huk is a sandy area divided by a natural overhanging rock covered with macroalgae. The grain size is somewhat coarse with patches of different particle sizes within the littoral zone. Nesset is a sheltered bay within a narrow zone at the inner part of the bay. In contrast to Huk, the sediment is mixed with mud and the sand grains are finer and the porosity and permeability of the sediment is small compared to Huk. The grain size in these two locations is illustrated in Figure 3-2.

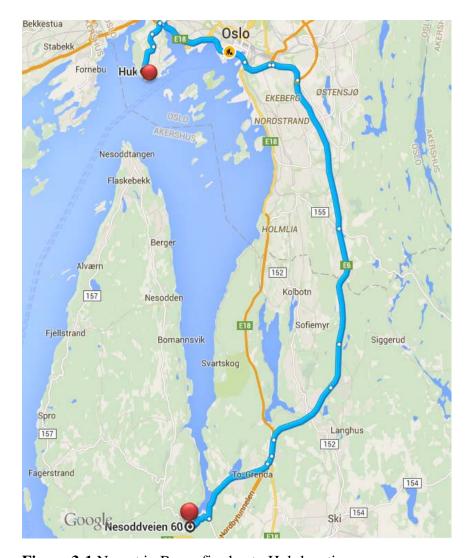


Figure 3-1 Nesset in Bunnefjorden to Huk location

a b



**Figure 3-2** Illustration of grain size at Huk (a) and Nesset (b), both pictures were taken of the same magnification of 7.5 times by means of LM-Nikon SMZ.

# 3.2 Preparation of the samples

Samples from the mentioned two locations were collected regularly during four seasons. The samples were collected with a bailer from the top centimeters of the littoral zone, which is a turbulent layer of water that washes up on the beach after an incoming wave has broken in exposed areas and less so in sheltered areas. The sand in this zone is moisten by the waves and tides, but not fully submerged. Figure 3-3 and Figure 3-4 shows Huk and Nesset fields during colleting. It was noted that there is a creek entering the sea at Nesset (as shown in Figure 3-4). This might result in a lower salinity compared to Huk.

The top ca 2 cm in the littoral zone of the beach was scooped up with a plastic bailer with a flat mouth, put into plastic bags and stored in a cooler for transport to the laboratory. The temperature was measured with a thermometer. The salinity was measured with a hand refractometer (ATAGO-S/Mill, Japan) immediately after moving the samples to the laboratory.



Figure 3-3 Sample collection at Huk



Figure 3-4 Sample collection at Nesset- A creek entering the sea is shown with arrow.

# 3.3 Microscopy

# 3.3.1 Light microscopy (LM)

A Nikon Microphot-FX LM (Japan) was used to take the pictures from samples (Figure 3-5). This microscope has two features, a phase contrast, and differential interpherence contrast (DIC). In the current study, we mainly used the 100, 400 phase contrasts and 200 and 400 DIC magnifications.



Figure 3-5 Light microscope

The collected sand from both locations was transferred to Petri dishes which were covered by the lens papers. The cover slips were then put on the lens papers as shown in Figure 3-6. After closing the petri dish lid, samples were stored in culture rooms at temperatures close to ambient temperature. The light intensity was ca 80-100 µmol photons m<sup>-2</sup> sec<sup>-1</sup>.



**Figure 3-6** Cover slips on the lens paper

The cover slips with the attached organisms were put on the LM slide. Then the observation process was started. Before taking pictures, the focus of the LM was adjusted to get clear pictures of the species.

The identification of species and taxa were based on direct identification in the LM and the pictures taken. The Identification was mostly based on the Herdman (1920, 1922, 1923 and 1924), Parke (1949), Hulburt (1957, 1969), Campell (1973), Dodge (1982), Kaas et al. (1985), Larsen and Patterson (1990), Ekebom et al. (1995), Hasle and Syvertsen (1996), Throndsen (1993, 1997), Throndsen and Eikrem (2010), Hoppenrath et.al (2014) and Algaebase website (http://www.algaebase.org), also other works if needed.

To cover the uncertainty and to reduce human errors, the procedure of taking pictures and identification were repeated a few times with several cover slips from each sample.

# 3.3.2 Scanning Electron Microscopy (SEM)

To study the cell shape, appendages, outer structures of the species and the morphology of microorganisms in details, the Scanning Electron Microscope (SEM), JEOL JSM 6400(JEOL Ltd., Japan) at the Biology laboratory, University of Oslo was used as shown in Figure 3-7.



Figure 3-7 Scanning Electron Microscope

In order to produce enough material for SEM the same procedure as for the petri dishes were carried out but in larger containers and microscopy slides were put on top of the lens paper. After 2-4 days the organisms were scraped off and into eppendorph tubes. An equivalent volume of glutaraldehyde (OCHC<sub>3</sub>H<sub>6</sub>HCO) and sample was prepared to a final concentration of 2 % was used to fix the specimens. A drop of this mixture was placed on a special SEM cover slip coated with poly-L-lysin where the organisms were allowed to sink and attach to the coverslip for a few hours. Then the cover slips were moved into SEM holders and dehydrated in an ethanol series starting at 30 %, followed by 50 %, 70 %, 90 % and 96 %. The procedure was concluded with four rinses in 100 %. All rinses lasted for 10 min.

At the end, the mixture was transferred to a Critical Point Drier (Figure 3-8) in order to dry the sample using CO<sub>2</sub> and Ethanol 100 %. The dried samples were mounted on stubs and coated with Platinum in a Sputter Coater (CRESSINGTON-308 UHR, England). Then samples were ready to be examined in the SEM.



**Figure 3-8** Critical Point Drier (BAL-TEC-CPD 030)

# 3.4 Statistical method for comparison of the species richness at the two locations

In order to compare the species diversity at Huk and Nesset, Ugland (Ugland et al., unpublished) modified a statistical test which he has developed in cooperation with some other marine botanists in a different study. Having performed a survey on two different sites, each species will contribute to one of the following four categories:

 $s_{10}$  = Number of species detected on only the first site (Huk).

 $s_{02}$  = Number of species detected on only the second site (Nesset).

 $s_{12}$  = Number of species detected on both sites.

 $s_{00}$  = Number of species detected in none of the sites.

The total number of observed species is the sum: 
$$s_{obs} = s_{10} + s_{02} + s_{12}$$
 (1)

Since we have no information on the number of species which has not been observed ( $s_{00}$ ), we must restrict the analysis to the conditional probability of the observed composition given the total number of observed species. In order to interpret the data in a probability model we need the fundamental parameter p: the average probability of detecting a species. Since the probability of not observing a given species is  $(1-p)^2$ , the data set  $\{s10, s02, s12\}$  is the result of the following probabilities:

$$p_{I} = \frac{p(1-p)}{1-(1-p)^{2}} = \frac{1-p}{2-p} =$$
(2)

- = Probability that an observed species was detected on only the first site (or only at the first of the two times)
- = Probability that an observed species was detected on only the second site (or only at the second of the two times).

$$p_{II} = \frac{p^2}{1 - (1 - p)^2} = \frac{p}{2 - p} =$$
(3)

= Probability that an observed species was detected on both sites (or at both of the two times).

 $2p_I$  is the probability that a species is only seen at one of the sites (at one of the times) and  $2p_I$  + $p_{II} = 1$ . Thus, given an observed species number of  $n = sobs = s_{10} + s_{02} + s_{12}$ , the number of species detected on only one of the sites (or only at one of the two times) has the conditional probability:

$$P(s_{10} + s_{02} = k \mid s_{obs} = n) = \binom{n}{k} (2p_I)^k p_{II}^{n-k}$$
(4)

Our purpose is to establish a test of the null hypothesis that the two sites (at the same time) contain the same species. If there are many species which are observed at only one of the sites (or at only one of the times) there are reasons to believe that the species composition at the two sites (times) are different. Define  $k_{crit}$  as the smallest integer such that:

$$P(s_{10} + s_{02} \ge k_{crit} \mid s_{obs} = n) < 0.05$$
(5)

We may therefore reject the null hypothesis at the 5% significance level and accept the alternative that the assemblages at the two sites (times) contain different species if the number of species detected on only one of the sites (one of the times) is greater or equal than kcrit. Unfortunately it is extremely difficult to estimate the average probability of detecting a species (p) when there is no information about the species that was not observed in the investigation. We shall therefore find the minimum value of the parameter p which induces rejection of the null hypothesis. It is then necessary to calculate the critical value kcrit for several alternatives of reasonable values for the parameter p. In order to have an estimate the average probability of species detection it was necessary to use additional data which may provide information on the value of the unknown parameter p. A useful data set would be a previous investigation on the

same site in order to measure the recurrence of the species. In this study we used data from Zubizarreta (2005) work. We then used an analogous model with the same parameters:

 $s_{10}$  = Number of species detected in only the first investigation (our work).

 $s_{02}$  = Number of species detected in only the second investigation (Zubizarreta work).

 $s_{12}$  = Number of species detected in both investigations.

 $s_{00}$  = Number of species detected in none of the investigations.

 $s_{obs} = s_{10} + s_{02} + s_{12}$ . = Number of observed species in both investigations

Since we have no information on the number of species which has not been observed (i.e.  $s_{00}$ ), we must restrict the analysis to the conditional probability:

P  $\{s10, s02 | sobs = s10 + s02 + s12\}$  = observed composition given the total number of observed species. Since the probability of not observing a given species is (1-p)2 the likelihood function

$$L = \left[\frac{p(1-p)}{1-(1-p)^2}\right]^{s_{10}} \left[\frac{(1-p)p}{1-(1-p)^2}\right]^{s_{02}} \left[\frac{p^2}{1-(1-p)^2}\right]^{s_{obs}-s_{10}-s_{02}} = \left[\frac{(1-p)}{2-p}\right]^{s_{10}+s_{02}} \left[\frac{p}{2-p}\right]^{s_{obs}-s_{10}-s_{02}}$$
(6)

So the log-likelihood function is

$$l(p) = (s_{10} + s_{02}) \ln(1-p) + (s_{obs} - s_{10} - s_{02}) \ln p - s_{obs} \ln(2-p).$$
(7)

and the maximum likelihood estimator of p is therefore the solution of the equation

$$\frac{dl(p)}{dp} = -\frac{s_{10} + s_{02}}{1 - p} + \frac{s_{obs} - s_{10} - s_{02}}{p} + \frac{s_{Obs}}{2 - p} = 0$$
(8)

$$\hat{p} = \frac{2s_{12}}{s_{obs} + s_{12}}$$

In order to derive a confidence interval for this estimator we notice that the conditional sum of species observed at only one of the investigations has a binomial distribution:

$$\binom{s_{obs}}{s_{10} + s_{02}} \left[ \frac{2p(1-p)}{1 - (1-p)^2} \right]^{s_{10} + s_{02}} \left[ \frac{p^2}{1 - (1-p)^2} \right]^{s_{obs} - s_{10} - s_{02}} = \binom{s_{obs}}{s_{10} + s_{02}} P^{s_{10} + s_{02}} (1-P)^{s_{obs} - s_{10} - s_{02}}$$

$$(9)$$

$$P = \frac{2(1-p)}{2-p}$$

From elementary probability theory it is known that the estimate and variance of the binomial probability P are given as:

$$\hat{P} = \frac{s_{10} + s_{02}}{s_{obs}}$$
 &  $sd(\hat{P}) = \frac{\hat{P}(1 - \hat{P})}{s_{obs}}$ 

so the 95% confidence interval for P is:

$$\hat{P}_{low} = \hat{P} - 2 \Box sd(\hat{P}) \qquad \& \qquad \hat{P}_{high} = \hat{P} + 2 \Box sd(\hat{P})$$

From the expression for P we get the estimator for the average detection probability:

$$\hat{p} = \frac{2 - 2\hat{P}}{2 - \hat{P}} = 1 - \frac{\hat{P}}{2 - \hat{P}}$$

The lower and upper 95% confidence limits for the average detection probably are finally obtained from the equations:

$$\hat{p}_{low} = 1 - \frac{\hat{P}_{high}}{2 - \hat{P}_{high}}$$
 &  $\hat{p}_{high} = 1 - \frac{\hat{P}_{low}}{2 - \hat{P}_{low}}$ 

This range of p was determined in the statistical assessments described above and used for the quantitative comparison between Huk and Nesset species diversity. Details are presented in Appendix B.

#### 4 RESULTS

Field sampling was started in August 2014 and ended in June 2015 as listed in Table 4-1.

**Table 4-1** Collected samples months

Month	Month order
August 2014	1
September 2014	2
October 2014	3
November 2014	4
December 2014	5
Feb. 2015	6
March 2015	7
April 2015	8
June 2015	9

The species (taxa) encountered are presented below followed by a description of the species:

#### Dinoflagellates

```
Class Dinophyceae Fritsch 1929
       Order Gymnodiniales Lemmermann 1910
               Genus Gymnodinium Stein 1883
                       Gymnodinium variabile Herdeman 1924
               Genus Amphidinium Claparede and Lachmann 1859
                      Amphidinium herdmanii Kosfoid and Swezy 1921
                      Amphidinium incoloratum P.H Campell 1973
                      \stackrel{\cdot}{Amphidinium} pellucidum Kosfoid and Swezy 1921
                      Amphidinium ovum E.C Herdman 1924
                      Amphidinium cf. trulla Jørgensen and Murray 2004
               Genus Togula Flø Jørgensen, Murray and Daugbjerg 2004
                       Togula britannica (E.C Herdmann) Flø Jørgensen, Murray and Daugbjerg
                       Togula jolla Flø Jørgensen, Murray et Daugbjerg 2004
               Genus Katodinium Fott 1957
                       Katodinium cf. Fungiforme Anissimova 1926
               Genus Chilodinium Massart 1920
                       Chilodinium cruciatum Massart 1920
       Order Suessiales
               Genus Biecheleria (Horiguchi and Pienaar) Mosetrup 2009
```

Biecheleria sp

#### Cryptomonads

Class Goniomonadeae Cavalier-Smith1993

Order Goniomonadales Novariono&Lucas1993

Genus Goniomonas Stein 1878

Goniomonas amphinema J. Larsen and Patterson 1990

Goniomonas pacifica Larsen and Patterson 1990

Class Cryptophyceae Fritsch 1927

Order Cryptomonadales Engler 1903

Genus Chroomonas Hansgirg 1885

Chroomonas diploccoca Butcher 1959

Genus Cryptomonas Ehrenberg 1832

Cryptomonas sp

Order Pyrenomenadales G. Nevavine & I.A.N. Lucas

Genus Rhodomonas Karsten 1898

#### Chlorophytes

Class Prasinephyceae T. Christensen 1926 ex Moestrup & Throndsen 1988

Order Chlorodendrales Fritsch 1917

Genus Pyramimonas Schmarda 1850

Pyramimonas sp1

Pyramimonas cf. disemata Butcher ex McFadden, Hill et Wetherbee 1986

Pyramimonas sp2

Pyramimonas sp3

Genus Nephroselmis Stein 1878

Nephroselmis rotunda (N. Carter) Ettl

Class Chlorophyceae Sensu Mattox and Stewart 1984

Order Volvocales Oltmanns 1904c

Genus Chlamydomonas Ehrenberg 1834

Chlamydomonas cf. nonpulsata Butcher 1959

Chlamydomonas sp1

#### **Euglenoids**

Class Bodonophyceae Silva 1986

Order Bodonales

Genus Bodo Ehrenberg 1832

Bodo saliens Larsen & Patterson 1990

Bodo designis Skuja 1948

Genus Rhynchomonas Klebs 1892

Rhynchomonasnasuta (Stokes, 1888) Klebs 1892

Genus *Cryptaulax* Skuja 1948

Cryptaulax cf. marina Throndsen 1969

Cryptaulax elegans Larsen Patterson 1990

Class Euglenophyceae Schoenichen 1925

Order Sphenomonadales Leedale 1967

Genus Anisonema Dujardin 1841

Anisonema cf. acinus Dujardin 1841

Anisonema prosgoebium Dujardin 1841

Genus Metanema Senn 1900

Metanema sp1

Metanema sp2

Genus Petalomonas Stein 1878

Petalomonas poosilla Larsen & Patterson 1990

Petalomonas cf. cantuscygni Cann and Pennick 1986

Petalomonas minor Larsen & Patterson 1990

P etalomonas abscissa (Dujardin 1841) Stein 1859

Genus Notosolenus Stokes 1884

Notosolenus urceolatus Larsen&Patterson 1990

Order Heteronematales Leedale 1967

Genus Dinema Perty 1852

Dinema litoralis Skuja 1939

Dinema valida Larsen & Patterson 1990

Genus Ploeoita Leedale 1969

Ploeoita pseudanisonema Larsen & Patterson 1990

Genus Urceolus Mereshkowsky 1879

Urceolus cornutus Larsen &Patterson 1990

Genus Heteronema Dujardin 1841

Peranema fusiforme Larsen 1987

Heteronema ovale Kahl 1928

Order Eutreptiales Sensu Leedale 1967

Genus Eutreptiella Cunha 1913

Eutreptiella cf. Eupharyngea Moestrup and Norris 1986

#### Haptophytes

Class Coccolithophyceae Rothmale

Order Prymnesiales Papenfuss 1955

Genus Prymnesium Massart & Conrad 1926

Prymnesium cf. nemamethecum

#### Heterokonts

Class Chrysophyceae Pascher 1914

Order Chromulinales Pascher 1914

Genus Paraphysomonas De Saedeleer 1930

Paraphysomonas sp

Class Biocosoecophyceae Fenchel & Patterson 1988

Order Biocosoecules Fenchel & Patterson 1988

Genus Cafeteria Fenchel & Patterson 1988

Cafeteria sp.

Class Dictyochophyceae(P.C Silva 1980) Silva 1982

Order Pedinellales Zimnermann Moestrup and Hallfors 1989

Genus Actinomonas Kent 1880/Pteridomonas Penard 1890

Actinomonas mirabilis

Class Raphidophyceae Chadefand ex P.C Silva 1989

Order Chattonellales Throndsen 1993

Genus *Olisthodiscus* N. Carter 1937

Olisthodiscus luteus N. Carter 1937

Olisthodiscus sp

Class Bacillariophycea Haeckel 1948

Order Naviculaes Bessey 1907

Genus Navicula Bory de Saint-Vincent 1822

Navicula sp

Order Naviculales Bessey

Genus Pleurosigma W. Smith 1852

Pleurosigma sp.

Order Mastoglolales D.G Mann

Genus Achnanthes Bory de Saint-Vincent 1822

Achnanthes sp.

Order Thalassiophyales D.G Mann

Genus Amphora F.T Kutzing 1844

Amphora sp.

Order Cocconeidales E.J Cox 2015

Genus Cocconeis Ehrenberg 1836

Cocconeis sp.

Order Rhopalodiales Ehrenberg 1845

Genus Entomoneidaceae Ehrenberg 1845

Entomoneis sp.

Class Fragilariophycea R.M. Crawford & D.G Mann, 1990

Order Fragilariales P.C Silva

Genus Ceratoneis Ehrenberg 1839

Ceratoneis closterium Ehrenberg 1839

#### Cercozoans

Class Imbricatea

Order Thaumatomonadida

Genus Protaspis Skuja 1939

Protaspis oblique Larsen and Patterson 1990

Protaspis tegere Larsen and Patterson 1990

Genus Metromonas (Griessmann 1913) Larsen and Patterson 1990

Metromonas simplex Larsen and Patterson 1990

#### Apusozoans

Class Amastigomonea

Order Amastigomonadales

Genus Amastigomonas De Saedeleer 1931

Amastigomonas mutabilis (Griessmann) Molina and Nera

#### Choanoflagellates

Class Choanoflagellatea E.Takahashi 1984

Order Acanthoecida Cavalier Smith 1997

Genus Acanthocorbis Hara and Takahashi 1984

Acanthocorbis sp.

#### Cyanobacteria

Class Cyanophyceae

Order Chroococcales Hansgirg 1892

Genus Chroococcus

Chroococcus sp

Genus Merismopedia Meyen 1839

Merismopedia sp

Genus Microcrocis P.G Richt 1892

Microcrocissabulicola (lagerh) Geitler 1942

Order: Oscillatoriales Vaucher ex Comont 1892

Genus Oscillatoria Vaucher ex Comont 1892

Oscillatoria sp

Genus Lyngbya Agardh ex Comont 1892

Lyngobya sp

Order Spirulimales Komoarek, Kastorstky, Mars and Johansen 2014

Genus Spirulina P.J.F Turpin ex M. Comont 1892

Spirulina subsalsa cf. versicolerccohn (Cohn ex Comont) Koster 1892

Order Synechococcales

Genus Pseudoanabaena Lauterborn 1915

Pseudoanabaena sp.

Order Thiotrichales

Genus Beggiatoa

Beggiatoa sp.

# 4.1 Results from light microscopy

The species (taxon) identifications and comments regarding the observation are presented in the following section.

**Dinoflagellates** 

**Class Dinophyceae** Fritsch 1929

**Order Gymnodiniales** Lemmermann 1910

Genus Gymnodinium Stein 1883

Gymnodinium variabile Herdman 1924

Figures are located in: Plate 4-1\_ a and Figure 4-1\_a

**<u>Description:</u>** Oval to more rounded cells with a dome shaped apex and dorsoventrally flattened.

A nucleus was observed in the cell center. The epicone and hypocone were of similar sizes, the

epicone a little bigger. The cingulum was seen slightly below the cell center. This species had a

yellow-brown chloroplast.

Size: 20-30μm long, 12-20μm wide

**Observation:** It was observed at Nesset in months 1 and 9 in low abundances. This species was

not observed in Huk.

Comments: The species swimming mode was mainly rotational, anticlockwise and on a circle

path. This observation is consistent with description of Daughjerg et al. (2000). Due to having a

high movement speed, identification of this species was challenging.

**Genus** *Amphidinium* Claparede and Lachmann 1859

Amphidinium herdmanii Kofoid and Swezy 1921

Figures are located in: Plate 4-1\_ b and Figure 4-1\_b, Figure 4-14\_a (SEM)

**Description:** Oval or quadrat-angle cells with a large triangle epicone, approximately 9 μm. The

epicone was much smaller than the hypocone. This species was asymmetrical around the cell

axis and being deflected towards the left. A Y-shape furrow was observed on the ventral side as

also reported by Hoppenrath (2014). The chloroplast was yellow-brown.

Size: 25-35μm long, 16-20μm wide

**Observation:** It was observed at Nesset in months 1, 2 and 9 in low abundances. At Huk it was

observed in months 1 and 8 in low abundances.

**Comments:** Due to having very slow movement, identification of this species was not very

difficult, except that this species might be confused with Amphidinium operculatum. Our

observation was in a good agreement with the species definition by Kofoid and Swezy (1921).

Amphidinium incoloratum P.H Campell 1973

Figures are located in: Plate 4-1\_ c and Figure 4-1\_c

**<u>Description:</u>** Symmetrical oval to ovoid shape. The cell was slightly straight at the left side

while tended to be convex at the right side. Sulcus was observed at the cell center, deflected a

bit to the left side. No chloroplast was visible.

**Size:** 22-32μm long, 18-20μm wide

**Observation:** It was observed at Nesset in months 2, 8 and 9 in low abundances. This species

was not observed in Huk.

**Comments:** The observed features of this species were more or less similar to the one described

by Campell (1973). This species was easily identified due to its specific non-symmetrical shape

and absence chloroplast.

*Amphidinium pellucidum* Kofoid and Swezy 1921

Figures are located in: Plate 4-1\_d and Figure 4-1\_d

**<u>Description:</u>** Oval shape, with a semi-circular hypocone and domed shaped epicone. Hypocone

was approximately three times larger than the epicone. The sulcus was narrow and originated

from the epicone. Nucleus was observed above the hypocone, about to 1/3 of the cell length

from the apex. The species had an orange-brown chloroplast.

**Size:** 28-35µm long, 22-28µm wide

**Observation:** It was observed at Nesset in months 1, 2 and 9 in low abundances. At Huk, this

species was only observed in month 9 in low abundance.

**Comments:** The observed species characteristics and structure were very similar, *Gymnodinium* 

myriopyrenoides, except that our observed species was substantially smaller in size compared to

Gymnodinium myriopyrenoides.

<u>Amphidinium ovum E.C Herdman 1924</u>

Figures are located in: Plate 4-1\_ e and Figure 4-1\_e

**<u>Description:</u>** Oval shape. Epicone was smaller than hypocone, also deflected slightly to the left.

The cell was asymmetrical. Sulcus spread from the left edge to the right side and

crossed/approached to the antapex. A brown-green chloroplast was also observed.

Size: 22-33μm long, 18-20μm wide

**Observation:** It was observed at Huk in month 1 in medium abundance. This species was not

observed in Nesset.

**Comments:** The epicone deflection to the left side and was very obvious, which also had been

commented by Herdman (1924). In his species description, nucleus was reported in the lower

part of the cell. Due to its rapid movement it was not possible to find the position of nucleus.

**Genus** *Amphidinium* Claparede and Lachmann 1859

Amphidinium cf. trulla Jørgensen and Murray 2004

Figures are located in: Plate 4-2\_f and Figure 4-1\_f

**Description:** Oval shape. The epicone was approximately 10 times smaller than the hypocone,

deflected a bit to the left side. The cingulum was started just below the apex. A yellow- brown

chloroplast was also observed in the cell center.

**Size:** 18-25µm long, 10-15µm wide

**Observation:** It was observed at Nesset in months 8 and 9 in medium abundances. This species

was not observed in Huk.

**Genus** *Chilodinium* Massart 1920

Chilodinium cruciatum Massart 1920

Figures are located in: Plate 4-2\_g and Figure 4-1\_g

**Description:** Oval shape. The epicone was around 1/3 cell length long. A long flagellum

coming out from the cell center was observed. The species had a longitudinal furrow which was

extended to the apex. In addition, the cell was naked and had not any specific colour. An eyespot was observed at the hypocone.

Size: 20-28μm long, 15-17μm wide

**Observation:** It was observed at Nesset in month 1 in medium abundance. This species was not observed in Huk.

<u>Comments:</u> One of the main/obvious species features was its eyespot in the hypocone, which also has been reported by Throndsen and Eikrem (2010).

**Genus** *Togula* Flø Jørgensen, Murray and Daugbjerg 2004

*Togula britannica* (E.C Herdmann) Flø Jørgensen, Murray and Daugbjerg 2004 Figures are located in: Plate 4-2\_h and Figure 4-1\_h

**<u>Description:</u>** Oval shape, dorsoventrally flattened. The cingulum descended in a way that the cells appeared asymmetrical. Its sulcus was slightly deflected to the right side of the cell. Nucleus was observed in the cell center. This species had many yellow-brown chloroplasts.

Size: 28-40μm long, 20-25μm wide

<u>Observation:</u> It was observed at Nesset in months 3, 4, 8 and 9 in medium abundances. This species was not observed in Huk.

<u>Comments:</u> The species movement was slow and with respect to the cell shape was very similar to *Togula jolla* Flø Jørgensen, Murray and Daugbjerg 2004.

# *Togula jolla* Flø Jørgensen, Murray et Daugbjerg 2004

Figures are located in: Plate 4-2\_i and Figure 4-1\_i1,2

**<u>Description:</u>** Oval shape, dorsoventrally flattened. The cingulum descended very much. The sulcus was slightly deflected to the right side of the cell. Nucleus was observed in the cell center.

Size: 26-37μm long, 25-30μm wide

**Observation:** It was observed at Nesset in month 3 in high abundance and in month 8 in low abundance. At Huk, it was only observed in month 9 in low abundance.

**Comments:** The species was very similar to *Togula britannica* (as discussed above) except its

small size.

**Genus** *Katodinium* Fott 1957

*Katodinium* cf. *fungiforme* Anissimova 1926

Figures are located in: Plate 4-2\_j and Figure 4-1\_j

**Description:** Ovoid shape, dorsoventrally flattened, no chloroplast. The hypocone was smaller

than epicone. Its epicone was very rounded. The cingulum descended about one cingulum width.

Two unequal flagella originating from the anterior surface were observed. The anterior

flagellum was about 0.5 cell lengths long while the posterior one was about 1.5 cell lengths.

Size: 10-12μm long, 8-10μm wide

**Observation:** It was observed at Nesset in months 8 and 9 in low abundances. This species was

not observed in Huk.

**Comment:** The species was similar in general to *Katodinium asymmetricum* Massart 1920. The

epicone outline of the species was broken by a small incision, slightly to the left from the mid

ventral line. This helped to differentiate between these two species.

**Order Suessiales** Fensome, Taylor, Norris, Sarjeant, Wharton and

Williams 1993

Genus Biecheleria Mosetrup, Lindberg and Daugberg 2009

*Biecheleria* sp.

Figures are located in: Plate 4-2\_k and Figure 4-1\_k

**Description:** Semi-circular cell. The epicone and hypocone sizes were the same. Its sulcus

reached to the antapex. A greenish chloroplast was observed at outer part of the cell. An orange

eyespot was present in the upper part of hypocone.

Size: 18-20µm long, 15µm wide

Observation: It was observed at Nesset in month 3 in low abundance. This taxon was not

observed in Huk.

**Comments:** An orange eyespot was the distinguishing characteristics of this taxon.

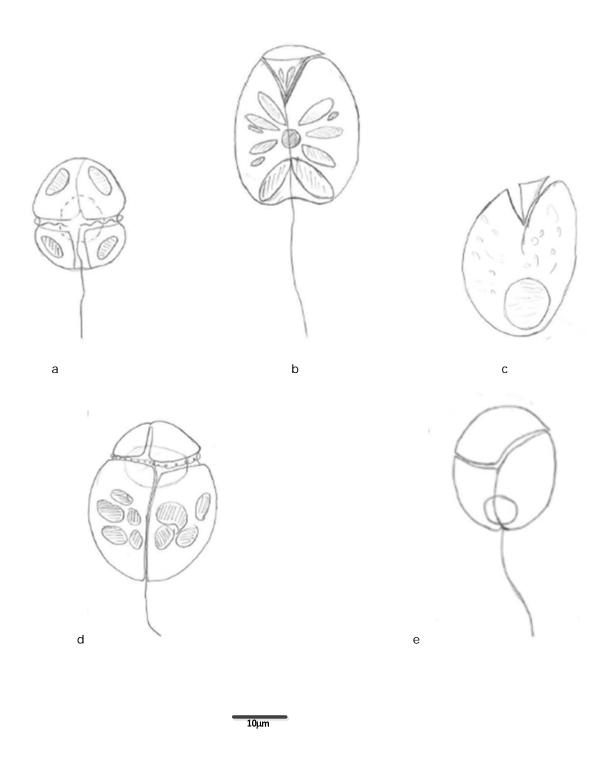
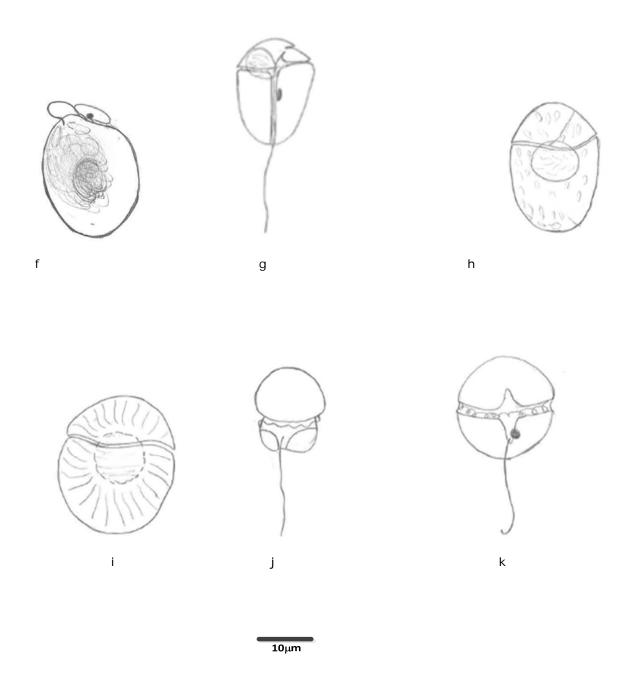


Plate 4-1 Gymnodinium, Amphidinium, ventral view except e

a) Gymnodinium variabile, b) Amphidinium herdmanii, c) Amphidinium incoloratum, d) Amphidinium pellucidum, e) Amphidinium ovum



**Plate 4-2** *Amphidinum, Chilodinium, Togula, Katodinium, Biecheleria*, ventral view except i f) *Amphidinium* cf. *trulla*, g) *Chilodinium cruciatum*, h) *Togula britannica*, i) *Togula jolla*, j) *Katodinium* cf. *fungiforme*, k) *Biecheleria* sp.

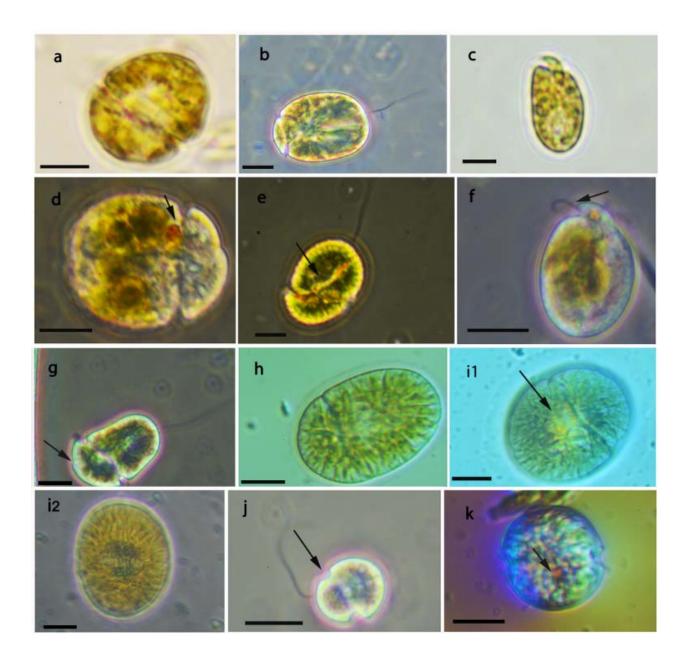


Figure 4-1 Gymnodium, Amphidinium, Chilodinium, Togula, Katodinium, Biecheleria - All figures are taken in the phase contrast.

a) Gymnodinium variabile, b) Amphidinium herdmanii, c) Amphidinium incoloratum, d) Amphidinium pellucidum, eyespot (arrow), e) Amphidinium ovum, ventral view (arrow), f) Amphidinium cf. trulla, transverse flagellum (arrow), g) Chilodinium cruciatum, apical furrow(arrow), h) Togula britannica, i) Togula jolla, i1: nucleus in the cell center (arrow), j) Katodinium cf. fungiforme, dorsal view(arrow), k) Biecheleria sp., eyespot(arrow)

Cryptomonads

**Class Goniomonadeae** Cavalier-Smith 1993

**Order Goniomonadales** Novariono & Lucas 1993

**Genus** *Goniomonas* Stein 1878

Goniomonas amphinema J. Larsen and Patterson 1990

Figures are located in: Plate 4-3\_a and Figure 4-2\_a1,2, Figure 4-14\_c (SEM)

**<u>Description:</u>** Oval shape with two unequal flagella; one directed anteriorly, other one

posteriorly. The longer flagellum was longer than the cell length. There was a transverse band of

ejectisomes near the anterior end. During swimming, the small flagellum was seen in the

straight position while the longer one was trailed behind. This species was heterotrophic and

with no chloroplast.

Size: 6-9µm long, 4-6µm wide

**Observation:** It was observed at Nesset in month 3 in low abundance. At Huk the species was

observed in month 1 in high abundance and in months 7 and 9 in medium abundances.

**Comments:** The species was easily identified due to its clear ejectisomes, and no chloroplast.

With respect to overall specifications, the species was consistent with the one described by

Larsen and Patterson (1990). Our observation under SEM also re-confirmed the identification

(See section 4.2).

Goniomonas pacifica Larsen and Patterson 1990

Figures are located in: Plate 4-3\_b and Figure 4-2\_b

**<u>Description:</u>** Colourless rounded cell. Since it was a heterotrophic species, there was no

chloroplast. The species had two equal flagella coming out from the upper side of the cell. It was

observed that, the flagella bends close to the dorsal surface during swimming, while the other

one bends toward the ventral side.

Size: 9-11µm long, 6-8µm wide

**Observation:** It was observed at Nesset in month 2 in low abundance. At Huk the species was

observed in month 7 in low abundance.

**Comments:** This species was very similar to *Goniomonas amphinema* as described above.

Larsen and Patterson (1990) have mentioned that Goniomonas pacifica and Goniomonas

amphinema could be differentiate by their size and the flagella length.

Class Cryptophyceae Fritsch 1927

**Order Cryptomonadales** Engler 1903

**Genus** *Chroomonas* Hansgirg 1885

Chroomonas diploccoca Butcher 1959

Figures are located in: Plate 4-3\_c and Figure 4-2\_c1,2,3, Figure 4-14\_d,e,f (SEM)

**Description:** Elliptical shape, with a blue-green colour chloroplast. A bar-shape gullet was

observed in the anterior part of the cell where the species' two flagella emerging from upper part

of the gullet. These two flagella were short, but approximately equal in length. In both sides of

the gullet, two rows of ejectisomes were observed. At lower part of the cell, a two-rings shape

was very obvious.

Size: 8-12μm long, 5-8μm wide

**Observation:** It was observed at Nesset in months 1 and 2 in high abundances, months 3 and 7

in medium abundances and months 8 and 9 in high abundances. At Huk the species was

observed in months 1, 6 and 7 in high abundances and in months 8 and 9 in low abundances.

**Comments:** Our observation showed a presence of very many plate on the outer surface of the

cell (the priplast) which was very similar to the species description by Throndsen and Eikrem

(2010) and Butcher (1952, 1967). We had a detailed study of this species under the SEM. Our

observation under SEM re-confirmed the species identification (See section 4.2).

**Genus** *Cryptomonas* Ehrenberg 1832

Cryptomonas sp.

Figures are located in: Plate 4-3\_d and Figure 4-2\_d1,2, Figure 4-14\_g (SEM)

**<u>Description:</u>** Ovoid shape, with a yellow-brown coloured chloroplast, green colour sometimes.

The species had two flagella (almost equal) emerging from a furrow. At least two rows of

ejectisomes were observed along this furrow.

Size: 25-50μm long, 12-20μm wide

**Observation:** It was observed at Nesset in months 1, 2, 4 and 7 in medium abundances, in month 8 in low abundance and in month 9 in high abundance. At Huk the taxon was only observed in month 9 in low abundance.

<u>Comments:</u> This taxon is often found in fresh water. It was found at Nesset where there is a creek entering to the sea. This taxon had been also reported by Zubizarreta (2005) in an area with fresh water flow.

## **Order Pyrenomenadales** Nevavine and Lucas

Genus Rhodomonas Karsten 1898

#### Rhodomonas baltica Karsten 1898

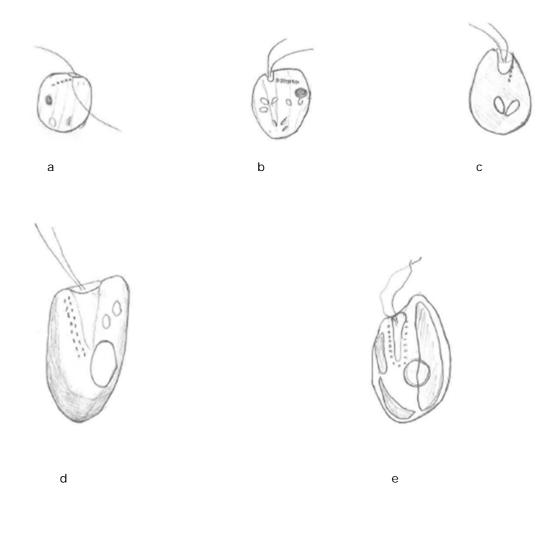
Figures are located in: Plate 4-3\_e and Figure 4-2\_e, Figure 4-14\_h (SEM)

**<u>Description:</u>** Ovoid shape, with a red-brown colored chloroplast. The species had two flagella coming out from a gullet. A short furrow extending posteriorly and two rows of ejectisomes around the gullet were observed. Nucleus was seen at the cell center, slightly to the right side.

**Size:** 13-25µm long, 11-14µm wide

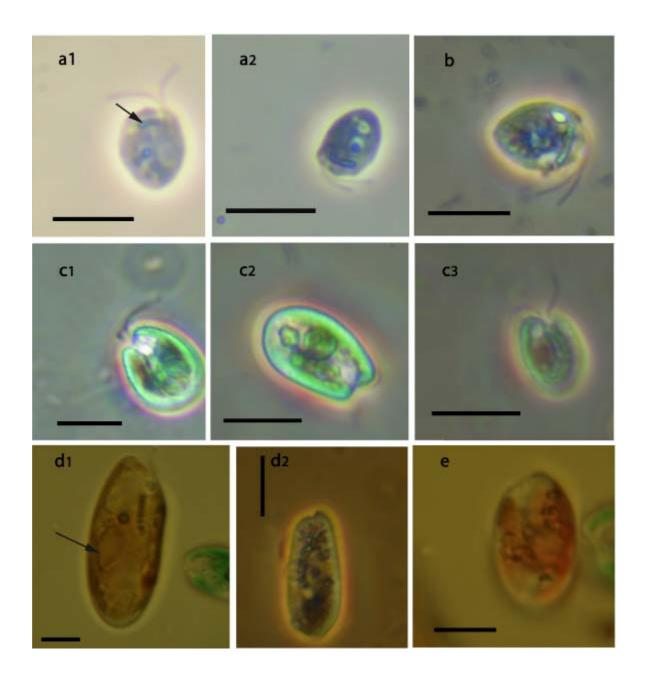
**Observation:** It was observed at Nesset in months 1 and 9 in low abundances. At Huk the species was observed in month 9 in low abundance.

<u>Comments:</u> This species was easily identified under the LM observations due to its colour. Our observation under SEM also re-confirmed the species identification (See section 4.2).



10µm

**Plate 4-3** *Rhodomonas, Chroomonas, Cryptomonas, Goniomonas*, ventral view a) *Goniomonas amphinema*, b) *Goniomonas pacifica*, c) *Chroomonas diploccoca*, d) *Cryptomonas* sp., e) *Rhodomonas baltica* 



 $\textbf{Figure 4-2} \ \textit{Rhodomonas}, \ \textit{Chroomonas}, \ \textit{Cryptomonas}, \ \textit{Goniomonas-} \ \textbf{All figures are taken in the phase contrast}.$ 

a) Goniomonas amphinema, ejectisomes (arrow), b) Goniomonas pacifica, c) Chroomonas diploccoca, d) Cryptomonas sp., d1: pyrenoid (arrow), e) Rhodomonas baltica

Chlorophytes

Class Prasinephyceae T. Christensen 1926 ex Moestrup & Throndsen

1988

**Order Chlorodendrales Fritsch 1917** 

**Genus** *Pyramimonas* Schmarda 1850

*Pyramimonas* sp1

Figures are located in: Plate 4-4\_a and Figure 4-3\_a

**Description:** Flat heart shape with two green chloroplasts, where the pyramid side was located

at the posterior section. Four long flagella were observed. A big double eyespot was obvious in

the cell center.

Size: 13-15μm long, 10-12μm wide

Observation: It was observed at Huk in month 5 in low abundance. This taxon was not

observed in Nesset.

**Comments:** The main taxon specification was a wide width at the upper side of the cell. Also

the eyespot helped for the genus identification, but still the species was not identified.

*Pyramimonas* cf. *disomata* Butcher ex McFadden, Hill et Wetherbee 1986

Figures are located in: Plate 4-4\_b and Figure 4-3\_b

**Description:** Pyramidal cell with square to round in the transverse section. Four flagella coming

out from the anterior part and emerging from a deep/and narrow hole were observed. A U-shape

green chloroplast was visible. One or two eyespots inside the plastid were one of the species

features.

Size: 5-10μm long, 3-6μm wide

**Observation:** It was observed at Nesset in months 2, 8 and 9 in high abundances. This species

was not observed in Huk.

Comments: Due to very fast and jumping type of species movements, it was very difficult to

take photo of this species.

*Pyramimonas* sp2

Figures are located in: Plate 4-4\_c and Figure 4-3\_c

**<u>Description:</u>** Rounded pyramid cell with four flagella emerging from the anterior and pointing

to two sides of the cell. The taxon had a green chloroplast. Pyrenoid was visible in the posterior

part. A double eyespot was observed in the cell center.

Size: 14-18μm long, 7-9μm wide

**Observation:** It was observed at Nesset in months 7 and 8 in low abundances. At Huk the taxon

was only observed in month 7 in low abundance.

Comments: The cell length of this species was slightly bigger than the other observed

Pyramimonas e.g P. disomata. In addition the movement was also slightly slower than the others.

*Pyramimonas* sp3

Figures are located in: Figure 4-3 d

**<u>Description:</u>** The cell shape was not evident since we looked at this species from top view.

From that view, the species appeared as a spider with 8 flagella. The swimming mode was

mainly jumping and gliding.

Size: 7-10µm long, 7-10µm wide.

**Observation:** At Nesset the species was observed in months 1 and 2 in high abundances and

months 8 and 9 in low abundances. This taxon was not observed in Huk.

**Genus** *Nephroselmis* Stein 1878

Nephroselmis rotunda (N. Carter) Ettl

Figures are located in: Plate 4-5\_d and Figure 4-3\_e, Figure 4-15\_c,d,e(SEM)

**Description:** A bean shape with a green chloroplast and a pyrenoid inside that. Two flagella

were observed, one small and sleeping on the cell side, and the other one was longer and

tangential to the anterior. A red eyespot was found in the cell center.

Size: 5µm long, 3µm wide

**Observation:** It was observed at Nesset in months 1, 2, 8 and 9 in high abundances. This

species was not observed in Huk.

**Comments:** The species was very similar to *Nephroselmis* cf. pyriformis (Carter 1937) and it

was almost impossible to differentiate between these two taxa. This species was investigated

under SEM and it was observed that the outer surface has been covered by very many single star

shaped scaled which are characteristic of N. rotunda, the same as reported by Abildhauge

(1992).

**Genus** *Tetraselmis* Stein 1878

Tetraselmis sp.

Figures are located in: Plate 4-5\_e and Figure 4-3\_f1,2

**Description:** Oval cell with a deep and narrow pit at the anterior part. Four flagella emerging

from that pit and pointing towards the posterior were observed. Pyrenoid was found in the

posterior part of the cell. The cell had a light green chloroplast.

Size: 16-20μm long, 13-15μm wide

**Observation:** It was observed at Nesset in month 1 in high abundance. This taxon was not

observed in Huk.

**Comments:** The species was similar to the one described in Throndsen and Eikrem (2010),

expect that a red-eyespot inside the chloroplast had been reported by them, however it was not

observed here.

**Class Chlorophyceae** Sensu Mattox and Stewart 1984

**Order Volvocales** Oltmanns 1904

**Genus** *Chlamydomonas* Ehrenberg 1834

Chlamydomonas cf. nonpulsata Butcher 1959

Figures are located in: Plate 4-5\_f and Figure 4-3\_g1,2

**Description:** Rounded to oval cell with a papilla at the apex. The species had two equal flagella,

shorter than the cell size, emerging from the mentioned papilla, same as described by Butcher

(1959). The cell was surrounded by a thin wall. The species had a green chloroplast and a big pyrenoid in the posterior part. A big red eyespot was observed in the plastid.

Size: 18-22μm long, 15-17μm wide

**Observation:** It was observed at Nesset in months 1, 2, 3, 4, 7, 8 and 9 in high abundances. This species was not observed in Huk.

**Comments:** The anterior papilla was very obvious in SEM.

# Chlamydomonas sp.

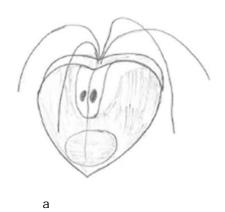
Figures are located in: Plate 4-5\_g and Figure 4-3\_h<sub>1,2,3</sub>

**Description:** Full circle cell with a thick wall. The cell surface appeared to have very many hexagonal shapes. In addition a big eyespot inside the plastid was observed. The taxon had two flagella slightly longer than the cell.

Size: 9-11µm long, 9-11µm wide

**Observation:** It was observed at Nesset in months 1, 2, 3, 7, 8 and 9 in high abundances. This taxon was not observed in Huk.

**Comments:** A thicker wall around cell helped for the genus identification.







**10**μm

# Plate 4-4 Pyramimonas

a) Pyramimonas sp1, b) Pyramimonas cf. disomata, c) Pyramimonas sp2

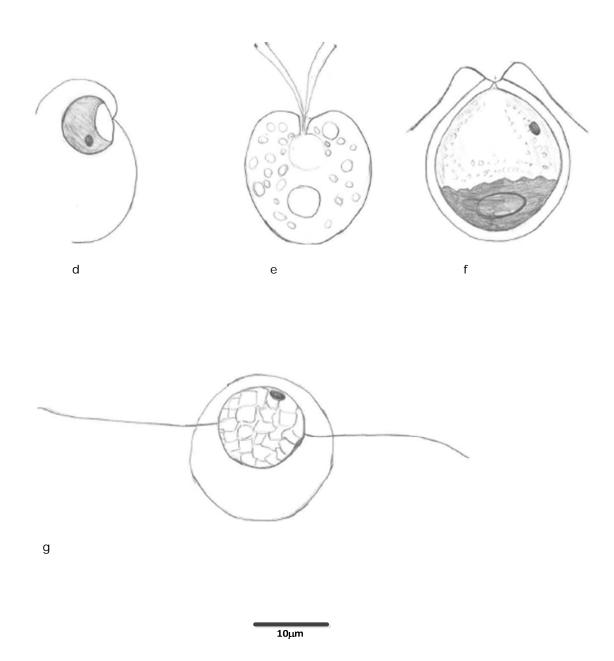


Plate 4-5 Nephroselmis, Chlamydomonas, Tetraselmis

d) Nephroselmis rotunda, e) Tetraselmis sp., f) Chlamydomonas cf. nonpulsata, g) Chlamydomonas sp.

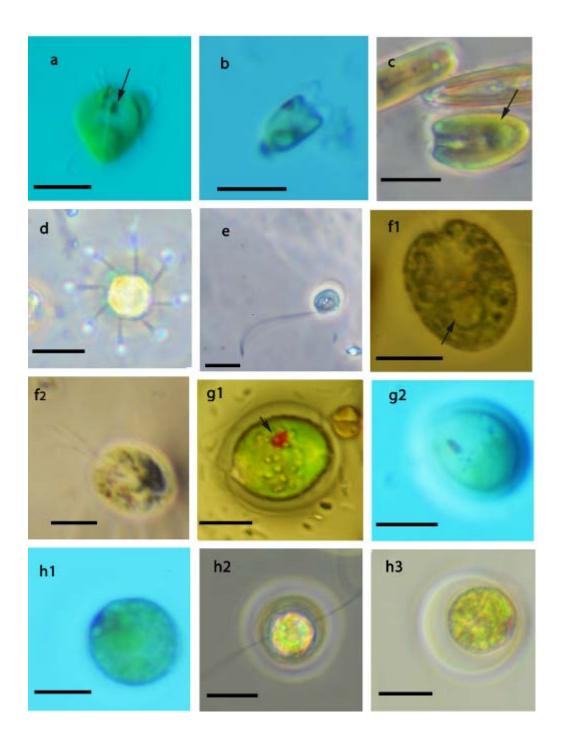


Figure 4-3 Pyramimonas, Chlamydomonas, Nephroselmis, Tetraselmis - All figures are taken in the phase contrast, except f2 and g2, where they are taken in the diffraction interference contrast.

a) *Pyramimonas* sp1, big double eyespot (arrow), b) *Pyramimonas* cf. *disomata*, c) *Pyramimonas* sp2, Pyrenoid in the posterior part (arrow), d) *Pyramimonas* sp3, (top view), e) *Nephroselmis rotunda*, f) *Tetraselmis* sp., f1:Pyrenoid (arrow), g) *Chlamydomonas* cf. *nonpulsata*, g1: a big eyespot (arrow), h) *Chlamydomonas* sp.

**Euglenoids** 

Class Bodonophyceae Silva 1986

**Order** Bodonales

**Genus** *Bodo* Ehrenberg 1832

**Bodo saliens** Larsen & Patterson 1990

Figures are located in: Plate 4-6\_a and Figure 4-4\_a

**<u>Description:</u>** Elongate ellipsoid with dark colour. The species had two unequal flagella, where

the posterior flagellum was smaller than the anterior one. Anterior flagellum had longer than cell

length. These two flagella originated from the cell groove and almost parallel cell axis. In the

swimming, anterior flagellum attached to the cover slips, hence the main force of the movement

came from the posterior flagellum.

Size: 9-12µm long, 5µm wide

**Observation:** It was observed at Nesset in months 1, 2 and 4 in high abundances and in month 9

in low abundance. At Huk, the species was observed in months 1, 5, 8 and 9 in high abundances.

**Comments:** This species was similar to *Bodo angusta* (Dujardin 1841) Butschli 1833 as also

referred by Larsen & Patterson 1990. Since this species was heterotrophic, a lot of bacteria were

observed around it.

*Bodo designis* Skuja 1948

Figures are located in: Plate 4-6\_b and Figure 4-4\_b1,2

**<u>Description:</u>** Elliptical shape with two unequal flagella. The apical part of the species was being

set off as a rostrum. Nucleus was observed at the cell center. The main modes of cell movements

were rotating, skidding and gliding.

Size: 8-11μm long, 3-5μm wide

**Observation:** It was observed at Nesset in months 1, 2, 4 and 7 in high abundances. At Huk, the

species was observed in months 1 and 5 in high abundances.

**Comments:** Since it was heterotrophic, many bacteria were observed around this species. The

main differentiate between Bodo designis and Bodo saliens was that apical part of the cell was

being set off as a rostrum in Bodo designis, which was not observed on Bodo saliens.

**Genus** *Rhynchomonas* Klebs 1892

Rhynchomonas nasuta (Stokes 1888) Klebs 1892

Figures are located in: Plate 4-6\_c and Figure 4-4\_c

**Description:** Ovoid cell with a motile snout at the lateral anterior margin of the cell. The cell

colour was dark without any chloroplast. A long flagellum was observed at the base of snout and

pointed to the posterior. This flagellum was in parallel with the cell axis. The flagellum was

about 3 cell lengths long. The cell movement was mainly gliding.

Size: 5-8μm long, 4-6μm wide

Observation: It was observed at Nesset in months 1, 2 and 3 in medium abundances and in

months 8 and 9 in low abundances. At Huk, the species was observed in month 3 in low

abundance.

**Comments:** Due to having a motile snout, it was not very difficult to identify this species. Only

a single flagellum was observed under LM, however according to Stokes (1888a,b) and Klebs

(1892), there should be a second flagellum, which we couldn't find under LM observation.

**Genus** *Cryptaulax* Skuja 1948

Cryptaulax cf. marina Throndsen 1969

Figures are located in: Plate 4-6\_d and Figure 4-4\_d

**<u>Description:</u>** Elongated oval cell with two flagella inserted in a groove near the anterior end.

The anterior flagellum was about a cell length long and usually was curve shaped. The posterior

flagellum was about two times of the cell length and twisted as a S-shape around the cell. A big

vacuole was observed close to the posterior end.

Size: 8-22μm long, 4-5μm wide

**Observation:** It was observed at Nesset in month 3 in low abundance. This species was not

observed in Huk.

Comments: A big vacuole was observed in our work, the same as reported by Throndsen and

Eikrem (2010).

Cryptaulax elegans Larsen & Patterson 1990

Figures are located in: Plate 4-6\_e and Figure 4-4\_e1,2

**Description:** Elongated elliptical cell with two flagella inserting near the anterior end of a

groove. Both flagella were pointing in the same direction.

Size: 5-8µm long

**Observation:** It was observed at Nesset in month 2 in low abundance. At Huk, the species was

observed in months 6, 7 in very low abundances.

Comments: The species was similar to Cryptaulax cf. marina except this species has no

vacuole; also the cell length was slightly shorter.

Class Euglenophyceae Schoenichen 1925

**Order Sphenomonadales** Leedale 1967

**Genus** *Anisonema* Dujardin 1841

Anisonema cf. acinus Dujardin 1841

Figures are located in: Plate 4-7\_f and Figure 4-4\_f1,2

**Description:** Elongated oval, dorsoventrally flattened. The species had a few visible pellicular

stripes on both the ventral and dorsal side. Two unequal flagella were observed; one was about

1.5 cell lengths long and the other one was thickened and a little longer. This longer flagellum

pointed towards the posterior. Both flagella originated from a ventral groove. The swimming

was mainly gliding and sliding. The species was able to change shape facilitating crawling

movements.

Size: 15-18μm long, 7-10μm wide

**Observation:** It was observed at Huk in months 6 and 7 in medium abundances. At Nesset, the

species was only observed in month 9 in low abundance.

**Comments:** Shape of this species was similar to Anisonema prosgeobium except that the

Anisonema acinus species was thinner and somewhat smaller than the other one (See next taxon

description).

**Genus** *Anisonema* Dujardin 1841

Anisonema prosgeobium Dujardin 1841

Figures are located in: Plate 4-7\_g and Figure 4-4\_g1,2

**Description:** Elongated oval dorsoventrally flattened. The species had two flagella. The

posterior flagellum was around 3 cell lengths long; the anterior approximately 1.5 cell lengths.

The species pellicula was smooth and was able to change shape facilitating crawling movements.

Size: 20-32μm long, 12-14μm wide

**Observation:** It was observed at Nesset in months 2, 3, 4 and 7 in medium abundances. At Huk

the species was observed in month 2 in medium abundance.

**Comments:** As mentioned before, the shape of this species was similar to *Anisonema acinus*. A

main difference between A. acinus and A. prosgeobium was its smooth pellicula and larger size.

**Genus** *Metanema* Senn 1900

*Metanema* sp1

Figures are located in: Plate 4-7\_h and Figure 4-5\_a1,2

**Description:** Apple shape, dorsoventrally flattened. Its pellicula had an inclination relative to

the cell axis. The taxon had two equal flagella, one pointing to the left side and the other

pointing to the right side. They were slightly longer than the cell itself. The main movement

mode was gliding.

Size: 17-22μm long, 12-15μm wide

**Observation:** It was observed at Nesset in months 2, 3 and 4 in low abundances. At Huk, the

taxon was observed in months 1 and 7 in medium abundances.

**Comments:** The cell shape and description was similar to the *Heteronema* and it was difficult to

distinguish between these two taxa.

*Metanema* sp2

Figures are located in: Plate 4-7\_i and Figure 4-5\_b1,2

**<u>Description:</u>** Apple shape, dorsoventrally flattened. Its pellicula had an inclination relative to

the cell axis. The taxon had two equal flagella, one pointing to the left side and the other

pointing to the right side. Their lengths were a little longer than the cell length. A large vacuole

was observed in the left side of the cell. The movement mode was gliding.

Size: 25-35µm long

**Observation:** It was observed at Nesset in months 2, 3, 7, 8 and 9 in high abundances. At Huk,

the taxon was observed in months 2 and 7 in low abundances.

**Comments:** The main difference between this and the previous *Metanema* sp1 was its bigger

size and its slower movement.

**Genus** *Petalomonas* Stein 1878

Petalomonas poosilla Larsen & Patterson 1990

Figures are located in: Plate 4-8\_j and Figure 4-5\_c

**Description:** Ellipsoidal shape, dorsoventrally flattened. The species had only one flagellum

emerging from the reservoir. Its length was about 1.5 cell lengths. Due to cell's dark colour, it

was almost impossible to observe the outer surface. The main movement mode was gliding.

Size: 5-6µm long, 2-3µm wide

**Observation:** It was observed at Nesset in months 1, 3, 4, 8 and 9 in high abundances. At Huk,

the species was observed in months 3, 8 and 9 in high abundances.

**Comments:** Small sizes, lack of furrow, invisible surface were the main features of this species.

*Petalomonas* cf. *cantuscygni* Cann and Pennick 1986

Figures are located in: Plate 4-8\_k and Figure 4-5\_d1,2,3

**Description:** Oval shape, pointed anterior and a semi-circle at the posterior, dorsoventrally

flattened. The species had only one flagellum emerging from a canal close to the anterior and

was approximately one cell length long. At the posterior an ingestion apparatus was observed.

Size: 10-12μm long, 7-9μm wide

**Observation:** It was observed at Nesset in months 7 and 8 in medium abundances and in low

abundance in month 9. This species was not observed in Huk.

**Comments:** The species was similar to *Notosolenus* and it was difficult to distinguish between

them.

*Petalomonas minor* Larsen & Patterson 1990

Figures are located in: Plate 4-8\_1 and Figure 4-5\_e

**<u>Description:</u>** The cell shape was close to ovate-rhomboid with a dorsal keel along the cell. Its

flagellum was approximately one cell length long.

Size: 7-9μm long, 3-5μm wide

**Observation:** It was observed at Huk in months 3 and 4 in low abundances. This species was

not observed in Nesset.

**Comments:** According to Larsen and Patterson (1990), this species is similar in shape to

Petalomonas lata Christien 1962, Petalomonas steinii Klebs 1892 and Petalomonas variabilis

Christian 1962. The main differentiating feature was the small size of this species compared to

the others.

Petalomonas abscissa (Dujardin 1841) Stein 1859

Figures are located in: Plate 4-8\_m and Figure 4-5\_f

**Description:** Triangular shape, narrow anteriorly and wider at the posterior. The species had

lateral hyaline flanges. A single flagellum emerged from a canal with the same length as cell.

Three keels, one at the right dorsal side and two on the ventral side were visible. The species

movement was mainly gliding.

**Size:** 12-15µm long, 9-11µm wide

**Observation:** It was observed at Nesset in months 8 and 9 in medium abundances. This species

was not observed in Huk.

**Comments:** Three keels, one at the right dorsal side and two on the ventral side were clearly

observed which enabled easy species identification.

**Genus** *Notosolenus* Stokes 1884

Notosolenus urceolatus Larsen & Patterson 1990

Figures are located in: Plate 4-8\_n and Figure 4-6\_a

**Description:** Pitcher shaped cell, narrow anteriorly with small neck around the flagella canal.

Two flagella were found; the anterior one was about one cell length and the posterior one was a

little smaller. Three dorsal keels and three ventral ridges were also visible.

Size: 18-20µm long, 16µm wide

Observation: It was observed at Huk in month 7 in low abundance. This species was not

observed in Nesset.

**Comments:** Due to very fast movement, it was impossible to observe the cell surface in detail.

Having three dorsal keels and three ventral ridges helped us to identify the species.

**Genus** *Dinema* Perty 1852

*Dinema litoralis* Skuja 1939

Figures are located in: Plate 4-9\_o and Figure 4-6\_b1,2,3

**<u>Description:</u>** Elongated oval cell, dorsoventrally flattened. The species pellicula striation was

longitudinal with an S-helix. Two flagella were observed; the anterior flagellum was about 1.5

cell lengths and the other one was about one cell length. Often, diatoms, as their food source,

were clearly visible within the cells. Gliding was the main movement mode and during

swimming the cell shape was changing.

**Size:** 35-50µm long, 18-30µm wide

**Observation:** It was observed at Nesset in months 7, 8 and 9 in high abundances. This species

was not observed in Huk.

**Comments:** Due to its large size and the pellicula striation, this species was easily identified.

*Dinema valida* Larsen & Patterson 1990

Figures are located in: Plate 4-9\_p and Figure 4-6\_c

**Description:** Elongated ovate, flattened cell. Pellicular striations were similar to an S-helix. The

nucleus was visible in the lower right part of the cell. The species had two flagella; the anterior

one was about the cell length and the posterior one was thicker and about two cell lengths. The

posterior flagellum looked like a hook.

Size: 25-30μm long, 10-13μm wide

**Observation:** It was observed at Nesset in months 1, 3, 4 in medium abundances and in month 8

and 9 in high abundances. At Huk, the species was observed in month 7 in low abundance.

**Comments:** This species was similar in shape to *Anisonema*. The main distinction between

them was presence of an S-helix shaped pellicula in *Dinema valida*.

**Genus** *Ploeotia* Leedale 1969

*Ploeotia pseudanisonema* Larsen & Patterson 1990

Figures are located in: Plate 4-9\_q and Figure 4-6\_d

**<u>Description:</u>** Oval shape and dorsoventrally flattened. Very many ridges were visible on the cell

surface. The nucleus was seen at the lower right of the cell. Two flagella were observed; one

was slightly longer than the cell length and the recurrent flagellum was about 3-4 cell lengths,

also thicker and located in the ventral furrow.

Size: 15-20µm long, 10µm wide

**Observation:** It was observed at Nesset in months 3, 8 and 9 in medium abundances. At Huk

the species was observed in month 3 in low abundance.

**Comments:** The main feature of this species was its very long posterior flagellum. This species

was more or less similar to Ploetia longifilum.

**Genus** *Urceolus* Mereshkowsky 1879

*Urceolus cornutus* Larsen & Patterson 1990

Figures are located in: Plate 4-9\_r and Figure 4-6\_e

**Description:** Sack shaped cell with a wide opening at the anterior collar. Pellicular striations

were closely spaced with S-helix shape. The species had only a single flagellum emerging from

inside of the anterior collar with the same length of the cell. The nucleus was found at the cell

center slightly to the posterior side. The species had the capability of changing the shape with

crawling.

Size: 40-45μm long, 10-20μm wide

Observation: It was observed at Nesset in months 7, 8 and 9 in low/medium abundances. At

Huk the species was not observed.

**Comments:** The anterior collar was the main characteristic of the species. This species was

similar to the one described by Larsen and Paterson (1990), except their reported cell size was

marginally smaller (about 40µm). Our measured size was however in agreement with the data

reported by Throndsen and Eikrem (2010), where they have found cells up to 50µm.

**Order Heteronematales** Leedale 1967

**Genus** *Peranema* Dujardin 1841

Peranema fusiforme Larsen 1987

Figures are located in: Plate 4-10\_s and Figure 4-7\_a

**<u>Description:</u>** Long sack shape cell with a sharp anterior and truncated posterior. The species had

two unequal flagella, where the anterior flagellum was thicker and longer (about two cell lengths

long) and the other one was slightly shorter. This species had a pellicula, which was very

difficult to see. The main movement was a combination of gliding and rotating, with a sharp

change of the cell shape.

**Size:** 35-40µm long, 15-20µm wide

Observation: It was observed at Nesset in months 1, 8 and 9 in medium abundance. This

species was not observed in Huk.

**Comments:** The species' main specification was its sack shape. This species was similar to

Peranema dolichonema as also mentioned by Larsen & Patterson (1990).

**Genus** *Heteronema* Dujardin 1841

*Heteronema ovale* Kahl 1928

Figures are located in: Plate 4-10\_t and Figure 4-7\_b

**<u>Description:</u>** Oval cell with a pointed posterior end, dorsoventrally flattened and no chloroplast.

Its pellicular striation consisted of many oblique S-helixes. It had two flagella, the posterior

flagellum being about twice as long as the anterior one. The main cell movement mode was

skidding.

Size: 10-19µm long

**Observation:** It was observed at Nesset in month 2, 3, 7, 8 and 9 in high abundances. At Huk,

this species was observed in months 1, 2, 3 and 4 in high abundances and in medium abundance

in month 8.

**Comments:** The main species characteristic was the pointed posterior as also reported by Kahl

(1928). However this species could be confused with *Metanema*.

**Order Eutreptiales** Sensu Leedale 1967

Genus Eutreptiella Cunha 1913

Eutreptiella cf. eupharyngea Moestrup and Norris 1986

Figures are located in: Plate 4-10\_u and Figure 4-7\_c1,2,3

**Description:** A sack shaped cell with pointed posterior. The species had two flagella originating

from a long, thin canal close to the apex. The anterior flagellum was about two times of cell

length while the other one was slightly smaller than the cell length and pointing towards the

posterior. The cells had green chloroplasts. A red eyespot was observed outside the plastid. A

large nucleus was found in the middle of the cell.

Size: 25-45µm long, varying in wide direction during movement

**Observation:** It was observed at Nesset in months 2, 7, 8 and 9 in medium abundances. At Huk, the species was observed in month 7 in low abundance.

<u>Comments:</u> The species was very active and had a flexible shape. During swimming, it looked like that its chloroplast was constantly moving inside the cell.

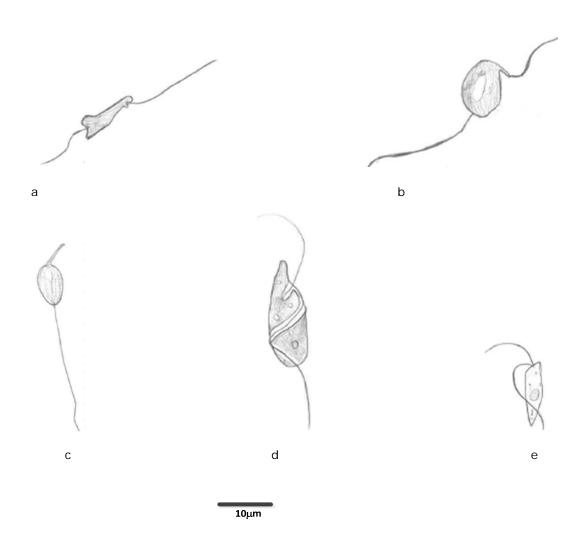


Plate 4-6 Bodo, Rhynchomonas, Cryptaulax

a) Bodo saliens, b) Bodo designis, c) Rhynchomonas nasuta, d) Cryptaulax cf. marina, e) Cryptaulax elegans

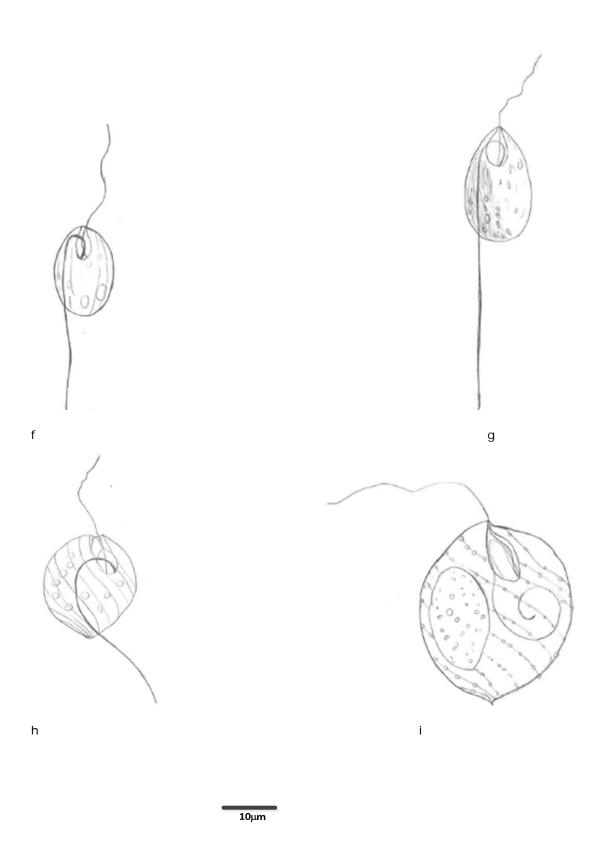


Plate 4-7 Anisonema, Metanema

f) Anisonema cf. acinus, g) Anisonema prosgeobium, h) Metanema sp1, i) Metanema sp2.

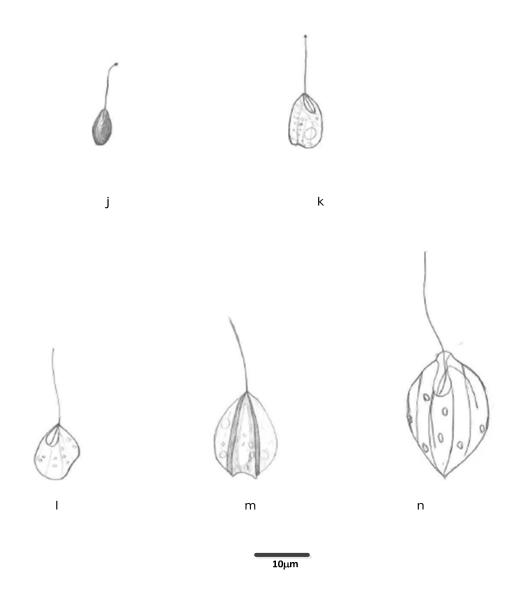


Plate 4-8 Petalomonas, Notosolenus

j) Petalomonas poosilla, k) Petalomonas cf. cantuscygni, l) Petalomonas minor, m) Petalomonas abscissa, n) Notosolenus urceolatus

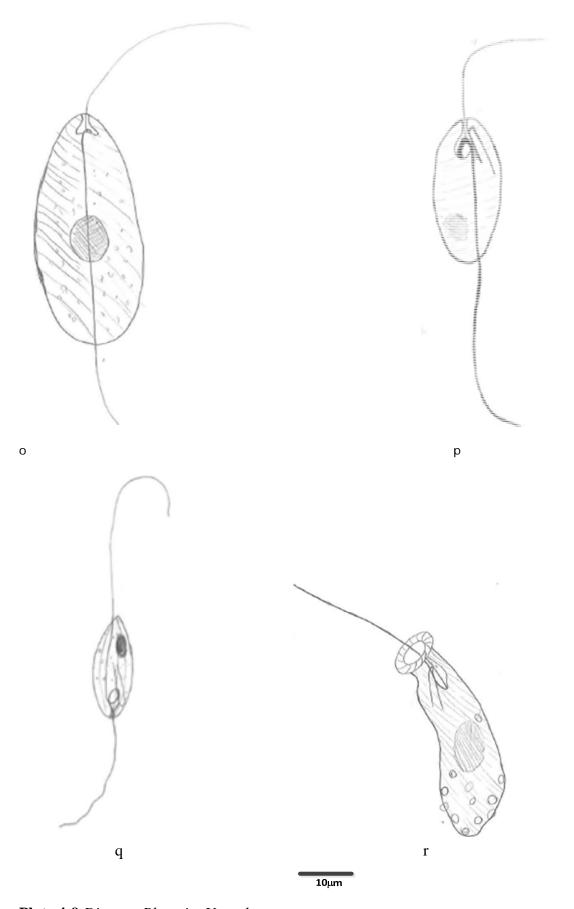


Plate 4-9 Dinema, Ploeotia, Urceolus

o) Dinema litoralis, p) Dinema valida, q) Ploeotia pseudanisonema, r) Urceolus cornutus

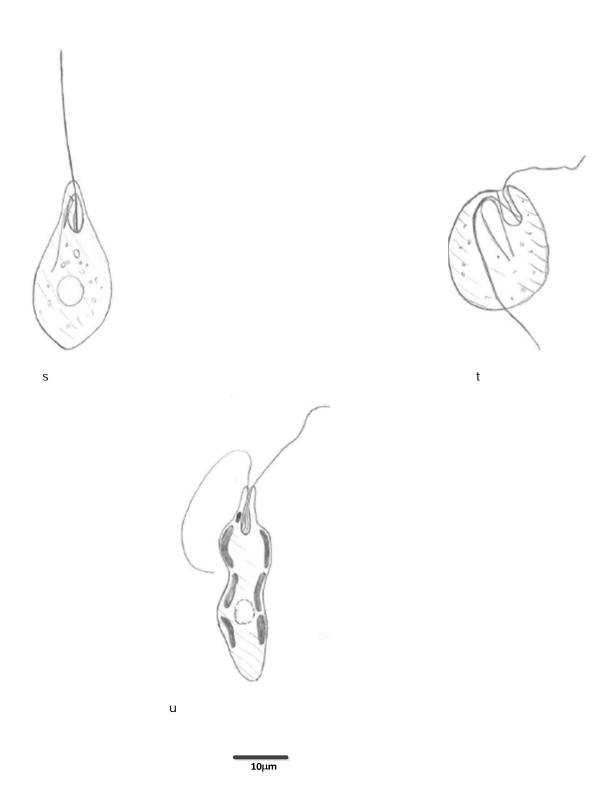


Plate 4-10 Peranema, Heteronema, Eutreptiella

s) Peranema fusiforme, t) Heteronema ovale, u) Eutreptiella eupharyngea

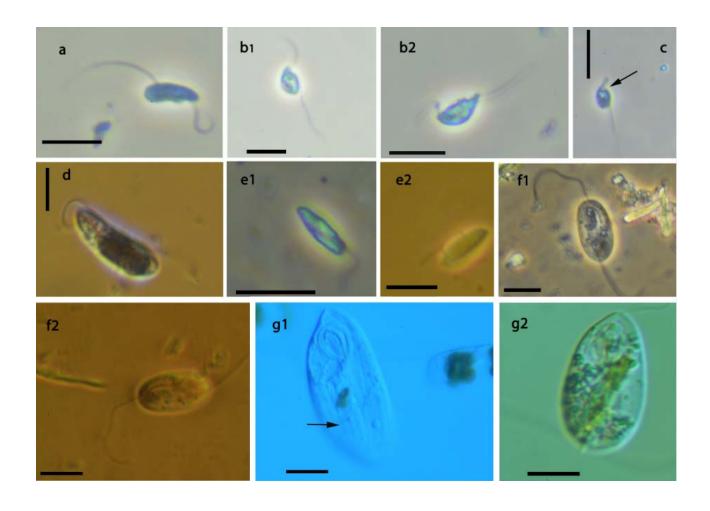


Figure 4-4 Bodo, Rhynchomonas, Cryptaulax Anisonema- All figures are taken in the phase contrast, except f2 and g1 where they are taken in the diffraction interference contrast.

a) Bodo saliens, b) Bodo designis, c) Rhynchomonas nasuta, a motile snout (arrow), d) Cryptaulax cf. marina, e) Cryptaulax elegans, f) Anisonema cf. acinus, g) Anisonema prosgeobium, g1: vertical pellicular stripes (arrow)

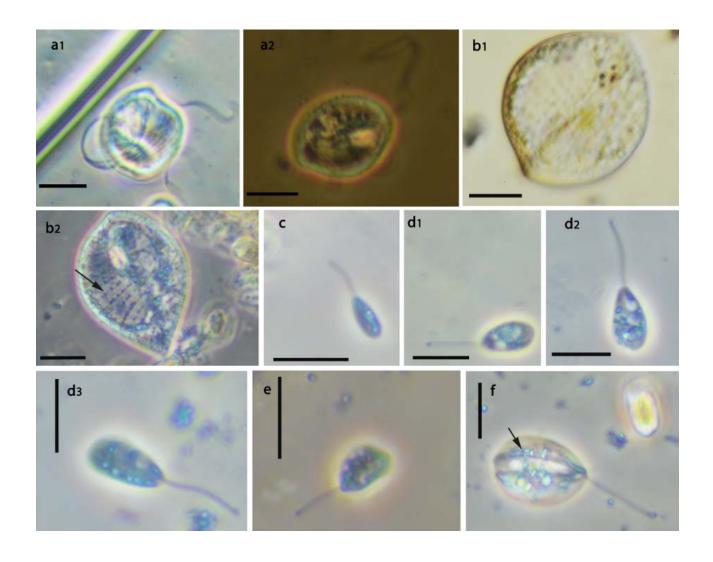


Figure 4-5 *Metanema*, *Petalomonas* - All figures are taken in the phase contrast, except b1, where it is taken in the diffraction interference contrast.

a) Metanema sp1, b) Metanema sp2, b2: a large vacuole was observed in the left side of the cell, the pellicular has an inclination relative to the cell axis (arrow), c) Petalomonas poosilla, d) Petalomonas cf. cantuscygni, e) Petalomonas minor, f) Petalomonas abscissa, hyaline flange (arrow)

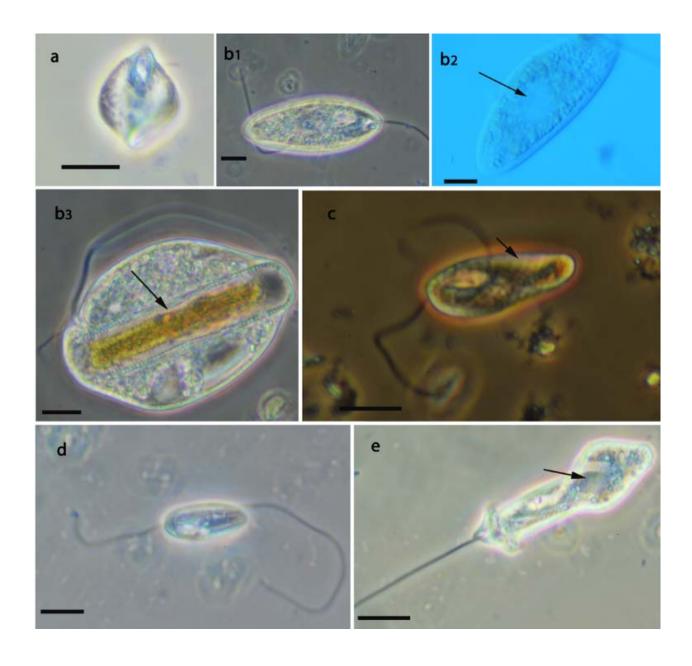
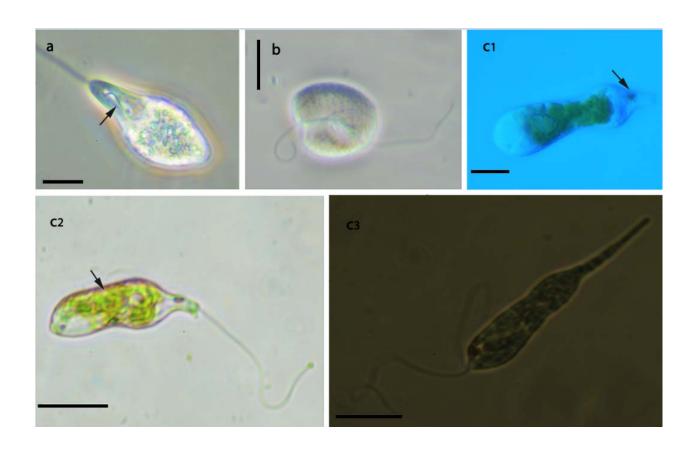


Figure 4-6 Notosolenus, Dinema, Ploeotia, Urceolus - All figures are taken in the phase contrast, except b2, where it is taken in the diffraction interference contrast.

a) *Notosolenus urceolatus*, b) *Dinema litoralis*, b2: Nucleus (arrow), b3: since this species is heterotrophic we observed that the species was eating diatom (arrow), c) *Dinema valida*, S-helix shape of Pellicular (arrow), d) *Ploeotia pseudanisonema*, e) *Urceolus cornutus*, nucleus (arrow)



 $\textbf{Figure 4-7} \ \textit{Peranema Heteronema}, \ \textit{Eutreptiella-} \ \textbf{All figures are taken in the phase contrast}, \\ \textbf{except b and c1, where it is taken in the diffraction interference contrast}. \\$ 

a) *Peranema fusiforme*, a short flagellum (arrow), b) *Heteronema ovale*, c) *Eutreptiella* cf. *eupharyngea*, c1: a red eyespot (arrow), c2: a green chloroplast (arrow)

**Haptophytes** 

**Class Coccolithophyceae Rothmaler 1951** 

**Order Prymnesiales** Papenfuss 1955

Genus Prymnesium Massart & Conrad 1926

Prymnesium cf. nemamethecum

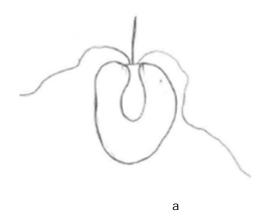
Figures are located in: Plate 4-11\_a and Figure 4-8\_a1, 2, Figure 4-18\_a,b (SEM)

Description: Egg shaped cell with two yellow-brown chloroplasts. This species had two flagella. One flagellum was slightly longer than the other one. During the swimming, these two flagella looked like the bird wings. The haptonema was observed in the center/slightly anterior to the center of the cell and was 3-5μm long. The movement direction was mainly toward the haptonema.

Size: 11-13μm long, 6-8μm wide

<u>Observation:</u> It was observed at Huk in month 1 with a high abundance and month 6 in low abundance and in medium abundances in months 7, 8 and 9. At Nesset, it was only observed in month 9 in low abundance.

**Comments:** The identifying species characteristic was the presence of a thick haptonema.



5µm

Plate 4-11 Prymnesium

a) Prymnesium cf. nemamethecum

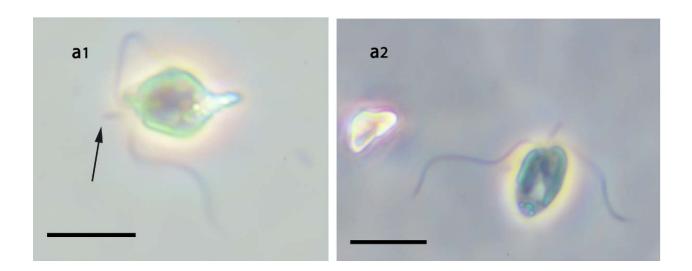


Figure 4-8 *Prymnesium*- All figures are taken in the phase contrast.

a) Prymnesium cf. nemamethecum, a1: a short haptonema (arrow)

## **Heterokonts**

Class Chrysophyceae Pascher 1914

**Order Chromulinales** Pascher 1914

**Genus** *Paraphysomonas* De Saedeleer 1930

## Paraphysomonas sp.

Figures are located in: Figure 4-18\_c (SEM)

**Description:** This taxon was not observed under LM.

Size: No data.

**Observation:** It was observed at Nesset in month 4 only by means of SEM. At Huk, the taxon was not observed.

Class Biocosoecophyceae Fenchel & Patterson 1988
Order Biocosoecules Fenchel & Patterson 1988
Genus Cafeteria Fenchel & Patterson 1988

### Cafeteria sp.

Figures are located in: Figure 4-9\_a1,2

<u>Description:</u> Circle, ovate or kidney-shaped cell with two unequal flagella. The recurrent flagellum was more or less straight. During swimming, the anterior flagellum was visible in the cell front and the posterior one trailed behind.

Size: 3-5µm long

**Observation:** It was observed at Nesset in months 1, 2, 4, 7, 8 and 9 in high abundances. At Huk, the taxon was observed in months 5, 6, 7, 8 and 9 in high abundances.

**Comment:** Due to very small size, the taxon identification was very difficult. One of the main taxon characteristic is the delicate shelf encircling the cell reported by Fenchel & Patterson (1988). We did not observe this shelf.

Class Dictyochophyceae (P.C Silva 1980) Silva 1982

**Order Pedinellales** Zimnermann Moestrup and Hallfors 1989

**Genus** Actinomonas Kent 1880/Pteridomonas Penard 1890

Actinomonas mirabilis

Figures are located in: Plate 4-12\_a and Figure 4-9\_b

**Description:** Spherical cell with a thick apical sinus shaped flagellum. The length of this

flagellum was about 15µm. The species had some thin arms arising from the anterior side. With

respect to the movement mode, it appeared that the cell swam along a very long arc.

Size: 4-5µm diameter

**Observation:** It was observed at Huk in months 3, 7 and 8 in medium abundance. This species

was not observed in Nesset.

Comment: The sinus-shaped apical flagellum was clearly observed. With respect to the cell

shape, the species was similar to Pteridomonas danica. In the LM observation it is however

impossible to differentiate between these two species.

**Class Raphidophyceae** Chadefand ex P.C Silva 1989

**Order Chattonellales** Throndsen 1993

**Genus** *Olisthodiscus* N. Carter 1937

Olisthodiscus luteus N. Carter 1937

Figures are located in: Plate 4-12\_b1,2 and Figure 4-9\_c1,2,3,4

**Description:** Elliptical cell with yellow-brown colour, dorsoventrally flattened with two equal

flagella about a cell length. Many chloroplasts (at least 6) were observed in the peripheral layer.

The main swimming mode was gliding without rotating.

Size: 18-26μm long, 13-18μm wide

**Observation:** It was observed at Nesset in months 2, 3, 4 and 8 in high abundances. At Huk the

species was observed in months 2, 4, 5, 6, 7, 8 and 9 in high abundances.

<u>Comment:</u> Due to the species contact with the substratum, no free swimming was observed. As mentioned by Zubizarreta (2005), this species was confused for a long time with *Heterosigma akashiwo* (Hara) Hara and Chihara (1987).

#### Olisthodiscus sp.

Figures are located in: Plate 4-12\_c and Figure 4-9\_d1,2

<u>Description:</u> Oval cell, dorsoventrally flattened with two equally thick flagella near the apex and a bit longer than the cell length. Cell colour was green-yellow. Many chloroplasts were observed in the cell periphery. The main swimming mode was gliding without rotating.

Size: 35-45μm long, 35-45μm wide

**Observation:** It was observed at Huk in months 5, 6, 7, 8 and 9 in medium abundances. This taxon was not observed in Nesset.

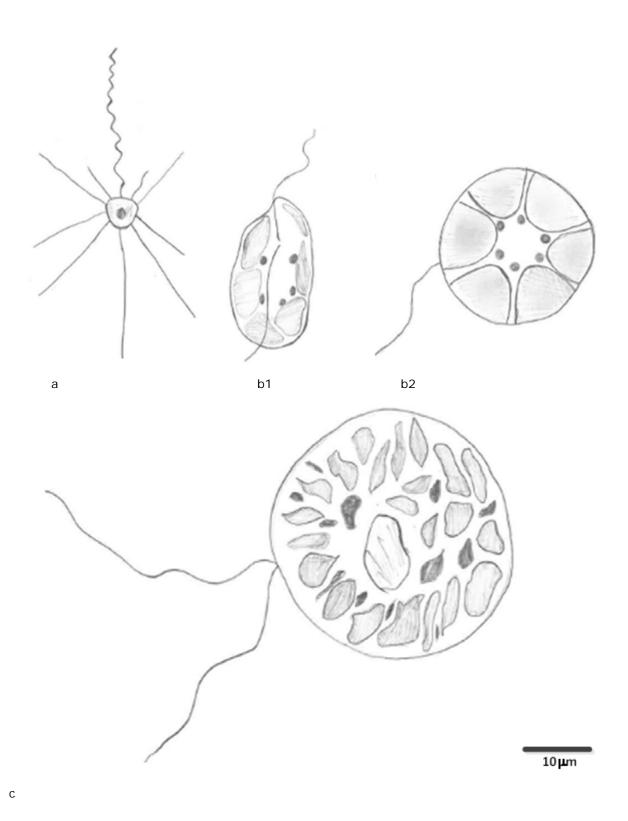


Plate 4-12 Actinomonas, Olisthodiscus

a) Actinomonas mirabilis, b) Olisthodiscus luteus 1) ventrally, 2) dorsally, c) Olisthodiscus sp.

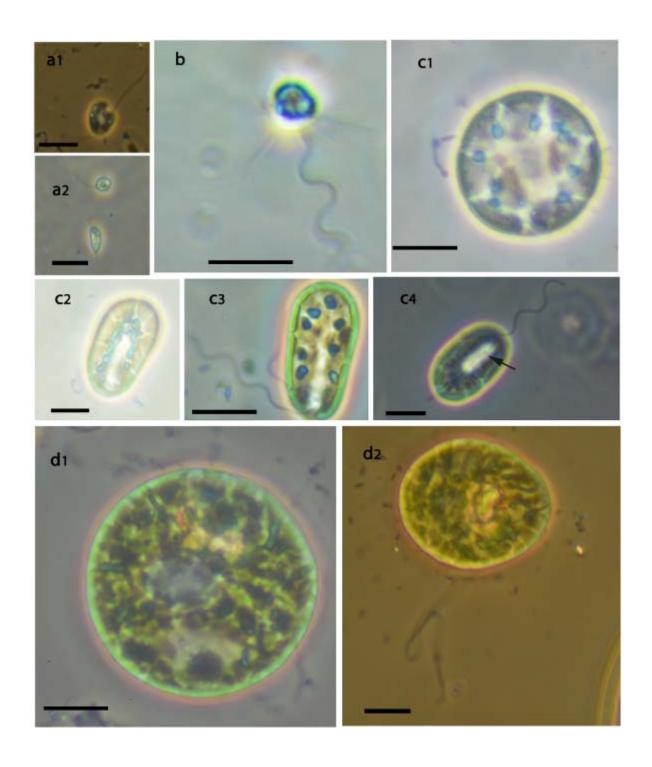


Figure 4-9 Cafeteria, Olisthodiscus, Actinomonas - All figures are taken in the phase contrast.

a) Cafeteria sp., b) Actinomonas mirabilis, c) Olisthodiscus luteus, c4: ventral view (arrow), d) Olisthodiscus sp.

Scale bar= 10µm

**Heterokonts (diatoms)** 

Class Bacillariophycea Haeckel 1948

Order Naviculaes Bessey 1907

Genus Navicula Bory de Saint-Vincent 1822

*Navicula* sp.

Figures are located in: Plate 4-13\_a1,2 and Figure 4-10\_a1,2,3, Figure 4-16\_a,b (SEM)

**Description:** A boat shaped (elliptical) cell, with two yellow-brown chloroplasts. The valves ends may capitated (this was observed in the SEM). The raphe was straight and the proximal raphe ends were deflected to one cell width the side.

Size: 20-65μm long, 5-20μm wide

**Observation:** It was observed at Nesset in months 1, 2, 3, 4, 7 and 8 in very high abundances. At Huk, the taxon was observed in all months in medium abundances.

**Order: Naviculales** Bessey

**Genus:** *Pleurosigma* W. Smith 1852

*Pleurosigma* sp.

Figures are located in: Plate 4-13\_b and Figure 4-10\_b

**<u>Description:</u>** Long cell with a gentle sigmoid (S-shape) and a sigmoid raphe. This raphe had some degrees of inclination relative to the longitudinal axis. Central raphe was clearly observed under LM. The taxon had two brown-green chloroplasts.

**Size:** 70-150μm

**Observation:** It was observed at Nesset in months 4 and 8 in low abundances. At Huk, the taxon was observed in months 1 and 8 in medium abundances.

#### **Order Mastogloiales** D.G Mann

Genus Achnanthes Bory de Saint-Vincent 1822

#### Achnanthes sp.

Figures are located in: Plate 4-13\_c and Figure 4-10\_c1,2, Figure 4-16\_d (SEM)

<u>Description:</u> Rectangular cell. Raphe valve was concave while the rapheles valve was convex. Two chloroplasts were located centrally in the cell center. The cell was brown-green in colour.

Size: 10-40μm long, 5-10μm wide

**Observation:** It was observed at Nesset in months 1, 2, 3, 7, 8 and 9 in very high abundances. At Huk, the taxon was observed in months 1, 2, 3, 6, 7, 8 and 9 in medium abundances.

### Order Thalassiophyales D.G Mann

Genus Amphora F.T Kutzing 1844

#### Amphora sp.

Figures are located in: Plate 4-14\_d and Figure 4-10\_d, Figure 4-17\_a,b (SEM)

<u>Description:</u> Oval cell, with two green-brown chloroplasts. The cell was symmetrical to the apical and ventral margins. Its raphe was not centric and it was positioned along the ventral margin. The raphe was more or less straight.

**Size:** 30-60μm

**Observation:** It was observed at Nesset in months 4, 7, 8 and 9 in medium abundances. At Huk, the taxon was observed in months 1, 7, 8 and 9 in medium abundances.

#### **Order Cocconeidales** E.J Cox 2015

**Genus** *Cocconeis* Ehrenberg 1836

#### Cocconeis sp.

Figures are located in: Plate 4-14\_e and Figure 4-10\_e1,2, Figure 4-16\_f (SEM)

**<u>Description:</u>** Oval/ovoid shape with transverse ribs, and a green/brown colour chloroplasts. A raphate valve was only observed on one occasion, otherwise raphes were not observed.

**Size:** 40-60μm long, 20-25μm wide

**Observation:** It was observed at Nesset in months 2 and 3 in medium abundances and in month

8 in low abundance. At Huk, the taxon was observed in month 2 in medium abundance.

**Order Rhopalodiales** Ehrenberg 1845

**Genus** *Entomoneidaceae* Ehrenberg 1845

Entomoneis sp.

Figures are located in: Figure 4-10\_f, Figure 4-17\_e (SEM)

**Description:** Cell constricted at the middle. The frustules of *Entomoneis* had numerous girdle

bands. Valves were linear with acute apices. The valve face was highly arched. The raphe was

observed at the outer edge of each keel.

**Size:** 45-60μm

**Observation:** It was observed at Nesset in months 1 and in low abundance. At Huk, the taxon

was not observed.

Class Fragilariophycea R.M. Crawford & D.G Mann, 1990

**Order Fragilariales** P.C Silva

**Genus** *Ceratoneis* Ehrenberg 1839

Ceratoneis closterium Ehrenberg 1839

Figures are located in: Plate 4-14\_f and Figure 4-10\_g1,2, Figure 4-16\_g,h,i (SEM)

**<u>Description:</u>** Cylinder cell with very sharp ends. The raphe of *Ceratoneis closterium* is seen to

be interrupted in the center, the fibulae were narrow, arcuate and fastened to the valve by short

cross-bars. This species had two green-brown chloroplasts.

Size: 30-100μm long, 2-5μm wide

**Observation:** It was observed at Nesset in months 1, 2, 3 in medium abundances and in months

7, 8 and 9 in very high abundances. At Huk, the species was observed in months 1, 3, 5, 8 and 9

in medium abundances.

Comment: The species size reported by Throndsen and Eikrem (2010) was between 32-260µm

while in our study we did not find a length more than 100µm. This however confirms a very

board range of cell sizes in this species.





a1 a2





10µm

Plate 4-13 Navicula, Pleurosigma, Achnanthes

a) Navicula sp.; a1) valve view, a2) gridle view, dorsally, b) Pleurosigma sp., c) Achnanthes sp.

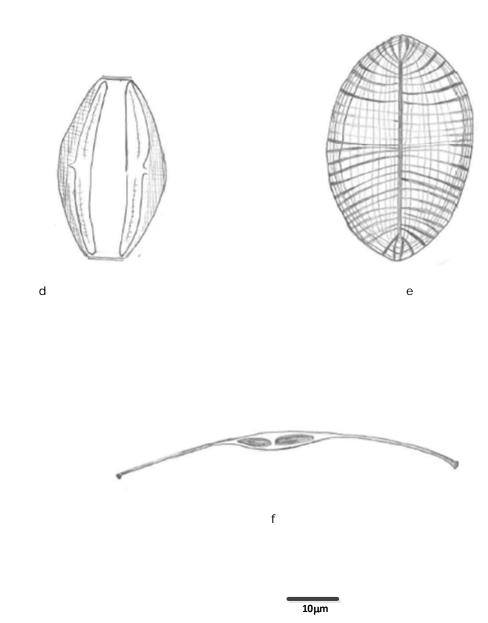


Plate 4-14 Amphora, Ceratoneis, Cocconeisd) Amphora sp., e) Cocconeis sp., f) Ceratoneis closterium

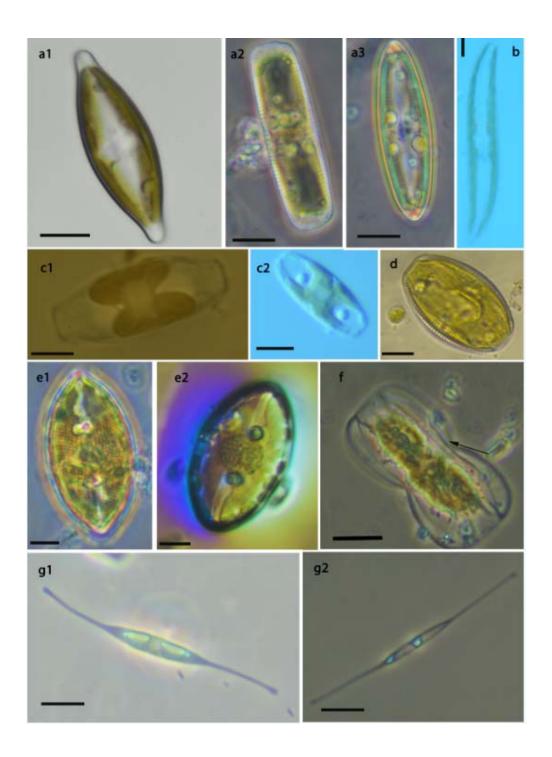


Figure 4-10 Navicula, Pleurosigma, Achnanthes, Amphora, Cocconeis, Ceratoneis- All figures are taken in the phase contrast, except b, c2 and e2 where it is taken in the diffraction interference contrast.

a) Navicula sp., a2: gridle view, a3: valve view, b) Pleurosigma sp., c) Achnanthes sp., d) Amphora sp., e) Cocconeis sp., f) Entomoneis sp., gridle view, the curved raphe was observed at the outer edge of each keel (arrow), g) Ceratoneis closterium,

#### Scale bar= 10µm

Cercozoans

**Class Imbricatea** 

**Order Thaumatomonadida** 

**Genus** *Protaspis* Skuja 1939

*Protaspis oblique* Larsen and Patterson 1990

Figures are located in: Plate 4-15\_a and Figure 4-11\_a1,2,3,4

**Description:** Oval shape, dorsoventrally flattened. The species had two unequal flagella inserted

near the cell apex. Anterior flagellum was about half of the cell length while the posterior

flagellum was about 1.5 cell lengths. The 5µm diameter, spherical nucleus was observed

centrally in cell, slightly closer to the anterior end. The apex looked like a U-shape. The species

had a slow gliding movement using its flagella.

Size: 16-25μm long, 10-14μm wide

**Observation:** It was observed at Nesset in months 1, 2, 3, 4, 7, 8 and 9 in medium abundances.

At Huk, it was observed in months 2, 4, 6, 7 and 8 in medium abundances.

**Comment:** This species was similar to *Protaspis glans*. The main difference between these two

species were the positioning of the nucleus and the asymmetrical appearance of *Protaspis* 

oblique due to an indentation, as explained by Larsen and Patterson (1990).

<u>Protaspis tegere Larsen and Patterson 1990</u>

Figures are located in: Plate 4-15\_b and Figure 4-11\_b

**Description:** Elongated oval (oblong) dorsoventrally flattened. The species had a longitudinal

groove starting from the flagella insertion point radiating toward the posterior side. Anterior

flagellum was about equal to the cell length while the posterior flagellum was a bit longer than

the cell length. Protaspis had a slow gliding movement using its flagella.

Size: 14-18µm long, 7µm wide

**Observation:** It was observed at Huk in months 2 and 3 in medium abundance. This species

was not observed in Nesset.

**Comment:** The species' main feature was the longitudinal groove.

Order incertaesedis

Genus Metromonas (Griessmann 1913) Larsen and Patterson 1990

*Metromonas simplex* Larsen and Patterson 1990

Figures are located in: Plate 4-15\_c

**Description:** Balloon shape, dorsoventrally compressed. Only one flagellum twice the length of

the cell was observed. The cell movement was along an arc from one side to the side like a

pendulum.

**Size:** 4-6μm

**Observation:** It was observed at Nesset in months 3 and 8 in high abundances. At Huk, the

species was observed in months 3 and 6 in medium abundances.

Comment: We took a picture from this species, but coincidently missed it. Throndsen and

Eikrem (2010) reported two flagella for this species. It is however mentioned by them that the

second flagellum was very small and not very visible which was the case here hence our

inability to find it.

**Apusozoans** 

Class Amastigomonea

**Order Amasigomonadales** 

**Genus** *Amastigomonas* De Saedeleer 1931

Amastigomonas mutabilis (Griessmann) Molina and Nerad

Figures are located in: Plate 4-15\_d and Figure 4-11\_c

**Description:** Elliptical and flexible cell, dorsoventrally flattened. Two unequal flagella inserting

from the anterior part were observed. Anterior flagellum was about 0.5 cell length while the

posterior flagellum was about 1.5 cell lengths. It looked like the posterior flagellum trailed

under the body.

Size: 10-18μm long, 3-5μm wide

Observation: It was observed at Nesset in months 1 and 8 in low abundance. At Huk, the

species was observed in month 7 in low abundance.

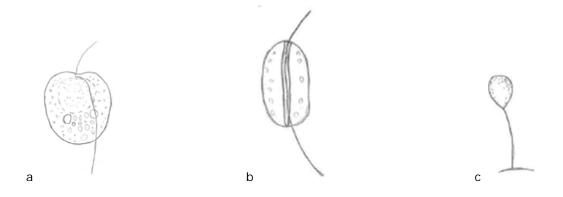
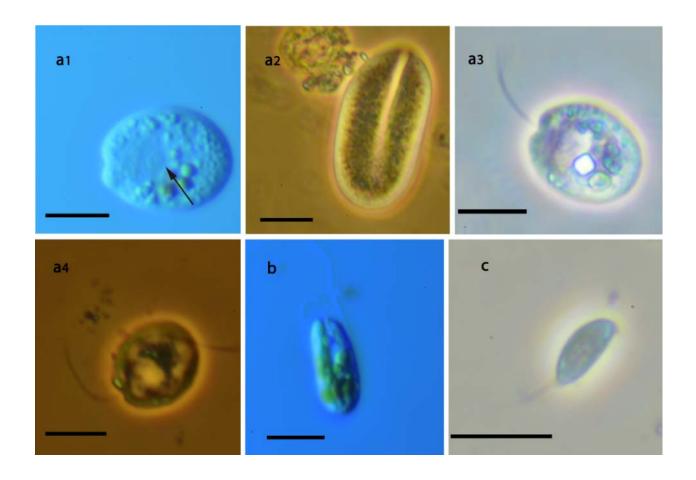




Plate 4-15 Protaspis, Metromonas, Amatigomonas

a) Protaspis oblique, b) Protaspis tegere, c) Metromonas simplex, d) Amastigomonas mutabilis

10μm



 $\textbf{Figure 4-11} \ \textit{Protaspis, Amatigomonas-} \ \textbf{All figures are taken in the phase contrast, except a1} \\ \textbf{and b, where they are taken in the diffraction interference contrast.}$ 

a) Protaspis oblique, a1: nucleus (arrow), b) Protaspis tegere, c) Amastigomonas mutabilis Scale bar= 10μm

# Choanoflagellates

Class Choanoflagellatea Takahashi 1984

**Order Acanthoecida** Cavalier Smith 1997

**Genus** *Acanthocorbis* Hara and Takahashi 1984

# Acanthocorbis sp.

Figures are located in: Figure 4-12\_a

<u>Description:</u> Conical shape or rod shape cell with anterior projections. The taxon had many numbers of longitudinal costae and a few transverse costae.

**Size:** 5-7μm

**Observation:** It was observed at Huk in months 8 and 9 in high abundances. This taxon was not observed in Nesset.

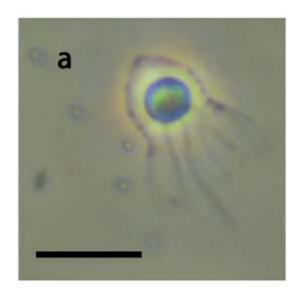


Figure 4-12 Acanthocorbis- All figures are taken in the phase contrast.

a) Acanthocorbis sp.

Scale bar= 10µm

Cyanobacteria

Class Cyanophyceae Schaffner 1909

**Order Chroococcales** HanSgirg 1892

**Genus** Chroococcus

Chroococcus sp.

Figures are located in: Plate 4-16\_a and Figure 4-13\_a

**<u>Description:</u>** Cells were in colonies of two cells with blue green colour. A colourless zone as a

center line was observed.

Size: Two celled colonies (10-10)μm× (10-10)μm

**Observation:** It was observed at Nesset in months 1, 8 and 9 in low abundances. This taxon was

not observed in Huk.

**Comments:** This taxon was similar in specification to *Chroococcus turgidus* (kutz) Nag.var

Kaas. However the cell size was half of their reported values.

**Genus** *Merismopedia* Meyen 1839

Merismopedia sp.

Figures are located in: Plate 4-16\_b and Figure 4-13\_b

**Description:** A combination of ovoid cells arranged in a form of rectangular shape. A

mucilaginous matrix held the colony together, but the resulting structure was not very flexible.

Cell colour was green.

Size: Size of each individual cell was about 1.5-3μm

**Observation:** It was observed at Nesset in months 1, 2, 3 and 4 in medium abundances and in

low abundance in month 9. This taxon was not observed in Huk.

**Genus** *Microcrocis* P.G Richt 1892

Microcrocis cf. sabulicola (lagerh) Geitler 1942

Figures are located in: Plate 4-16\_c and Figure 4-13\_c

**Description:** Colonized plate with green colour. Each cell was oblong in front view and all cells

were densely packed in a plate form.

Size: The plate diameter was more than 100μm, where each cell size was about 3×3μm

Observation: It was observed at Nesset in months 3 and 9 in low abundances. At Huk, the

species was observed in month 3 in low abundance.

**Order: Oscillatoriales** Vaucher ex Comont 1892

**Genus:** Oscillatoria Vaucher ex Comont 1892

*Oscillatoria* sp.

Figures are located in: Plate 4-16\_d and Figure 4-13\_d, Figure 4-18\_f (SEM)

**Description:** Long straight and cylinder filament with a dome shaped apex and smooth layered

strata. The cell colour was usually blue-green but sometimes tended brownish. Sliding/gliding

by helps of filaments was the main modes of movement.

Size: 10-30μm wide, could be hundred μm long

**Observation:** It was observed at Nesset in months 1, 2, 3, 4, 7, 8 and 9 in very high abundances.

This taxon was not observed in Huk.

Genus: Lyngbya Agardh ex Comont 1892

*Lyngbya* sp.

Figures are located in: Plate 4-16\_e and Figure 4-13\_e

**Description:** It was a long, un-branching filament inside a rigid mucilage sheath. The filament

colour was blue green.

Size: 6-8µm wide and could be much more than this in long

**Observation:** It was observed at Nesset in months 3 and 9 in low abundances. This taxon was not observed in Huk.

**Order Spirulinales** Komarek, Kastorstky, Mars and Johansen 2014 **Genus** *Spirulina* P.J.F Turpin ex M. Comont 1892

Spirulina cf. subsalsa (Cohn ex Comont) Koster 1892

Figures are located in: Plate 4-16\_f and Figure 4-13\_f

<u>Description:</u> This species looked like a coiled spring with blue-green colour. The main movement was gliding.

<u>Size:</u> Size of each individual cell was about 3-6 $\mu$ m wide and could be more than hundred  $\mu$ m long.

<u>Observation:</u> It was observed at Nesset in months 1, 2, 3, 8 and 9 in medium abundances. This species was not observed in Huk.

# **Order Synechococcales**

**Genus** *Pseudanabaena* Lauterborn 1915

#### Pseudanabaena sp.

Figures are located in: Plate 4-16\_g and Figure 4-13\_g1,2, Figure 4-18\_d,e (SEM)

**<u>Description:</u>** Filamentous, cylinder shape and trichome chains with mucus surrounding the outer side of the cell.

<u>Size</u>: Size of each individual cell was about 2-7µm and could be much more than this in long.

<u>Observation:</u> It was observed at Nesset in months 3, 4, 7, 8 and 9 in medium abundances. This taxon was not observed in Huk.

**Order: Thiotrichales** 

**Genus:** *Beggiatoa* Trevisan 1842

Beggiatoa sp.

Figures are located in: Figure 4-13\_h

Description: A cylindrical cell shape, a long filaments with a cell of around 1-2 μm and

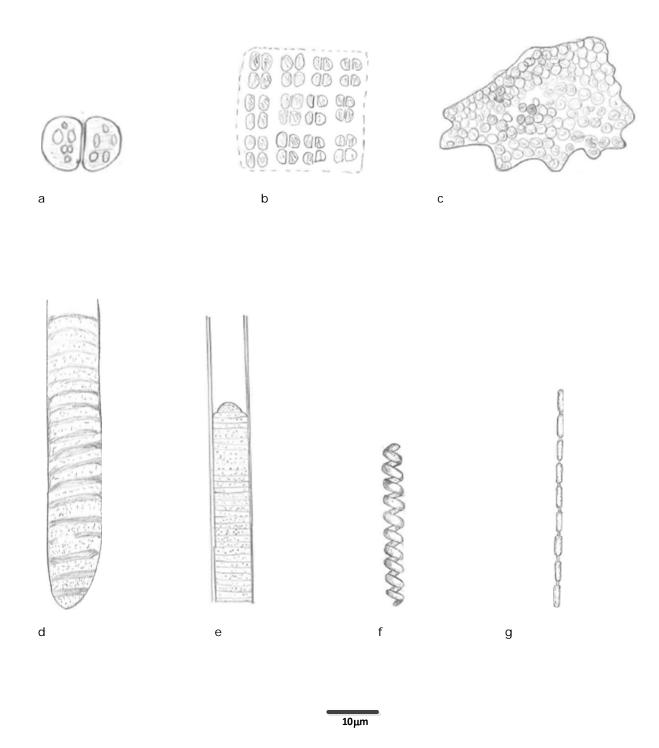
colourless. Although it formed long chains, it did not appear as a straight line, and looked like

twisted such coils.

Size: 1–2μm wide and could be much more than 100μm long

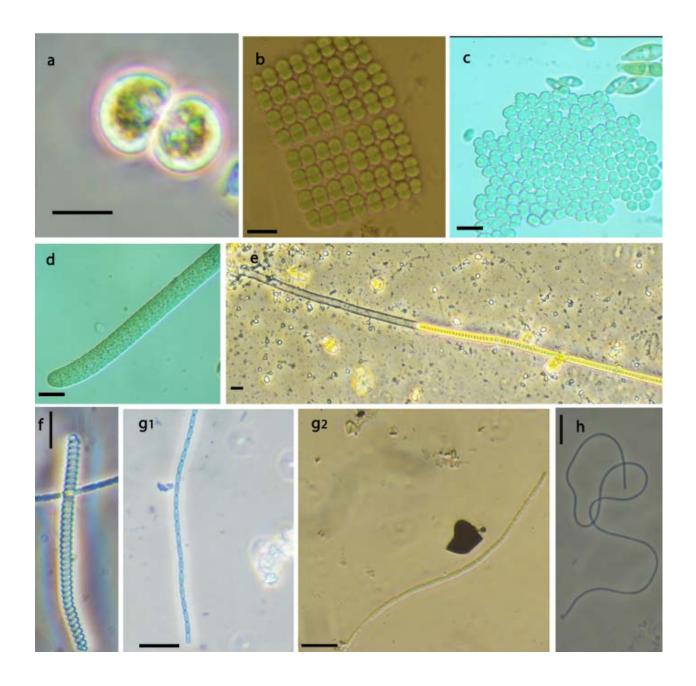
Observation: It was observed at Nesset in months 1, 2, 8 and 9 in low abundances. At Huk the

taxon was only found in month 9 in low abundance.



**Plate 4-16** Chroococcus, Merismopedia, Microcrocis, Oscillatoria, Lyngbya, Spirulina, Pseudanabaena

a) Chroococcus sp, b) Merismopedia sp., c) Microcrocis cf. sabulicola, d) Oscillatoria sp., e) Lyngbya sp, f) Spirulina cf. subsalsa, g) Pseudanabaena sp.



**Figure 4-13** Chroococcus, Merismopedia, Microcrocis, Oscillatoria, Lyngbya, Spirulina, Pseudanabaena, Beggiatoa-- All figures are taken in the phase contrast, except b, where it is taken in the diffraction interference contrast.

a) Chroococcus sp., b) Merismopedia sp., c) Microcrocis cf. sabulicola, d) Oscillatoria sp., e) Lyngbya sp., f) Spirulina cf. subsalsa, g) Pseudanabaena sp., h) Beggiatoa sp.

Scale bar= 10µm

# 4.2 Results from the Scanning Electron Microscope

To prepare a sample for Scanning Electron Microscope (SEM) inspection, a very long and multi staged preparation procedure was needed. This procedure caused the majority of our species to be washed from the cover slips. For the few remaining species, some species suffered sufficient structural damage so as to make identification impossible. In addition, since the species were fixed to the cover slip, identification of the species based on their movement was not possible. These were the main SEM limitations compared to the LM observations. In this research, some groups of diatoms, dinoflagellates, cryptomonads, chlorophytes, euglenoids, heterokonts and cyanobacteria were identified down to genus/species level under SEM observations. It should be emphasized that a very high level of experience needed to obtain more accurate results from the SEM.

Examples of pictures taken in the SEM of the mentioned groups are presented in Figure 4-14 through Figure 4-18 along with their descriptions.

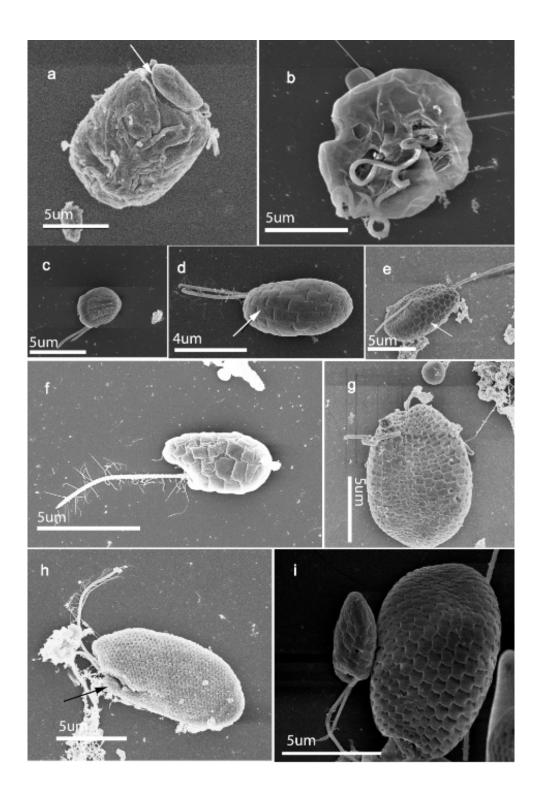


Figure 4-14 dinoflagellates (a and b) and cryptomonads (c to i) in the SEM

a) Amphidinium herdmanii, epicone (arrow), b) unknown taxon, c) Goniomonas amphinema, d-f) Chroomonas diploccoca, with rectangular plates (arrow in d), e) Chroomonas sp., hexagonal periplast plate (arrow), g) Cryptomonas sp., h) Rhodomonas baltica, furrwo (arrow), i) unknown taxon, two species with different sizes.

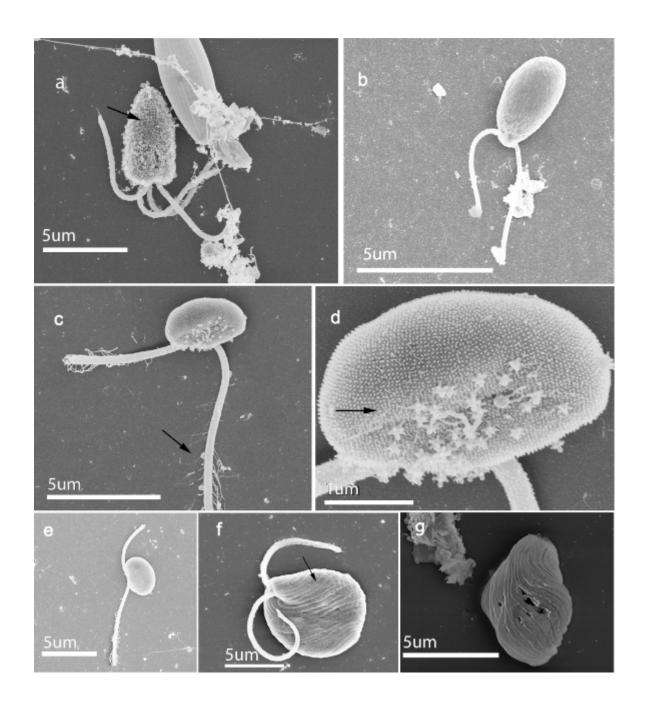


Figure 4-15 chlorophytes (a to e) and euglenoids (f to g) in the SEM

a) *Pyramimonas* sp, box scales (arrow), b) *Chlamydomonas* sp., c-e) *Nephroselmis rotunda*, flagellar hair scales (arrow in c), higher magnification and star shape scales (arrow in case d), f-g) *Eutreptiella* sp., pellicullar (arrow in f)

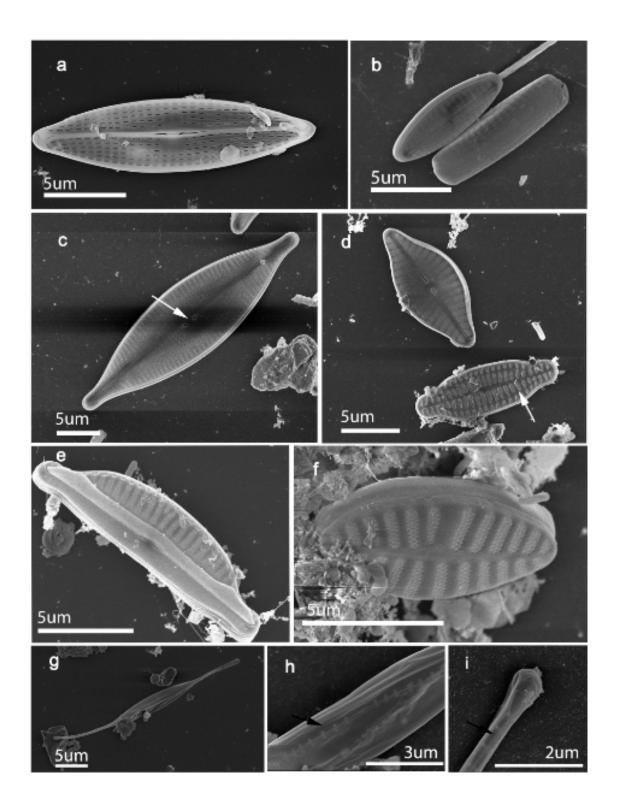


Figure 4-16 diatoms in the SEM

a-b) *Navicula* sp., b: valve and gridle views, c) diatom sp., simple raphe (arrow), d) *Achnanthes* sp1., investigated taxon (arrow), e) *Amphora* sp1, f) *Cocconeis* sp., g-i) *Ceratoneis closterium*, h: raphe with central nodulus, the fibulae are narrow arcuate and fastened to the valve by short cross-bars, i: end of valve

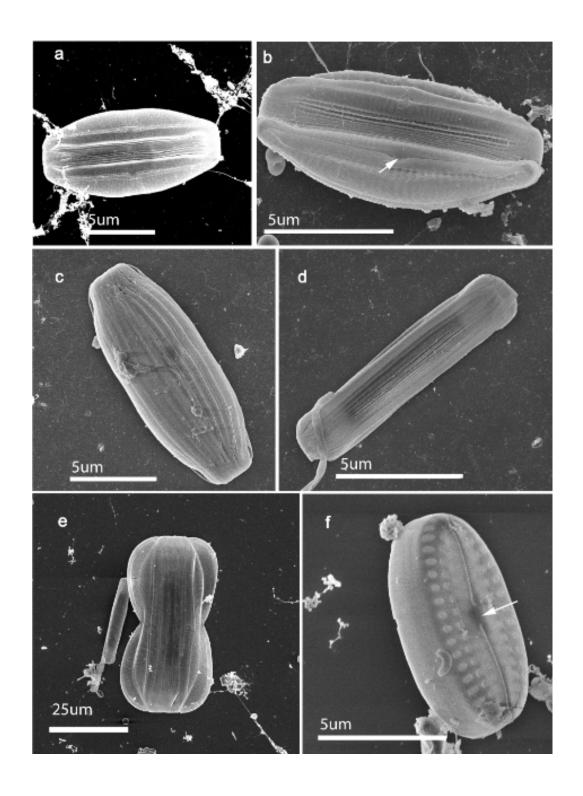


Figure 4-17 diatoms in the SEM

a-b) Amphora sp1, c-d) Amphora sp2, e) Entomoneis sp., f) diatom sp., central nodulus (arrow)

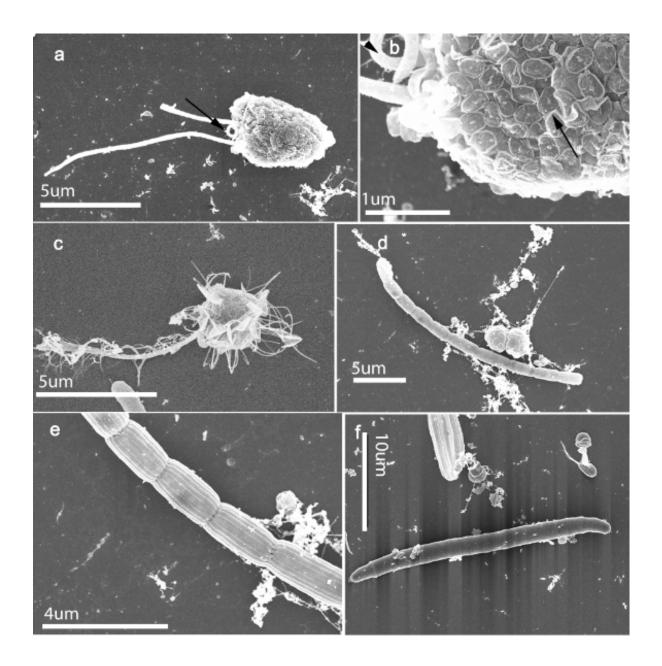


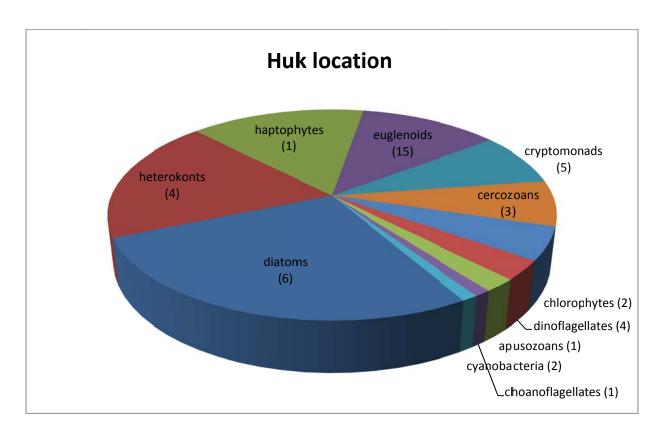
Figure 4-18 haptophytes (a-b), heterokont (c), cyanobacteria (d-f) in the SEM

a-b) *Prymnesium* cf. *nemamethecum*, a short haptonema in a (arrow), b is the same taxon with a higher magnification, organic scales (arrow), c) *Paraphysomonas* sp., This speceis was only obsrved in SEM. Due to very small size of this speceis and similarity to *Cafeteria*, it was impossible to identify it by LM observation, d-e) *Pseudanabaena* sp., e is the same taxon with a higher magnification, f) *Oscillatoria* sp.

# 4.3 A relative comparison between species abundances at each location

With the available methods of species fixation (fixation of coverslips with Lugol's solution and a hemocytometer), it was not possible to count the observed taxa. Despite the lack of quantitative data, it was possible to perform a rough estimate by eye-impression of the relative abundances of the main groups and species. This is illustrated in Figure 4-19 through Figure 4-22. Number of observed taxa is shown inside parenthesis in Figure 4-19 and Figure 4-21.

In general, diatoms, heterokonts (Olisthodiscus), haptohytes and euglenoids in Huk and diatoms, cyanobacteria, euglenoids, cryptomonads and chlorophytes in Nesset were the most frequent taxa.



**Figure 4-19** Relative abundances of the observed groups at Huk- number of observed taxa in parenthesis

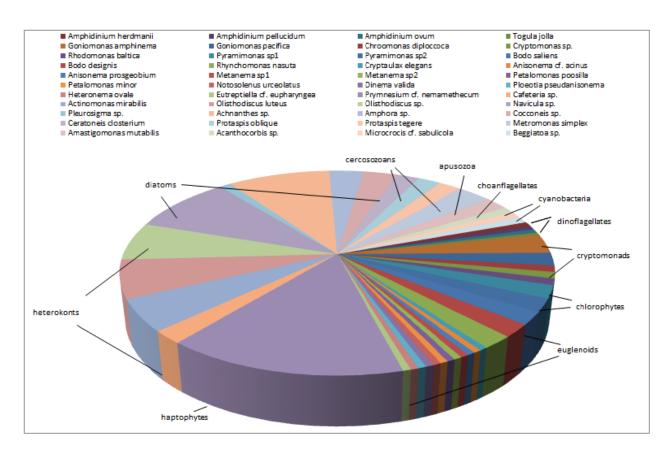
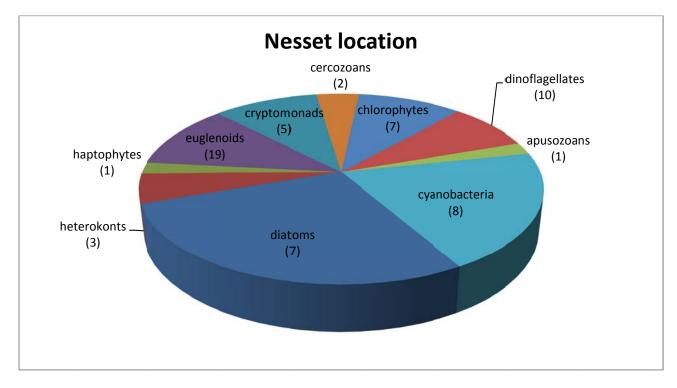


Figure 4-20 Taxa diversity at Huk – range of each groups is illustrated by two lines.



**Figure 4-21** Relative abundances of the observed groups at Nesset- number of observed taxa in parenthesis

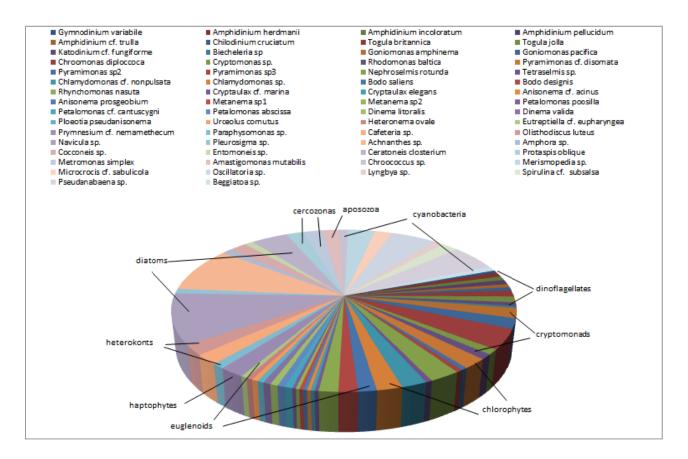


Figure 4-22 Taxa diversity at Nesset - range of each groups is illustrated by two lines.

#### 4.4 Probabilistic assessment

#### 4.4.1 Statistical test (calibration)

One of our goals in this thesis was to compare Huk and Nesset locations with regard to species diversities. If there are many species which are observed at only one of the sites (or at only one of the times) there are reasons to believe that the species composition at the two sites (times) are different. To estimate the average probability of detecting a species, previous data at Huk (Zubizarreta, 2005) was used for calibration. In these two surveys (Zubizarreta, 2005 and the current study), a total of 57 species (the flagellates) were observed, of which 24 were observed by in both cases ( $s_{12} = 24$ ).

This assessment gave the following estimate of the average species detection probability (Section 3.4):

$$\dot{P} = \frac{2 \times 24}{57 + 24} = 0.59$$

Applying a 95% confidence interval, a range between [0.43, 0.70] was found a proper range to be used in our assessments.

We concluded that at Huk, the sampling effort by us is at a level where the average probability of species detection is of the order 50%. There is no previous data set for Nesset, however since the sampling was done very similar on Huk and Nesset (same person and same technique and same effort), we took this as an acceptable indication that also at Nesset the average probability of species detection is of the order 50%. Detail of the species, together with their observed months and probabilistic assessments are presented in Appendix B.

#### 4.4.2 Probabilistic comparison between Huk and Nesset

As disused in the previous section, a lower and higher range of average probability of species detection was calculated and used in the diversity comparisons between Huk and Nesset locations. We calculated the critical species numbers based on :

- the mentioned range for p which is [0.43, 0.70],
- sum of species numbers observed at each location only (S10+S02) and
- 5% confidence level as a basis for rejection of null hypothesis.

This is illustrated in Figure 4-23 through Figure 4-26.

In these figures, the horizontal bold black line is 5% significance level and the vertical bold red line is (S10+S02). Cross point between the significance level and (S10+S02) with the coloured curves shows the minimum average probability that two locations might have same species for that specific month. In other words, we may therefore reject the null hypothesis at the 5% significance level and accept the alternative that the assemblages at the two sites contain different species if the number of species detected on only one of the sites is greater or equal than k<sub>crit</sub>. Looking at Figure 4-23 through Figure 4-26, we can conclude that in general and in all months, two locations have different species compositions if the average probability of detecting a species is about 0.5-0.6 or larger. In other words, rejection of the null hypothesis of equal species compositions requires that one may say that the sampling intensity was so large that approximately one of 2 species was detected. Details of calculation are presented in Appendix C.

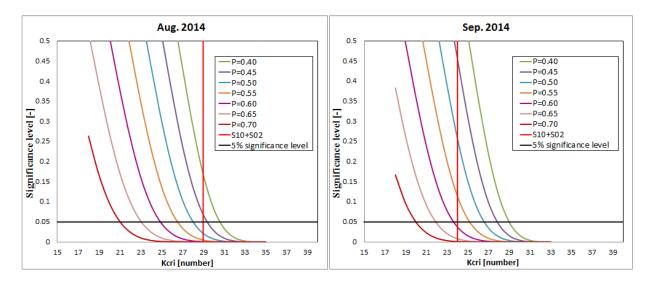


Figure 4-23 Species critical number at months Aug. and Sep. 2014

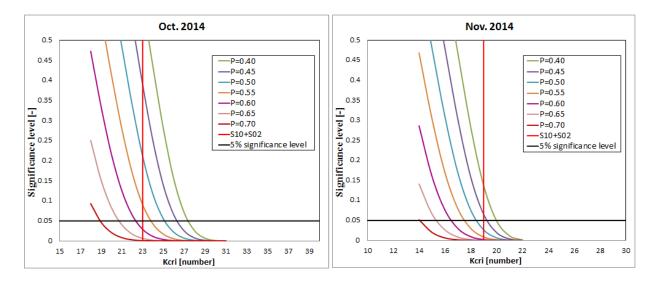


Figure 4-24 Species critical number at months Oct. and Nov. 2014

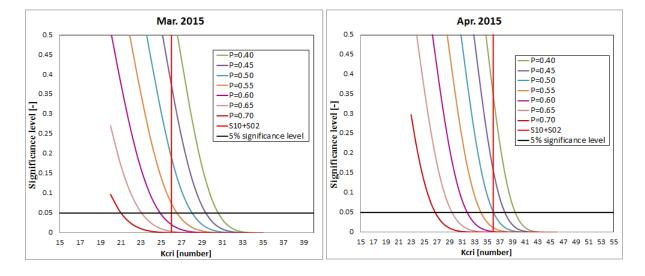


Figure 4-25 Species critical number at months Mar. and Apr. 2015

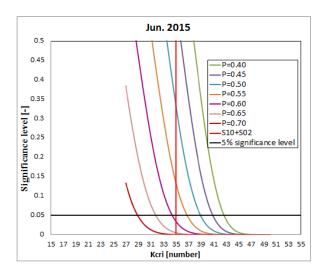


Figure 4-26 Species critical number at month Jun. 2015

## 4.5 Hydrography

During sampling, both temperature and salinity were measured. This is illustrated in Figure 4-27 and Figure 4-28 based on month order from Table 4-1. At both location, while the temperature increases from cold seasons (fall and winter) to mild/warm seasons (spring and summer) as expected, the salinity does not follow any specific trend and looks like a sinusoidal curve. It is however seen the salinity at Nesset is low almost in all months compared to ones at Huk due a creek entering at Nesset location. It is noted due to frozen surface in months December, January and February, there are no data for those months at Nesset.

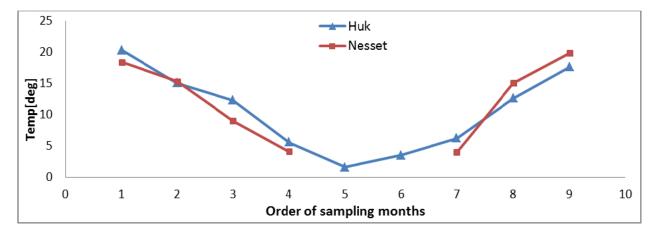


Figure 4-27 Temperaturer at Huk and Nesset locations

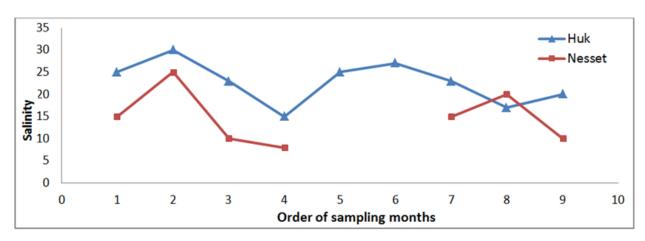


Figure 4-28 Salinity (psu) at Huk and Nesset locations

#### 5 DISCUSSIONS

#### 5.1 Comments on material and methods

In the current study, the focus was on comparing the diversity of flagellate protists (dinoflagellates, haptohytes, cryptomonads, chlorophytes, euglenoids, heterokonts, diatoms, cercozoans, apusozoans, and choanoflagellates) and cyanobacteria of two sandy shores in the inner part of Oslofjorden.

As mentioned in chapter 3.2, sampling was done in the top centimeter of the beech. Kingston (1999) suggests that this layer hosts the greatest population of protists within the sediment. The main reason could be light and photosynthesis. In some cases at Nesset, the quality of sampling was to some occasions affected of the presence of a brown to black color (low oxygen/anoxic) in the top layer and care was made to avoid this.

The extraction of organism from the samples can also be commented. Extraction was done by means of coverslips. In order to use the samples for a longer period, collected samples had to be retained in the growth-culture chambers. A disadvantage of this method is that the moisture may evaporate after some time and cause some species mortality. In addition, longer time to start the observations, more changes in samples composition occurs, e.g an increase in bacteria population with time (especially at Nesset) was recorded and favored heterotrophs. In the current study and in order to reduce this problem, our LM observations were started from the first available moment after sampling, typically a couple of hours after the collection.

Further, it should be mentioned that only species that attached to the cover slips were observed, considering the fact that some species might not attach to the cover slips during incubation; hence they would not be recorded. The focus and discussion herein is then limited to the species that attached to cover slip and was clearly visible under the light microscope.

Final comment is related to the observation technique. Since the swimming mode and the accurate cell morphology could only be demonstrated by living species, no preserved material was used before species identification in LM. This was the main reason why we were not able to count the number of individuals of the species.

#### 5.2 Species identification

Taxa were identified to the lowest possible level and identifications were based on cell morphology, movements and also the special specifications. Some taxa like *Dinema*, cyanobacteria and some of *Cryptomonas* were easily identified due to their large size or their slow swimming mode or no movement at all as in the case of many cyanobacteria. In some cases, for example *Amphidinium*, *Anisonema*, *Chlamydomonas*, *Pyramimonas*, *Protaspis*, *Rhynchomonas*, *Chroomonas* and *Goniomonas*, despite their fast swimming, identification to genera was not very complicated, as these taxa possess some special features such as e.g. the presence of eyespots, different cell coverings, ejectisomes, and number and morphology of flagella which is reflected in the different modes of swimming within different groups. Different swimming modes are described below:

<u>In dinoflagellates</u>, such taxa *Gymnodinium* and *Katodinium* had very fast rolling around and gliding movements. The transverse flagellum allowed them to roll around and the longitudinal flagellum was used for the gliding. The main movement mode for *Amphidinium* was gliding over the cover slip surface with a low speed. This is in agreement with the previous observations by Kofoid and Swezy (1921), and Kaas et al. (1985).

<u>In cryptomonads</u>, some genera such as *Chroomonas* had a very high speed and demonstrated a dislike for mixing with the others. In this group, *Goniomonas* had a fast swimming around a circle/spiral shape which is consistent with the one described by Larsen and Patterson (1990).

<u>In chlorophytes</u>, quick swimming was observed in *Pyramimonas* with occasional jumping movements caused by released ejectisomes. Contrastingly, *Chlamydomonas* from this group exhibited a slow gliding/rolling.

<u>In euglenoids</u>, cells mainly swam with attachment of their posterior flagellum to the surface and sliding forward with the other one. An example taxon of this swimming type was *Bodo*, as also discussed by Dujardin (1841). Many of the other species of this group had a capability of changing their shape and also crawling movements. Examples of taxa with this swimming mode were *Anisonema*, *Urceolus*, *Heteronema* and *Dinema* (Kahl 1928; Larsen and Paterson 1990).

<u>In haptohytes</u>, gliding and rotating by means of their flagella along their cell sides were the main movement. The swimming speed was low and movement direction was with the haptonema directed forwardly. An example of this group was *Prymnesium*.

In heterokonts, *Olisthodiscus* had not any rotation but smoothly glided while swimming.

<u>In diatoms</u>, movements were mainly gliding using mucus from the raphe. The speed was low.

<u>In cercozoans</u>, *Protaspis* had a slow gliding movement using its flagella, while in *Metromonas*, the long flagellum was attached to the surface and the cell moving from side to side as a pendulum. This is consistent with the description by Larsen and Patterson (1990).

<u>In apusozoans</u>, the movement was a slow gliding movement using flagella.

<u>In cyanobacteria</u>, a gliding type of swimming by help of filaments was the main movement. The *Oscillatoria* was an example of this swimming type.

Since some taxa were very similar to each other with respect to cell shape and movement, the genus identification was not straight forward. *Heteronema*, *Metanema* and *Gymnodinium* were examples of such groups, as was commented on in the results section. In order to identify these complex groups, observation should be confirmed by SEM and/or TEM. In our study, the SEM was used to aid species identification. For example, one specimen was initially identified as *Nephroselmis* sp. under the LM. Further examination of the organic scales covering the cell surface in SEM resulted in the identification of *Nephroselmis rotunda* (Abildhauge 1992).

Regarding diatom identification, although distinguishing between diatom and others groups was not very difficult, identification to the genus and species level may be challenging as many of the required morphological features (e.g. raphe and areola) are covered by organic material. This needs to be removed by an acid cleaning procedure (Hasle 1978) to become visible. The acid cleaning method requires a lot of material (e.g. net hauls) and could not be applied to the coverslips and small amount of sample scraped off the microscope slides.

With the current method of species fixation (fixation of coverslips with Lugol's solution and a hemocytometer) it was not possible to count the observed taxa. Despite the lack of quantitative data, it was however possible to perform a rough estimate by eye-impression of the relative abundances of the main groups (Figure 4-19 and Figure 4-21). In general, diatoms, heterokonts (*Olisthodiscus*), haptohytes and euglenoids in Huk and diatoms, cyanobacteria, euglenoids, cryptomonads and chlorophytes in Nesset were the most frequent taxa. The cover slips from Nesset were always full of diatoms, especially *Navicula* and *Achnanthes*. This is in agreement with Mcciathechie et al. (1982) and Admiraal et al. (1984) where they have mentioned mudflats are often dominated by diatoms. Most of the cyanobacteria were also found mainly in Nesset, this group was however rarely observed at the Huk location.

# 5.3 Why is species richness and abundance higher at Nesset compared to Huk?

Based on the microscopy observation and the performed probabilistic assessment (Section 4.4), it was found that in general, the species abundances in Nesset were much higher than in Huk. It is seen from Figure 4-19 and Figure 4-21, that the main differences between these two locations are presence of more cyanobacteria and diatoms at Nesset.

According to Macintyre et al. (1996) and Kaas (1987), microbenthic productivity is depended on many variables including chlorophyll and nutrient content in the sediment, temperature, irradiance, sediment moisture content, salinity, duration of tidal and waves. Below, these variables are considered in more detail:

- The place we had selected for sampling in Nesset was close to a creek where there was a continuous water flow entering to the sea at that location, while in Huk there was not any fresh water flow. The Nesset location might have a higher level of nutrient due to this fresh water flow, which might be one of the reasons for higher species abundance. Furthermore, it is known that certain bacteria such as cyanobacteria are capable of fixing nitrogen. Appreciable N-fixation has also been measured in several marine areas (Jones 1974; Whitney et al. 1975). In this process, nitrogen gas (N2) is converted to ammonium (NH4+), a form of nitrogen that is biologically available to plants. Based on our LM observations, abundance of cyanobacteria is high at Nesset. This causes the nutrient level of surrounding sediments would be kept more or less constant. According to Hussain et al. (2014), NH4-N is a basic source of nitrogen for most of microalgae groups' growth and abundance. This might be one of reasons to have numerous species at Nesset compared to Huk location.
- It is seen from Figure 4-28, the salinity level is low at Nesset compared to Huk in all seasons. This is mainly due to presence of creek there. The lower salinity at Nesset may be one of the reasons for the high diversity of cyanobacteria in this location.
- Another important parameter influencing the species abundances is the sediment grain size. We compared the difference in grain size distribution under a light microscope at a low magnification of 7.5 times (Figure 3-2). It is seen that the grain size in Nesset was fine compared to the one at Huk. This results in lower porosity and permeability at Nesset. Previous investigations reported an inverse relationship between the particle size

and organic matter concentration and bacterial abundance (Longbottom 1970; Hargrave 1972; Dale 1974). They have shown that, as a general rule, the fine-grained sediments provide a richer food sources than coarse-grained sediments. This means the finer material has a higher content of dissolved organic matter, and therefore sustains a higher species number as observed in Nesset.

- The Huk location is more exposed to wave action. Powerful waves washing the beech results in a more unstable substrate. The coarser sand at Huk compared to Nesset results in a higher porosity. In a higher porosity, sand particles can move more easily. Hence the waves can easily wash the material surface and reduce the species abundance and diversity.
- Admiraal and Peletier (1980) have emphasized that temperature and irradiance level has a high impact on the spatial distribution of benthic microalgae. However, due to the short distance between these locations, it is likely these factors have the same influence on these sites (See Figure 4-27) and therefore play an insignificant role for the observed differences.

# 5.4 Statistical comparison between Huk and Nesset with respect to the species diversity

One of our goals in this thesis was to compare Huk and Nesset locations with regard to species diversity. If there are many species which are observed at only one of the sites (or at only one of the times) there are reasons to believe that the species composition at the two sites (times) are different.

Based on available information from Zubizarreta (2005) and comparison with our data at Huk, a best estimate of the average species detection probability was calculated to 0.59 and with a 95% confidence interval, p= [0.43, 0.70]. These relative high probabilities allowed us to conclude that the species compositions were significant different at Huk and Nesset.

### 5.5 Seasonal species diversity

At both location, temperature increases from cold seasons (fall and winter) to mild/warm seasons (spring and summer) as shown in Figure 4-27. Increase in the light intensity and day length increase the species photosynthetic capacity. This result in higher abundances for the phototrophs species in the spring and summer times compared to the autumn and winter seasons. Our observations at both locations confirm this fact. During the winter months, abundances and number of species at Huk was in a very low level. In fact, some months, the organism needed some days to multiply after inoculation before they could be observed in LM. In contrast, identification of the species was difficult at Nesset due to presence of very many species (especially in spring and summer seasons).

For heterotrophic species, seasonal changes had limited impact on the species abundances. Those species abundance were more or less constant during the entire year.

#### 6 SUMMARY AND CONCLUSION

The main emphasis of this work was to identify the diversity and relative abundance of protist flagellates and cyanobacteria of two sandy shore locations in Oslofjorden. The two investigated locations were the intertidal zone in Huk on the Bygdøy Peninsula and Nesset in Bunnefjorden. The sampling was started in August 2014 and ended June 2015. Huk was sampled nine times whereas Nesset was only sampled seven times due to freezing in the winter season. During sampling two hydrographical parameters, temperature and salinity, were recorded. After transferring the samples to the laboratory at the University of Oslo, they were incubated in growth chambers and investigated by means of light and scanning electron microscopes (LM and SEM). The main findings are summarized below:

- Both the selected biotopes in the Oslofjorden had a high taxa number (especially at Nesset). However, it should be kept in mind that if the investigation had been running for a longer time period, more species would likely have been observed. The result is also biased by human observation error and preparation methods.
- In total, we found 71 different taxa in these two locations. The main protist flagellate groups recorded were dinoflagellates, haptohytes, cryptomonads, chlorophytes, euglenoids, heterokonts, diatoms, cercozoans, apusozoans and choanoflagellates. In addition a large number of cyanobacteria were observed, especially at Nesset.
- Of these 71 taxa, 63 were recorded at the Nesset and 44 were recorded at Huk. Of these 71 taxa, 30 at Huk and 42 at Nesset were identified to species level by means of light microscope.
- Of these 71 taxa, 18 were investigated by means of scanning electron microscope (SEM) to confirm/correct the suspected groups in the LM observations. Of these 18 samples, 8 were identified to the species level.
- Of the 71 species, 30 were heterotrophs and 41 were phototrophs. Due to the light, the phototrophs diversity and abundances were much higher in the spring and summer times compared to the autumn and winter seasons. These parameters had negligible impact on the heterotrophs abundances which exhibited similar relative abundances all the time.
- Based on the results obtained from probabilistic analyses, we conclude that the two assemblages (Huk and Nesset locations) have different species compositions if the average probability of detecting species is 0.5 or larger. In other words, rejection of the null hypothesis of equal species compositions requires that one may say that the sampling intensity was so large that approximately one of 2 species was detected.

#### 7 PROPOSED WORK FOR FUTURE

This thesis presents a relative and a quantitative comparison of species diversity and abundance at the Huk and Nesset shores. Species quantification was not possible due to the lack of good methods and damage to the cells. In order to conduct an accurate comparison, it is necessary to improve preservation method and thus improve species counting. A fixation method without any major damage to cells that only slowed down the species would be highly beneficial. By quantifying the species numbers and using some mathematical tools like the Shanon index, it would be possible to obtain a clearer view of species abundance and diversity.

Another goal for future studies could be to improve the preparation methods, and conduct a more detailed study of the species in SEM and TEM. Species should be chosen for more detailed investigation, including molecular and chemical methods which involve analysis of DNA, RNA, protein, and lipid. In the future with the program of molecular methods more accurate tools for species identification and enumeration might be developed.

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# Appendix A- Summary tables-Taxa observations at Huk and Nesset

#### X= observed species

## Huk location

		month	aug.14	sep.14	okt.14	nov.14	des.14	feb.15	mar.15	apr.15	jun.15
Phylum	genus	Taxa	1	2	3	4	5	6	7	8	9
	a. Gymnodinium	a1. G variabile									
	b. Amphidinium	b1. A herdmanii	x							x	
		b2. A incoloratum									
		b3. A pellucidum									х
Dinoflagellates		b4. A ovum	x								
Dinonagenates		b5. A trulla									
	c. Chilodinium	c. C cruciatum									
	d. Biecheleina	d. B sp									
	e. Tagula	e1. T Britannica									
		e2. T jolla									x
	g. Katodinnium	f. K c.f fungiforme									
Haptophytes	a. Prymnesium	a1. P sp	x					x	x	x	X
	a. Rhodomonas	a1. P baltica									x
	b. Chroomonas	b1. C.diplococca	x					x	x	х	X
Cryptomonads	c. Cryptomonas	c1. C sp									x
	d. Goniomonas	d1. G amphinema	х						х		х
		d2. G pacifica							x		
	a. Pyramimonas	a1.P disomata									
		a2. P sp1					x				
		a3.P sp2							x		
Chlananhata		a4. P sp3									
Chlorophytes	b. Tetraselmis	b1.T F. Stein									
	c. Chlamydomonas	c1. C cf nonpulsata									
		c2. C sp1									
	d. Nephroselmis	d1. N pyriformis									
	a. Paranema	a1. P fusiforme									
	b. Rhynchomonas	b1. R nasuta			x						
	c. Anisonema	c1. A prosgeobium		x							
		c2. A acinus						x	x		
	d. Bodo	d1. B saliens	x				x			x	X
		d2. B designis	x				x				
	e. Metanema	e1. M sp1	x						x		
		e2. M sp2		x					x		
	f. Euterpiella	f1. E sp							x		
	g. Heteronema	g1. H. ovale	x	х	x					x	
Euglenoids	h. Cryptaulax	h1. C. elegans						х	х		
		h2. C cf. marina									
	i. Petalomonas	i1. P abscissa									
		i2. P cantuscygni									
		i3. P poosilla			х					х	x
		i4. P minor			х	х					
	j. Dinema	j1. Dinema litoralis									
		j2. D valida							X		
	k. Ploeotia	k1. P pseudanusonema									
	I.Notosolenus	I1 N urceolatus							X		
	m. Urceolus	m1. U cornutus									

	a. Choorococcu	a1. C cf									
	b. Oscillatoria	b1. 0 sp	+								
	c. Merismopedia	c1. M sp									
_	d. Spirulina	d1. S subsalsa									
Cyanobacteria	e. Microcrocis	e1. M sabulicola			х						
	f. Pseudoabaena	f1. P sp									
	g. Lyngbya	g1. L sp									
	h. Microsclla	h1. cf Beggiatoa									х
	a. Achnanthes	a1. A dispersa	x	x	x			x	X	x	x
	b. Navicula	b1. N cf	х	x	x	х	X	х	X	х	x
	c. Ceratoneis	c1. C closterium	x		x		x			х	x
	d. Cocconeis	d1. C sp		x							
	e. Pleurosigma	e1. sp	x							х	
Heterokonts	f. Amphora	f1. A sp	х						х	х	x
neterokonts	g. Olisthodiscus	g1. O luteus		x		х	х	х	х	х	x
		g2. O sp					x	x	X	x	x
	h.Paraphysomonas	h1. P sp									
	i. Actinomonas	i. A sp			x				X		
	j. Acanthocorbis	j1. A sp								x	x
	k. Cafeteria	k1. C sp					x	x	x	x	x
	a. Protaspis	a1. P obligua		х		х		х	х	х	
Cercozoans		a2. P tegere		X	X						
	b. Metromonas	b1. M simplex									
Apusozoans	a. Amastigomonas								X		
Choanoflagellates	a. Acanthocorbis sp	a1 . A sp.									x

#### **Nesset location**

Nesset 100		month	aug.14	sep.14	okt.14	nov.14	des.14	feb.15	mar.15	apr.15	jun.15
Phylum	genus	Taxa	1	2	3	4	5	6	7	8	9
	a. Gymnodinium	a1. G variabile	х								x
	b. Amphidinium	b1. A herdmanii	х	х							x
		b2. A incoloratum		x						x	x
		b3. A pellucidum	x	х							x
Din effections		b4. A ovum									
Dinoflagellates		b5. A trulla								x	x
	c. Chilodinium	c. C cruciatum	х								
	d. Biecheleina	d. B sp			x						
	e. Tagula	e1. T Britannica			x	х				x	x
		e2. T jolla			x					x	
	g. Katodinnium	f. K c.f fungiforme								x	x
Haptophytes	a. Prymnesium	a1. P sp	х	х							
	a. Rhodomonas	a1. P baltica	х								x
	b. Chroomonas	b1. C. diploccoca	x	х	х					x	x
Cryptomonads	c. Cryptomonas	c1. C sp	х	х		х			х	х	х
	d. Goniomonas	d1. G amphinema			х						
		d2. G pacifica		х							
	a. Pyramimonas	a1.P disomata		х						х	x
		a2. P sp1									
		a3.P sp2							x	x	
		a4. P sp3	х	x						x	x
Chlorophytes	b. Tetraselmis	b1.T F. Stein	х								
	c. Chlamydomonas	c1. C cf nonpulsata	х	х	х	x			x	x	х
		c2. C sp1	х	x	x				х	x	х
	d. Nephroselmis	d1. N pyriformis		х						х	x

	a. Paranema	a1. P fusiforme	х				X		х	х
	ar r ar ar rema									
	b. Rhynchomonas	b1. R nasuta	x	x	х				х	х
	c. Anisonema	c1. A prosgeobium		x	х	х		х		
		c2. A acinus								х
	d. Bodo	d1. B saliens	x	x		х				х
		d2. B designis	x	x		х		х		
	e. Metanema	e1. M sp1		x	х	х				
		e2. M sp2		x	х			х	х	х
	f. Euterpiella	f1. E sp		x				х	х	х
F	h. Heteronema	h1. H. Ovale		x	х			х	х	х
Euglenoids	j. Cryptaulax	j1. C. Eelegans		x						
		j2. C cf. marina			х					
	k. Petalomonas	k1. P abscissa							х	х
		k2. P cantuscygni						х	х	х
		k3. P poosilla	x		х	х			х	х
		k4. P minor								
	I. Dinema	l1. Dinema litoralis						x	х	х
		I2. D valida	x		х	х			х	х
	m. Ploeotia	m1. P pseudanusonema			х				х	х
	N.Notosolenus	n1 N urceolatus								
	o. Urceolus	o1. U cornutus						х	x	х
	a. Choorococcu	a1. C cf	x	İ					х	х
	b. Oscillatoria	b1. O sp	x	x	х	х		х	х	х
	c. Merismopedia	c1. M sp	х	х	х	х				х
l	d. Spirulina	d1. S subsalsa	х	х	х				х	х
Cyanobacteria	e. Microcrocis	e1. M sabulicola			х					х
	f. Pseudoabaena	f1. P sp			х	х		х	х	х
	g. Lyngbya	g1. L sp			х					х
	h. Microsclla	h1. cf Beggiatoa	x	x					х	х
	a. Achnanthes	a1. A dispersa	x	x	x			х	х	х
	b. Navicula	b1. N cf	x	x	x	х		x	х	х
	c. Ceratoneis	c1. C closterium	x	X	х	х		х	х	х
	d. Cocconeis	d1. C sp		x	х				х	
	e. Pleurosigma	e1. sp				х		х	х	х
	f. Amphora	f1. A sp				х		х	х	х
Heterokonts	g. Entomoneis	g1. E sp	x							
	h. Olisthodiscus	h1. O luteus		x	x	х			х	
		h2. O sp								
	i.Paraphysomonas	i1. P sp				х				
	j. Actinomonas	j. A sp								
	k. Acanthocorbis	k1. A sp								
	I. Cafeteria	I1. C sp	x	x		x		х	х	x
	a. Protaspis	a1. P obligua	x	x	х	×		x	x	x
Cercozoans	a. Frotaspis	a2. P tegere		-				•		
	b. Metromonas	b1. M simplex			х				х	
Apusozoans	a. Amastigomonas	a1 . A mutabilis	x							

## Appendix B- Statistical test- our work and Zubizarreta 2005- Huk

1= species is observed

0= species is not observed

		92																																							_
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		month	Таха	a1. G variabile	b1. A herdmanii	b3. A pellucidum	b4. A ovum	b5. A trulla	c1. T jolla	d1. G rugatum	g2. K glandula	h1. P cf. Lima	a1. P sp	a2. Dicrateria gilva	a1. P baltica	b1. C.diplococa	c1. C sp	d1. G amphinema	d2. G pacifica	e1. H sp	a1.P dismata	a2. P sp1	a3.P sp2	b1.T F. Stein	c1. C cf nonpulsata	a1. P fusiforme	b1. R nasuta	c1. A prosgeobium	c2. A acinus	d1. B saliens	d2. B designis	e1. M sp1	e2. M sp2	f1. E sp	h1. H. Ovale	j1. C. Elegans	k2. P cantuscygni	k3. P possila	k4. P minor	k5. P volitans	14 Dinama liforalia
			Class	a. Gymnodinium	b. Amphidinium				c. Togula	d. Galeidinium	f. Katadinium	h. Prorocentrum	a. Prymensium		a. Rhodomonas	b. Chroomonas	c. Cryptomonas	d. Goniomonas		e. Hemiselmis	a. Pyramimonas			b. Tetraselmis	c. Chlamydomonas	a. Paranema	b. Rhynchomonas	c. Anisonema		d. Bodo		e. Metanema		f. Euterpiella	h. Heteronema	j. Cryptaula1	k. Petalomoonas				1 Dinama
			Div.	Dinophyta									Haptophyta		Cryptophyta						Cholorophyta					Euglenophyta															
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			m. Ploeotia				N.Notosolenus	o. Urceolus	p. Jenningsia	g. Olisthodiscus		j. Actinomonas	k. Acanthocorbis	j. Cafeteria	a. Protaspis			b. Metromonas	The second secon
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Processed data					
	dec/jan	feb	mar	apr	june
	Huk	Huk	Huk	Huk	Huk
Sobs = Istak OR Zubizarreta	11	17	29	20	38
S10 = Only Istak	7	9	16	9	8
S02 = Only Zubizarreta	4	7	10	7	24
S12 = Both Istak & Zubizarreta	0	1	3	4	6
Diff = S10 - S02	3	2	6	2	-16
p = 2*\$12 / (Sobs+\$12)	0.00	0.11	0.19	0.33	0.27

S12	24
Sobs	57
P = (s10 + s02)/sobs	0.579
sd = sqrt( P(1-P)/Sobs )	0.065
P low	0.462
P high	0.723
p = 2*24/(57+24)	0.59259
p = 1 - P/(2-P)	0.59259
p low	0.43
p high	0.70

Calculated range for p: 0.43 to 0.7

## Appendix C- Probabilistic analyses results of all sampled months-Huk and Nesset locations

#### Processed data from appendix A

	aug.14	sep.14	Oct. 2014	no <b>v.14</b>	mar.15	apr.15	jun.15
Sobs = Nesset or Huk	36	34	32	23	36	47	51
S10 = Only Nesset	22	23	20	17	13	29	30
S02 = Only Huk	7	1	3	2	13	7	5
S12 = Both Nesset and Huk	7	10	9	4	10	11	16
		Г					
S10+S02	29	24	23	19	26	36	35

#### Probabilistic analyses results in August 2014

aug.14									
n	36	36	36	36	36	36	36	36	36
р	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7
2pl	0.8235	0.7879	0.7500	0.7097	0.6667	0.6207	0.5714	0.5185	0.4615
K <sub>cri</sub>									
18	1.000	1.000	0.999	0.994	0.972	0.905	0.758	0.523	0.264
19	1.000	1.000	0.997	0.984	0.942	0.836	0.643	0.391	0.167
20	1.000	0.999	0.991	0.965	0.891	0.739	0.513	0.271	0.097
21	0.999	0.996	0.979	0.928	0.813	0.619	0.380	0.172	0.051
22	0.998	0.988	0.954	0.868	0.707	0.484	0.260	0.100	0.025
23	0.994	0.971	0.908	0.777	0.578	0.350	0.162	0.052	0.011
24	0.983	0.937	0.833	0.658	0.438	0.232	0.091	0.025	0.004
25	0.959	0.877	0.725	0.517	0.303	0.139	0.046	0.010	0.001
26	0.911	0.781	0.588	0.372	0.190	0.074	0.021	0.004	0.000
27	0.828	0.650	0.436	0.241	0.105	0.035	0.008	0.001	0.000
28	0.704	0.494	0.290	0.138	0.051	0.014	0.003	0.000	0.000
29	0.545	0.333	0.168	0.068	0.022	0.005	0.001	0.000	0.000
30	0.371	0.195	0.083	0.029	0.008	0.001	0.000	0.000	0.000
31	0.214	0.095	0.034	0.010	0.002	0.000	0.000	0.000	0.000
32	0.099	0.037	0.011	0.003	0.000	0.000	0.000	0.000	0.000
33	0.035	0.011	0.003	0.001	0.000	0.000	0.000	0.000	0.000
34	0.008	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
35	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Probabilistic analyses results in September 2014

sep.14									
n	34	34	34	34	34	34	34	34	34
р	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7
2pl	0.8235	0.7879	0.7500	0.7097	0.6667	0.6207	0.5714	0.5185	0.4615
K <sub>cri</sub>									
18	1.000	0.999	0.996	0.980	0.933	0.822	0.629	0.384	0.167
19	1.000	0.998	0.988	0.956	0.874	0.718	0.493	0.261	0.095
20	0.999	0.993	0.972	0.912	0.787	0.590	0.358	0.162	0.049
21	0.996	0.982	0.939	0.840	0.670	0.450	0.238	0.091	0.023
22	0.989	0.958	0.881	0.736	0.532	0.315	0.143	0.046	0.009
23	0.972	0.912	0.789	0.603	0.388	0.200	0.077	0.021	0.003
24	0.936	0.833	0.664	0.455	0.256	0.113	0.037	0.008	0.001
25	0.868	0.715	0.513	0.310	0.151	0.057	0.016	0.003	0.000
26	0.758	0.564	0.357	0.187	0.078	0.025	0.006	0.001	0.000
27	0.607	0.397	0.218	0.098	0.035	0.009	0.002	0.000	0.000
28	0.430	0.243	0.114	0.043	0.013	0.003	0.000	0.000	0.000
29	0.259	0.124	0.049	0.016	0.004	0.001	0.000	0.000	0.000
30	0.126	0.051	0.017	0.004	0.001	0.000	0.000	0.000	0.000
31	0.046	0.015	0.004	0.001	0.000	0.000	0.000	0.000	0.000
32	0.011	0.003	0.001	0.000	0.000	0.000	0.000	0.000	0.000
33	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

## Probabilistic analyses results in October 2014

okt.14									
n	32	32	32	32	32	32	32	32	32
р	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7
2pl	0.8235	0.7879	0.7500	0.7097	0.6667	0.6207	0.5714	0.5185	0.4615
K <sub>cri</sub>									
18	0.999	0.997	0.984	0.946	0.856	0.694	0.473	0.251	0.093
19	0.998	0.990	0.962	0.892	0.757	0.558	0.335	0.152	0.047
20	0.994	0.974	0.920	0.807	0.630	0.414	0.216	0.083	0.021
21	0.983	0.940	0.846	0.688	0.484	0.279	0.125	0.040	0.008
22	0.956	0.877	0.737	0.543	0.338	0.169	0.064	0.017	0.003
23	0.903	0.776	0.594	0.389	0.211	0.090	0.029	0.006	0.001
24	0.809	0.635	0.432	0.248	0.115	0.042	0.011	0.002	0.000
25	0.669	0.467	0.278	0.137	0.054	0.017	0.004	0.001	0.000
26	0.493	0.299	0.153	0.064	0.022	0.006	0.001	0.000	0.000
27	0.311	0.161	0.070	0.025	0.007	0.001	0.000	0.000	0.000
28	0.159	0.069	0.025	0.007	0.002	0.000	0.000	0.000	0.000
29	0.061	0.022	0.007	0.002	0.000	0.000	0.000	0.000	0.000
30	0.016	0.005	0.001	0.000	0.000	0.000	0.000	0.000	0.000
31	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

#### Probabilistic analyses results in November 2014

nov.14									
n	23	23	23	23	23	23	23	23	23
р	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7
2pl	0.8235	0.7879	0.7500	0.7097	0.6667	0.6207	0.5714	0.5185	0.4615
K <sub>cri</sub>									
14	0.988	0.961	0.904	0.801	0.651	0.469	0.287	0.141	0.052
15	0.962	0.905	0.804	0.657	0.481	0.304	0.160	0.067	0.020
16	0.904	0.801	0.654	0.481	0.310	0.170	0.076	0.026	0.007
17	0.792	0.641	0.468	0.303	0.169	0.080	0.030	0.009	0.002
18	0.617	0.443	0.283	0.158	0.076	0.030	0.010	0.002	0.000
19	0.402	0.250	0.137	0.065	0.026	0.009	0.002	0.000	0.000
20	0.202	0.106	0.049	0.020	0.007	0.002	0.000	0.000	0.000
21	0.068	0.030	0.012	0.004	0.001	0.000	0.000	0.000	0.000
22	0.011	0.004	0.001	0.000	0.000	0.000	0.000	0.000	0.000

## Probabilistic analyses results in March 2015

mar.15									
n	36	36	36	36	36	36	36	36	36
р	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7
2pl	0.8235	0.7879	0.7500	0.7097	0.6667	0.6207	0.5714	0.5185	0.4615
K <sub>cri</sub>									
20	1.000	0.999	0.991	0.965	0.891	0.739	0.513	0.271	0.097
21	0.999	0.996	0.979	0.928	0.813	0.619	0.380	0.172	0.051
22	0.998	0.988	0.954	0.868	0.707	0.484	0.260	0.100	0.025
23	0.994	0.971	0.908	0.777	0.578	0.350	0.162	0.052	0.011
24	0.983	0.937	0.833	0.658	0.438	0.232	0.091	0.025	0.004
25	0.959	0.877	0.725	0.517	0.303	0.139	0.046	0.010	0.001
26	0.911	0.781	0.588	0.372	0.190	0.074	0.021	0.004	0.000
27	0.828	0.650	0.436	0.241	0.105	0.035	0.008	0.001	0.000
28	0.704	0.494	0.290	0.138	0.051	0.014	0.003	0.000	0.000
29	0.545	0.333	0.168	0.068	0.022	0.005	0.001	0.000	0.000
30	0.371	0.195	0.083	0.029	0.008	0.001	0.000	0.000	0.000
31	0.214	0.095	0.034	0.010	0.002	0.000	0.000	0.000	0.000
32	0.099	0.037	0.011	0.003	0.000	0.000	0.000	0.000	0.000
33	0.035	0.011	0.003	0.001	0.000	0.000	0.000	0.000	0.000
34	0.008	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.000
35	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Probabilistic analyses results in April 2015

apr.15				April 20					
n	47	47	47	47	47	47	47	47	47
р	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7
2pl	0.8235	0.7879	0.7500	0.7097	0.6667	0.6207	0.5714	0.5185	0.4615
K <sub>cri</sub>									
23	1.000	1.000	1.000	0.999	0.991	0.954	0.839	0.601	0.298
24	1.000	1.000	1.000	0.997	0.981	0.919	0.757	0.486	0.205
25	1.000	1.000	0.999	0.993	0.962	0.865	0.657	0.372	0.133
26	1.000	1.000	0.997	0.984	0.930	0.790	0.545	0.268	0.080
27	1.000	0.999	0.994	0.967	0.881	0.695	0.428	0.181	0.045
28	1.000	0.998	0.986	0.938	0.811	0.585	0.316	0.114	0.023
29	0.999	0.994	0.970	0.891	0.718	0.466	0.219	0.066	0.011
30	0.998	0.987	0.941	0.822	0.608	0.349	0.141	0.036	0.005
31	0.995	0.971	0.894	0.729	0.486	0.244	0.084	0.018	0.002
32	0.987	0.942	0.824	0.616	0.365	0.159	0.046	0.008	0.001
33	0.971	0.893	0.728	0.490	0.255	0.095	0.023	0.003	0.000
34	0.941	0.819	0.610	0.364	0.164	0.052	0.011	0.001	0.000
35	0.887	0.716	0.478	0.249	0.096	0.026	0.004	0.000	0.000
36	0.804	0.588	0.346	0.156	0.051	0.012	0.002	0.000	0.000
37	0.689	0.447	0.228	0.088	0.024	0.005	0.001	0.000	0.000
38	0.548	0.309	0.135	0.044	0.010	0.002	0.000	0.000	0.000
39	0.396	0.191	0.071	0.019	0.004	0.001	0.000	0.000	0.000
40	0.254	0.103	0.032	0.007	0.001	0.000	0.000	0.000	0.000
41	0.140	0.048	0.012	0.002	0.000	0.000	0.000	0.000	0.000
42	0.065	0.018	0.004	0.001	0.000	0.000	0.000	0.000	0.000
43	0.024	0.006	0.001	0.000	0.000	0.000	0.000	0.000	0.000
44	0.007	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
45	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
46	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Probabilistic analyses results in June 2015

jun.15			Suits III s						
n	51	51	51	51	51	51	51	51	51
р	0.3	0.35	0.4	0.45	0.5	0.55	0.6	0.65	0.7
2pl	0.8235	0.7879	0.7500	0.7097	0.6667	0.6207	0.5714	0.5185	0.4615
K <sub>cri</sub>									
27	1.000	1.000	0.999	0.995	0.971	0.884	0.681	0.384	0.133
28	1.000	1.000	0.999	0.989	0.947	0.819	0.575	0.283	0.082
29	1.000	1.000	0.996	0.978	0.908	0.735	0.462	0.196	0.047
30	1.000	0.999	0.992	0.957	0.851	0.634	0.353	0.128	0.025
31	1.000	0.997	0.982	0.923	0.773	0.522	0.254	0.078	0.013
32	0.999	0.994	0.965	0.872	0.677	0.408	0.171	0.044	0.006
33	0.998	0.986	0.934	0.799	0.565	0.300	0.108	0.023	0.003
34	0.995	0.970	0.885	0.705	0.448	0.207	0.063	0.011	0.001
35	0.988	0.941	0.815	0.593	0.333	0.133	0.034	0.005	0.000
36	0.973	0.894	0.720	0.471	0.231	0.079	0.017	0.002	0.000
37	0.945	0.822	0.606	0.350	0.149	0.043	0.008	0.001	0.000
38	0.897	0.725	0.479	0.242	0.088	0.022	0.003	0.000	0.000
39	0.823	0.604	0.352	0.153	0.048	0.010	0.001	0.000	0.000
40	0.718	0.470	0.238	0.089	0.023	0.004	0.000	0.000	0.000
41	0.588	0.336	0.146	0.046	0.010	0.001	0.000	0.000	0.000
42	0.443	0.217	0.080	0.021	0.004	0.000	0.000	0.000	0.000
43	0.301	0.125	0.039	0.009	0.001	0.000	0.000	0.000	0.000
44	0.181	0.063	0.016	0.003	0.000	0.000	0.000	0.000	0.000
45	0.093	0.027	0.006	0.001	0.000	0.000	0.000	0.000	0.000
46	0.040	0.010	0.002	0.000	0.000	0.000	0.000	0.000	0.000
47	0.014	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
48	0.004	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000
49	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
50	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

# Appendix D- example photos of ciliates

