Dinoflagellate diversity and dynamics in Outer Oslofjorden as revealed by molecular methods

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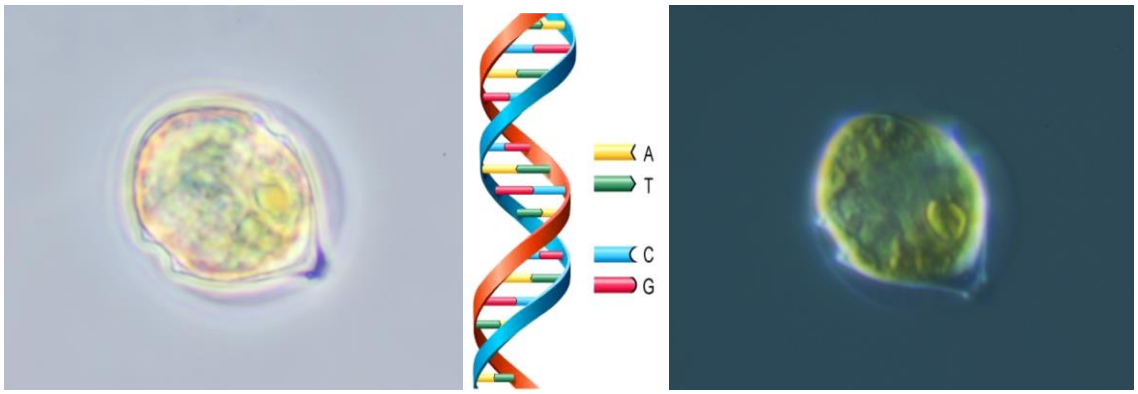

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SUMMARY

The Dinoflagellates are an intriguing and diverse protist group in terms of morphology, ecology and geographical distribution amongst others. The proportion of heterotrophic and photosynthetic taxa are rougly even. However, many of the photosynthetic taxa are actually mixotrophic. Together with the diatoms and haptophytes dinoflagellates vastly contributes to the primary production in marine environments. Besides this positive feature, many dinoflagellate taxa can produce potent toxins leading to sea-food borne dieseases and fish kills when they occur in high concentration. Given the importance of dinoflagellates they are vigorously studied from many aspects. Molecular biology and bioinformatics development are utilized by researchers of dinoflagellates. In this work diversity and seasonal occurence is investigated; yearly dynamics is discussed based on 12 selected OTU (Operational Taxonomic Unit).

Samples were collected between September 2009 and June 2011 in Outer Oslofjorden, OF2 station. A single cast of CTD rosette with Nishkin bottles attached was done with monthly intervals. Fluorescence, nutrients (Nitrogen, Silica, Phosphorous), chlorophyll-a, as well as irradiance was measured on each occasion. Water samples obtained by Nishkin bottles from 1 m depth were filtered with 45 μm and 3 μm filters in order to get the nanoplankton size fraction. Then, the total ribosomal RNA of the SSU V4 region was extracted and converted to cDNA. The SSU V4 rDNA region was sequenced. The DNA was extracted from culture stocks of strains UiO306, UiO307 and UiO312 and the SSU and LSU rDNA regions were sequenced. Data from the 454-pyrosequencing was processed using Qiime. rDNA sequences of the cultured species were edited in BioEdit. RaxML phylogenetic tree was built to unravel their identity.

In total 374 dinoflagellate OTUs were detected by the 454-pyrosequencing approach at station OF2 in Outer Oslofjorden during the period September 2009-June 2011. The most abundant taxa, based on read abundance, were Gymnodiniales and Peridiniales, which had representatives in all samples. Syndiniales, Dinophysiales and Gonyaulacales were also important components of the dinoflagellate communities. Strains UiO306 and UiO312 were identified as *Pelagodinium béii*, whereas UiO307 was *Scrippsiella donghaiensis*. Dinoflagellate diversity showed seasonal variations, being the most diverse in autumn and the lowest in the late winter/early spring. Both *P. béii* and *S. donghaiensis* were detected in Outer Oslofjorden thanks to the 454-pyrosequencing data.

The applied methods have flaws, which are important to be aware of. The main source of errors of 454-pyrosequencing is homopolymers and the mistakes made during the PCR (single base errors). Collection of samples assumed heterogeneous phytoplankton distribution in the water column, whereas phytoplankton communities have rather patchy pattern. *Scrippsiella donghaiensis* occurred more frequently than *P. béii* in Outer Oslofjorden.

Using more sampling occasions, including sequencing of additional depths and including more genetic information (LSU rDNA, ITS) would help to provide a more reliable and exhaustive desciption of the detected seasonality of dinoflagellate diversity and dynamics.

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1. INTRODUCTION

1.1. Outer Oslofjorden and Skagerrak

The approximately 100 km long Oslofjorden can be subdivided into an inner, middle and outer part, separated from each other by a sill (Alve & Nagy 1990). The northernmost part called Inner Oslofjorden is located south from Oslo, which has a harbour along its northern coastline and is further partitioned to Bunnefjorden and Vestfjorden. Outer Oslofjorden lies south of Drøbaksundet, which is the narrowest segment of the Oslofjorden with a width of 1 km only. There is a sill situated at this 17 km long part. The water depth thus is shallow, no more than 19 m deep. From the south at Fulehuk-Missingen Oslofjorden is bordered by the Skagerrak. Outer Oslofjorden has an irregular bottom topology with depth ranging from 200-350 m, finding the deepest water column in the adjacent Skagerrak is ca. 700 m (Norskerenna). The boundary between the Skagerrak and the main part of the North Sea runs from Hanstholm on Jutland, the west coast of Denmark, to Lindesnes, on the south coast of Norway (Hostyeva 2011).

Transport of water masses in and out of the Outer Oslofjorden happens through the Skagerrak, which is influenced by the Baltic Sea through the Kattegat and the North Sea as well as Atlantic Ocean. Due to the properties (salinity, temperature and density among others) of various water supplies, which meet one another, result in a heterogeneous environment. The neighbouring Skagerrak is a highly productive area with a yearly fish production of 7 g m^{-2}, which is almost double as in the North Sea (Danielssen et al. 1997). Therefore the region is of great economic importance. While tidal forces control the hydrographic conditions in the North Sea, these are mainly ruled by meteorological variables in the Outer Oslofjorden and Skagerrak area.

The North Atlantic current bifurcates and sends a warm and highly saline branch to the North Sea, which reaches the Skagerrak region. The Atlantic water encounters the low salinity Baltic current on its way from the Baltic Sea along the Swedish east coast. From the German coast a high salinity current called Jutland current enters the North Sea and eventually meets with the Atlantic current moving together towards the Baltic current. These three flows unite and take an anticlockwise turn proceeding towards the Norwegian Sea along the Norwegian coast as the Norwegian Coastal Current (Pederstad et al. 1993). Due to the anticlockwise

circulation, upwelling occurs in the Skagerrak region supporting a significant primary production (Rodhe 1996).

The Oslofjorden is essentially an estuary of great hydrographic stability except in the winter months when the stratification tends to weaken (Alve & Nagy 1990). Weakened stratification enables mixing of water layers. This phenomenon results in nutrient mixing up to surface layers, which has significant implications on phytoplankton ecology. The low saline stratified surface layer is confined to the upper narrow water column. The thickness of this layer and the stratification is greater close to the Swedish and Norwegian coasts than to the open Skagerrak. The main reason is the river outflow from the Glomma, Skienselva and Göta rivers to the Oslofjorden and the Northern Skagerrak (Pederstad et al. 1993). At the onset of the spring, melting ice accounts for a huge amount of freshwater outflow since the Outer Oslofjorden is the drainage area to South East Norway.

1.1. Dinoflagellates

1.1.1. Phylogenetic location in domain Eukaryota

Dinoflagellates is a highly diverse monophyletic group of Protists that forms one of the major lineages of domain Eukaryota (Baldauf 2008). Protists are considered unicellular or colonial eukaryotic organisms with polyphyletic phylogeny. Indeed we may encounter with protists in all major eukaryotic lineages including Rhizaria, Alveolata, Stramenopila Archaeplastida, Excavata and Hacrobia (Keeling 2013). According to Keeling 2013 dinoflagellates are placed within the supergroup Stramenopila-Alveolata-Rhizaria (SAR) rejecting the previous concept of Chromalveolates, where dinoflagellates were believed to belong. The supergroup SAR is obviously a significant lineage of eukaryotic organisms since they accounts for about half of the recognized species of protists and algae (Cavalier-Smith 2004). Together with the Ciliates and the Apicomplexans, Dinoflagellates build up the Alveolates group (Harper et al. 2005). Molecular phylogeny and the presence of cortical alveoli underlying their plasma membranes unite these groups of protists (Fast et al. 2002).

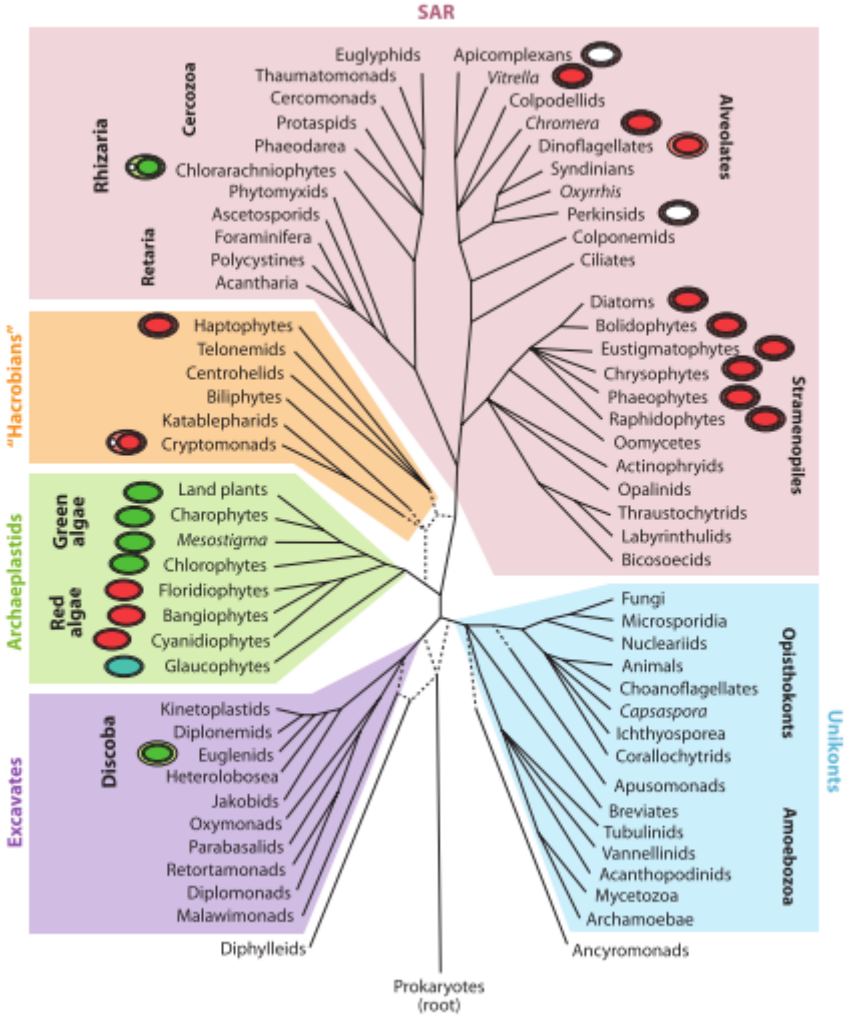


Figure 1.1: Consensus phylogenetic relationship within domain Eukaryota according to Keeling, 2013.

1.1.2. Basic morphology of dinoflagellates

Traditionally classification of Dinoflagellates relies on vegetative cell and cyst morphology, plate tabulation and ultrastructure (Zardoya et al. 1995). Based on traditional plate tabulation gymnodinoid, suessioid, peridinioid, gonyaulacoid, dinophysoid, and prorocentroid dinoflagellates can be distinguished (Taylor 1987). Morphological classification utilizes the different pattern, shape and number of amphiesmal plates. The dinoflagellate cell covering or amphiesma basically consists of a continuous outermost membrane, the plasma membrane. This membrane is underlain by a single layer of flattened vesicles (amphiesmal vesicles) that are usually appressed at their edges (Höhfeld & Melkonian, 1992). The amphiesmal vesicles may appear empty or with cellulosic plates and cells are marked as naked (athecate) or

armored (thecate) respectively (Eaton 1980). Gymnodinioids possess no thecal plates, thus for identification other features are used such as shape of apical furrow, displacement of the cingulum and dimensions of the cell among others. The rest of the groups exhibit species with defined amphiesmal plates. The plate pattern varies greatly among groups and to a different extent within groups as well. Suessoids have numerous, irregularly shaped plates. Peridinioids and Gonyalucoids are covered by 4 rows of thecal plates (apical, precingular, cingular, postcingular, antapical) and various pore plate complexes may be present. Dinophysoids have 4 big plates, two-two at the hypotheca (posterior part) and epitheca (anterior part) respectively. While Prorocentroid dinoflagellates show a highly reduced plate tabulation possessing 2 large plates. Nomenclature of dinoflagellate plate tabulation is summarized in the Figure 1.2.

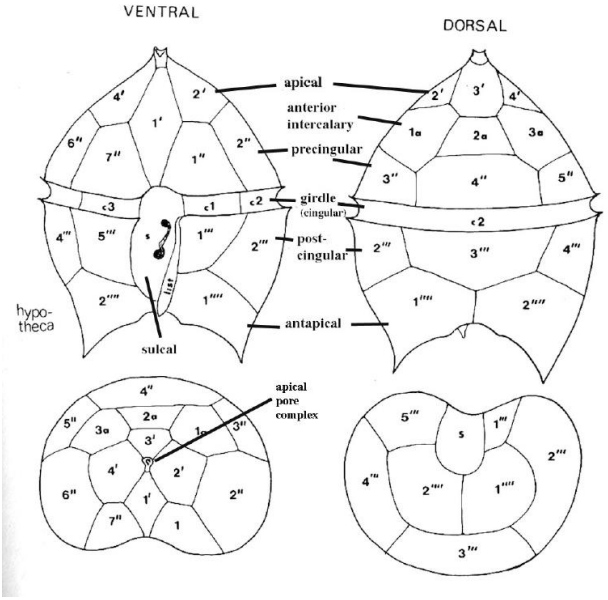


Figure 1.2: Illustration of a standard tabulation pattern of a thecate dinoflagellate cell with kofoidian nomenclature. Upper part of the figure shows the different dimensions of a dinoflagellate. Ventral view is shown on the left, and dorsal view is shown on the right. Down the apical view on the left and the antapical view on the right is drawn. Plates are grouped together in rings from apical to antapical end. The particular plates are named as apical pore complex (Po), apical (´), anterior intercalary (a), precingular (´´), girdle cingular (c), postcingular (´´´), sulcal (s) and antapical (´´´´) plates. Numbering goes round from left to right using Arabic numbers. Tabulation pattern varies from taxa to taxa.

Besides the amphiesma dinoflagellates also own other remarkable morphological and ultrastructural features. A girdle (cingulum) divides the cell into an epitheca and a hypotheca. Their size (equal, unequal) and shape (cone, spherical or flattened) is variable among species. Cells may have one or more protrusion such as species of the genus *Ceratium* or *Protoperidinium*. Cingulum can be placed median, more posteriorly or anteriorly.

Displacement is also an important feature to consider for identification. Dinoflagellates have two dissimilar flagella emerging anteriorly (desmokont) or ventrally (dinokont). Dinoflagellates with desmokont flagella lack furrow. Dinokont dinoflagellates have a longitudinal flagellum, which emerges from the sulcus and helps to propel the cell forward. A transverse ribbon-shape flagellum with hairs resting in the cingulum gives a rotatory movement and also moves the cell forward. Having flagella, they are greatly motile creatures (Graham et al. 2008). This enables them to actively change position in order to find the most optimal conditions to utilize the nutrients (N, P,) and micronutrients (Se, Fe) essential for growth.

The characteristic nucleus called dinokaryon is regarded one of the most extraordinary ultrastructural compartment in dinoflagellate cells. It hosts a huge amount of DNA and chromosomes are condensed trough the life cycle except during the DNA replication. Despite the findings of Fensome et al. 1999 that dinoflagellates completely lack histone proteins, Wong et al. 2003 found evidence for existence of histone-like proteins . DNA content of *Alexandrium tamarense* nucleus reaches 200 pg·cell-1. The human cell has 3.2 pg·cell-1 DNA (Hackett et al. 2005). Mitosis in dinoflagellates is termed amitosis. Here, the nuclear envelope remains intact and the spindle is extra-nuclear. When nuclear division occurs microtubule bundles enter the nucleus trough channels and chromosomes attach to them through the nuclear membrane. Nuclear envelope constricts and microtubule bundles pull the nucleus apart to two daughter nuclei. Dinoflagellates lack nucleosomes as well.

About one-half of the extant species (ca. 2000) are autotrophic meaning that they bear photosynthetic plastids and pigments. The commonest plastid type has thylakoids in groups of three and is surrounded by a triple-membrane envelope. However, those present in the genus *Dinophysis* are surrounded by only 'a pair of membranes and have thylakoids with dense, granular lumens in pairs (Fensome et al. 1999). Similar to most eukaryotes chlorophyll-a is the antennae pigment of the Light Harvesting system (LHC) (Iglesias & Trench 1997). Peridinin is the main accessory pigment together with chlorophyll-c (Yoon et al. 2002; Durnford et al. 1999; Iglesias & Trench 1997). In addition to the peridinin containing chloroplast, other types of chloroplasts with various pigments have also been reported from dinoflagellates. Such pigments are pennate diatom originated fucoxanthin in *Peridinium balticum,* haptophyte derived 19'hexanoyloxy fucoxanthin in the famous Florida red tide causing *Karenia brevis,* phycobilins in Dinophysis species obtained from cryptomonads by

kleptoplastidy are examples (Tengs et al. 2000). In some Dinoflagellates such as *Gymnodinium chlorophorum* chloroplast without chlorophyll-c but with chlorophyll-b can be observed.

1.1.3. Ecology of dinoflagellates

Dinoflagellate diversity is astonishing regarding not only morphological but also biological and ecological features, as well as spatio-temporal distribution (Not et al. 2012). Various trophies have been described. As mentioned before, one half of dinoflagellates have chloroplasts, thus they are photosynthetic organisms. However, most of these species are capable of feeding by heterotrophic means being mixotrophic organisms. Remaining proportion is obligatory heterotrophs. This means that they do not utilize the energy from the sun, but feed on particles, other dinoflagellates and other planktonic organisms. Due to complexity in nutrition sources dinoflagellates take up various ecological niches. Photosynthetic and/or mixotrophic genera are important component of both marine and freshwater phytoplankton contributing greatly to primary production, energy flux, and nutrient cycles. Heterotrophic dinoflagellates can also have significant effect on phytoplankton community through grazing. Since these types are hard or impossible to culture we know relatively little of their impact (Stern et al. 2010; Burkholder et al. 2006). Thus importance of heterotrophic orders may be overlooked as well as significance of the parasitoid forms (Blastodineans, Syndinians). Furthermore algae that have been called zooxanthellae include members of the classes Bacillariophyceae, Cryptophyceae, Dinophyceae, and Rhodophyceae have been reported. At least 8 genera of Dinophyceae are known to have symbiotic members (Rowan 1998). The most studied genus is *Symbiodinium*. These dinoflagellates mainly have been described from tropical and subtropical reef building cnidarians in shallow waters. Together with the host they are responsible for the so-called warm water coral reefs (Caribbean coral reefs, Great Barrier Reef at East-Australia). These coral reefs host an enormous diversity of life ranging from protists to vertebrates. Thus these ecosystems accounts for huge amount of carbon fixation playing role in shaping the climate of Earth. *Symbiodinium* species can be found in symbiotic association with Platyhelminthes, molluscs and protist as well (Lajeunesse 2001; Baker 2003).

However, dinoflagellates inhabit many different habitats thanks to their complexity (Yamaguchi et al. 2011). They reach the highest abundance in estuaries and coastal-waters, where the human interaction with marine environment is the most prevalent. Besides tropical and temperate species, a number of dinoflagellates has been recorded from snow and sea ice (Taylor et al. 2007; Marret & Zonneveld 2003; Okolodkov & Dodgeb 1996). Many dinoflagellates produce resting spores called cysts, which can survive for substantial amount of time and disturbance. Cysts may be transported with ballast water of cargo vessels together with other algae (Hallegraeff & Bloch 1992), influencing the biogeography and algae spread.

1.1.4. Harmful dinoflagellates

Besides the positive influences mentioned before, some species produce potent toxins which can be amassed in the food web (Abdenadher et al. 2012; Faust & Gulledge 2002). Marine algal toxins are responsible for an array of human illnesses associated with consumption of seafood and, in some cases, respiratory exposure to aerosolized toxins (Van Dolah 2000). In addition to hazardous public- health effects, algal toxins are responsible for extensive die-offs of fish and shellfish and have been implicated in the episodic mortalities of marine mammals, birds, and other animals dependent on the marine food web (Van Dolah 2000).

Toxin producing algae are usually present in low cell densities. In such instances they are not sources of nuisance, with exception of highly toxic species. However when environmental conditions, both biotic and abiotic, become favourable to toxic species they suddenly reach high cell numbers. Such events are labelled harmful algal blooms (HAB) by researchers and as red tides in public notions (Gilbert et al. 2005). The term red tide is misleading since non-toxic species may discolour waters in high cell density without harmful effect (Anderson et al. 2012). At HABs conditions toxin concentration exceeds the safe level, as cell density increases and accumulates in the food webs. This type of HAB is deleterious to humans upon consumption of food sources concentrating algal toxins. Most often filter feeding organisms, including shellfish, become vector for diseases caused by algal toxins. In addition fishes and vertebrates can be concerned as well. Two further kinds of HABs are known to exist. First the bloom forming algae is non-toxic, but it can reach such an extreme cell densities that threaten other life forms (Backer & Mcgillicuddy 2006). Finally the algae is harmful to fishes and invertebrates, but not to humans. *Karenia mikimotoi* (as *Gyrodinium aureolum*) is an example

of dinoflagellates releasing ichthyotoxins (Blasco et al. 1996). These toxins act on biological membranes and the ichthyotoxic effect is assumed to be due to increased permeability in fish gills resulting in disturbed ion balance (Edvardsen & Imai 2006). The last type of HAB leads to losses of aquaculture and have economic concerns.

Dinoflagellates are the most common deleterious blooming organisms in the marine environment (Shankle et al. 2004; Hakanen et al. 2012). Approximately 2 % of the described algal species are reported to form HABs. Dinoflagellates exhibit most of the bloom forming species. Paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), neurotoxic shellfish poisoning (NSP), ciguatera fish poisoning (CFP), azaspiracid poisoning (AZP) and Pfiesteria and Estuary-associated syndrome can be distinguished and are caused by dinoflagellate species (Hallegraeff et al. 2003; Touzet et al. 2010; Plumley 1997).

Our knowledge on the biochemistry of toxic species, HABs, and seafood borne diseases increase as researcher find new toxins. Spirolides (cyclic imine), azaspiracids, yessotoxins and pinnatoxins (Cyclic imine) produced by *Alexandrium ostenfeldii, Azadinium spinosum, Protoceratium reticulatum, Vulcanodinium rugosum* respectively are examples of newly found harmful chemicals from dinoflagellates.

1.2. Molecular biology and Bioinformatics

Research has been conducted on phytoplankton including dinoflagellates for a century. In its infancy and even more recent scientific works were mainly based on morphological and ecological characters such as e.g. morphology of thecal plates. The problem with morphological identification is that many species, especially pico- and nanoflagellates, lack distinctive features (Savin et al. 2004). Presence of cryptic species has also been documented as a hindering phenomenon for species level identification. Molecular biology started to develop tremendously in the 1990's. The methods have advantages that may overcome the problems mentioned above. Phycologists also took advantage of an array of molecular procedures to approach scientific questions; PCR (polymerase chain reaction), microarrays, dot-blot, hybridizations, flow cytometry, DNA sequencing and molecular probes are examples of widely used molecular methods in phytoplankton investigations.

Recently, sequencing technologies have gained huge importance. Next Generation Sequencing (NGS) technologies giving rise to high-throughput sequencing methods have gradually replaced their forerunner the so-called automated Sanger sequencing (Metzker 2010; Voelkerding et al. 2009). Sanger sequencing is useful to discover sequences of isolated individual genes. The NGS technologies allow researchers to explore diversity, and microbial community composition (such as marine, soil and animal intestinal environments), as well as detect rare or novel species at a lower cost than Sanger sequencing of environmental clone libraries. NGS also allows whole genome sequencing of an organism at a much higher rate and lower cost than with Sanger sequencing.

Since the essence of all phylogenetic analysis is a proper alignment, programs capable of sequence editing, and alignment execution (such as ClustalW, Mafft or T-coffe) are essential and have improved the last decade. Phylogenetic tree building programs have also become faster and more easy to use, and have favoured scientific research within systematics. Mega5, BioEdit, Jalview are software widely available with such features. However, the amount of available packages is high and some may be preferred over others regarding specific tasks or based merely on personal preferences. By the development of computational resources, scientists have also better access to execute research using main phylogenetic approaches (maximum likelihood, Mr Bayes, Neighbour joining, Maximum parsimony etc.) on large datasets. In our society, based on information technology, we can surely expect further developments and significant refinement of research in environment, technology, health and many more fields.

1.3. The aim of the study

In marine ecosystems algae have a function similar to vascular plants in terrestrial ecosystems. Oceans and coastal communities are based on microalgae; mainly pico-, nano-, and microphytoplankton as primary producers. Dinoflagellates are among these essential organisms. Due to their morphological, ecological and biogeographical complexity they make up a significant part of phytoplankton communities besides Stramenopiles and Haptophytes. Therefore it is no wonder that this taxon is one of the most studied organisms. However, we have only rough estimates of the true diversity up to date. Also species identification is often problematic due to cryptic species or lack of distinctive morphological characters. Since their

ecological importance and the fact that many dinoflagellate species produce potent toxins causing concerns of human health and economy, it is of great importance to develop further our knowledge of taxonomy, biodiversity, biochemistry and ecology of dinoflagellates.

The primary goal of this MS project is to explore the diversity and seasonal dynamics of small dinoflagellate taxa in Outer Oslofjorden with the help of molecular DNA techniques such as 454 pyrosequencing and Sanger sequencing of newly isolated strains. The study focuses on the dinoflagellate community composition, diversity through seasons and environmental variables. Available data (454 pyrosequencing and environmental data) from monthly samples taken in 2009-2011 was used, and also own sequence data from three investigated strains (UiO306, UiO307, UiO312). A phylogenetic overview of the strains is also included.

The following questions are addressed: I. which dinoflagellate taxa are present in Outer Oslofjorden (station OF2) during a two years period as revealed by 454-pyrosequencing? II. When are the different taxa present during the year? Are there seasonal variations? Are some taxa there all year around? III. At what environmental conditions (temperature, salinity, nutrient concentration, stratification, and light) are certain dinoflagellates present? IV. How do light microscopic analyses conducted on the same samples compare with the results from the 454? V. What is the phylogenetic and taxonomic placement of the cultured strains UiO312, UiO306 and UiO307? VI. Are these cultured species found in Outer Oslofjorden according to the 454-pyrosequencing data? If applicable, when are they present?

2. MATERIALS AND METHODS

2.1. Sampling areas

The environmental data (salinity, temperature, density), water and biological samples, were collected in the framework of the projects HAPTODIV and BioMarks by others, mainly Elianne Sirnæs Egge. The samples were taken at OF2 station (59.18666 N, 10.691667 E), outer Oslofjorden between May 2009 and June 2011.

Figure 2.1: Location of OF2 station is shown on the broader area (on the left) and in a closer view (on the right). Pictures were obtained from Bing maps. Coordinates are given in decimals.

Information on the origin of the three cultured strains is provided in Table 3.1. Strain UiO306 was collected from Raunefjorden, West Norway; UiO307 from Flekkefjord, South Norway and UiO312 from Oslofjorden, station OF2, South Norway.

2.2. Sampling and origin of environmental and 454-sequence data

Single cast of CTD (Conductivity, Temperature, Depth) rosette was performed on each sampling cruise to measure the salinity, temperature and density by depth along the water column. The rosette was also equipped with a fluorometer that measured the fluorescence. The CTD collected data was sent to a computer on-board Trygve Braarud. Water samples were obtained with Niskin bottles attached to the rosette from 1m depth.

To measure the irradiance a LI-192 Underwater Quantum Sensor and a deck sensor connected to LI-250A Light Meters 157 (LI-COR, NE, USA) were used. The irradiance level ($\mu E\ m^{-2}\ s^{-1}$) was measured on the deck as well as underwater each meter from surface to 18m depth. Normalized underwater values were calculated with the formula:

(Max deck sensor value/deck sensor value)*underwater value

Log irradiance was plotted against depth and the 1% light depth was interpolated from the logarithmic irradiance curve. This depth estimates the boundary of the euphotic zone where photosynthesis is still possible, by a standard light attenuation curve.

For nutrient analysis water samples from various depths (0, 1, 2, 4, 8, 12, 16, 20, 40, 60, 100m) were collected by Niskin bottles For each depth 20 mL of the sampled water was transfered to plastic scintillation vials. The bottles were accordingly labeled with date, depth, station, and project. Samples were then kept frozen until laboratory analysis.

Analysis of nutrients in the laboratory was conducted by Berit Kaasa on an Autoanalyzer (Bran Luebbe AutoAnalyzer 3). Nitrate, nitrite, phosphate and silicate concentrations were determined. The values are calculated by a computer which is the terminal part of the system.

500 mL of water sample from depths 0, 1, 2, 4, 8, 12, 16, 20 m were tapped for Chlorophyll-a in vitro analysis. 2x250 ml water sample from each depth was filtered with GF/F-filters. The filters were stored frozen in glass vials and kept in dark conditions (for this purpose we covered them with aluminium foil). The vials were frozen until measurement was performed at UiO, to avoid chlorophyll-a degradation.

To extract the chlorophyll the filtres in the glass vials were incubated for 30-60 min in 10 mL 90% acetone. Values were then measured using Turner design fluorometer. The fluorometer emits blue light at 440nm which chlorophyll-a absorbs. Chlorophyll-a then emits red light at ca. 665 nm which the fluorometer detects and giving an estimate of chlorophyll-a in vitro.

Protists were obtained with Niskin bottles collecting water from 1m depth. 20 L water was first prefiltered by 45 µm GF/C plankton tissue. The prefiltered water was then filtered by two Isopore polycarbonate membrane filters 3µm and 0.8 µm pore size respectively to retain the nano-size fraction and pico-size fraction. Picoplankton and femtoplankton fraction was not

included in this study. Filters were frozen in liquid nitrogen and stored at -80 °C until total RNA extraction at UiO.

2.3. Cultures and origin of sequence data

Three different dinoflagellate species already available in cultures at the University of Oslo culture collection were included in this study. Information of these strains is given in Table 3.1. The identity of the species was uncertain and was determined by molecular methods described at the DNA Lab work section below.

Table 3.1: Summary of information of cultures included in this study. Code specifies the name as registered in the culture collection of University of Oslo. Data on collection is also shown. Culturing conditions are also presented.

Strains			
code	UiO306	UiO307	UiO312
collected from	Raunefjorden, West Norway	Flekkefjord, South Norway	Oslofjorden, OF2, South Norway
Species identity	*Pelagodinium beii*	*Scrippsiella donghaiensis*	*Pelagodinium beii*
collection date	2011	2011	2012
isolation date	08.2011	10.2011	01.06.2012
isolated by	Wenche Eikrem	Wenche Eikrem, Vladyslava Hostyeva	Wenche Eikrem
culturing conditions			
medium	IMR 1/2	IMR 1/2	IMR 1/2
growth temperature (°C)	16	16	12
salinity (PSU)	30	30	30
light intensity (µmol photons m-2s-1)	95	95	85

The cultures were grown in borosilicate test tubes sealed with a plastic cap. IMR ½ medium with 30 Partial Salinity Unit (PSU) was used for all the three strains. UiO306 and UiO307 was grown in 16 °C, whereas UiO312 was grown in 12 °C climate room at the Marine Biology section, UiO. The number of the microalgal cells in the test tubes increases exponentially through time, becoming too dense for further growth. Density of cultures was checked by inverted microscopy at weekly intervals. In order to maintain the cultures, I diluted them and provided them with new nutrients. Between 5-10 drops were transferred to new 30 PSU IMR ½ medium in new test tubes. Transfer was performed with sterile Pasteur glass pipettes for each transfer in a sterile room equipped with UV light. A flame was applied to prevent contamination. Transfer dates depended on the species cultured, due to the

difference in growth rates. As strains UiO306 and UiO307 showed fast growth, transfer interval was usually every 2-4 weeks. The strain UiO312 developed slowly, thus it was transferred less often.

2.4. DNA lab works

2.4.1. Environmental samples extraction

Total RNA was extracted from 142 mm isopore polycarbonate membrane filters by E.S. Egge as described in Egge et al. 2013. ½ filter, cut with sterilized scissor, was transferred to 15 mL RNase free tubes containing 2.1 mL lysis buffer and 21 µl β-mercaptoethanol. After closing, the tubes were turned upside-down to ensure that the filter was fully soaked in buffer. The tubes were placed on ice and then were shaken in a FastPrep-24 bead-beater with a TeenPrep adapter (MP Biomedicals, Illkirch, France) at 2 x 20 sec 4000 rpm, with a 20 sec break, without beads added to the tubes. Samples that were processed later were frozen at -20 °C. 700 µl lysate was transferred to three extraction columns. Total RNA was extracted according to protocol RNA NucleoSpin II (Macherey-Nagel, Düren, Germany). RNA was eluted in 50-60 µl RNase-free water per column, which was run through the column twice, and the eluates were pooled. RNA concentration was determined using a NanoDrop spectrophotometer (Wilmington, DE, USA) (Egge et al. 2013).

The RNA eluates were checked for residual DNA by running standard PCR with universal eukaryote partial SSU rDNA primers 1F and 300R, annealing temperature 50 °C and 35 cycles. If a PCR product could be detected by gel electrophoresis, the RNA eluate was treated with additional DNase (TURBO DNase kit, Ambion, Austin, TX, USA), according to the protocol from the manufacturer. cDNA was reverse-transcribed from RNA using High-Fidelity 1st Strand cDNA Synthesis Kit (Agilent, Santa Clara, CA, USA) with random primers, according to the protocol from the manufacturer. For the synthesis reaction approximately 100 ng of RNA was added to a mix containing 2.0 µl AccuScript RT Buffer 10x, 3 µl random primers (0.1µg µl-1), 0.8 µl dNTP (final conc. 25 mM of each dNTP) and RNase-free water to a total volume of 16.5 µl. The mix was incubated at 65° C for 5 min before annealing at room temperature for 5 min. Subsequently 2 µl 100 mM DTT (dithiothreitol, reducing agent), 1 µl AccuScript Reverse Transcriptase and 0.5 µl RNase Block ribonuclease inhibitor were added. The reaction was incubated at 25° C for 10 min

before cDNA synthesis took place at 42° C for 75 min in a thermocycler (Mastercycler ep gradient S, Eppendorf, Hamburg, Germany). The synthesis reaction was terminated by incubation at 70° C for 15 min (Egge et al. 2013). The process was performed by E.S. Egge.

2.4.2. Culture samples extraction

The DNA was extracted from the cultures as described below. The SSU (whole sequence) and LSU (domains D1 and D2) ribosomal DNA (rDNA) region were then amplified by Polymerase Chain Reaction (PCR) and sequenced. 12 ml of liquid culture material were transferred into 15 ml centrifuge tubes and were centrifuged for 3 minutes at 2000 rpm at 4°C with an Eppendorf 5810R centrifuge to collect dinoflagellate cells in pellet at the bottom of the tubes. Liquid was discarded and only the pellet was kept. For DNA isolation from the strain UiO312 two times 14.5 ml liquid culture material (due to smaller cell density compared to UiO306 and UiO307) was centrifuged.

DNA extraction of the stock cultures was performed according to the Nucleospin plant II Genomic DNA kit (Macherey-Nagel, Düren, Germany) protocol and isolates were stored frozen in the freezer at -20°C. The SSU and LSU rDNA were amplified by PCR (Mastercycler ep gradient S, Eppendorf, Hamburg, Germany). 25 µl reaction volume was prepared for each PCR tube in accordance with the GoTaq green master mix protocol. 1F (forward) and 1528R (reverse) primers for the SSU, DIR (forward) and D2C (reverse) primers for the LSU with concentration of 5 nM each were used to amplify the target region of the template (Table 3.2). When amplifying the SSU rDNA region, following an initial denaturation step at 94 °C for 3 minutes, thermocycler (Mastercycler ep gradient S, Eppendorf, Hamburg, Germany) was set for 35 cycles at 94°C (denaturation) for 45 seconds, 50°C (annealing) for 45 seconds, 72°C (elongation) for 2 minutes, and a final extension at 72°C for 5 min. When amplifying LSU rDNA region thermocycler was set for a single denaturation step at 95°C for 2 min followed by 30 cycle at 95 °C for 45 seconds, 58°C for 45 seconds and 73°C for 30 seconds, and a final extension step at 73°C for 5 min. Negative controls (template was replaced by water) were run on PCR.

Table 3.2: Oligonucleotide primers used for PCR and sequencing of SSU and LSU rDNA. Sd = synthesis direction: F = forward; R = reverse (Edvardsen et al. 2003).

Code	PCR	Sd	Nucleotide sequence 5´ to 3´	Position based on the *Saccharomyces cerevisiae* numbering system[a]
1F	SSU	F	AACCTGGTTGATCCTGCCAGT	1-21 in SSU rDNA
528F	SSU	F	CGGTAATTCCAGCTCC	575-590 in SSU rDNA
1055F	SSU	F	GGTGGTGCATGGCCG	1263-1277 in SSU rDNA
300R	SSU	R	TCAGGCTCCCTCTCCGG	397-381 in SSU rDNA
1055R	SSU	R	CGGCCATGCACCACC	1277-1263 in SSU rDNA
1528R	SSU	R	TGATCCTTCTGCAGGTTCACCTAC	1795-1772 in SSU rDNA
DIR-F	LSU	F	ACCCGCTGAATTTAAGCATA	184-203 in LSU rRNA
D2C-R	LSU	R	CCTTGGTCCGTGTTTCAAGA	816-797 in LSU rRNA

The PCR products (both SSU and LSU rDNA amplicons) were verified on a 0.8% agarose gel. Gelred Nucleic acid stain, that unlike the Ethidium Bromide is not carcinogenic, was used to stain DNA in the agarose gel. PCR products of 5 µl (gel loading buffer is included in the GoTaq green master mix) was filled to each well on the agarose gel. 2 µl GeneRuler Express DNA ladder was used to estimate the length of the fragments on the gel electrophoresis image. The DNA bands were visualized by GENE Genius Bio Imaging System and Genesnap software from Syngene.

Only PCR products showing single, strong bands were processed for sequencing. The products were cleaned with Exosap-IT reagent. For PCR product clean-up 30 µl PCR grade water and 2 µl Exosap-IT reagent were added to 20 µl of each product. The thermocycler (Mastercycler ep gradient S, Eppendorf, Hamburg, Germany) was set as: 37°C for 30 minutes, 80°C for 15 minutes.

Samples were sent to the ABI lab at Department of Biosciences, University of Oslo for sequencing in accordance with the instructions given at the website of the laboratory. Prior to sequencing, the reaction mix was prepared in 8 racked-sequencing tubes. The reaction mix included 8 µl cleaned PCR product and 2 µl primer in each sequencing tube. The primers used are as follows: 1F, 528F, 1055F, 1528R, 1055R, 300R to cover the full length of the SSU rDNA; DIR and D2C to sequence LSU rDNA from 218 bp to 816 bp (Edvardsen et al. 2003). Samples were sequenced by Applied Biosystems 3730 DNA analyser.

2.5. Bioinformatical data analysis

2.5.1. Analysis of 454-pyrosequencing data

Filtering of 454-pyrosequencing data was executed with Qiime version 1.6.0, 64 kbit as described by Egge et al. 2013 and performed by Sandra Gran Stadniczeñko. The „.sff" (Standard Flowgram File) files containing all environmental sequences of all eukaryotic protist in the size fraction 3-45 µm from OF2 station sampled between 2009 and 2011 was obtained from Elianne S. Egge and further processed by Sandra G. Stadniczeñko. Demultiplexing and quality check was done with AmpliconNoise. OTUs were clustered with 99% similarity threshold value followed by picking a representative sequence set for each OTU group. Taxonomic assignment was done using BLAST against Protist ribosomal reference database (PR2). An e-value of 0.00001 was set as expectation value for taxonomic assignment.

The dinoflagellate reads were selected and further processed as described below. BLAST (Basic Local Alignment Search Tool) search was executed on the NCBI (National Center for Biotechnology Information) BLAST server using the processed SSU V4 rDNA reads to identify the OTUs at the lowest possible taxonomic level. The OTUs were then grouped together according to order level classification (Gymnodiniales, Peridiniales, Syndiniales, Dinophysiales, Gonyaulacales, Suessiales, Prorocentrales, Noctilucales, Blastodiniales, other dinoflagellates). In order to describe the dynamics of the most abundant species, the 12 most represented dinoflagellate OTUs were selected based on the read abundance. 18S rDNA reference alignment obtained from Russel J. Orr was used to construct the RaxML trees. The alignment was done with Mafft version 7 multiple alignment on its online server (http://mafft.cbrc.jp/alignment/server/) and checked in Bioedit 7.2.5. RaxML phylogenetic trees were built using Lifeportal, research interface of Universitetet i Oslo and Abel computing resources. Bootstrapping was run 1000 times with substitution model GTRCAT, 7 random seed, and non-parametric value for bootstrapping (b) 9. Output file from Abel server was wieved and edited in Figtree version 1.4.0 followed by final editing in Adobe Illustrator v. 6 to adjust the branch labels.

2.5.2. Analysis of sequence data from cultures

All SSU rDNA sequences from the cultures were edited in BioEdit, including trimming the ends. Sequence fragments were placed in appropriate order in the editor. Contig sequence was generated. Bases of the contig sequence were checked and edited according to the chromatograms (that visually represents the sequenced DNA sample) considering DNA ambiguity code table. Non necessary bases, that appeared in the contig sequences but not on the chromatograms, were removed, ambigous bases were checked and edited. The terminal ending of the contig sequences were removed where the signal became noisy and assembly became hindered. The final contig sequences were saved as fasta files.

The sequences were submitted in fasta format for a preliminary BLAST search on the NCBI webpage to discover the closest relative sequences to the organism based on similarity. Nucleotide BLAST (BLASTn) was used with default settings.
The most similar sequences were downloaded in fasta format for an alignment and phylogenetic analysis. Sequences giving lower similarity (e.g <90 % identity) were also added to the alignment. *Perkinsus marinus* ribosomal sequences was used in the alignment to form an outgroup. Alignment was created as described in chapter 2.5.1. RaxML phylogenetic trees were built as previously described in chapter 2.5.1 except that the bootstrapping was run 500 times.

2.5.3. Statistical approach

To reveal the seasonality of dinoflagellate communities in Outer Oslofjorden, station OF2 Non-metric Multidimensional Scaling (NMSD) plot was constructed for the 454-pyrosequencing data using the R version 3.0.3 and illustrated on Figure 3.11. Shannon diversity index [H’] was also calculated for each month (Figure 3.10).

3. RESULTS

3.1. The physical environment

Temperature and salinity curves are illustrated on Figure 3.1. Both variables showed seasonal variability through the sampling period. The highest temperature values were observed in August 2010 (18,44 °C) and June 2011 (16,06°C), whereas the coldest temperatures were measured during the winter period. In January and February 2010 the cruise recorded colder surface waters (-1.25 and -1.12 °C respectively) than during the winter 2010/11. However, in the winter of 2010/11 the surface water remained cold until March 2011, when -0,26 °C was measured compared to relatively warm water of 5,26°C in March 2010. The highest salinities were recorded on October 2009 (31,41 PSU) and March 2010 (32,75 PSU), while the lowest value was observed in April 2011 (16,09 PSU). Density was not plotted, because it followed exactly the salinity curve.

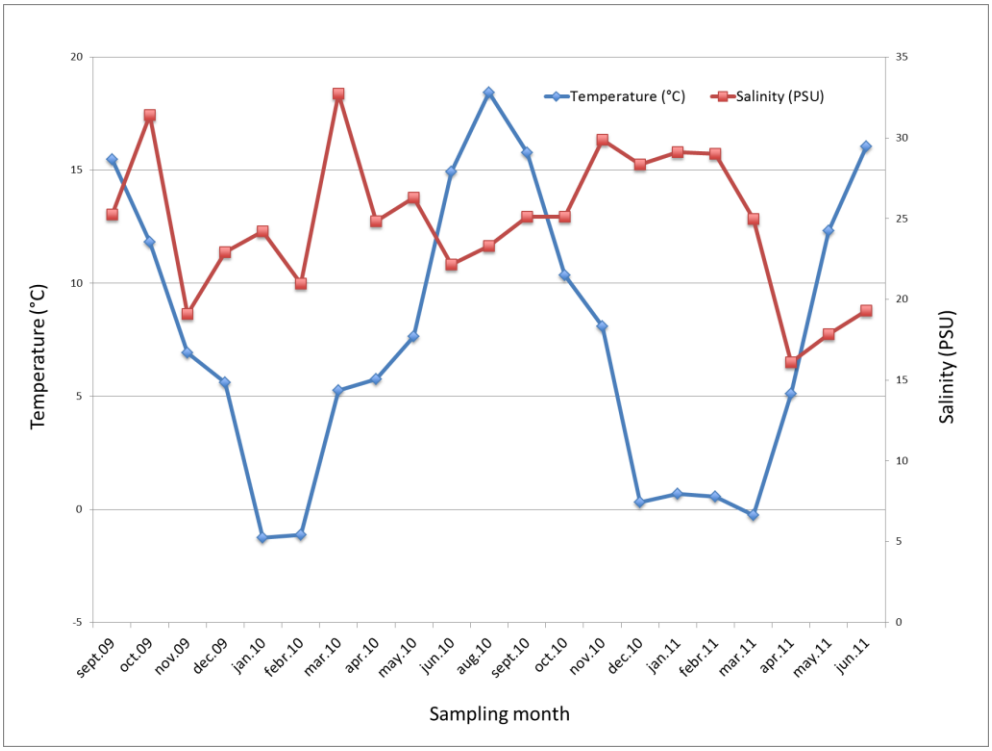


Figure 3.1: Change of temperature and salinity over time at the surface layer (1m depth) in Outer Oslofjorden, station OF2 between September 2009 and June 2011.

As pictured in Figure 3.2 high irradiance values were recorded during the summer, while the lowest radiation reaching the sea surface was observed during the winter (November-

February). The irradiance curve illustrates nearly even oscillation in the amount solar radiation. The presence of chlorophyll was also measured both in vivo (Fluorescence) and in vitro (Chlorophyll-a concetration) and drawn in Figure 3.2. The two curves followed a similar pattern. There were two peak values recorded during the two years period, one in January 2010 and another smaller in February 2010. Both chlorophyll-a and fluorescence were also relatively high almost reaching 3 µg/L in September 2009.

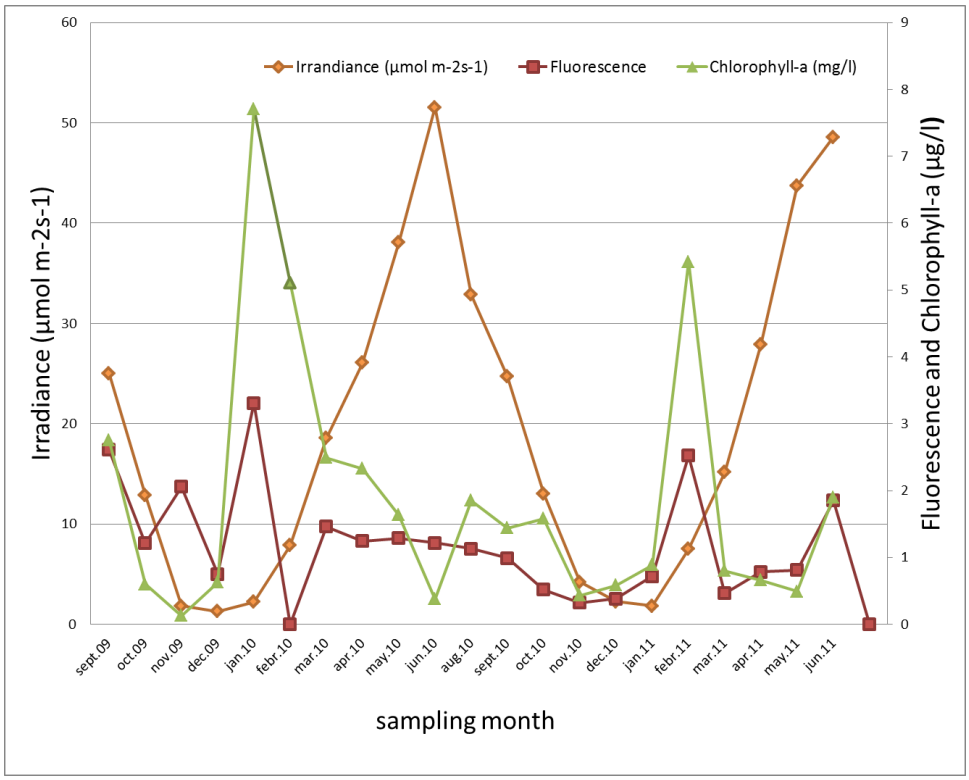


Figure 3.2: Irradiance, fluorescence and chlorophyll-a concentrations over time at the surface layer (1m depth) in Outer Oslofjorden, station OF2 between September 2009 and June 2011.

The changes in nutrient (N, Si, P and total P) concentrations are represented in Figure 3.3. There were no measurements performed in February 2010 and July 2010. The highest N and Si concentrations were observed in December 2009 with 8,92 and 14,71 µg/L respectively. In general N and Si concentrations were low in summer and in late winter. However, in May and June 2011 Si reached high values of 6,16 and 13,15 µg/L. The P and total-P showed less oscillation during the study period. Total-P was highest in December 2010 with 9,52 µg/L and in April 2011 with 3,92 µg/L.

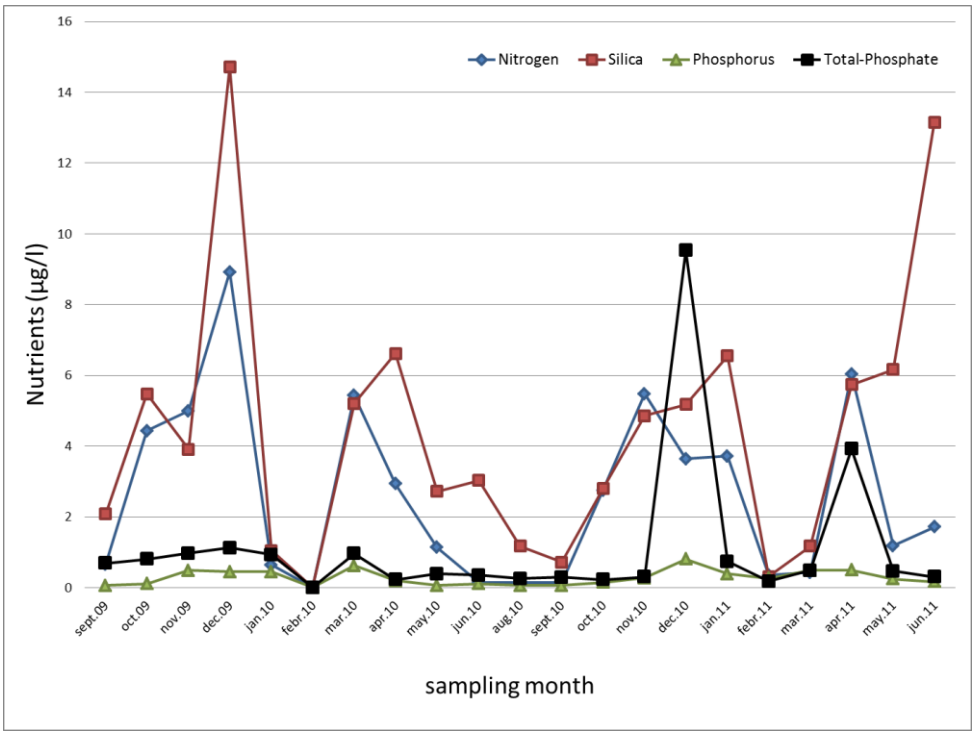


Figure 3.3: Nutrient concentrations over time at the surface layer (1m depth) in Outer Oslofjorden, station OF2 between September 2009 and June 2011.

3.2. The Cultured material

Using maximum likelihood (ML, RAxML) phylogenetic analyses to unravel the identity and phylogeny of the cultured species (strains UiO306, UiO307, UiO312) we found that we actually had only two species. Both UiO306 and UiO312 were suggested to be *Pelagodinium béii*. On the basis of the SSU and LSU rDNA data RAxML trees placed these ribosomal nucleotide sequences close to each other on the respective phylograms. The sequence U37367 of *Gymnodinium beii* also turned up as a candidate for the identity of the strain UiO312. Sequence EF492490 of *Karlodinium micrum* was placed close to UiO306 and UiO312 on the SSU rDNA trees. DNA distance analysis, executed by the algorithm DNAdist in the program Bioedit, pointed out zero distance between EF492490 *Karlodinium micrum*, UiO306 and UiO312. JF791096 *Karlodinium micrum* was included to test the reliability of EF492490 *Karlodinium micrum* since the last sequence was highly similar to those originated from the studied strains. The distance matrix reveals that there is significant difference between the *K. micrum* sequences. (Table 3.1.).

Table 3.1. DNA distance matrix of UiO306, UiO312 and the most related sequences of the small subunit ribosomal DNA region. JF791096 karlodinium micrum sequence was also included.

	EF492490	JF791096	KF422623	U37367	UiO306	Uio312
EF492490 *Karlodinium micrum*	-	0.0500	0.0006	0.0050	0.0000	0.0000
JF791096 *Karlodinium micrum*	0.0500	-	0.0523	0.0535	0.0500	0.0518
KF422623 *Pelagodinium beii*	0.0006	0.0523	-	0.0055	0.0006	0.0000
U37367 *Gymnodinium beii*	0.0050	0.0535	0.0055	-	0.0051	0.0053
UiO306 DinoBergen	0.0000	0.0500	0.0006	0.0051	-	0.0000
Uio312 DinoOslofjorden	0.0000	0.0518	0.0000	0.0053	0.0000	-

The third strain with code UiO307 was confirmed to belong to the genus *Scrippsiella.* The LSU rDNA phylograms placed UiO307 together with *Scrippsiella donghaiensis* with strong support (bootstrap value of 95). DNA distance matrix firmly supports that the organism under investigation is *Scrippsiella donghaienis* (Table 3.2.), since there was no distance between sequences JN982374, JN982387 and UiO307. Figures 3.6 - 3.9 below illustrate the phylogenetic relationships of these species in our cultures.

Table 3.2. DNA distance matrix of four strains of *Scrippsiella* 23S rDNA sequences. The values indicate the distance between the DNA sequences

	AY685011 Scrippsiella sp.	JN982374 Scrippsiella donghaiensis	JN982387 Scrippsiella donghaiensis	UiO307
AY685011 *Scrippsiella sp.*	-	0.0156	0.0156	0.0216
JN982374 *Scrippsiella donghaiensis*	0.0156	-	0.0000	0.0000
JN982387 *Scrippsiella donghaiensis*	0.0156	0.0000	-	0.0000
UiO307	0.0216	0.0000	0.0000	-

The closest relatives of UiO307, determined by Maximum likelihood phylogenetic analyses, were selected for this distance analysis. The matrix reveals that the two *Scrippsiella donghaiensis* sequence are identical, whereas the one with accession number AY685011 is the most distant from my *Scrippsiella* sequence of UiO307.

3.2.1. *Pelagodinium béii* and *Scrippsiella donghaiensis*

In this study both *Scrippsiella donghaiensis* and *Pelagodinium béii* were detected in the SSU V4 rDNA 454-pyrosequencing data based on sequence match. Dynamics of *P. béii* and *S. donghaiensis* is depicted on the Figure 3.4 and Figure 3.5 below. *S. donghaiensis* was represented with a total of 3609 sequences, which was the 4th highest count among the 357 dinoflagellate OTUs, in 17 out of 21 samples. The *P. béii* was present in 7 samples and exhibited total 918 sequences.

The dynamics of these two dinoflagellates are discussed in terms of read abundance and presented in Figure 3.4. Both species were the most abundant in September 2009. *S. donghaiensis* had 1315 and *P. béii* 577 reads from September 2009. However, there was not observed such vast abundance of dinoflagellates in the sample from September 2010. From october 2009 to may 2010 *P. béii* was absent, whereas *S. donghaiensis* was only represented with low abundance (>200 reads) in this period. The last mentioned species did not appear in February 2010. There was, however an increasing trend of *S. donghaiensis* in Spring 2010 with the highest value (184 reads) observed in May. After the increase in Spring 2010 the detected number of *S. donghaiensis* reads dropped to zero by June 2010 and remained low until its second big peak in October 2010. This time 575 reads were recorded from this species. On the other hand, *P. béii* reached its second and last peak (221 reads) in June 2010. Then a gradual decrease followed and *P. béii* was missing from the sequence pool from October 2010 onwards. *S. donghaiense* was absent from the sample in November 2010. It peaked again in March 2011 with 727 reads, but in April 2011 only 14 reads were found from this species. The abundance of S. donghaiensis reads remained low in the last two sampling months as well (59 reads in May and 38 reads in June).

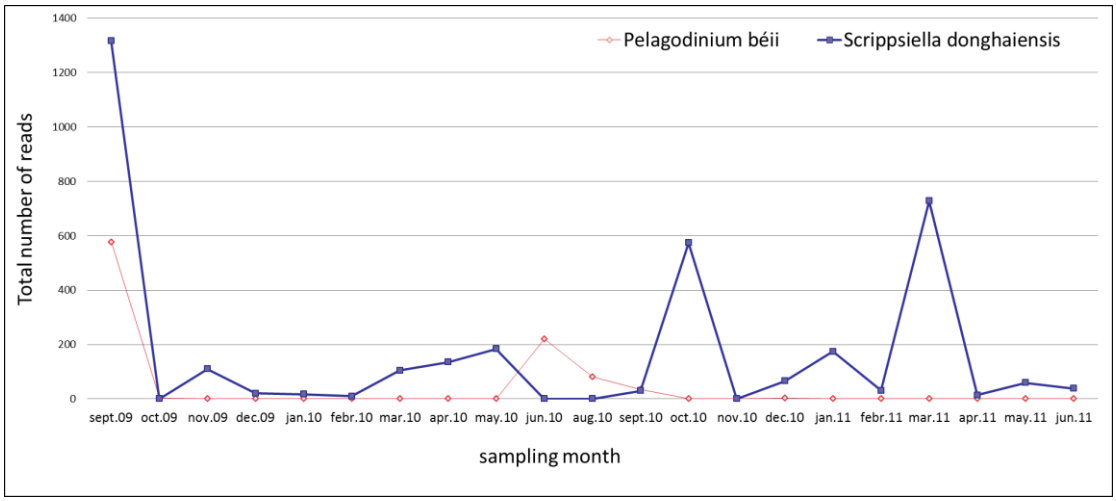


Figure 3.4: Read richness for *Pelagodinium béii* and *Scrippsiella donghaiensis* from the 454-pyrosequencing dataset between September 2009 to June 2011.

Considering the relative read abundance, which is portrayed below in Figure 3.5, a very similar profile for both species can be described. The occurrences of *P. béii* happened in the same fashion as revealed by looking in the total read abundances. *P. béii* reached its highest contribution to the dinoflagellate community in June 2010 with a 14,55%. *S. donghaiensis* deliniated also similar trend with some variances. The first peak at September 2009 seems much more moderate when compared to the peak observed on the total number of reads (Figure 3.4). Also the period from September 2009 to June 2010 was characterised by remarkably greater oscillation in recorded reads that it was suggested from total read abundance data. Despite the magnitude of peaks and the more oscillatory pattern the dynamics is alike as described above based on total read abundance.

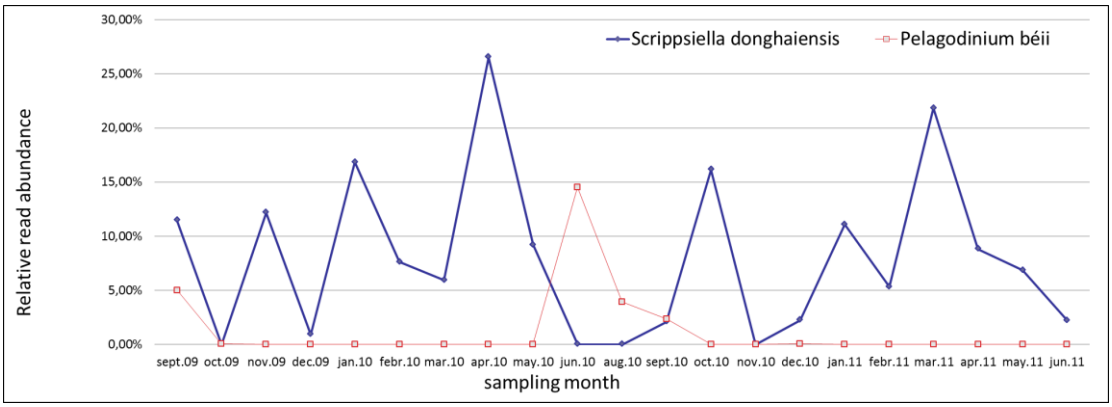


Figure 3.5: Relative read abundance for *Pelagodinium béii* and *Scrippsiella donghaiensis* for each month.

Figure 3.6: Maximum likelihood tree (RaxML) presenting the phylogenetic relationships of strains UiO306, UiO307 and UiO312 in broad taxon sampling based on small subunit ribosomal DNA (SSU rDNA). RaxML tree and bootstrap values from 500 resamplings are marked at the nodes. Perkinsus marinus was used as outgrup.

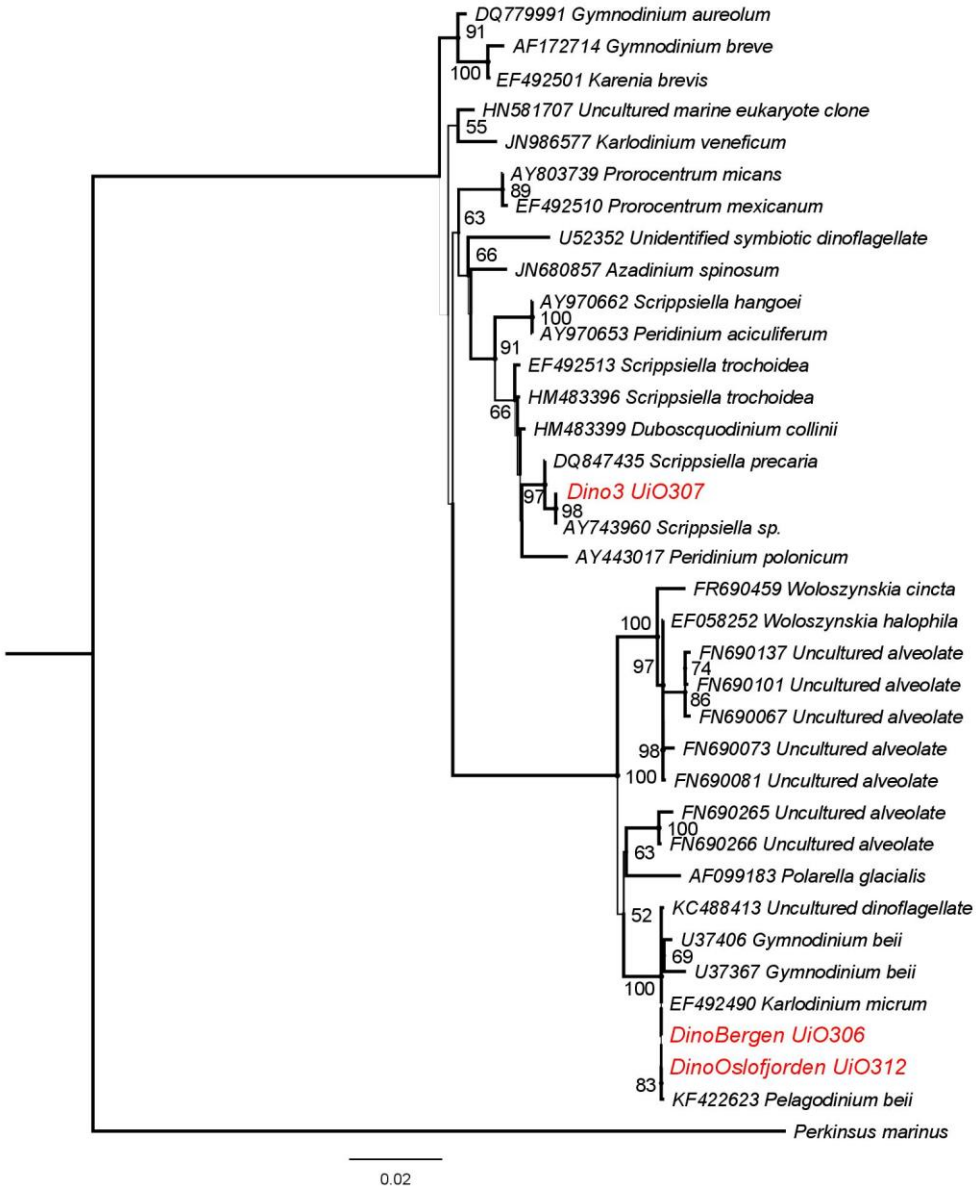


Figure 3.7: Maximum likelihood tree (RaxML) displaying the phylogenetic relationships of strains UiO306, UiO307 and UiO312 in narrow taxon sampling based on small subunit ribosomal DNA (SSU rDNA). RaxML tree and bootstrap values from 500 resamplings are marked at the nodes. Perkinsus marinus was used as outgrup. Bootstrap values greater than 50 are displayed.

Figure 3.8: Maximum likelihood tree (RaxML) showing the phylogenetic relationships of strains UiO306, UiO307 and UiO312 in broad taxon sampling based on large subunit ribosomal DNA (LSU rDNA). RaxML tree and bootstrap values from 500 resamplings are marked at the nodes. Perkinsus marinus was used as outgrup. Bootstrap values greater than 50 are displayed.

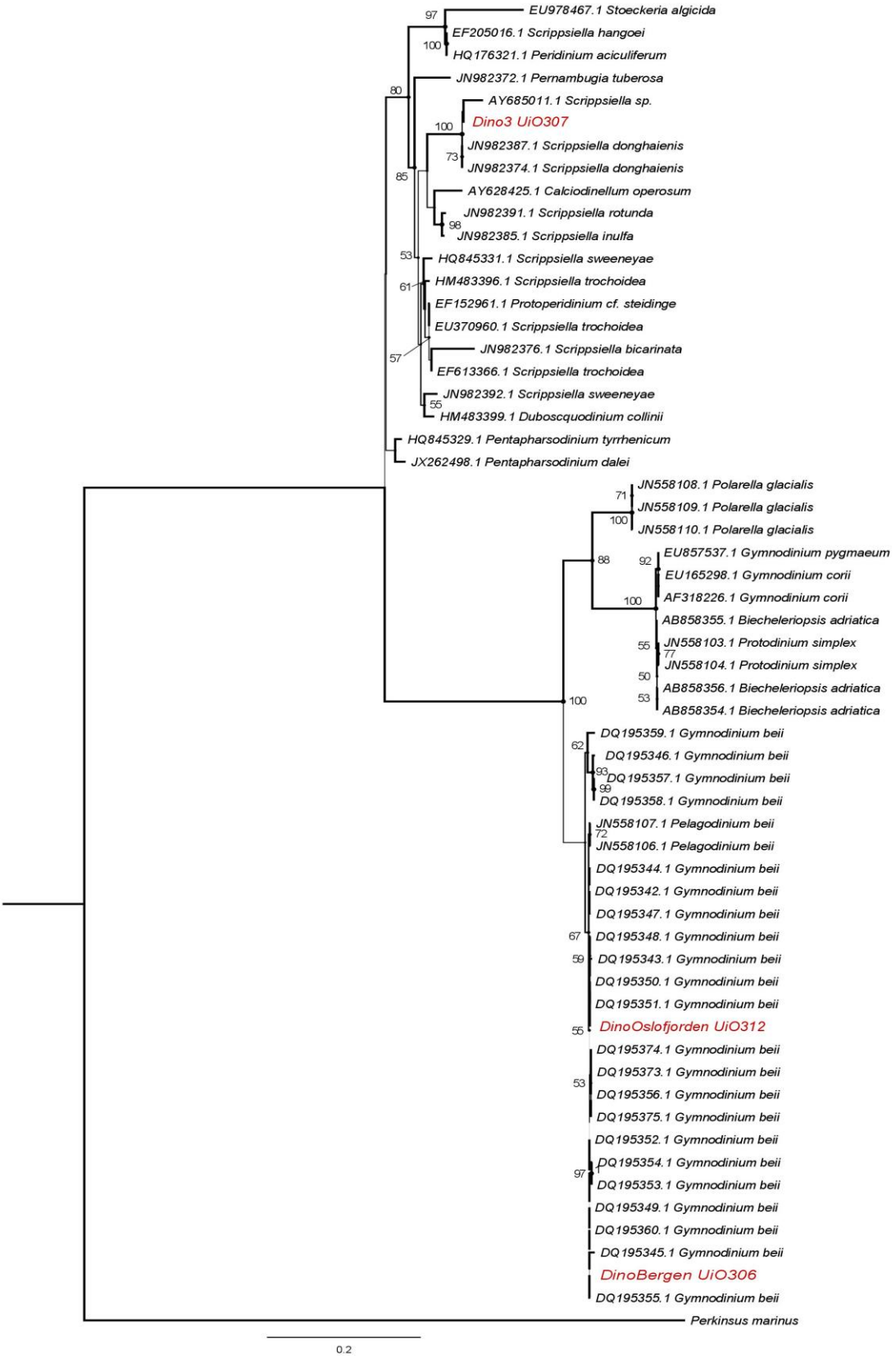


Figure 3.9: Maximum likelihood tree (RaxML) displaying the phylogenetic relationships of strains UiO306, UiO307 and UiO312 in narrow taxon sampling based on large subunit ribosomal DNA (LSU rDNA). RaxML tree and bootstrap values from 500 resamplings are marked at the nodes. *Perkinsus marinus* was used as outgrup. Bootstrap values greater than 50 are displayed.

3.3. Biodiversity and seasonality of Dinoflagellates

3.3.1. Diversity patterns

Dinoflagellate biodiversity followed a fluctuating profile in terms of calculated Shannon diversity index [H]. The Shannon-diversity indices of each sampling month are plotted below in the Figure 3.10. The Shannon diversity index is used to describe species diversity in a given community and made possible of comparision our 21 individual samples with each other. It considers both abundance and evenness. H' values mostly varied in the range 1,25-2,15. The lowest indices were observed on October 2009 and June 2011 with values 0,86 and 0,97 respectively. On the other hand June 2010 and September 2010 were the most diverse with values 2,38 and 2,59 respectively.

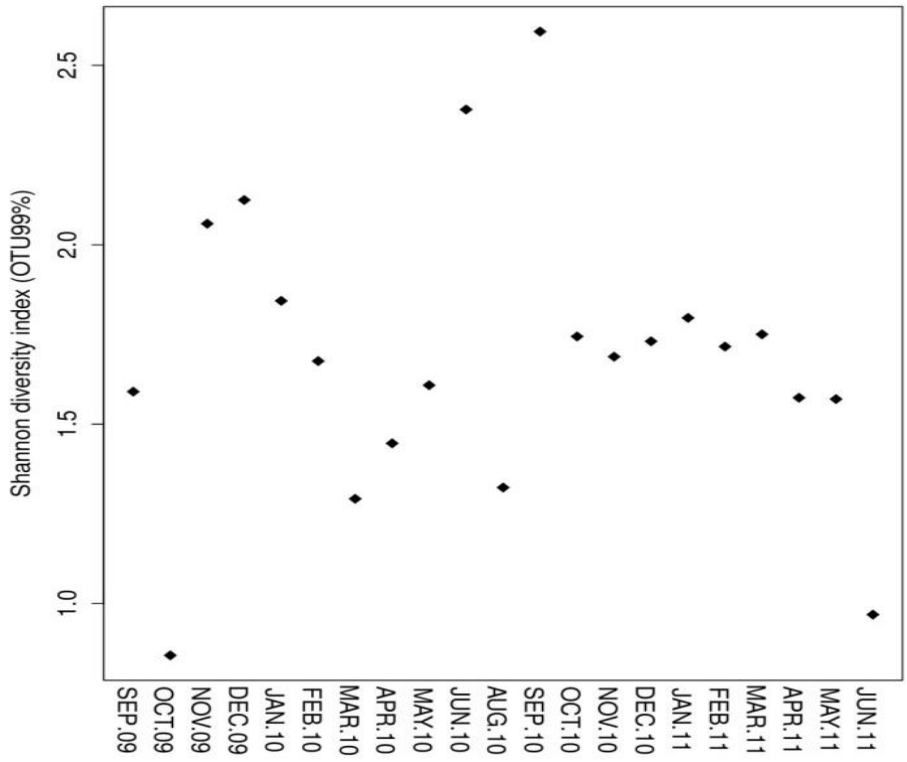


Figure 3.10: Shannon diversity index [H'] values plotted agains the sampling dates. The number of SSU V4 rDNA reads was normalized in order to be able to compare the individual months to each other.

Based on Shannon's diversity index we could point to a seasonal trend in the dinoflagellate community at OF2 station. The inferred dinoflagellate diversity pattern showed a tendency to

be greater in the late autumn-winter months. November to February in 2009/2010 and September to March in 2010/2011 were diverse periods. The winter period of 2009/2010 was characterized by larger variation in diversity indices, but comparable to those obtained in 2009/2010. Spring months (March to May in 2010 and April to June in 2011), however, were the least diverse. Unfortunately inferring a summer trend was hindered because there was no samples available from July 2010, no summer sampling in 2009 and in June 2011 the project was terminated.

Non-metric Multidimensional Scaling (NMDS) also supported the seasonality of dinoflagellate communities in Outer Oslofjorden (Figure 3.11). Samples from a particular season showed similarity to each other. Despite the seasonality some samples were more similar to those that were obtained in the previous season in the same year. March 2011 was comparable to winter (February 2011), December 2009 to autumn (November 2009) and September 2010 to summer (August 2010) samples. The similarity may be explained by that the seasons usually does not have sharp beginnings and ends. They rather progress towards the consecutive one with different delay due to the variability of weather patterns over time.

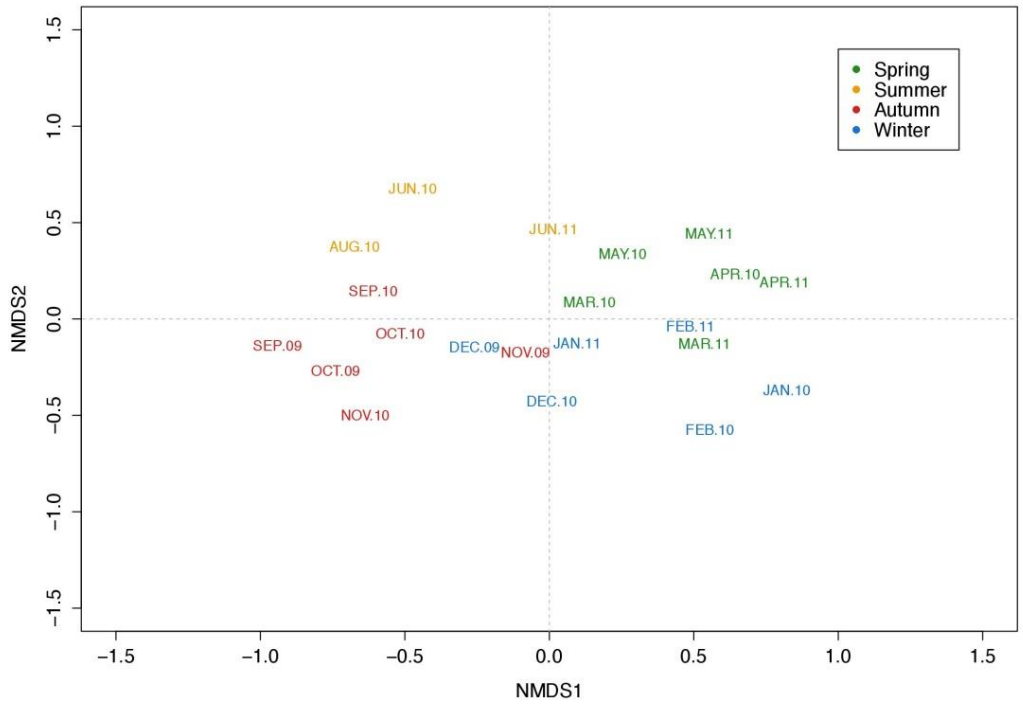


Figure 3.11: Non-metric Multidimensional Scaling (NMSD) of the dinoflagellate data obtained over the two years period (2009-2011) in outer Oslofjorden, station OF2. Samples are marked with green, yellow, red and blue from spring, summer, autumn, and winter seasons respectively.

A total of 374 unique Operational Taxonomic Units (OTU) assigned to Dinophyta were detected in the sampling period, after a rigorous cleaning process and clustering at 99% similarity level (see chapter 2.5.1). Figure 3.12 illustrates the proportional distribution of the represented orders in the total 454-pyrosequencing dataset. The three orders in Outer Oslofjorden represented by the most OTUs were Gymnodiniales, Syndiniales, and Peridiniales contributing with the 32%, 22% and 17% of the observed OTU numbers respectively. Dinophysiales, Gonyaulacales and Suessiales were also OTU rich components of the dinoflagellate community contributing with the 8%, 7% and 4% of the OTUs respectively. Blastodiniales, Prorocentrales and Noctilucales occurred with only few OTUs across the data compared to the previously mentioned orders. The occurrence of *N. scintillans* was low with 21 being the highest assigned reads in December 2009. Note that sequences which were not possible to assign to any of these orders were pooled under name of Other dinoflagellates. Many dinoflagellates are difficult to culture and execute extensive studies on them (genetical, morphological, ecological and biochemical for example). The group Other dinoflagellates must contain such species without any or correct data entry in the databases

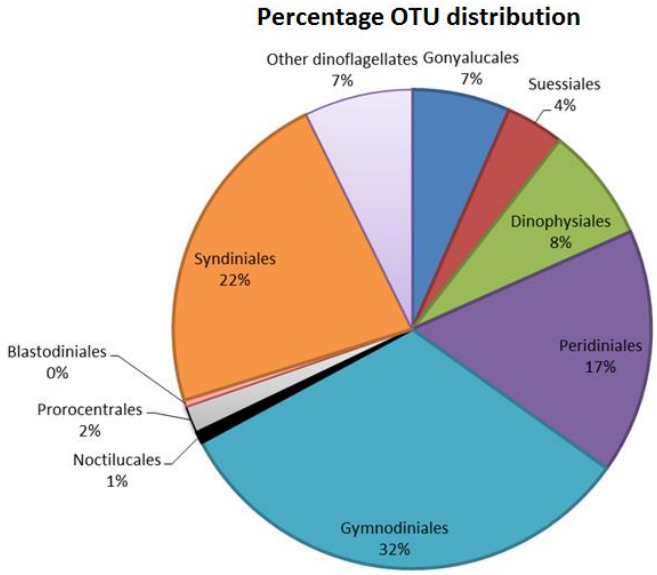


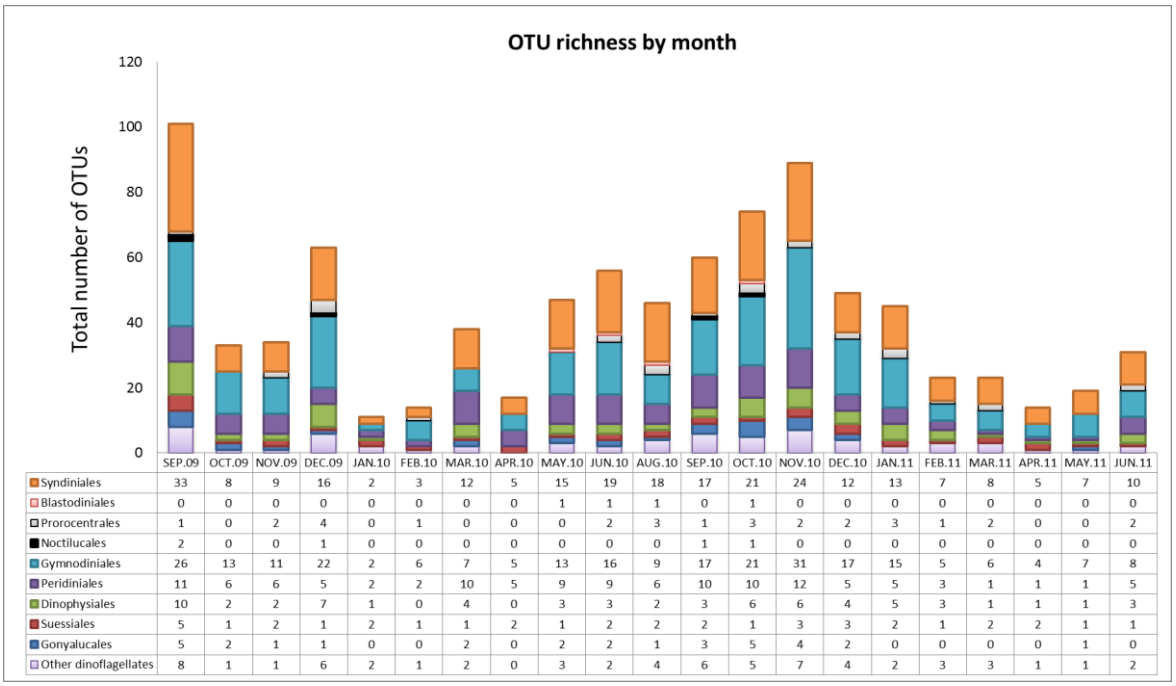
Figure 3.12: Distribution of observed dinoflagellate OTUs among the 10 main dinoflagellate orders (Gymnodiniales, Syndiniales, Peridiniales Dinophysiales, Suessiales, Gonyaulacales, Blastodiniales, Prorocentrales, Noctilucales, other dinoflagellates) in the 21 sampled months from September 2009 to June 2011.

The OTU richness (number of OTUs in normalised subsample) showed a certain seasonality throughout the sampling months. The Figure 3.13 illustrates the numbers of OTUs found in each sampling occasion classified to the main orders of dinoflagellates.

The general trend was similar to the one indicated from Shannon diversity index, but with some differences. In both years the lowest OTU richness was detected during the late winter/spring. Lowest richness was found in January and February in 2010 and April in 2011 with 13, 14 and 14 observed OTUs respectively. However, March 2010 was comparable with October and November 2009. In the next year significantly higher OTU values were observed for these months.

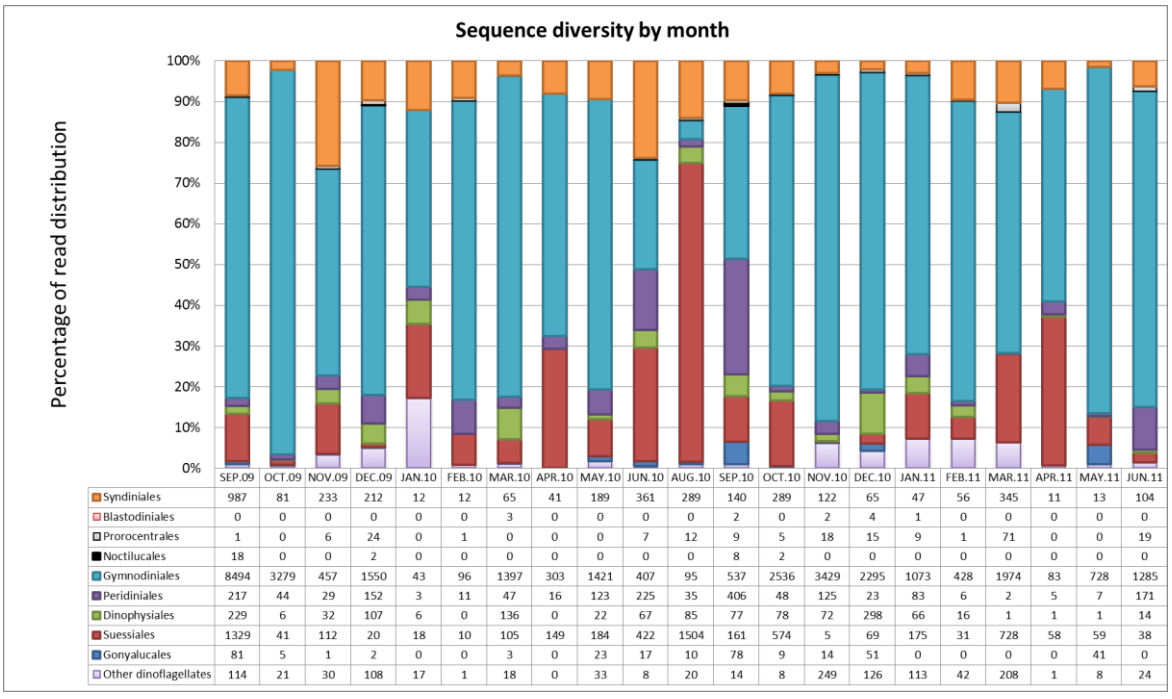
Over the sampling period Gymnodiniales and Peridiniales were the only orders that were represented in all 21 samples. In regard of sequence read Gymnodiniales and Peridiniales dominated the dinoflagellate communities all year round. Exception was August 2010 when a suessoid dinoflagellate reached bulky proportion in regard with the sequence abundance There was, however, a big decrease in the OTU richness in the summer 2010. In august 2010 sequences originated from Gymnodiniales reached its lowest OTU richness proportion. At this time a single OTU from the order Suessiales reached high abundance (1502 sequence reads) accounting to the dominating proportion of the order at that time Figure 3.14. From May to December 2010 consecutive peaks over 1000 reads of *Kareni*a sp. (May 2010), suessoid dinoflagellate (August 2010), *Karenia* sp. again (October 2010), and *Akashiwo sanguinea* (December 2010) were observed in terms of total read abundance. In November *Karenia* sp.and *Akashiwo sanguinea* co-occurred with similar read abundance both OTU counting more than 1000 reads. The potentially toxic species of the genera *Dinophysis* sp. was recorded by 454-pyrosequencing approach in May 2010 with over 200 reads but was entirely missing from the sequence pool until January 2011 with 192 reads.

The proportions of the detected dinoflagellate orders by each month, based on OTU and read number, are supplemented in the Appendix A.2-A.7.



	SEP.09	OCT.09	NOV.09	DEC.09	JAN.10	FEB.10	MAR.10	APR.10	MAY.10	JUN.10	AUG.10	SEP.10	OCT.10	NOV.10	DEC.10	JAN.11	FEB.11	MAR.11	APR.11	MAY.11	JUN.11
Syndiniales	33	8	9	16	2	3	12	5	15	19	18	17	21	24	12	13	7	8	5	7	10
Blastodiniales	0	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	0	0
Prorocentrales	1	0	2	4	0	1	0	0	0	2	3	1	3	2	2	3	1	2	0	0	2
Noctilucales	2	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
Gymnodiniales	26	13	11	22	2	6	7	5	13	16	9	17	21	31	17	15	5	6	4	7	8
Peridiniales	11	6	6	5	2	2	10	5	9	9	6	10	10	12	5	5	3	1	1	1	5
Dinophysiales	10	2	2	7	1	0	4	0	3	3	2	3	6	6	4	5	3	1	1	1	3
Suessiales	5	1	2	1	2	1	1	2	1	2	2	2	1	3	3	2	1	2	2	1	1
Gonyalucales	5	2	1	1	0	0	2	0	2	2	1	3	5	4	2	0	0	0	0	1	0
Other dinoflagellates	8	1	1	6	2	1	2	0	3	2	4	6	5	7	4	2	3	3	1	1	2

Figure 3.13: OTU abundance by sampling months broken down between the 9 main orders and other dinoflagellates. The columns show detected OTUs by each month in a stacked style. The table part introduces the number of OTUs found from each month by taxa.



	SEP.09	OCT.09	NOV.09	DEC.09	JAN.10	FEB.10	MAR.10	APR.10	MAY.10	JUN.10	AUG.10	SEP.10	OCT.10	NOV.10	DEC.10	JAN.11	FEB.11	MAR.11	APR.11	MAY.11	JUN.11
Syndiniales	987	81	233	212	12	12	65	41	189	361	289	140	289	122	65	47	56	345	11	13	104
Blastodiniales	0	0	0	0	0	0	3	0	0	0	0	2	0	2	4	1	0	0	0	0	0
Prorocentrales	1	0	6	24	0	1	0	0	0	7	12	9	5	18	15	9	1	71	0	0	19
Noctilucales	18	0	0	2	0	0	0	0	0	0	0	8	2	0	0	0	0	0	0	0	0
Gymnodiniales	8494	3279	457	1550	43	96	1397	303	1421	407	95	537	2536	3429	2295	1073	428	1974	83	728	1285
Peridiniales	217	44	29	152	3	11	47	16	123	225	35	406	48	125	23	83	6	2	5	7	171
Dinophysiales	229	6	32	107	6	0	136	0	22	67	85	77	78	72	298	66	16	1	1	1	14
Suessiales	1329	41	112	20	18	10	105	149	184	422	1504	161	574	5	69	175	31	728	58	59	38
Gonyalucales	81	5	1	2	0	0	3	0	23	17	10	78	9	14	51	0	0	0	0	41	0
Other dinoflagellates	114	21	30	108	17	1	18	0	33	8	20	14	8	249	126	113	42	208	1	8	24

Figure 3.14: Distribution of SSU V4 rDNA reads among the 9 dinoflagellate orders and the other dinoflagellates by each month. Column diagram shows proportion in percentage, whereas the table part below introduces the recorded reads from the respective order by each month.

3.3.2. The most represented species

The 12 most frequently occurring dinoflagellate species were selected from the 374 OTUs and displayed in Figure 3.15. This was done to reveal if there was a pattern in the dinoflagellate community composition over the seasons. In most of the cases the most frequent species were also the most abundant, as assessed from read proportion. An exception was *Lepidodinium* sp. which was represented by a massive 7300 reads in September 2009 followed by a decrease. *Lepidodinium* sp. had total 11358 reads being the second highest read number preceeded by *Karenia* sp. (11591 reads). It was, however, detected only in 7 samples. *Lepidodinium* sp. was detected also in September, October, November and December 2010 with 88, 576, 598 and 71 reads respectively.

There were 6 dinoflagellates chosen, based on the contribution to the total sequence pool, that had at least two sequence count over 300 reads a month. The most frequent species picked from this subgroup were *Lepidodinium* sp., *Karenia* sp., *Akashiwo sanguinea*, *Scrippsiella donghaiensis*, and *Gyrodinium* sp. and the suessoid species. This suessoid species had only one significant peak over 1000 reads, which was comparable with other OTUs in this subgroup, I found it consistent to illustrate its dynamics on the Figure 3.15a.

In September 2009 the overall dinoflagellate read abundance was enormous. At this time the previously mentioned *Lepidodinium* sp. had the highest abundance. The *Scrippsiella donghaiensis* and *Karenia* sp. were examples of those species that appeared with high read number. As these populations declined *Akashiwo sanguinea* could reach its first peak with 868 reads in December 2009. In January and February 2010, the growth of the dinoflagellate community was restricted and the abundances remained low. In March 2010 *Gyrodinium* sp and *Karenia* sp. dominated the community. By April both species decreased in numbers, but the next month we detected *Karenia* sp. with significant sequence richness. The consecutive months brought changes again in favour for a Suessoid dinoflagellate. This species started to develop, whereas *Karenia sp*. collapsed again. Finally a suessoid dinoflagellate bloomed in the late summer period in august followed by the comeback of *Karenia* sp. which got a peak in October 2010. The dynamics of these dinoflagellates happened in a similar fashion in the rest of the sampling period.

A second subgroup of dinoflagellate OTUs (Figure 3.15b) were formed based on the criterium that those species were still abundant but read numbers were not greater than 300. These

OTUs were the following ones, *Pelagodinium béii*, *Warnowia sp.*, *Gyrodinium sp.*, *Dinophysis sp.*, *Gymnodinium sp.* and *Biecheleria cincta*. However *P. béii* had a peak in September 2009 exceeding 300 reads, it was placed into this subgroup, since its read numbers were comparable with other species from this subgroup. The observed seasonal pattern was similar to the one drawn above at the first category. However, the magnitude of the scale was more moderate in this instance.

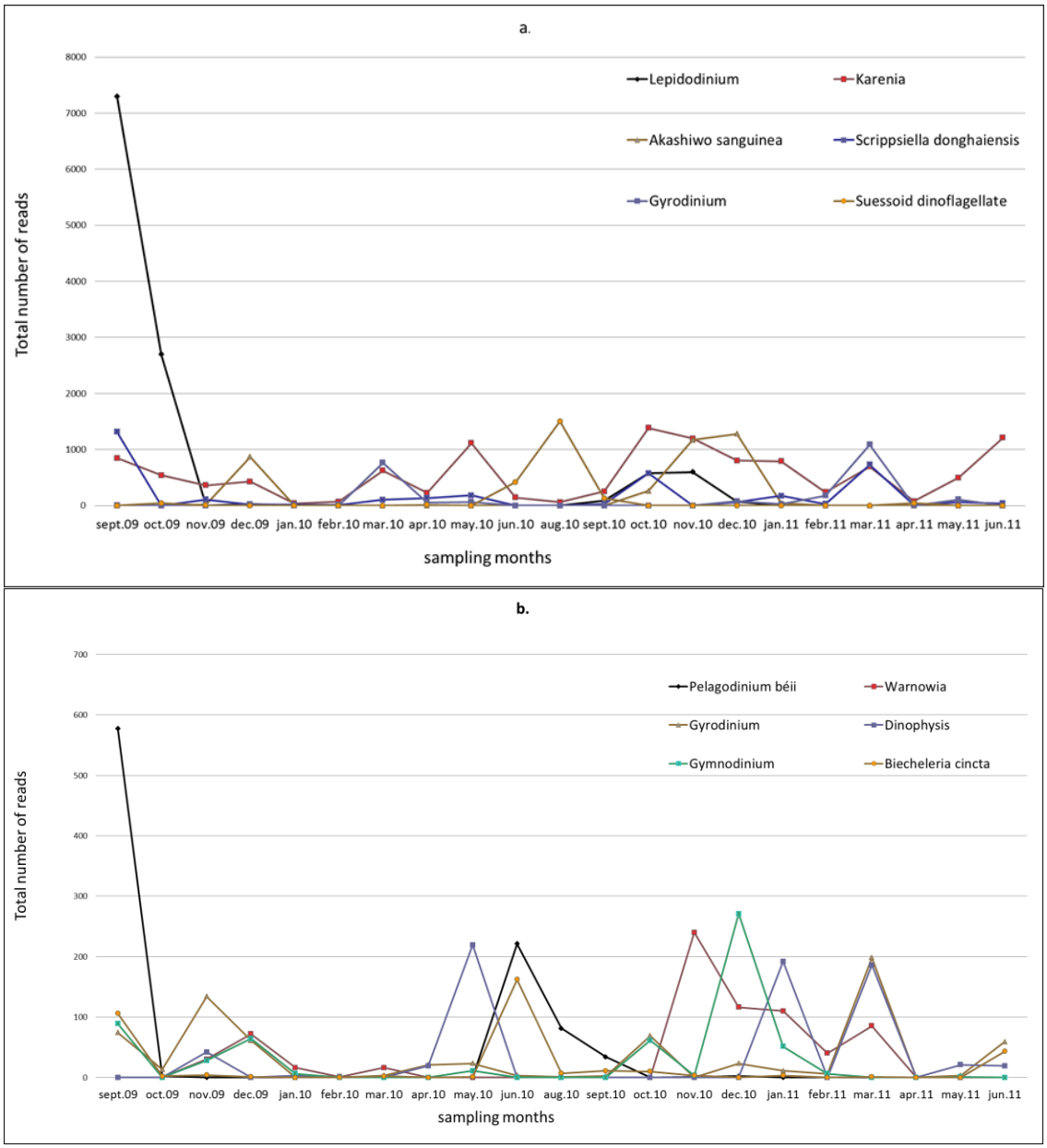


Figure 3.15a and b. showing the most frequent and abundant dinoflagellate OTUs in Outer Oslofjorden from September 2009 to June 2011.

4. DISCUSSION

4.1. Evaluation of the materials and methods

Before discussing the results obtained from this study it is meaningful to mention potential flaws in the applied methods.

Obtaining water samples were conducted by Niskin attached to a CTD rosette as described in chapter 2.2. This method assumes random phytoplankton distribution in the water column, which is not the case (Wroblewski & O'Brien 1976; Hostyeva 2011). On the contrary, phytoplankton has no random but patchy distribution in the sea (Wroblewski & O'Brien 1976). The scale of the heterogeneity may vary from milimeters to hundreds of kilometers and from milliseconds to even weeks in terms of spatial and temporal distribution respectively. Lovejoy, et al. 2001 also noted that the distribution is the result of biological (grazing and vertical migration) and physical (wind, currents, mixing and turbulence) phenomena.

Mainly dinoflagellates in the size fraction 3-45 μm from 1m depth are investigated in this study, which covers the nanoplankton (2-20 μm) and a certain proportion of microplankton (20-200 μm), due to filtering conditions as described in chapter 2.2. Therefore, it involves a limitation on this study attempting the seasonal dynamics and diversity of dinoflagellates in outer Oslofjorden. The investigation was missing larger dinoflagellates such as species belonging to the genus *Ceratium*, *Protoperidinium*, and toxic and potentially toxic species containing genus *Dinophysis* (*D. acuta, D. norvegica, D. caudata*). But their presence in this data cannot be excluded, because some larger cell could have got through the filters. Thus, we may infer the diversity and dynamics of dinoflagellates that fall between 3-45 μm. Also sequence data was only obtained from water samples at 1 m, meaning that inferring community composition and seasonality is merely based on surface water. Thus, providing an insight of the whole range dinoflagellate community at the study area in the study period was not possible.

While comparing the results of this study to the observations made by Hostyeva 2011 from 2009 to 2011 in Outer Oslofjorden (OF2 station) I have to point out differences in the applied methodologies of these works. Hostyeva 2011 investigated the phytoplankton size fraction

greater than 20 µm and focused on *Pseudo-nitzschia* (diatom) species, but also included records on the occurrence of 40 dinoflagellate species (36 identified and 4 unidentified species) between June 2009 and June 2010. In addition to the 1m depth samples Hostyeva 2011 took vertical net hauls increasing the description to thicker water column. Light microscopic approach was used in her work opposed to metagenomic approach applied in this present study.

The 454-pyrosequencing platform from the company Roche, which is one of the Next Generation Sequencing (NSG) technologies, is widely used in biodiversity and microbiome researches amongst others. With the GS FLX 454 platform ~400.000 realtively long reads (250bp) can be generated per single run (Shendure & Ji 2008; Quince et al. 2011). With the Titanium FLX 454 platform, as used in this study up to 400 bp reads can be obtained. However, the main source of error to consider is related to homopolymers, which are consecutive instance of the same bases (e.g TTTT or GGG). The length of the homopolymers may be inferred from the strength of the emitted light signal only, since there is no terminating moiety preventing multiple consecutive additions at a given cycle (Shendure & Ji 2008). This involves that the major error with application of the 454-pyrosequencing platform are insertions-deletions. As this sequencing platform uses emulsion PCR, one must consider errors occurring during the PCR amplification as well (Quince et al. 2009). For example, single-base errors and chimeras (hybrid of two or more different sequences, which can then be misidentified as a novel OTU) are frequent errors during PCR reactions. Also noteworthy to mention that the cost per read base is the highest amongst the available NGS technologies.

The mistakes mentioned previously that may be introduced to the sequence data by either PCR or pyrosequencing error must be treated. Otherwise the estimation of OTU numbers and classification can be biased. Amplicon-Noise is a robust set of sequence error removing algorithms that is capable to reduce noise by one-third to a half in a given dataset (Quince et al. 2011). According to Quince et al., 2011 the application of Amplicon-Noise is not only faster than PyroNoise, but it also outperforms the other denoising options according to the author's experiment. Still there is an important side effect of using Amplicon-Noise as for denoising procedure according to Quince et al., 2011. Amplicon-Noise may make changes to sequence reads that are inconsistent with simply removing noise (Gaspar & Thomas 2012).

Despite of the rapid development of sequencing and sequence processing resources, inaccurate or missing information from sequence databases may be another obstacle for

researchers. Biodiversity, metagenomics and also phylogenetics studies among others vastly rely on the sequence informations already available on various databases. The accessible genetic data has increased tremendously in the past years. Computational and molecular biological resources allow gathering and storing huge amount of data. Besides the explosive growth of such data, there is still a vast proportion of organisms that are regarded as unculturable. We may know sequences from such organisms, but we lack additional data such as source organism, morphology, biochemistry or ecology.

4.2. The dinoflagellate community structure and dynamics

Using 454-pyrosequencing and environmental data to explore dinoflagellate diversity and seasonal cycle, main patterns in diversity and community structure were unfolded. Seasonality was detected and the dinoflagellate community showed a heterogeneous, dynamic profile throughout the seasons most probably due to the variability of the hydrographical factors (irradiance, temperature, salinity and nutrients). Both the hydrographical conditions and concentration of nutrients in the uppermost layer had seasonal patterns. Seasonality of the dinoflagellate community showed the most co-variation of the irradiance, but nutrient concentration can also be an important factor. Fluorescence (chlorophyll in vivo) and chlorophyll-a (chlorophyll in vitro) concentration followed a similar trend to each other with certain variance. The observed variance may be due to the different methods of measuring chlorophyll-a concentration (Hostyeva 2011). Worth to note that there was a slight shift in peak values of chlorophyll-a measurements and nutrient concentrations. These factors drew an up-down dynamic when they were plotted against the sampling months (Figure 3.2). The change in the amount of nutrients (Si, N, P and total P) over the months happened in a trend alike to that described by Paasche (2005).

Irradiance, temperature, salinity and density showed a clear seasonality. Irradiance corresponds with the length of days when solar radiation can reach the sea surface. Thus the best light conditions occurred in the summer (the highest irradiance was measured in June 2010). Whereas the worst light conditions were recorded during the winter months (November-January) when the sunny hours were few. The curve of the surface temperature over time was similar to the curve of irradiance since temperature is mainly the factor of energy received through solar radiation. However the lowest temperature recorded occurred in

January and February 2010 (-1,25 and -1,11 °C respectively) chlorophyll measurements (both in vivo and in vitro) took up relatively high values (see Figure 3.2) indicating a phytoplankton blooming event as early as in January. The abundance of dinoflagellate sequence reads in January 2010 was the lowest, followed by February 2010, despite the significant chlorophyll-a values. Hostyeva 2011 reported a *Pseudo-nitzschia* (diatoms) bloom in January 2010, which can explain the high chlorophyll values. The winter 2010/2011 was slight milder than the previous, but depression of temperature lasted longer until March. In March probably a spring bloom occurred with lesser magnitude than in the previous year considering the chlorophyll curves. However, samples were taken once in a month, thus it is possible that the peak of a blooming event was missed. The 454-pyrosequencing data, however, revealed the opposite (more prevalent dinoflagellate presence in March 2011 than in March 2010). According to Paasche 2005 phytoplankton vernal blooming may be expected in the outer Oslofjorden and north Skagerrak area during February and March. Paasche (2005) regards to vernal phytoplankton bloom incorporating all photosynthetic planktonic groups with a dominance of diatoms. Also the 454-pyrosequencing data suggest that dinoflagellates are not the main contributors of the vernal phytoplankton blooms in Outer Oslofjorden.

Providing a detailed and all inclusive description of dinoflagellates at species level (often even at genus level) was highly restricted. Only SSU V4 rDNA data did not give enough support for making reliable and comprehensive distinction on lower taxonomic levels. Thus I decided to choose the most abundant OTUs to describe the dynamics of dinoflagellate communities in Outer Oslofjorden. Given the shortness (233 bp) and the fact that dinoflagellates are very similar on the sequenced SSU V4 rDNA I could mostly identify the selected reads to genus level.

In our dataset only one OTU was found to occur in all the 21 samples. Unfortunately this species was not possible to identify accurately from the SSU V4 rDNA sequence read. The OTU most likely covers a *Karenia* sp. based on RaxML phylogeny and may be a potentially toxic dinoflagellate species. Figure 3.15a includes the recorded read abundance from *Karenia* sp. Moreover, this OTU counted the most sequences in total 11591 reads.

At the study area the dinoflagellate community structure is influenced by abiotic factors (irradiance, temperature salinity, stratification, nutrients, currents and meteorological conditions). As visualized in the Figures 3.15a and b „gain-loss" dynamics was a typical pattern based on the sequence abundances, which is described in more detail below. Another

feature to point out was that there were co-occurring groups of species competing with each other for the same resources. Some species are more competitive in a given space and time at certain environmental conditions than others and can then become dominating.

At the beginning of the study period in September 2009 the highest number of dinoflagellate OTUs were detected through the two years period. In spite of this the Shannon diversity index (Figure 3.10) suggested the lowest diversity in 2009. A possible explanation might be a *Lepidodinium sp.* (Gymnodiniales) towering presence in terms of sequence reads. The northern North Sea bloom of dinoflagellates is known to occur usually in September according to Reid et al. 1990 , which is consistent with the findings presented here. The hydrographic conditions (relatively warm, stratified surface water, still good light conditions) seemed to favour a phytoplankton bloom in late summer-early autumn as seen in Figure 3.1-3.3. Hostyeva 2011 also noted from previous rapport (DNV 2006) that autumn phytoplankton blooms are frequent events in outer Oslofjorden. Slightly declining water temperature and still good light conditions coupled with salinity 25 PSU indeed can be favourable conditions for a bloom. The chlorophyll-a concentration reached 4-13 mg L^{-1} in the upper 20 m, which was consistent with the observed high cell density (>1,2x10^6 cell/L) by Hostyeva 2011. According to Hostyeva 2011 the autumn bloom in 2009 was dominated by diatoms. A diverse *Chaetoceros* community, *Skeletonema* spp. and *Pseudo-nitzschia* spp. was observed. Hostyeva 2011 found that the genus *Prorocentrum* (especially *P. micans* and *P. gracile* were notable in abundance) dominated the dinoflagellate community in September 2009.

In opposition to results of Hostyeva 2011 about the *Prorocentrum* dominance, 454-data could not reveal high abundance of this genus based on neither OTU numbers nor sequence reads. In September 2009 an OTU *Lepidodinium* sp. turned out to be present with the most sequence reads (7300). Since there seemed to be favourable conditions at this time that this species could utilize and reach high read numbers along with other dinoflagellate species. *Lepidodinium* sp. however is a small species with diameters less than 20 μm according to nordicmicroalgae.org. Thus, it is possible that the majority of *Lepidodinium* sp. cells escaped the plankton net and were not possible to identify in light microscopy from water samples either. An OTU identified as *Karenia* sp., which was abundant in sequence reads and was observed in all the 21 samples, was also significant contribution to the community in September 2009. However, DNA distance matrix revealed that the sequence may be associated with *Prorocentrum* sp. since these two candidate genus came extremely close in

SSU rDNA similarity. There is a possibility that this OTU in fact covers a *Prorocentrum* species.

After the autumn bloom from September 2009 we detected decreasing trend of dinoflagellates based on both sequence reads and OTU numbers as the season was shifting to winter. Between September 2009 and January 2010 the amount of sunlight (irradiance were gradually decreasing) was reducing. Reducing irradiance coincided with the declining trend of the dinoflagellate community. From February 2010 irradiance started increasing again reaching its top value in June 2010. The trend was more defined when considering the read abundance. One possible explanation can be that the photosynthetic dinoflagellates gradually became limited in growth due to poor light conditions. In December 2009 higher dinoflagellate diversity was recorded than that would have been expected from the trend described above. Nutrients (Si 15µg/L, N 8 µg/L) values were high and CTD data testified the presence of a stratified surface layer at this sampling date, which could prevent the vegetative cells to drift below the compensation depth where respiratory loss would exceed the photosynthetic gain (Sverdrup 1935).

In December 2009 *Akashiwo sanguinea* and *Karenia* sp. contributed the most to the dinoflagellate community. However, Hostyeva (2011) counted only 200 *Akashiwo sanguinea* cells per litre. We also found *Warnowia* sp., *Gyrodinium* sp. and *Gymnodinium* sp. with lower abundance (>100 reads). Hostyeva (2011) also had records of unidentified small dinoflagellates, which can include *Karenia* and the previously mentioned species as well

Light conditions remained poor in January and February 2010. Water temperature reached its lowest value of -1.2 °C and sampling was not possible in February due to ice cover. Despite these conditions, the chlorophyll-a measured were the greatest in January 2010 in the study period (September 2009-June 2011). Dinoflagellates were represented by only 13 and 14 OTUs in January 2010 and February 2010 respectively opposed to the OTU rich periods of summer and autumn (58 in June, 97 in September 2009, 87 in November 2010). Thus the observed photosynthetic activity was probably because of the overwhelming dominance of diatom species in January 2010, which was supported by high cell counts of diatoms by Hostyeva 2011.

Progressing towards the spring more light could penetrate into the sea and nutrients were high throughout the water column due to vertical mixing during the winter. These conditions

together may have made the development of dinoflagellate communities possible. The spring was also characterized by low abundance of dinoflagellates (Figure 3.14), with gradual increase through the spring and also the summer based on OTU richness and sequence reads richness as well. The amount of encountered dinoflagellate sequences showed even slighter increase than OTUs. The light conditions are getting significantly better in favour for photosynthetic organisms. In March 2010 *Gyrodinium* sp. and *Karenia* sp. were in the greatest abundance amongst the dinoflagellates in the size fraction 3-45 μm. In April 2010 dinoflagellate community was featured by low read and OTU numbers, *Karenia* sp. accounting for half of the dinoflagellate reads. *Akashiwo sanguinea* was not detected in April 2010 using 454-pyrosequencing approach in spite of the 100 cell counted per liter by Hostyeva 2011. *Dinophysis* spp. were present indeed in accordance with Hostyeva 2011, but the species identification was problematic because *Dynophisis* species are very similar on SSU V4 rDNA region. Hostyeva found *D. acuminata* and *D. norvegica*. Of these two species *D. acuminata* could be most likely incuded in this study since its small measurements according to nordicmicroalgae.org. The later Dinophysis species is too big for this size fraction. However, as mentioned earlier, the presence of larger Dinophysis species cannot be excluded as well. Dinophysis species were too similar on SSU V4 rDNA region in order to make distinction between them.

The data from summer and autumn (June-November) months showed relatively high abundance of dinoflagellates in the surface water. Reid et al. 1990 regarded dinoflagellates as important and abundant components of the summer phytoplankton community all across the North Sea. This is consistent with our results to a great extent since high OTU and read numbers were recorded in the period June-November. Light conditions were good for photosynthesis and nutrient supply was also adequate for algal growth. Steady stratification developed by May 2010 and lasted until November 2010, during this period the water collum pictured a homogeneous profile, in the upper 10 m. These hydrographic conditions may support thriving phytoplankton communities at the studied area.

The toxic species containing genera *Dinophysis* was recorded by 454-pyrosequencing approach in May 2010, but the OTU was entirely missing from the sequence pool until January 2011. Hostyeva 2011 also reported *Dinophysis* species in May (*D. acuminata, D. acuta, and D. norvegica*) and June 2010 (*D. acuminata, D. norvegica* and *Phalacroma rotundata* [*D. rotundata* as synonym]). The order Syndiniales is also worth to mention since

its representative OUT numbers were comparable to Gymnodiniales and Peridiniales, but the contribution to the sequence pool was low. It may be inferred that syndiniales had fair number of OTUs but they were presented with low abundance. The order Syndiniales contains parasitic species, which can be also an obstacle to assess their true diversity. In terms of reads Dinophysiales and Gonyalucales can be considered more abundant than the Syndiniales.

A similar pattern could be described regarding the hydrographic conditions and dynamics of dinoflagellates in the rest of the study period (December 2010-June2011).

4.3. The cultured material

The identity of strains UiO306, UiO307 and UiO312 was successfully determined by applying RaxML phylogenetics approach. Dinoflagellates are greatly similar to each other on the SSU rDNA sequence, which implies that the identification is hindered and one must be careful with conclusions from merely SSU rDNA data. Therefore researchers frequently use SSU, LSU and the ITS (Internal Transcriber Spacer) region of the ribosomal DNA combined for phylogenetics investigations amongst others. Thus, for species level identification of the strains under investigation both SSU and LSU rDNA sequences were used. Strains UiO306 and UiO312 turned out to be *Pelagodinium béii*, while UiO307 identified as *Scrippsiella donghaiensis*.

Pelagodinium béii was recently classified to the order Suessiales by (Siano et al. 2010). *Gymnodinium béii,* which is a free-living stage of the endosymbiotic dinoflagellate from the foraminifer *Orbulina universa,* is regarded as the basionym of *Pelagodinium béii* by the Algaebase. Siano et al. 2010 found inconsistency between the morphological observation of *G. béii* and the results from genetical (LSU rDNA) studies of their examined strain. Despite the morphology was identical to *G. béii*, LSU rDNA data clustered together with clades of suessiales and *G. béii* sequences. Due to works of Siano et al. 2010 a new genus *Pelagodinium* was erected within the order Suessiales.

The phylogenetic study conducted on strains UiO306 and UiO312 are in accordance with results of Siano et al. 2010. As seen on Figure 3.6-3.9 strains UiO306 and UiO312 are found together with other *G. béii* sequences and others belonging to the order Suessiales (*Baldinia*, *Polarella*, *Symbiodinium* and *Biecheleria*) on SSU and LSU RaxML trees as well. The most

related sequences were those named as *G. béii*. However, the SSU rDNA Maximum Likelihood trees placed a *Karlodinium micrum* (EF492490) sequence next to UiO306 and UiO312. DNA distance matrix revealed that EF492490 was identical to our two sequences. On the other hand, another *K. micrum* (JF791096) sequence showed significant distance from EF492490. Thus, I concluded that the *K. micrum* (EF492490) may be a misidentified entry in the NCBI nucleotide database and most probably belongs to *P. béii.*

Scrippsiella donghaiensis, which belongs to the order Peridiniales, was first described by Gu et al. 2008. In the original publication it is named as *S. donghaienis*, but in the Algaebase *S. donghaiensis* is the accepted taxonomic name. One explaination may be that Gu et. al (2008) may have applied the latin name incorrectly and later it was corrected, or it is wrong in Algaebase. In this work I use species names according to the Algaebase. The sequence was placed together with AY685011 *Scrippsiella* sp. on both the LSU and SSU rDNA RaxML tree with high bootsrap support (Figure 3.6-3.9). LSU RaxML trees also clustered our UiO307 sequence with other *Scrippsiella donghaiensis* sequences (see Figure 3.8-3.9). The lastly mentioned *Scrippsiella* species was not found on SSU rDNA trees, because *S. donghaiensis* SSU rDNA sequence is not available on the NCBI nucleotide database. On the basis of robust bootstrapping values and zero distance between UiO307 and the two other *Scrippsiella donghaiensis* sequence revealed by the DNA distance matrix the UiO307 is most probably *Scrippsiella donghaiensis.* I cannot exclude, however, based on one partial gene sequence that it belongs to a similar species, which has not yet been sequenced.

Scrippsiella donghaiensis was first recorded in Scandinavian waters by Gottschlin & Kirsch in 2003.while no reports of *Pelagodiunium beii* has been published previously from Scandinavian waters. The symbiotic *Pelagodinium béii* was first isolated from Orbulina universa, which was sampled off the coast of Puerto Rico, Caribbean Sea (Spero 1987), while the *Scrippsiella donghaiensis* was first isolated from the East Chinese Sea, China (Gu et al. 2008). In the framework of this work, sequences from both species were observed in Outer Oslofjorden. Hostyeva 2011 had reported the occurence of *Scrippsiella trochoidea* but not *S. donghaiensis.*

5. CONCLUSIONS

The 454-pyrosequencing approach made it possible to obtain a very high number of sequences from the environmental samples, but due to its limitations the results obtained must be considered with awareness. Dinoflagellates are very similar regarding the sequenced SSU V4 rDNA region, which makes identification of OTUs to species level highly restricted. To base a study on this region may be problematic. In order to provide an exhaustive assay on diversity and community dynamics using more than only SSU rDNA (e.g ITS, LSU) would be desired. Also including sequencing of samples from additional depths could increase the depth of the research. The study, however, did reveal a heterogeneous dinoflagellate community at the surface water showing seasonal variations in Outer Oslofjorden. The dinoflagellates reached their highest read abundance in late autumn. Gymnodiniales were the most dominant order amongst the dinoflagellates in the size fraction 3-45 μm. The strains were determined as *Pelagodinium béii* (UiO306, UiO312) and *Scrippsiella donghaiensis* (UiO307) and detected in the 454-pyrosequencing dataset. *Pelagodinium* seemed to occur only in the summer and early autumn, whereas *Scrippsiella* was present almost all year round reaching the highest read numbers in autumn.

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A. APPENDIX

Figure A.1: RaxML phylogenetic tree with the most represented OTUs inferred from SSU rDNA. The most represented(read abundance) OTUs are shown with red colour. Bootstrap values greater than 50 are displayed.

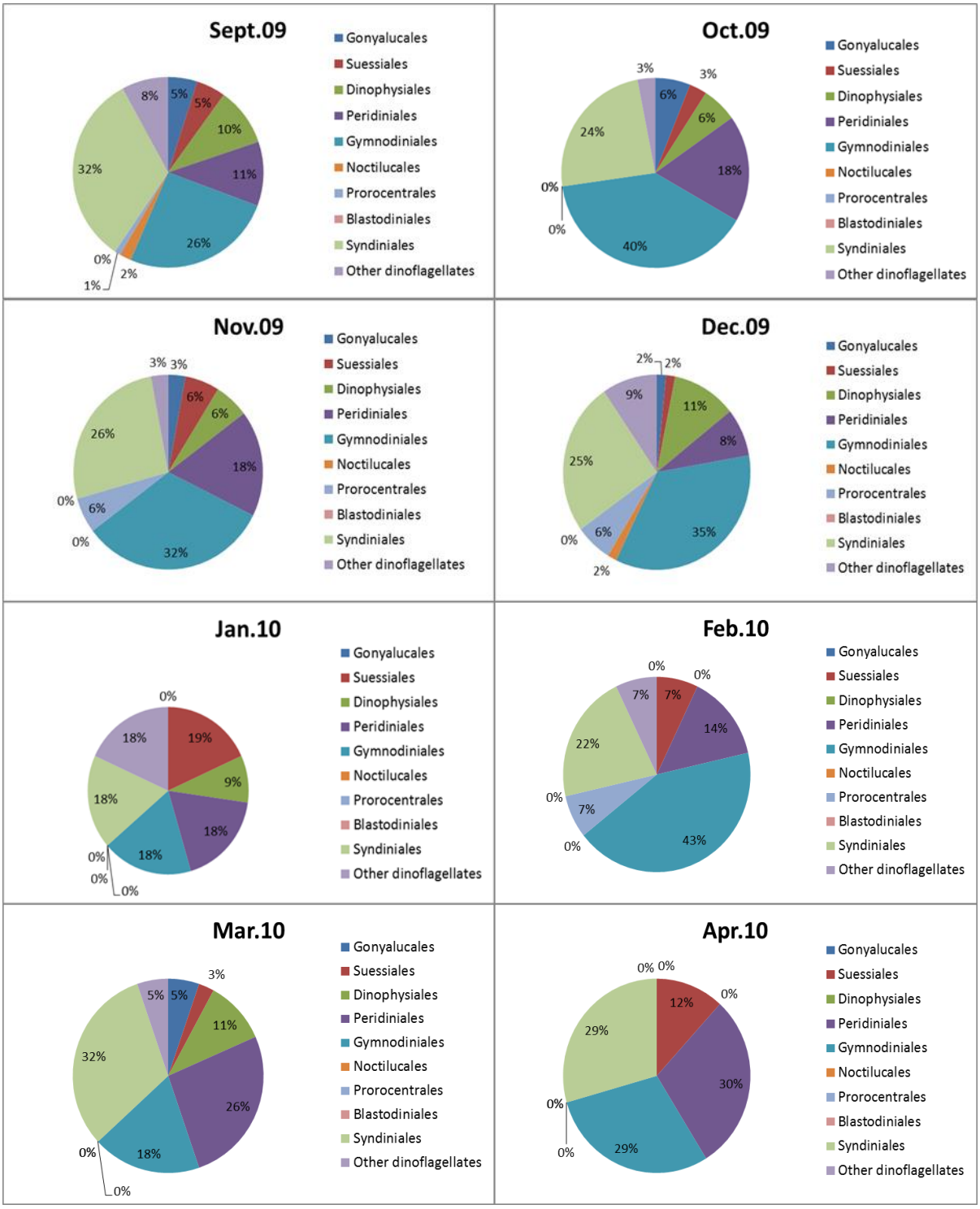

Figure A.2: Illustration of monthly OTU distribution amongst the main dinoflagellate orders and the Other dinoflagellates (September 2009-April 2010).

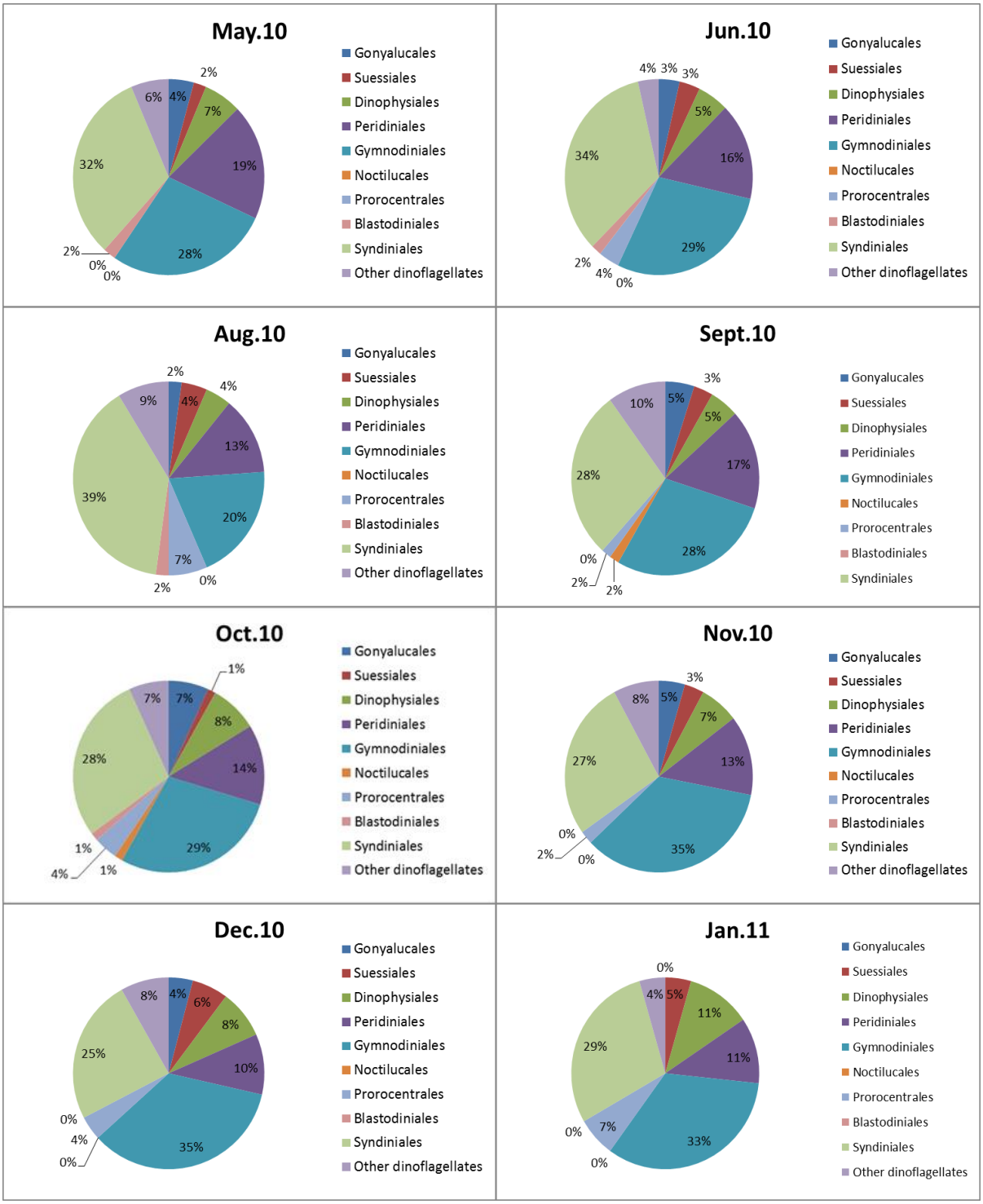

Figure A.3: Illustration of monthly OTU distribution amongst the main dinoflagellate orders and the Other dinoflagellates (May 2010-January 2011).

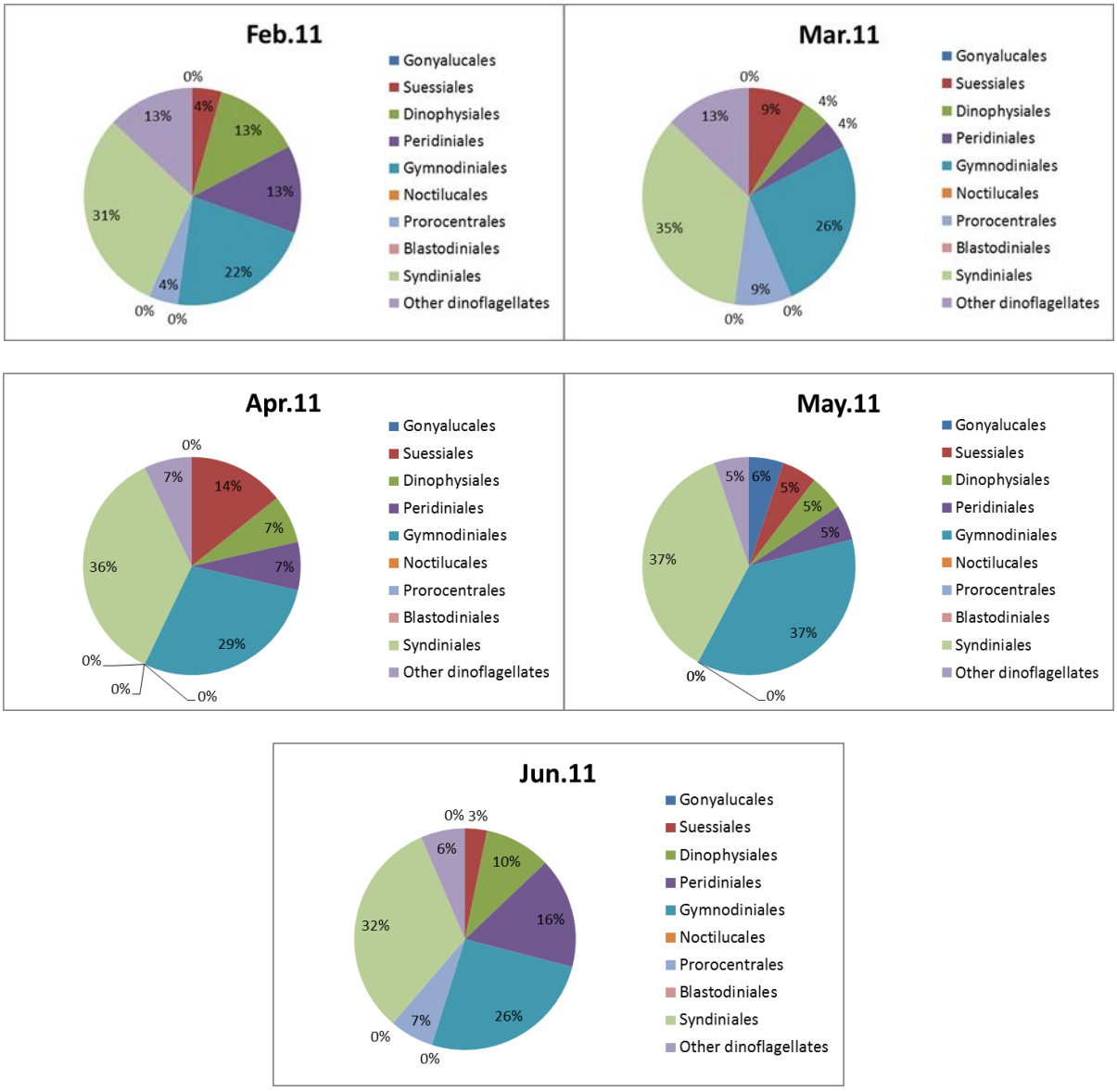

Figure A.4: Illustration of monthly OTU distribution amongst the main dinoflagellate orders and the Other dinoflagellates (February 2011-June 2011).

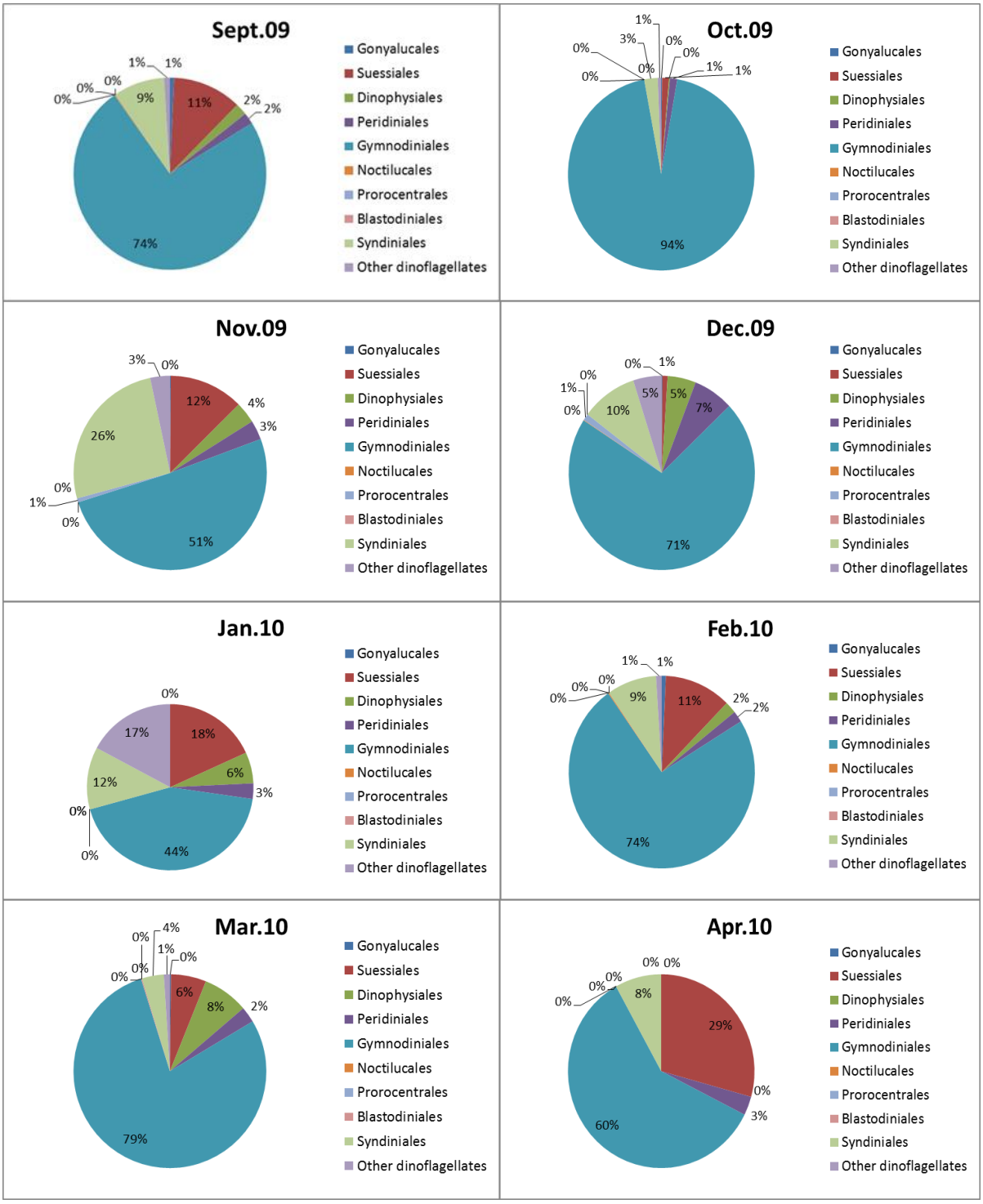

Figure A.5: Illustration of monthly sequence read distribution amongst the main dinoflagellate orders and the Other dinoflagellates (September 2009-April 2010).

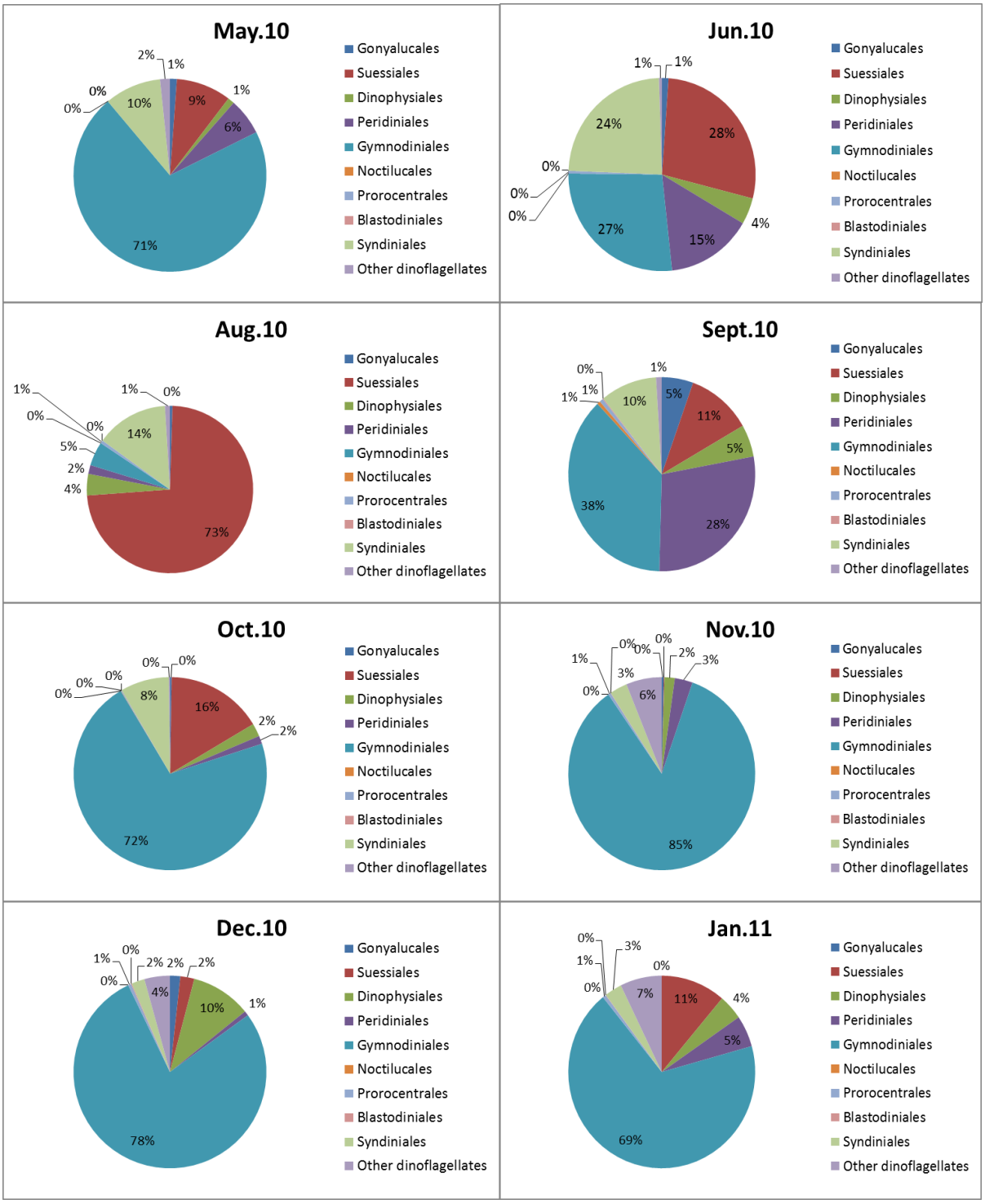

Figure A.6: Illustration of monthly sequence read abundance distribution amongst the main dinoflagellate orders and the Other dinoflagellates (May 2010-January 2011).

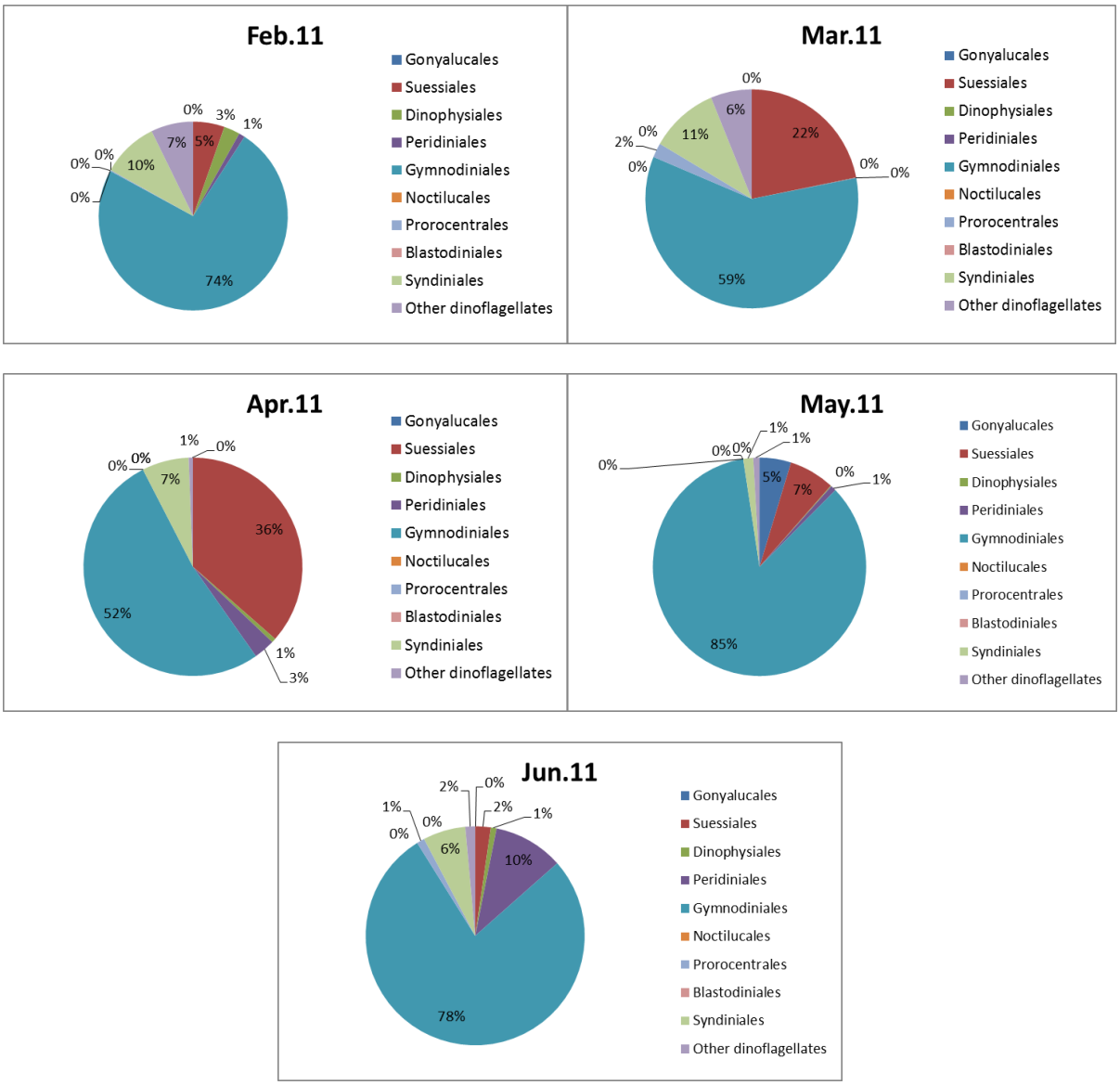

Figure A.7: Illustration of monthly sequence read distribution amongst the main dinoflagellate orders and the Other dinoflagellates (February 2011-June 2011).