



Ecological Characteristics of Dune
Heath Lakes in Thy National Park –
Threats and future Management

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Abstract

Thy National Park comprise the largest continuous dune areas in Denmark. Especially in low-lying dune slacks exists shallow dune- and heath lakes, which are home to unique plant communities. Rare Isoetids as *Littorella uniflora*, *Lobelia dortmanna*, *Isoetes lacustris* and *Isoetes echinospora* thrive in many of these lakes. However, many of the species are in rapid decline. Several lakes that were clear-watered in the past are now threatened by encroachment by *Sphagnum* sp. and *Juncus bulbosus*, high loadings of organic matter, brown coloring and acidification.

In 50 representative lakes in Thy National Park I examined the catchment land-use, water chemical factors and plant life. Organic material (FBOM) from the soil was compared with sources in the catchment using isotopic fingerprints. In order to identify possible links between lake water chemistry and land-use, hydrological catchments were classified in ArcMap. The plant species richness and relative frequencies of species were determined using eight transects in each lake. A PCA was used to analyze connections between physico-chemical factors and a DCA was used for the ordination of species data. The seed bank of a degraded lake was examined for germinating seeds to test the potential for restoration.

A strong causal link could not be established between catchment and water chemistry. Only plantations and bogs in the catchment could be compared to high nitrogen concentrations. Alkalinity and pH were the main explanatory factor for the species richness and distribution of plant communities. The vegetation in highly acidified lakes was dominated by species that thrive at high ammonium concentrations. Additionally, FBOM showed a negative influence on isoetids. The isotopic signal for FBOM was closely associated with the terrestrial organic soil around the lakes, suggesting that the highly organic sediment of the lakes originates from surface runoff with organic matter from the nearest surrounding areas. The sediment in the degraded lake showed signs of a species rich seed bank.

Primarily I found that many lakes in Thy National Park and their associated unique plant life are threatened by acidification, sedimentation of organic matter (FBOM) and encroachment by *Juncus bulbosus* and *Sphagnum* sp., which to some extent can be explained by acidification, nitrogen fallout, plantations and that the dune landscape has gradually become less dynamic. Restoring past dynamics and limiting vegetation in the dune landscape, together with continuous lake restoration, is suggested as strategies to alleviate the negative development in the lake systems.



Danish Abstract

Nationalpark Thy skaber rammen for de største sammenhængende klitområder i Danmark. Især i lavtliggende klitområder findes lavvandede klit- og hedesøer, som er hjemsted for et helt særligt planteliv. Sjældne isoetider som *Littorella uniflora*, *Lobelia dortmanna*, *Isoetes lacustris* og *Isoetes echinospora* trives i mange af disse søer. Dog er mange af arterne i kraftig tilbagegang. Flere søer der før i tiden var rene og klarvandede, trues nu af tilgroning med *Sphagnum* og *Juncus bulbosus*, tilslamning af labilt organisk materiale, brunfarvning og forsuring.

Vi har for 50 repræsentative søer i Nationalpark Thy undersøgt oplandstyper, vandkemiske parametre samt planteliv. Organisk materiale (FBOM) fra søers bund blev sammenlignet med eventuelle oplandsmæssige kilder vha. en analyse af isotopiske signaler. For at afdække eventuelle sammenhænge mellem søernes vandkemi og deres oplandstyper blev hydrologiske oplande klassificeret i ArcMap. Artsrigdom og relativ hyppighed af vegetationen blev kortlagt vha. otte transekter i hver sø. En PCA blev brugt til at analysere sammenhænge mellem fysisk-kemiske parametre og en DCA blev brugt til ordinationen af artsdata. Frøpuljen af en degraderet sø blev undersøgt i et spiringsforsøg for at teste potentialet for genopretning.

Der kunne ikke fastlægges en stærk årsagssammenhæng mellem opland og vandkemi. Kun plantager og moser i oplandet kunne sammenholdes med høje kvælstofkoncentrationer. Alkalinitet og pH var de vigtigste forklarende faktor for artsrigdom og fordeling af plantesamfund, og vegetationen i stærkt forsurede søer var domineret af arter, der trives ved høje ammoniumkoncentrationer. Derudover viste FBOM en negativ påvirkning på grundskudsplanter. Det isotopiske signal for FBOM havde en tæt sammenligning med terrestrisk organisk substrat omkring søerne, hvilket tyder på at tilslamning af søerne skyldes overflade afstrømning med organisk stof fra de nærmeste omkringliggende arealer. Sedimentet i den degraderede sø viste tegn på en artsrig frøpulje.

Overordnet fandt vi, at mange søer i Thy Nationalpark og deres tilhørende unikke planteliv er truet af forsuring, sedimentering af organisk materiale (FBOM) og tilgroning af *Juncus bulbosus* og *Sphagnum*, hvilket i nogen grad kan forklares ved forsuring, kvælstofnedfald, tilplantning af området og at klitlandskabet med tiden er blevet mindre dynamisk. En reetablering af tidligere tiders dynamik og begrænsning af vegetation i klitlandskabet sammen med løbende sø restaurering foreslås som strategier til at afhjælpe den negative udvikling.



Introduction

Western Jutland features a vast continuous coastal dune landscape. It is a diverse landscape with mosaics of several different successional stages providing habitats for a great number of specialized flora and fauna [1]. Many of the Danish dune habitats are highly threatened and listed as a priority in the European Union Habitats Directive [2] as they make up a third of the total coastal dune landscapes in northern Europe [3]. The different habitats are made up of exposed sandy beaches, embryonal dunes, fixed dunes, dune slacks and dune heath [4]. It is an aeolian landscape created by thousands of years of wind dynamics constantly impacting the formation of sandy dunes, with the first formations appearing around 7000 years ago [5]. The intensity of sand drift responsible for dune formation has been highly variable through history with several periods of fixation followed by remobilization [5], [6]. In especially dry periods, plant cover becomes scarce and sand will remobilize. This, in turn, makes it possible for sand deflation to occur, which creates depressions sometimes extending below the mean level of the water table. When wet conditions return, these depressions will become inundated and sometimes become shallow dune lakes. As a result, a lot of the entirely wind formed lakes are extremely transitory [7].

In Thy National Park in northwestern Jutland there are over 200 dune lakes or ponds of which the majority are wind formed. In addition, there exist a few other lake types inland from the dune heath. These deeper lakes are either solution type lakes resulting from depressions left by dissolution of the calcium rich bedrock in some areas, or they are kettle lakes created by depressions of melted ice blocks left behind during the last ice age [7]. Thus, the geological history of the landscape plays an essential role in the composition of lakes found in the area.

To evaluate the present status and future of the dune lakes in Thy National Park, there is great need for a labor intensive and thorough investigation of the area! In the present study I will focus on the abundance and composition of plant communities in dune lakes and evaluate the main threats and controlling factors of ecological conditions.

Threats to Coastal Dune Systems

Like the Danish inland freshwater systems, dune lakes and hinterland have experienced a myriad of anthropogenic stresses and influences through the years. Dune landscapes has historically been used for grazing by livestock, primitive agriculture and harvest of peat turf for firewood [3], [8]. However, since the massive sand drift periods in the 15-1700's, in part caused by the rough exploitation of the areas, the outlook on the natural dynamics changed [8]. The agricultural use of the dune landscapes



was subsequently discontinued to counteract the disastrous consequences of sand drift. New laws ensured the stabilization of the landscape by establishing pine plantations and *Ammophila arenaria* patches which helped fixate the dunes [9]. This paradigm shift in the once extremely dynamic aeolian systems severely changed the habitats and landscapes where these rare dune lakes are situated.

Eutrophication is a common threat to freshwater lakes in Denmark. It can lead to an increased growth of caulescent nymphaeids, helophytes and algae [10]. This in turn leads to shading and decline of isoetid macrophyte species. Eutrophication leading to an increase in primary production, will cause the accumulation of organic matter in the top sediment layer [11].

Lake acidification has previously been an important factor in the ecological decline of freshwater systems. In Europe the rates of lake acidification increased rapidly in the 1940's as a result of sulphur and NO_x emissions from fossil fuel combustion facilities, metal smelters, power plants and other industrial sources [12]. Since then, emissions have decreased significantly as a result of clean-air policies, closure of smelters and advancements in sulphur-scrubbing technologies [13], [14]. However, some areas still suffer from the consequences of the severe acid rain deposition that took place several decades ago.

Dissolved organic carbon (DOC) is an important controlling factor in light attenuation through the water column [15]. This is a result of the highly chromophoric properties of the organic molecules constituting DOC which in turn is highly correlated with chromophoric dissolved organic matter (CDOM) [16], [17]. The absorption of light by CDOM is strongest towards the lower end of the PAR spectrum (400-700 nm) [18], thus impacting the blue part of the PAR spectrum (400-500 nm); wavelengths by which absorption by several photosynthetic pigments peak [19]. Therefore, it can be expected that a high concentration of DOC, and in turn CDOM in the water column, can have a significant impact on the distribution of isoetid macrophytes.

Plant Communities in Dune Lakes

Coastal wetlands are home to several different plant communities. A plant community is often defined as a collection of plant species growing together in a particular environment and having a definite association with each other. Certain species are thus found growing together in certain locations more frequently than would be expected by chance [20]. Different plant species assemble in these communities because they have similar growth requirements in terms of both abiotic and biotic factors. They may prefer the same substrate, pH, alkalinity, nutrient levels and light (abiotic) or may require a high amount of disturbance from grazing, burning or trampling, reducing competition from

other species (biotic). Thus, it can be expected to see a greater species related similarity between lakes with similar water chemistry.



Figure 1: *Isoetes echinospora*. Photo: Michael Straarup Nielsen

Each species will have a growth optimum for a given environmental factor. If the abundance of a species is plotted against a variation of any given environmental factor it will likely approximate to a normal curve. This variation of species abundance across a varying environmental factor is called an environmental gradient. These gradients vary between environmental factors for the single species and varies between species.

The lakes in Thy National Park are home to a wide variety of highly specialized aquatic macrophytes. The shallow lakes contain rare isoetid species such as *Littorella uniflora* [21], *Isoetes lacustris*, *Isoetes echinospora* (Figure 1), *Lobelia dortmanna* (Figure 2), *Pilularia globulifera*, *Eleocharis spp.* and until recently *Subularia aquatica*. Some deeper lakes contain a wide variety of elodeid species as well, including several *Potamogeton*, *Myriophyllum* and *Ranunculus* species [22]. Many of these species, especially of the isoetid type, are threatened in Denmark and in the rest of their European distribution range [23]. Additionally, many of the lakes home to these rare species have experienced a serious decline in both macrophyte cover and water quality [24], [25].



Figure 2: Flowering *Lobelia dortmanna*. Photo: Michael Straarup Nielsen

Several studies suggest that isoetid species respond negatively on changing sediment conditions. Preferring an open firm and sandy substrate from which they derive most of their CO₂, many isoetids are sensitive to sediment accumulation of organic matter [26]–[28]. Especially root anchorage and seed germination is negatively affected by increasing organic sediment loads [27], [29]. Although factors controlling the distributions of hydrophytes are well known, the dynamics affecting their habitats, the rare dune lakes, are poorly understood.

The aims of the study are to:

- Determine how the water-chemical factors in the lakes are impacted by watershed land-use. Specifically identify the origin of FBOM in the lakes.



- Identify and describe macrophyte communities in the sampled lakes.
- Examine how distribution of macrophyte communities is related to physico-chemical conditions in the lakes during summer and autumn.
- Discuss the main threats to the lake ecology in Thy National Park. In addition, outline future implications for the aquatic macrophyte communities and the ecological status of the lakes in general with the changing climatic and ecological regimes in the dune landscape. Lastly, test the sediment seed bank of degraded lakes as an evaluation of restoration potential.

Methods

Study site and sampling design

Field studies were conducted in Thy National Park, North-Western Jutland, Denmark (56°56'00.6"N 8°20'44.1"E)(Figure 3). The park comprises the largest continuous coastal dune and heather landscape in Denmark, a total of 244 km² including vast stretches of pine plantations. Vegetation data was collected between the 1st and 31st of July 2017, where most plants were in an active phase and identification was possible. Water-chemical data was collected between the 7th and 8th of August for summer, and between the 4th and 8th of November for fall. Samples were collected in both August and November to examine chemical differences between summer and autumn. Only 20 of a total of 50 lakes could be sampled in August as many of the lakes are highly transient and dry out in summer.

During field work in July the weather was mainly sunny to partly cloudy and dry. Rain only occurred for a total of about 10 days during July, and winds were mainly blowing with speeds of around 5-7 m/s. The diurnal mean temperature was about 15 °C. Many of the lakes were completely dried out at this time.

August was mostly cloudy and rainy. Like in July the winds were blowing with speeds of 5-7 m/s and the diurnal mean temperature was around 15 °C.

Weather during the November sampling was mostly cloudy and dry, with a single rainy day. Windspeeds were around 4-5 m/s and the mean diurnal temperature around 6 °C.

Selecting lakes for sampling

The map in Figure 3, shows the extent and distribution of sampled lakes in Thy National Park. Lakes in the area are generally shallow (see Figure 4), slightly acidic and oligo- to dystrophic.



0 0,4 0,8 1,6 2,4
Kilometers

Figure 3: Map showing areas with 50 examined lakes in Thy National Park in North-western Jutland. Maps from Kortforsyningen and Google Maps.

However, a few lakes situated in the northern part of the park can be considered of the karst lake type because of large amounts of limestone in the underground in specific areas. In our data set I aimed to include lakes of all types in a dataset of 50 lakes. The selection includes lakes distributed from the northernmost to the southernmost part of the park. Lake sizes range from 0,0015 km² to 0,39 km². The amount of pine plantation, heather, bog and agriculture in the catchment varies greatly between the sampled lakes.



Figure 4: Shallow lake near Tved Klitplantage. Photo: Michael Straarup Nielsen

Four newly established lakes were also selected for sampling. Three of the lakes, dug in 2014, were located in a dry heathland near Stenbjerg Dune Plantation. I call these St_n_l_w, St_n_l_e and St_n_l_s (abbr. for Stenbjerg new lake west, east and south. The newest lake, dug in 2016, were located in the edge of the boggy wetlands of Ålvand Dune Heath. I call this one Ål_n_l (abbr. for Ålvand new lake).

Vegetation sampling

Vegetation data was collected using a transect method. Eight transects were evenly distributed around each lake using a field compass to determine the eight corners of the world (see Figure 5). Each transect was positioned perpendicular to the lake shore and extended either 25 meters into the lake, until the middle was reached or until vegetation cover became absent. For each meter of the transect a plot of 30x30 cm was examined for plant species. Some deeper lakes only had a narrow littoral zone of a few meters wide. In those lakes, plots were placed for each half meter of the transect.

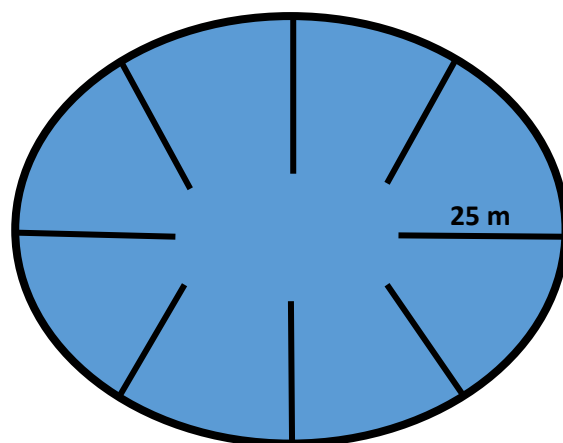


Figure 5: Transect method used for vegetation sampling, showing eight transects, one in each of the eight corners of the world.

Higher plants were identified on location, while characeans were collected for examination under microscope. All species were identified using ‘Danmarks Vandplanter’ by Schou *et al.* 2017 [30]. Species were registered by presence/absence in a transect table printed on waterproof paper. Additional species found outside the transects were noted as well for a complete species list.



Relative frequencies of plant species are calculated as:

$$\text{Relative frequency} = \frac{\text{number of plots containing a specific species}}{\text{number of plots in the lake}}$$

Physico-chemical Sampling

Water-chemical samples were collected in both August and November to examine chemical differences between summer and autumn.

Organic Matter

Water samples were collected at the east side of the lakes approximately five meters from the lake shore. Water was collected at 10 cm below the water surface. Samples for chromophoric dissolved organic matter (CDOM) and dissolved organic matter (DOC) were filtered through Whatman GF/F filters and refrigerated in the dark in pre-combusted glass vials until analysis. Samples for total organic carbon (TOC) were collected unfiltered in TPP centrifuge tubes from a depth of approx. 10 cm. TOC was analyzed on a Shimadzu TOC-V CPH total organic carbon analyzer. I used Specific Ultraviolet Absorbance at 254 nm (SUVA 254) as an indicator for CDOM. SUVA 254 is defined as the UV absorbance at 254 nanometers measured in inverse meters (m^{-1}) divided by the DOC concentration in milligrams pr. liter. (mg L^{-1}) [31].

The amount of fine benthic organic matter (FBOM), were estimated in the littoral zone of each lake. For each plot along the transects the depth of labile organic matter were measured with a ruler on a wooden stick, which was pushed through the organic layer until a firm sandy bottom was reached. The value of FBOM is given as the mean of the thickness of labile organic matter in the littoral zone in a specific lake.

Nutrients and Trace Elements

Samples for NO_3^- , NH_4^+ and PO_4^{3-} were filtered through Whatman 1,2 μm GF/C filters and stored in 50 ml polypropylene vials in the freezer until analysis. The samples were analyzed using a Lachat Quikchem 8500 flow injection analyzer.

Water samples for total phosphorous (TP), and total nitrogen (TN) were collected unfiltered in TPP centrifuge tubes from a depth of approx. 10 cm. Samples for TN collected in august however, were



filtered through a Whatman GF/C filter, thus did not include particulate nitrogen. All samples were stored in the freezer until analysis.

Samples for TP were analyzed using a Lachat Quikchem 8500 flow injection analyzer, while samples for TN were analyzed on a Shimadzu TNM-1 total nitrogen measuring unit.

To determine trace elements, water samples were filtered through Whatman GF/F filters and stored in 50 ml acid washed TPP centrifuge tubes in the freezer until analysis. The analysis was performed for elemental sodium, sulphur and iron using an Agilent 720 Inductive Coupled Plasma Optical Emission Spectrometer.

Chlorophyll-a

Chlorophyll-a samples were collected using Whatman 1,2 µm GF/C filters. Lake water was pumped through the filters until clogging, and filtrate volume was recorded. The filters were stored in small plastic vials and frozen until analysis.

For analysis, filters were submerged in 6 ml of 96% ethanol for extraction of chlorophyll-a. The samples were measured for absorbance at wavelengths 665 nm and 750 nm on a Shimadzu UV-1700 Spectrophotometer fixed to a flow-through pump. The spectrophotometer was recalibrated at each ten samples.

Chlorophyll-a concentration was determined using the formula:

$$\text{Chlorophyll } a = \frac{(Abs_{665} - Abs_{750}) * E * 10^3}{83,4 * V}$$

where Abs_{665} and Abs_{750} are absorbances at respective wavelengths, E is the volume (ml) of the extraction agent (ethanol), V is the filtrate volume (l) and 83,4 (l/g/cm) is the absorbance coefficient of chlorophyll in ethanol [32].

Alkalinity and pH

Alkalinity and pH samples were collected in 200 ml amber glass bottles and refrigerated until analysis. Two samples were collected for each lake. Bottles were flushed three times *in situ* and filled under water until no bubbles were left, to avoid any CO₂ contamination. Alkalinity of the samples were determined using the Gran-titration method using a Radiometer Analytical TIM850 Titration Manager [33]. Values for alkalinity are given in milliequivalent pr. L (meq/L). Samples for pH were measured on a Radiometer Copenhagen PHM 92 lab pH-meter.



Stable Isotope Analysis

In order to assess the origin of FBOM in the lakes I analyzed the content of the stable isotopes ^{13}C and ^{15}N in the main biomass components in and around three of the lakes (12_6, 12_28 and 12_21). All three lakes are situated in Ålvand Dune Heath. Lake 12_6 and 12_28 are situated in open heath/bog with *Erica tetralix*, *Myrica gale* and *Molinia caerulea* among the dominating terrestrial plant species. Lake 12_21 is situated in part pine plantation and part heath with *Erica tetralix*, *Calluna vulgaris*, *Molinia caerulea*, *Pinus contorta* and *Myrica gale* among the dominating terrestrial plant species.

Samples were measured for nitrogen and carbon levels on a Thermo Electronics Corporation FlashEA® 1112 series Nitrogen and Carbon Analyzer, and the isotope ratios $^{12}\text{C}/^{13}\text{C}$ and $^{14}\text{N}/^{15}\text{N}$ were analyzed on a Thermo Scientific Delta V™ plus Isotope Ratio Mass Spectrometer.

Germination of Seed Banks

I chose three lakes for testing the potential of viable seeds in the sediment seed bank. The three lakes were of different ecological states. The first one, lake 19_4, has previously been a pristine clear water lake containing rare species like *Isoetes echinospora*, *Isoetes lacustris*, *Lobelia dortmanna*, *Littorella uniflora* and *Elatine hexandra* [25]. To this day, none of the species remains and the lake is in a highly degraded condition.

The second lake, A_108, also known as Tvorup Hul, has previously contained all the same species as in lake 19_4 including the extremely rare isoetid, *Subularia aquatica*, found last time in 1998. Today only about half of the species remains, and the lake is in a partly degraded condition.

The last lake, 4_9 also known as Kokkær Vand, is still a system of very high ecological quality. The lake sustains a healthy population of *Lobelia dortmanna*, *Littorella uniflora*, *Isoetes echinospora* and even patches of the rare aquatic fern, *Pilularia globulifera*.

Samples were collected on June 11th, 2018. Sediment cores was collected using acrylic pipes 'kajakrør', which was pushed 10 cm into the sediment. Samples were collected in three different spots on the eastern side of each lake.

Sediment was placed in plastic boxes in a greenhouse and frequently watered with demineralized water, so the sediment was kept wet. Upon germination identifiable seedlings were counted and noted in a table.



Multivariate Methods

Environmental gradients are a core principle in multivariate analysis, that is, that different species has unique distributions across on or more environmental factors characteristic to the habitat.

Gradient analyzes can be divided into ‘indirect’ and ‘direct’ methods. A direct gradient analysis is used to display the variation of vegetation in relation to environmental factors while directly using environmental data to order vegetation samples. Direct methods thus assume that underlying environmental gradients are known.

While using an indirect method the vegetation data is ordinated independently of environmental data. Only in a second stage in the analysis environmental or biotic data are applied in order to explain variation in the vegetation. This method can especially be used when underlying environmental gradients are unknown or unclear [34].

For the multivariat analysis of physical and chemical factors of the lakes I used the ordination method, PCA (Principal Component Analysis).

For the multivariate analysis of plant communities of different lakes, I used the indirect method, DCA (Detreted Correspondence Analysis). In the ordination, axes were rescaled, and rare species were downweighed. When rescaling, the gradient length of the axes can be obtained. These indirect gradients of the DCA is a measure for beta diversity, which is the differentiation in species among habitats [35].

The PCA and DCA were performed in the ordination software package PC-ORD 6 by Wild Blueberry Media LLC.

The Spearman’s Rank correlation coefficient (Spearman’s Rho, ρ) was used as a measure of correlation between physico-chemical factors and ordination axes.

Isotopic fingerprints were visualized in a hierarchical clustering using group average method in JMP 14 by SAS.

Geographic Information System

A geographic information system (GIS) is a system designed to analyze and delineate spatial data. This data is mostly based remote sensing data obtained by satellites. Among other things it can be used for classification of land cover and land use, calculation of biomass in a specific area and determination of hydrological basins and watersheds.



GIS-data of watersheds draining to specific lakes in Thy National Park were delineated by DMU (Danmarks Miljøundersøgelser) and provided by Thy National Park. The digital watersheds are created by using a digital elevation model (DEM). The DEM is a three-dimensional representation of a specific area's surface and is created by a remote sensing satellite using a LIDAR to measure the distance to earth's surface. By analyzing the topography of the DEM, the flow direction of the water can be determined with high accuracy, which is subsequently used for delineating the watershed area.

GIS-data for habitat types were obtained from Danmarks Miljøportal and Kortforsyningen (Styrelsen for Dataforsyning og Effektivisering). The maps contain spatial data for agriculture, forest/plantation, dunes, heath, bog, fens, lakes and grasslands.

Results

Physico-chemical Analysis

All variables examined for the 20 permanent lakes in August and all 50 lakes in November are summarized in Table 1. The complete data matrices are found in sAppendix 1 and Appendix 2 respectively.

The dataset comprise a wide range of lake types. The range in alkalinity is broad, however the mean buffer capacity for the lakes is low (= 0,11 meq/L in November). The same goes for pH, where most of the lakes are below neutral (pH = 7). The values of organic carbon as well as CDOM is highly variable between lakes and the means relatively high (SUVA = 5,27 for

Table 1: Summary of physico-chemical factors from 20 lakes in August and 50 lakes in November.

August		
Factor	Range	Mean±SD
Alkalinity (meq/L)	0,03-1,44	0,28±0,35
pH	4,91-8,0	5,95±0,74
DOC (mg/L)	5,57-19,65	10,59±3,54
TOC (mg/L)	3,34-17,64	10,38±3,57
TN (dissolved) (mg/L)	0,22-2,24	0,63±0,49
Chlorophyll-a (mg/L)	1,10-34,36	10,82±10,09
CDOM	1,35-3,87	2,70±0,64
PO ₄ ³⁻ (mg/L)	1,00E-3-6,00E-3,1	2,95E-3±1,28E-3
NH ₄ ⁺ (mg/L)	0,03-1,71	0,18±0,40
NO ₃ ⁻ (mg/L)	0,01-0,35	0,05±0,08
Na (mg/L)	7,63-61,03	29,48±13,21
Fe (mg/L)	0-4,40	0,65±1,09
S (mg/L)	0,36-5,83	2,17±1,39
Area (m2)	1530-3,93E+5,9	5,49E+4±9,12E+4
FBOM	0,03-56,32	7,17±12,25
%Fens	0-14,20	2,39±4,80
%Heath	0,50-74,90	41,41±27,03
%Pasture	0-9,80	0,73±2,21
%Plantation	1,60-77,40	26,06±26,46
%Bog	0-38,30	12,79±11,72
%Agriculture	0-37,80	3,72±8,76
Distance to plantation (m)	0-586,02	161,57±173,43
November		
Factor	Range	Mean±SD
Alkalinity (meq/L)	0,002-0,68	0,11±0,17
pH	4,88-7,60	5,84±0,65
DOC (mg/L)	6,45-35,97	12,31±5,24
TOC (mg/L)	6,77-42,90	14,12±6,09
TN (mg/L)	0,27-2,26	0,89±0,43
Chlorophyll-a (mg/L)	0-5,80E-2	6,46E-3±9,20E-3
CDOM	3,01-9,02	5,27±1,44
PO ₄ ³⁻ (mg/L)	2,00E-3-2,20E-2	5,18E-3±3,27E-3
NH ₄ ⁺ (mg/L)	0,02-0,64	0,17±0,18
NO ₃ ⁻ (mg/L)	0,002-0,53	0,10±0,11
TP (mg/L)	0,02-0,13	0,05±0,03
Na (mg/L)	6,19-55,40	22,95±7,95
Fe (mg/L)	0-2,14	0,33±0,34
S (mg/L)	0,50-5,50	2,25±0,98
Area (m2)	1348-3,93E+5	2,66E+4±6,15E+4
FBOM	0-56,315	6,47±8,36
%Fens	0-14,2	1,26±3,33
%Heath	0,5-80,3	48,34±24,64
%Pasture	0-9,8	0,30±1,42
%Plantation	0,9-83,2	25,43±25,98
%Bog	0-38,3	12,25±10,69
%Agriculture	0-38,5	2,96±7,95
Distance to plantation (m)	0-1084,584	249,056±306,44



November). Nutrient measures (TN, PO_4^{3-} , NH_4^+ and NO_3^-) varies markedly between lakes, but are generally low to moderate. Some lakes have a high concentration of potassium (Na), especially lake 6_3 with a concentration of 61,03 mg/L for August. This lake is located about 200 meters from the sea.

Some of the ranges and means for the dataset collected in November are marginally different from the ones from August. Especially the range and mean of alkalinity is much lower, as well as the measures for organic carbon. However, the nutrient measures PO_4^{3-} and NO_3^- , both means and range are higher for November than August. This is not merely due to the dataset being smaller for August, for example the lakes 12_12 and 14_2 have a difference in concentration for NO_3^- of +0,13 mg/L and +0,32 mg/L respectively. The values for Na, Fe and S are highly variable in each lake between the months. Some are lower in November compared to August and some are higher.

The percentage of fens, pasture and agriculture in the catchment were generally very low to absent and are not widely considered in the analysis.

Lake Conditions in Relation to Land-use

The result of the PCA-analysis calculated on 22 environmental variables in 20 shallow lakes of Thy National Park in August 2017 is shown with axes 1 and 2 in Figure 6. It shows the relationships between physico-chemical variables in the lakes and land-use in the catchment.

There is a pronounced variation in physico-chemical conditions between the lakes in both August and November. In August axis 1 explains 19.37% of the total variation between the lakes. Distribution of lakes along axis 1 is strongest

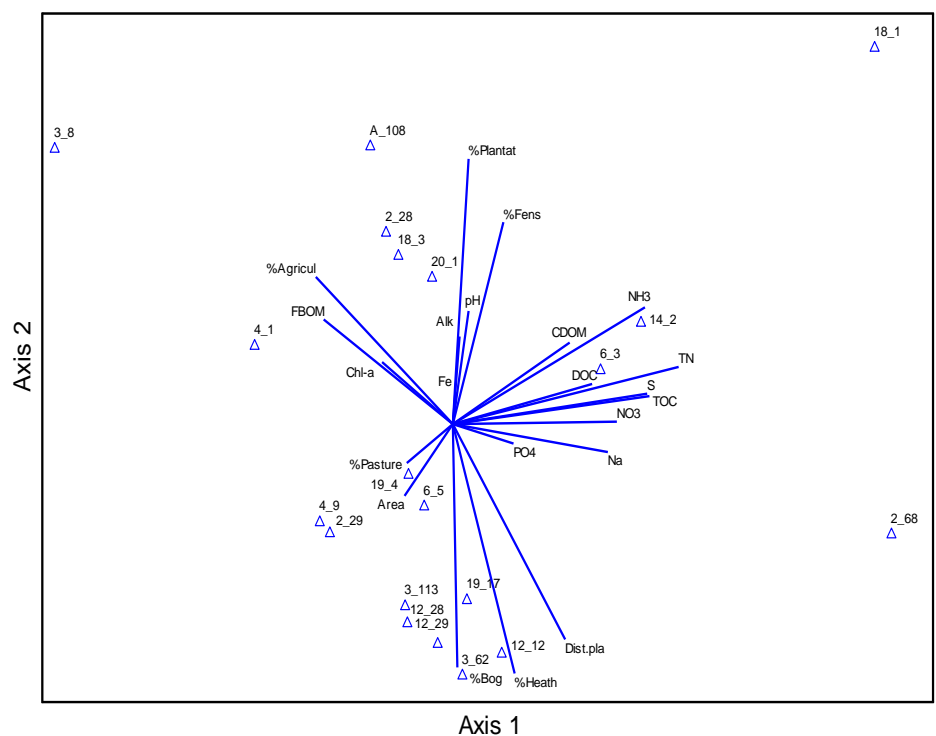


Figure 6: PCA ordination of 22 physical, chemical and catchment related variables on 20 lakes in August. Explanatory values: axis 1 = 19,37%, axis 2 = 17,18%, axis 3 = 14,33%.



Alkalinity, pH, percent plantation and percent heath in the watershed are the most important factors correlated with the second axis. This axis thus represents a gradient from acidous heath lakes to more alkaline lakes situated in or near plantations. The second axis explains 14,47% of the total variation between the lakes.

Lake area and Na are the most strongly correlated with the third axis, with a small effect from Chlorophyll-a. Thus, the third axis represents a gradient from small sodium poor and chlorophyll rich lakes to larger lakes with more sodium and less chlorophyll. This axis explains 9,55% of the total variation between the lakes. Thus, the cumulative explanatory value of the first three vectors is 49,70%.

Spearman's Rank Correlations

August

The most relevant correlations between specific variables are highlighted here. Spearman's Rank Correlations between all physico-chemical variables in August are shown in appendix 3. The most significant correlation was between alkalinity and pH ($\rho=0,96$, $p<0,0005$) which is also obvious from the diagram. The two measures for organic carbon, TOC and DOC are also significantly correlated ($\rho=0,61$, $p<0,005$), while TOC is also correlated with TN ($\rho=0,581$, $p<0,01$). Higher values of NO_3^- corresponds with higher values of NH_4^+ ($\rho=0,62$, $p<0,005$). Higher levels of Fe are significantly correlated with higher values of light absorption (SUVA 254/CDOM) ($\rho=0,57$, $p<0,01$).

November

The Spearman's Rank Correlations for all physico-chemical variables in November are shown in appendix 4. TOC and DOC are positively correlated column ($\rho=0,89$, $p<0,0001$), suggesting that a large portion of the total carbon is dissolved in the water column. Also, CDOM and DOC are strongly correlated ($\rho=0,54$, $p<0,0001$). NH_4^+ was strongly positively correlated with TN ($\rho=0,86$, $p<0,0001$) and CDOM ($\rho=0,44$, $p<0,005$). NO_3^- was also strongly positively correlated with TN ($\rho=0,619$, $p<0,0001$) and slightly but significantly with CDOM ($\rho=0,317$, $p<0,05$). Total phosphorus (TP) was positively correlated with TOC ($\rho=0,474$, $p=0,0005$), chlorophyll-a ($\rho=0,399$, $p=0,005$) and CDOM ($\rho=0,612$, $p<0,0001$). Lake area was negatively correlated with DOC ($\rho=-0,420$, $p<0,005$), TOC ($\rho=-0,315$, $p > 0,05$) thus the smaller the lake area the higher values for DOC and TOC gets.

The percentage of plantations in the watershed correlated significantly with alkalinity ($\rho=0,309$, $p<0,05$) and pH ($\rho=0,383$, $p<0,01$). Thus, lakes with a large percentage of plantations in the watershed

had a higher alkalinity and pH. The percentage of plantations was negatively correlated with iron (Fe) ($\rho=-0,412$, $p<0,005$).

Stable Isotope Analysis

The isotopic fingerprints of plants samples, terrestrial substrate and FBOM for lake 12_6, 12_28 and 12_21 are found in Appendix 5. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of plant samples for lake 12_6 ranged from -31,24‰ to -26,42‰ for $\delta^{13}\text{C}$ and from -1,06‰ to -0,43‰ for $\delta^{15}\text{N}$.

Samples of terrestrial substrate ranged from -28,38‰ to -27,97‰ for $\delta^{13}\text{C}$ and from -5,65‰ to -1,37‰ for $\delta^{15}\text{N}$. Samples of fine benthic organic matter (FBOM) ranged from -28,14‰ to -27,62‰ for $\delta^{13}\text{C}$ and from -2,62‰ to -1,59‰ for $\delta^{15}\text{N}$. The different plant sources of different distances (1m/10m), terrestrial substrate of different distances from the lake (1m/10m) and FBOM are clustered using Euclidean distance measure and the group average method in Figure 8.

If dissected at a level with about 70% information retained, I end up with seven small groups in lake 12_6. In one group, FBOM, terrestrial substrate (1m), *Molinia caerulea* (10m) and terrestrial substrate (10m) branch out in very low distance from each other.

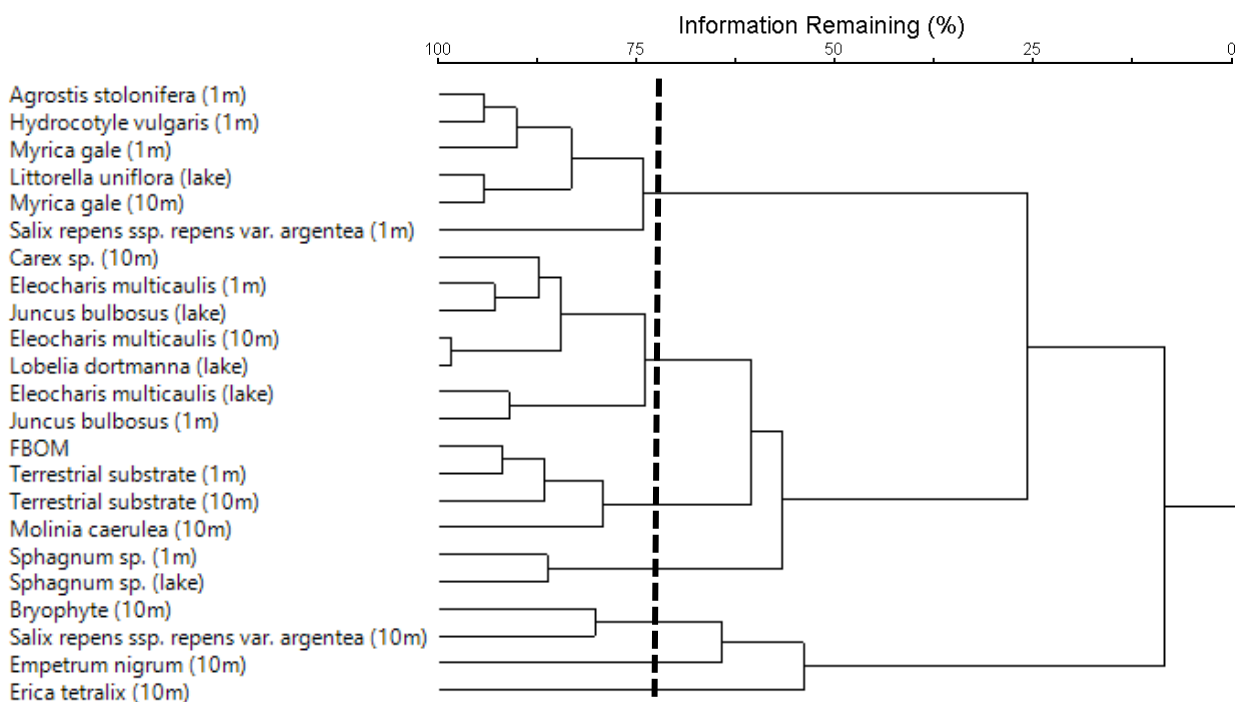


Figure 8: Clustering of isotopic fingerprints of lake 12_6.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of plant samples for lake 12_28 ranged from -32,54‰ to -22,41‰ for $\delta^{13}\text{C}$ and from -3,81‰ to 0,99‰ for $\delta^{15}\text{N}$.

In lake 12_28, samples of terrestrial substrate ranged from -30,13‰ to -27,54‰ for $\delta^{13}\text{C}$ and from -3,90‰ to -1,12‰ for $\delta^{15}\text{N}$. Samples of fine benthic organic matter (FBOM) ranged from -28,82‰ to

-27,95‰ for $\delta^{13}\text{C}$ and from -1,32‰ to -0,88‰ for $\delta^{15}\text{N}$. Like before, the different sources are visualized in a group average clustering using Euclidean distance measure in Figure 9.

If dissected at a level with about 77% information retained, I end up with seven small groups in lake 12_28. In one group, FBOM is closely grouped together with *Eleocharis palustris* ssp. *vulgaris* (lake). Subsequently the similarity is close to terrestrial substrate (1m), *Sphagnum* (1m and 10m), *Carex* sp. (1m) and terrestrial substrate (10m).

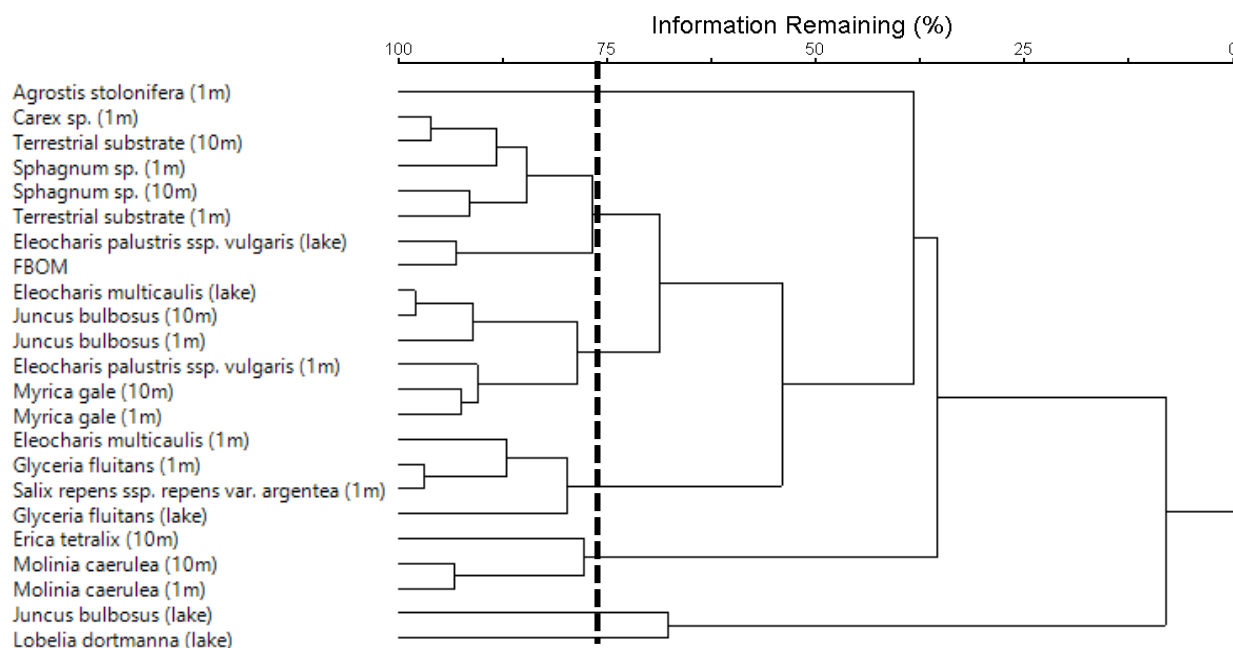


Figure 9: Clustering of isotopic fingerprints of lake 12_28.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of plant samples for this lake ranged from -33,21‰ to -25,70‰ for $\delta^{13}\text{C}$ and from -7,73‰ to 2,18‰ for $\delta^{15}\text{N}$.

Lake 12_21 samples of terrestrial substrate ranged from -31,21‰ to -28,31‰ for $\delta^{13}\text{C}$ and from -3,99‰ to -0,50‰ for $\delta^{15}\text{N}$. Samples of fine benthic organic matter (FBOM) ranged from -30,20‰ to -28,32‰ for $\delta^{13}\text{C}$ and from -2,87‰ to -1,77‰ for $\delta^{15}\text{N}$. Like before, the different sources are visualized in a group average clustering using Euclidean distance measure in Figure 10.

If dissected at a level with about 75% information retained, I end up with nine small groups in lake 12_21. In one group, FBOM is closely grouped together with *Sphagnum* sp. (10m), terrestrial substrate (10m) and terrestrial substrate (1m).

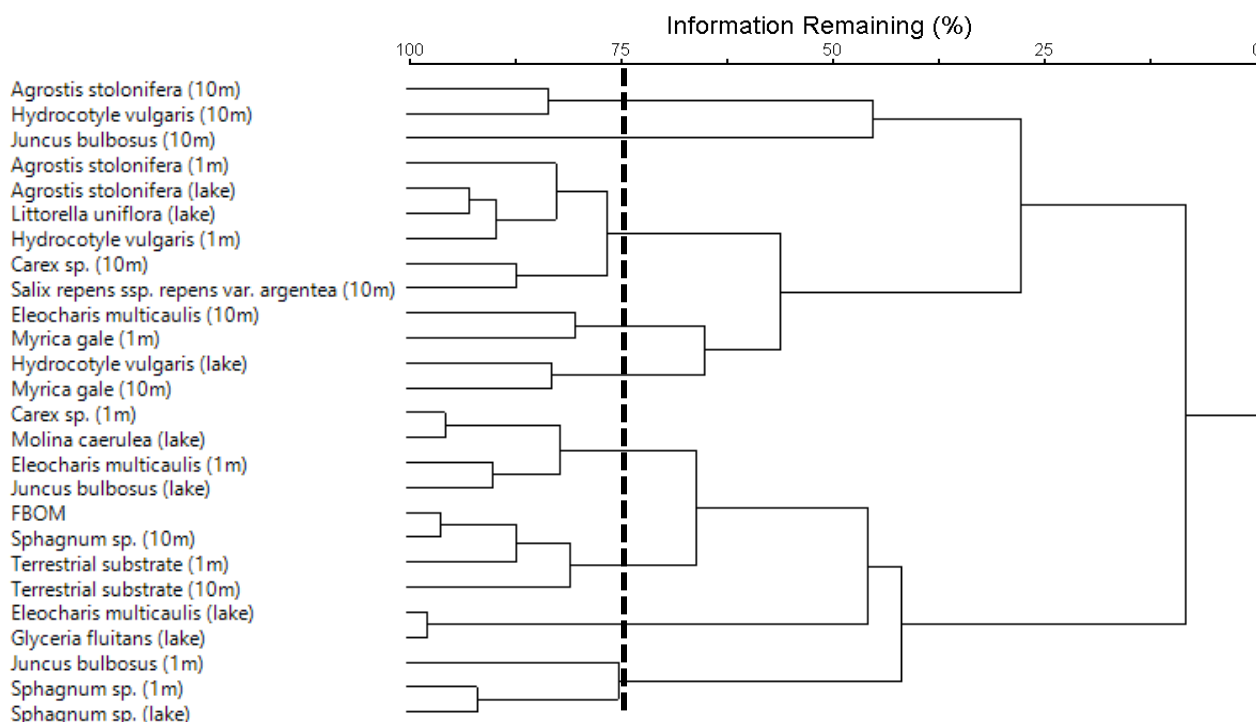


Figure 10: Clustering of isotopic fingerprints of lake 12_21.

Species Diversity

The number of plant species and the plant species diversity indices for the 50 study lakes are summarized in Table 2. The full table with values for species richness, evenness, Shannon and Simpson diversity are found in Appendix 6. A total of 65 different of macrophyte species (including *Spongilla palustris*) were found across the 50 lakes. The full species lists for each lake are found in Appendix 6.

The species richness is highly variable between lakes. Only four species were found in lake Ål_n_1, which is an artificial lake dug in late 2016. Another lake, 2_28, had a very high species richness of 26 species with one of the highest values for evenness of 0,85, the highest Shannon diversity index of 2,76 and the highest Simpson diversity index of 0,91. Most of the lakes are relatively species poor, thus the mean species richness is 11,70.

Table 2: Aquatic macrophyte diversity indices of 50 lakes in Thy National Park.

	Mean	Stand.Dev.	Minimum	Maximum
Species	11,70	4,45	4	26
Evenness	0,76	0,10	0,49	0,91
Shannon	1,82	0,40	0,86	2,76
Simpson	0,77	0,12	0,40	0,91

The 20 most common species are shown in Table 3. The complete species lists are found in Appendix 8.

Table 3: The 20 most common plant species found in lakes of Thy National Park.

Species	n lakes
<i>Hydrocotyle vulgaris</i>	47
<i>Juncus bulbosus</i>	46
<i>Glyceria fluitans</i>	44
<i>Carex nigra var. nigra</i>	37
<i>Sphagnum sp.</i>	36
<i>Littorella uniflora</i>	36
<i>Eleocharis palustris ssp. vulgaris</i>	35
<i>Agrostis stolonifera</i>	33
<i>Eleocharis multicaulis</i>	33
<i>Potamogeton polygonifolia</i>	33
<i>Persicaria amphibia</i>	29
<i>Comarum palustre</i>	29
<i>Lobelia dortmanna</i>	24
<i>Ranunculus flammula</i>	24
<i>Phragmites australis</i>	18
<i>Isolepis fluitans</i>	16
<i>Sparganium angustifolium</i>	15
<i>Juncus articulatus</i>	14
<i>Apium inundatum</i>	14
<i>Juncus effesus</i>	13

The most common plants in the study were mainly *Hydrocotyle vulgaris*, *Juncus bulbosus* and *Glyceria fluitans*. However, isoetids like *Littorella uniflora*, *Eleocharis multicaulis* and *Lobelia dortmanna* were relatively common. Thus, many lakes were dominated by plants of the isoetid type, while only a few lakes had a considerable amount of elodeid, nymphaeid and helophytic type plants. Of the elodeid types, *Potamogeton polygonifolia* and *Potamogeton natans* were the most common. Of the nymphaeid types, *Persicaria amphibia* was very common while *Nuphar lutea* was only found in a few lakes. Of the helophytes, *Glyceria fluitans*, *Eleocharis palustris ssp. vulgaris* and *Phragmites australis* were the most common species.

Included in the species list, although not a macrophyte, is the common poriferan *Spongilla lacustris*, as it was widely distributed in the examined lakes. Also the charophytes *Chara virgata* and *Nitella sp.* were included.

Only a few terrestrial/amphibian plants were included, like *Bidens sp.*, *Lysimachia sp.*, *Carex sp.*, *Comarum palustre*, *Ranunculus flammula* and a few common *Juncus* species besides *Juncus bulbosus*.

Vegetation distribution

The DCA analysis of 50 lakes in Thy National Park, shows that the lakes separated along two axes based on plant species distribution (Figure 11). The relative frequencies of macrophyte species in each lake are shown in Appendix 7.



Isoetes lacustris are found here. In the right end of axis 1 more elodeids start to appear. Especially different *Potamogeton* species, *Myriophyllum alterniflorum* and *Elodea canadensis*.

DCA Explained by Physico-chemical Gradients

The Spearman's Rank Correlation coefficient values from the indirect analysis of the DCA axes on

Table 4: Spearman's Rank correlation coefficients between DCA-axes and physico-chemical factors of 50 lakes in November, 2017.

	DCA1	DCA2	DCA3
Alkalinity	0,50**	0,20	-0,19
pH	0,51**	0,28*	-0,13
DOC	-0,19	-0,10	-0,18
TOC	-0,21	-0,11	-0,14
TN	-0,08	-0,28	-0,09
Chlorophyll-a	-0,01	-0,16	0,26
CDOM	0,01	-0,21	-0,01
PO ₄ ³⁻	0,17	-0,22	-0,13
NH ₄ ⁺	-0,04	-0,34*	-0,13
NO ₃ ⁻	0,03	-0,27	0,01
TP	0,12	-0,21	0,17
Na	0,22	0,18	-0,18
Fe	-0,08	-0,16	-0,04
S	0,03	0,23	-0,23
Area	0,42**	-0,15	0,07
FBOM	0,25	-0,329*	-0,24
%Fens	0,28*	-0,19	-0,04
%Heath	-0,02	0,07	-0,12
%Pasture	0,27	-0,04	-0,11
%Plantation	0,38**	-0,12	-0,24
%Bog	-0,05	-0,05	0,01
%Agriculture	0,27	-0,23	-0,18
Distance to plantation	-0,07	0,02	0,16
PCA1	-0,18	-0,23	0,02
PCA2	0,32*	-0,13	-0,19
PCA3	0,51**	0,13	-0,11

the PCA axes and physico-chemical variables are shown in Table 4.

The first axis, DCA1, is very strongly correlated with alkalinity ($\rho = 0,50$, $p > 0,0005$) and pH ($\rho = 0,51$, $p > 0,0005$). The variation of DCA1 is also partly explained by percentage of plantation in the catchment ($\rho = 0,38$, $p > 0,01$) and percentage of fens in the catchment ($\rho = 0,28$, $p > 0,05$). DCA1 is also correlated with PCA2 ($\rho = 0,32$, $p > 0,05$), which represents catchment land-use, and PCA3 ($\rho = 0,51$, $p > 0,0005$) which represents alkalinity, pH, area and Na.

The DCA2 is significantly negatively correlated with FBOM ($\rho = -0,33$, $p > 0,05$). Thus, the plants in the lower part of the axis are found in areas heavily loaded with organic matter. Some of the variation on DCA2 is explained by pH ($\rho = 0,28$, $p > 0,05$) and NH₄⁺ ($\rho = -0,34$, $p > 0,05$).

None of the variation in DCA3 was significantly explained by the physico-chemical variables examined in this study.

Germination of Seed Banks

The number of identified macrophyte species germinated from seeds and spores in sediment samples from the three selected lakes is shown in Table 5.

Table 5: Identifiable plant species found in sediment samples of three lakes in Thy National Park. Sediment volume in each sample is given in brackets.

Lake	<i>Littorella uniflora</i>	<i>Juncus bulbosus</i>	<i>Eleocharis multicaulis</i>	<i>Juncus articulatus</i>	<i>Eleocharis palustris</i> ssp. <i>palustris</i>	<i>Lemna minor</i>	<i>Carex</i> sp.	<i>Potamogeton</i> cf. <i>polygonifolia</i>	<i>Glyceria fluitans</i>
4_9 no. 1 (565 cm ³)		89	7	1	1	10			
4_9 no. 2 (565 cm ³)		13	2				1		
4_9 no. 3 (565 cm ³)									
4_9 no. 4 (565 cm ³)									
19_4 no. 1 (565 cm ³)		13	50					96	
19_4 no. 2 (565 cm ³)	1		3	1					
19_4 no. 3 (565 cm ³)									
19_4 no. 4 (565 cm ³)			20						
A_108 no. 1 (565 cm ³)									
A_108 no. 2 (565 cm ³)									
A_108 no. 3 (565 cm ³)									
A_108 no. 4 (565 cm ³)									1

The most fertile of the samples come from lake 4_9 and 19_4. The first sample from lake 4_9 was dominated by *Juncus bulbosus*, which is very common in most of the lakes in Thy National Park. The first sample from lake 19_4 was dominated by *Potamogeton*, possibly *P. polygonifolia*. The fourth sample from lake 19_4 only contained *Eleocharis multicaulis*.

The second sample of lake 19_4 contained a single seedling of *Littorella uniflora*. Samples from lake A_108 were almost devoid of plants, however a single *Glyceria fluitans* was found in the fourth sample.

Discussion

Land-use Effects on Lake Chemical Conditions

Overall, I did not find strong relationships between catchment land-use and chemical properties of the lakes in the PCA analysis (Figure 6 and Figure 7), however, I found many weaker correlations between pH/alkalinity, nitrogen, TOC, Na and Fe with different land-uses.

Alkalinity and pH ranged from very low to intermediate in the present study [38]. Higher alkalinity ($\rho = 0,31$, $p < 0,05$) and pH ($\rho = 0,38$, $p < 0,01$) were correlated with percentage plantation in the catchment. Lakes with more heath in the catchment tended to be more acidic. This is a result of the lakes being situated in an area with high organic content and *Sphagnum* vegetation and a low content of CaCO₃ [7]. The higher alkalinity found in plantation lakes is contrary to previous findings, that regularly harvested plantations can be a source of substrate acidification [39]. This is likely a result of the high-alkalinity lakes being found in the northernmost plantations in Thy National Park, where they are affected by large deposits of Campanian-Maastrichtian and Danien chalk [40].



The nutrients were, although very variable between lakes, not explained very well by catchment land-use. Percentage of plantations in the catchment had a significant impact on the level of TN and NH_4^+ while bogs additionally corresponded to NO_3^- levels. This is likely due to the relatively high mineralization rates in boggy *Sphagnum*-dominated environments [41]. A higher explanatory power in the PCA would have been obtainable through a higher variation in catchment land-use. However, fens, pasture and agriculture were almost absent in Thy National Park, thus they can hardly have any impact on overall lake ecology in the area. Søndergaard et al. (2005) have proposed a classification scheme for Danish lakes [38]. According to their classification, shallow lakes with a TN and TP concentration $<0,05$ mgP/L and $<1,40$ mgN/L can be classified as a lake of moderate ecological condition, along with other parameters. Thus, the mean TP/TN values for the examined lakes are exactly at the moderate level. A couple of lakes with TP/TN values under 0,025 mgP/L and under 1000 mgN/L are classified as lakes of high ecological condition with respect to these parameters. The concentrations of PO_4^{3-} , NH_4^+ and NO_3^- were significantly correlated with TP and TN respectively and thus follows the variation of those.

Chlorophyll-a was not directly associated with any catchment land-use. However, chlorophyll levels were highly influenced by seasonality, as the concentrations became relatively high in some lakes through August while concentrations were miniscule in November. Also, chlorophyll-a was significantly and positively correlated with TP ($\rho = 0,40$, $p < 0,01$), although this parameter was only included in the November sample, where chlorophyll-a levels were low. Thus, the association may be different in August. The results is in line with well-known nutrient dynamics in fresh water lakes, where phosphorous often is a limiting factor for phytoplankton growth [10]. As NO_3^- and NH_4^+ levels did not impact chlorophyll-a concentrations, phosphorous is likely the limiting factor of phytoplankton in many of the lakes in Thy National Park.

Overall organic carbon levels were relatively high throughout, yet there were no clear relationships to catchment land-use. Only the percentage of plantation in the catchment had a slight negative impact on DOC. The impacts of different catchment land-uses are hard to distinguish, as the dominating types, bog, heath and plantation, all are known to contribute allochthonous input of dissolved organic carbon [42]–[45]. As a rule, DOC concentrations were especially high in most lakes (ranging from 6,45 to 35,97 mg/L) and were significantly correlated ($\rho = 0,71$, $p < 0,0001$) with TOC levels in the November data set. Additionally, levels of DOC and TOC were in November very close, and therefore most of the organic carbon was in dissolved form at the time. DOC also had a profound effect on water color, as it was significantly correlated with CDOM. This effect of DOC on water color is a well-documented phenomenon [16], [17]. The compounds accounting for this coloring are



mostly complex humic substances, which effectively absorbs UV and visible radiation [7]. Iron was also found to influence the absorbance of light, which is in accordance with previous studies[31]. DOC, CDOM and possibly iron levels are in some lakes highly influenced by inflow from drainage canals. This is especially evident in lakes 19_4, 8_1 and A_108 although canals leading in to 8_1 and A_108 have been recently closed (Signe Kappel, personal communication, 2017). Lake area was also an important factor in explaining DOC ($\rho = -0,420$, $p > 0,01$) and TOC ($\rho = -0,315$, $p > 0,05$). Thus, smaller lakes were higher impacts by organic matter.

Fine benthic organic matter, FBOM, was a pronounced characteristic of many of the lakes in Thy National Park, but none of the catchment land-use types significantly explained the distribution of FBOM between the lakes. This labile organic matter mostly settled on the western side of the lakes, probably as a result of the strong westerlies moving water towards the eastern shores where the sandy lake bed gets exposed. The analysis of stable isotopes of different organic carbon sources revealed a clear pattern among three examined heathland lakes. The analyzes suggest that a large part of FBOM in the lakes consist of terrestrial substrate, especially the substrate/soil close to the lakes. This suggests that most of the FBOM is allochthonous. This is often the case with small unproductive acidic lakes, however autochthonous sources can also have a significant impact [46]. It has previously been demonstrated that DOC contributes significantly to sediment FBOM by flocculation [47], [48]. This may also be the case for parts of the organic matter in the studied dune lakes. In a thorough examination of lake A_108 in 2015, a lake which had previously had a drainage canal supplying DOC-rich water to the lake, a huge amount of labile aggregates of organic matter was found covering the sandy sediment [49].

The overall low relationship between catchment land-use and chemical properties of the lakes can partly also be explained by a relative low range in some of the key parameters among the lakes in Nationalpark Thy. No lakes were highly impacted by nutrient loads and all lakes can be classified as being oligo- to mesotrophic. This is not surprising due to the low variation in catchment land-use and substrate types. A wider range of lake types would have been possible to obtain if the lakes were distributed over a larger part of the country [50].

Relationship between lake chemical conditions and vegetation distribution

Some physical and chemical parameters had a clear impact on vegetation distribution and species richness in the lakes. Alkalinity was a highly important factor in respect to this. This is clear as the more species poor lakes are plotted on the left side on the first DCA axis (which is highly correlated with pH and alkalinity), and the species rich lakes are plotted on the right side of the axis. This



spectrum of species richness vs. alkalinity is thus firmly represented by the first DCA axis although it only ranges from low to intermediate alkalinity. Also, plant types were highly dependent on alkalinity. On the species ordination in the DCA (Figure 11), the first axis shows this clearly. *Sphagnum*, *Juncus* and isoetids were more common in low alkalinity lakes while *Myriophyllum*, *Elodea* and *Potamogeton* were more common in the more alkaline lakes. It is well known that alkalinity is an important factor in explaining species richness in freshwater lakes [50]–[53], especially because alkalinity determines the form of inorganic carbon in the water column. Isoetids utilize CO₂ from sediments which is the dominant inorganic carbon species in acid, low alkalinity lakes, while elodeids are capable of assimilating HCO₃⁻ for photosynthesis in alkaline waters, thus elodeids outcompete isoetids.

Nutrient availability may also be a contributing factor in species distribution. The second DCA axis was negatively correlated with NH₄⁺ ($\rho = -0,36$, $p > 0,05$). In highly acidic lakes nitrification occurs slowly, while NH₄⁺ is available in excess [7], thus ammonia becomes the dominant source of nitrogen. Isoetids more readily assimilate NO₃⁻ than NH₄⁺ [54] and an excess of the latter can result in domination by *Sphagnum* and *Juncus bulbosus*, as was seen in some of the lakes. This poses a problem for isoetids, as they will be outcompeted by *Sphagnum* and *Juncus bulbosus*, which have an advantage in ammonium rich waters.

As previously stated, high amounts of CDOM may result in a poor growing environment for submerged plant species. Although lake colorization was high in many of the lakes, it alone did not explain the species distribution in the DCA. This is likely due to the shallowness of the lakes, which allows enough light reaching the bottom for submerged plants to thrive. Especially isoetid-type plants are some of the most demanding in light availability, and are sensitive to an increase in light attenuation [55]. CDOM is likely to be of importance in macrophyte depth distribution in deeper lakes, like lakes 2_29 and A_108 (with a max depth of 6 meters), which had narrow littoral zones relative to the lake size.

Another important factor in explaining plant species distribution and richness is the cover of organic matter, FBOM, in the lakes. The second axis in the DCA was partly explained by the amount of FBOM in the littoral zone. Plants associated with lakes strongly affected by FBOM were mainly helophytes, elodeids and nymphaeids like *Sparganium angustifolium*, *Potamogeton natans*, *Nuphar lutea*, *Bidens sp.*, *Phragmites australis* and *Myriophyllum alterniflorum*. Lakes with low FBOM content were dominated by isoetids and low growing plants, especially *Lobelia dortmanna*, *Pillularia globulifera*, *Ranunculus repens*, *Littorella uniflora* and *Eleocharis acicularis*. Some of the rarer

Potamogeton species were also found in these low-FBOM areas. This is in accordance with previous studies, where isoetids are most often found on exposed sandy sediment [27], [28], [56]. Organic sediments can create a poor root anchorage for isoetids causing uprooting. Also, the reduced conditions of nutrient rich anaerobic organic sediments can result in iron and manganese plaques on the roots because of the reduced metals affinity for the oxidized rhizosphere. This in turn will especially inhibit the plants uptake of phosphorous [57].

Despite being an important factor for isoetids, not all low-growing plants showed a negative relationship to FBOM in the DCA. Both species of *Isoetes* are plotted in the intermediate range of the DCA2 axis as well is the rare and minuscule *Elatine hexandra*. This is likely due to the high amounts of organic matter found in the few lakes where these plants grow. These lakes were housing several rare species growing on small exposed patches in the eastern side of the lakes, where FBOM were not yet abundant due to wave action. The western shores were, however, highly threatened by organic matter loadings, often as a result of old drainage canals supplying the lake with DOC (e.g. lake 8_1/ Grube Vande).

Further Threats to Dune Lakes in Thy National Park

Coastal dune landscapes are naturally dynamic systems with high disturbance, but in Thy the natural dynamic of the systems on the Danish west coast have been rapidly changing towards less dynamic in the past 100 years. Where dunes and lakes once frequently were formed by the wind moving sand, today the landscape has become fixed by vegetation [58]. Figure 12 shows ortophotos of the central

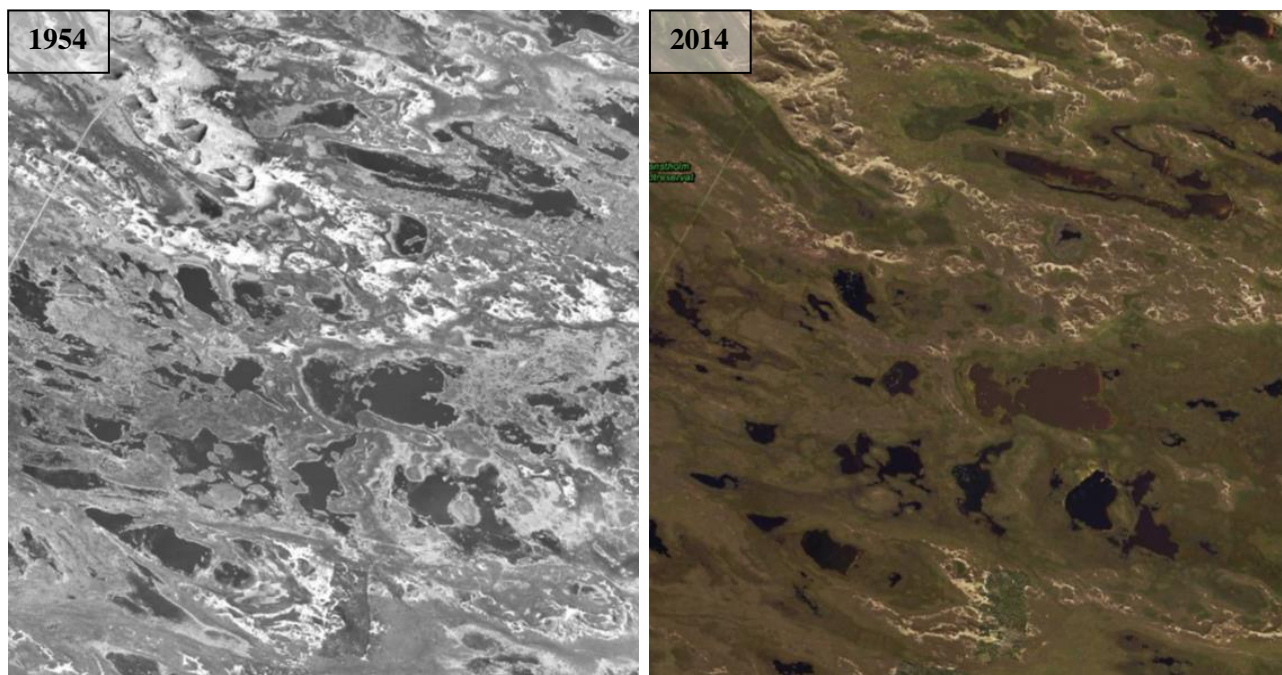


Figure 12: Ortophotos showing part of Hanstholm Game Reserve in 1954 and 2014 respectively. Source: Kraks Kort.



part of Denmark's largest continuous dune heath, Hanstholm Game Reserve in Thy National Park. The left photo shows the landscape in 1954 with extensive sandy wind breaches, large wetlands and larger lake areas. The photo on the right shows the same area in 60 years later, 2014. Wind breaches have become notably scarcer and more overgrown and most importantly, the lakes and ponds have become significantly smaller. This happens gradually as vegetation encroaches on the lake areas, resulting in a succession towards transitional bogs and poor fens.

The more stable conditions in the dune lakes are caused by several factors. First it is partly due to a directional succession where nutrients and organic matter accumulate in dune slacks as a natural process [59]. But this natural succession is likely influenced by a range of anthropogenic factors. As previously mentioned, especially pine plantations and planting of *Ammophila arenaria* have contributed significantly to the stabilization and subsequent overgrowing of dunes [9]. Second, increased nitrogen deposition, although relatively low in Thy National Park [60], may have a general accelerating effect on plant growth in these nutrient limited habitats [61], [62]. Lack of trampling and grazing by domestic megafauna further reduces the possibility for dune destabilization and thus new successional stages to be created [61]. Furthermore, migratory waterfowl may play a significant role in the eutrophication of inland freshwaters by acting as vectors for nutrients between agricultural land and lakes [63], [64]. A study on the effect of migratory geese on aquatic food webs in New Mexico found that isotope signatures of ^{15}N in the system were lower with high bird densities [65]. Possibly, there is a measurable isotopic signature in the vegetation and organic matter of lakes with high annual bird densities. Although geese were present in Thy National Park, I were not able to obtain guano for isotope analysis, as parts of the park is closed during the breeding season, when the bird density is high.

Climate change will likely have a significant impact on the future of dune landscapes and lakes in Thy National Park. Precipitation is expected to increase in Northwestern Jutland with warming of the climate [66]. Water is a main limiting factor in dune landscapes [1], and additional precipitation will create conditions for further plant growth and in turn dune fixation and accumulation of organic matter.

I suggest that all these factors, in addition to catchment land-use, contribute to the rapid change of these previously dynamic and heterogenic systems. Ultimately the increasing stabilization removes the process by which new dune lakes are formed. It has previously been shown how encroachment by dense vegetation is detrimental to the biodiversity of dune habitats [67]. This overall change in dunes landscapes is very likely negatively impacting dune and heath lake biodiversity as well through



the accumulation of organic matter and nutrients. Although sedimentation and accumulation in oligotrophic systems is generally low and carbon recycling nearly complete [7], the lakes in Thy National Park are small and my results show that they are also very sensitive to inputs of organic matter.

Future Management of Dune Lakes

The lakes in Thy National Park are still part of Denmark's most precious and pristine aquatic systems. The lakes in the area are home to some of the largest populations of rare isoetid species in the country [30]. However, many of the lakes have experienced degrading of ecological conditions since former times, resulting in loss of habitat for many aquatic species [25]. Several approaches can be used in re-establishing of the lake vegetation.

In order to evaluate the potential of species re-establishment in dune lakes, I examined the viability of seed banks in the degraded lake 19_4. Although the lakes had a very poor macrophyte cover, sediment samples yielded a high concentration of *Eleocharis multicaulis* and *Potamogeton* seed. These species were also found growing in the lake. A single seedling of *Littorella uniflora* appeared in a sample from lake 19_4, revealing a potential for at least some isoetid vegetation to recover, since this species was not found growing in the lake. Lake A_108 which is in seemingly better condition, had almost no viable seeds in the sediment samples. Rare isoetids species found in lakes A_108 and 4_9 may not have appeared as seeds in the sediment samples, as isoetid seed often benefit from desiccation and cold stratification treatments [68], [69].

Liming has been proposed as a possible restoration method for highly acidified lakes. However, there are several problems with this method. Liming has been tested in several lakes in The Netherlands, where lakes quickly reacidified to pH 4 within a year [11]. Furthermore, liming was not efficient in ameliorating the domination of *Juncus bulbosus*. In other instances liming resulted in increased coloration of humic substances in shallow parts of the lakes [11]. On the contrary, liming of the catchment has proven more effective in lakes with short retention times, however repeated treatments were still required [70]. Powdered limestone was spread by helicopter to the terrestrial areas. This might have adverse effects on the specialist vegetation should it be implemented in the dune heaths of Thy and should only be considered for severely acidified lakes in combination with other measures, like mud removal. I will therefore not recommend liming to be widely used in the lakes in Thy National Park.



A possible strategy for securing dune lake habitats in the future is by artificial lake construction. In Thy National Park, four of the examined lakes in the present study (St_n_l_e, St_n_l_w, St_n_l_s and Ål_n_l) have been recently dug, between 2014 and 2016. Also in Husby Klitplantage, several new transient lakes and dune slacks have been established [71]. In the newly dug lakes in Thy I did not find any rare species, and they were mainly dominated by *Juncus* and *Sphagnum*, were very acidic and had a high humic content. Future studies should follow the succession in these lakes to evaluate the success of establishing new dune lakes for securing lakes in different successional states and the associated vegetation. The construction of new lakes will probably demand a large-scale operation to be successful, as small and shallow lakes will quickly be a subject of encroachment by amphibious wetland species. Either there will be a need for larger lakes to be constructed, so that organic matter loadings will not be an immediate problem, or smaller lakes needs to be maintained regularly.

When dune systems reach a stabilized, vegetated state, remobilization by natural aeolian forces becomes more and more unlikely. This will result in a state of hysteresis where a much higher wind power is needed for remobilization, than what is normally limiting for plant growth [72]. In highly fixated areas, it takes tremendous forces to restore a state, where wind and drought naturally limits vegetation. An ameliorating strategy may be to limit the growth, and thus accumulation of organic matter, in the dune landscape. In the Netherlands, there are plenty of coastal dunes similar to the ones in Western Jutland. Several projects in the Dutch coastal dune systems aim to stimulate aeolian activity by artificially increasing erosion and deposition and hereby create possibilities for pioneer stages [73], [74]. The projects have resulted in the creation of large-scale deflation planes and sand drift. In some areas, sand deflation reached the ground water and small wet patches and transitory lakes emerged [73]. However, researchers recognize that these areas re-stabilize and that these projects likely are highly scale-dependent. I suggest that the vast fixated dunes in Thy National Park present several possibilities for experimenting with these methods, as has been done in Husby.

Conclusion

Catchment land-use did not provide an exhaustive explanation for water chemistry, organic matter levels and floristic status in heath and dune lakes in Nationalpark Thy, although some linkages were identified. Bogs and plantations were associated with increased concentrations of TN and NH_4^+ including NO_3^- for bogs. Additionally, PO_4^{3-} seemed to be the limiting factor of phytoplankton. Although not distinguishable in the analysis, the dominating catchment types of pine plantations and dune heath in Thy National Park, are likely both important as a source of allochthonous organic matter in the lakes. No direct source of organic matter was found in the analysis of land-use, however, stable



isotope fingerprints showed that lake FBOM was closely linked to macrophytes and terrestrial substrate close to the lakes.

I found a 65 different plant species (including *Spongilla lacustris*) in the dune lakes of Thy National Park. Although species like *Hydrocotyle vulgaris*, *Juncus bulbosus* and *Glyceria fluitans* were the most frequently found, the isoetids *Littorella uniflora* and *Lobelia dortmanna* were relatively common. The beautiful *L. dortmanna* was found in nearly half of the examined lakes. The more elusive *Isoetes* spp. were however, as expected, very rare.

Plant species distribution was mainly controlled by alkalinity and pH, which is a well-known association in plant ecology. Lakes of intermediate alkalinity were the most species rich, while encroachment by *Eleocharis multicaulis*, *Juncus bulbosus* and *Sphagnum* was characteristic of highly acidic lakes. Secondly fine benthic organic matter (FBOM) was found to especially limit the growth of rare isoetids in many of the lakes. Lakes with a thick layer of organic sediment were mostly dominated by common species of helophytes and elodeids like *Spharganium* sp. and *Potamogeton natans* and mosses like *Sphagnum* sp.

Organic matter and acidification are interpreted as the main drivers of degradation of dune lakes in Thy National Park. Nitrogen deposition and increased precipitation with climate change is expected to enhance vegetation encroachment. Although the future of dune lakes in Thy National Park, and European dune lakes in general, is unclear, without further intervention the dune landscape will remain stabilized which in turn will allow further encroachment and accumulation of organic matter. Seed/spore pools showed some potential for restoring a degraded lake and could be explored with further tests of the sediment seed bank. As large-scale experiments with remobilization of aeolian processes to reduce vegetation encroachment has been partly successful in the past, this is a possible strategy, along with lake construction, in combatting the ever-increasing fixation of the once majestic sand dune landscape.



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Appendix 1: Physico-chemical factors, August 2017

Lake	Alkalinity (meqv/l)	pH	DOC (mg/L)	TOC (mg/L)	TN (mg/L)	Chlorophyll-a (µg/l)	CDOM (Suva 254) l/mg/cm ²	o-phosphat (mg/l)	ammonia (mg/l)	nitrate (mg/l)	Mg	Na	K	Ca	Mn	Fe	Zn	B	S	Si	Area (m ²)	Mean FROM-layer (cm)
12_12	0.030	4.909	11.373	10.979	0.589	6.795	2.008	0.001	0.049	0.017	4.784	50.140	3.797	2.062	0.000	0.000	0.087	0.074	2.634	0.000	13871.728	1.468
12_28	0.075	5.463	7.919	11.473	0.601	12.070	2.004	0.004	0.054	0.017	2.035	17.709	1.084	1.041	0.000	0.174	0.000	0.000	1.546	0.000	7963.609	5.586
12_29	0.058	5.217	10.330	8.072	0.568	11.339	2.339	0.002	0.042	0.021	3.498	32.849	2.154	1.810	0.000	0.222	0.000	0.000	2.723	0.000	102337.360	5.949
14_2	0.660	6.769	16.773	14.810	0.790	5.146	3.869	0.005	0.046	0.018	4.112	46.841	3.201	16.688	0.000	0.421	0.111	0.064	3.216	1.216	11431.090	0.179
18_1	0.058	5.557	10.644	15.070	2.241	6.045	3.163	0.003	1.714	0.108	3.876	36.818	2.102	3.780	0.034	0.174	0.000	0.000	5.832	0.000	31015.981	7.638
18_3	0.080	5.577	19.650	17.640	0.345	33.973	1.345	0.002	0.032	0.023	2.247	20.929	1.191	1.162	0.000	0.000	0.000	0.000	1.086	0.000	5434.430	0.688
19_17	0.093	5.465	11.073	10.173	0.579	2.598	3.245	0.006	0.081	0.036	2.700	26.102	1.539	1.274	0.000	0.788	0.000	0.000	0.701	0.000	1529.791	7.384
19_4	0.095	5.727	10.425	10.250	0.320	7.261	3.223	0.003	0.037	0.034	3.379	29.898	1.928	2.449	0.000	4.395	0.276	0.091	1.565	0.000	24641.962	16.234
2_28	0.684	6.675	10.198	9.335	0.498	12.470	3.537	0.003	0.042	0.010	3.043	23.708	1.627	12.546	0.000	0.059	0.096	0.069	1.745	0.000	120319.109	10.012
2_29	0.655	6.869	8.383	7.726	0.361	2.898	2.485	0.002	0.057	0.025	2.777	22.255	1.279	12.990	0.000	0.000	0.000	0.000	2.146	0.000	392841.623	0.025
2_68	0.119	5.948	16.837	16.073	1.681	13.749	3.479	0.004	0.855	0.345	3.633	30.720	1.825	2.444	0.000	0.692	0.000	0.000	2.472	0.000	19687.625	1.015
20_1	0.324	6.463	10.471	8.874	0.525	2.248	2.884	0.004	0.074	0.023	2.799	36.361	1.677	7.956	0.000	2.768	0.000	0.000	0.707	0.000	3209.348	5.161
3_113	0.064	5.462	10.147	8.874	0.641	3.697	2.211	0.004	0.097	0.027	3.054	27.025	1.654	2.680	0.000	0.201	0.000	0.000	2.783	0.000	156457.359	2.178
3_62	0.070	5.644	10.662	10.046	0.608	13.156	2.285	0.003	0.038	0.023	3.551	30.926	1.575	1.923	0.000	0.260	0.000	0.000	2.332	0.000	89069.317	3.563
3_8	0.484	6.423	7.354	4.638	0.253	21.683	2.702	0.003	0.074	0.033	1.506	14.160	1.095	5.095	0.000	0.229	0.182	0.081	0.363	0.000	9591.553	56.315
4_1	0.032	5.257	7.019	3.335	0.220	1.699	2.484	0.002	0.192	0.087	1.204	15.311	1.914	0.928	0.000	0.111	0.099	0.000	1.058	0.000	12377.469	3.200
4_9	0.068	5.360	6.470	11.490	0.644	34.359	2.014	0.003	0.032	0.009	0.812	7.629	0.511	0.585	0.000	0.000	0.000	0.000	1.081	0.000	50381.120	2.706
6_3	1.435	8.000	9.863	11.688	0.387	2.398	3.065	0.002	0.072	0.020	5.412	61.030	2.090	35.579	0.000	0.741	0.000	0.000	5.091	0.000	4237.539	5.944
6_5	0.219	6.230	5.572	7.274	0.244	1.099	3.207	0.001	0.045	0.022	2.710	40.760	0.798	5.410	0.000	0.339	0.000	0.000	2.610	0.000	3052.453	7.915
A_108	0.200	5.994	10.663	9.838	0.428	21.763	2.517	0.002	0.043	0.020	2.389	18.497	1.115	2.514	0.000	1.406	0.000	0.000	1.602	0.000	37930.350	0.310



Appendix 2: Physico-chemical factors, November 2017

Lake	Alkalinity (meq/L)	pH	DOC (mg/L)	TOC (mg/L)	TN (mg/L)	filtered TN (mg/L)	unfiltered TN (mg/L)	Chlorophyll-a (mg/L)	CDOM (Suva 254) (L/mg/cm)	o-phosphate (mg/L)	ammonia (mg/L)	nitrate (mg/L)	TP (mg/L)	Mg	Na	K	Ca	Fe	Zn	B	S	Si	Area (m ²)	Mean FFDW-layer (cm)
12_12	0.032	5.430	16.090	18.010	1.429	0.638	1.570	0.007	6.010	0.007	0.589	0.248	0.110	2.879	27.914	2.316	1.442	0.468	0.132	0.080	2.148	0.000	13871.728	1.468
12_16	0.002	4.877	17.300	18.010	0.628	0.298	0.732	0.002	4.216	0.003	0.048	0.021	0.037	1.833	18.168	1.437	1.498	0.352	0.166	0.104	1.884	0.000	1581.423	1.000
12_17	0.024	5.396	10.647	15.320	0.508	0.885	0.004	0.004	5.661	0.004	0.053	0.019	0.030	2.972	19.411	0.837	2.222	0.315	0.000	0.000	3.260	0.139	8357.833	9.225
12_21	0.023	5.399	10.004	11.330	0.392	0.652	0.011	0.011	3.932	0.003	0.021	0.002	0.033	2.614	22.556	0.940	1.649	0.121	0.000	0.000	2.008	0.000	11471.467	7.256
12_28	0.029	5.641	8.928	9.723	0.695	0.890	0.007	0.007	4.006	0.004	0.203	0.065	0.027	2.455	17.665	1.624	1.638	0.191	0.087	0.068	2.008	0.000	7963.609	5.599
12_29	0.028	5.461	10.687	12.960	1.583	1.752	0.023	0.023	5.783	0.007	0.500	0.146	0.115	2.923	26.618	1.815	1.579	0.284	0.000	0.000	2.590	0.000	10237.360	5.949
12_6	0.018	5.261	13.150	12.460	1.008	0.953	0.002	0.002	4.122	0.003	0.363	0.085	0.034	3.139	28.219	1.765	1.717	0.255	0.129	0.000	2.968	0.000	5728.845	4.197
12_7	0.040	5.588	14.600	19.020	1.294	1.592	0.011	0.011	6.438	0.003	0.628	0.048	0.103	2.808	25.270	1.533	1.356	0.494	0.078	0.000	2.276	0.000	6536.174	4.408
12_8	0.030	5.416	13.730	15.910	0.792	0.953	0.002	0.002	6.621	0.003	0.206	0.083	0.059	2.376	22.197	1.463	1.338	0.499	0.000	0.000	1.424	0.000	5600.324	6.311
14_2	0.016	7.025	15.760	16.290	1.336	1.344	0.002	0.002	5.764	0.011	0.157	0.339	0.082	3.275	35.470	2.398	16.288	0.528	0.000	0.000	1.862	0.000	11431.090	0.179
16_1	0.016	5.218	15.997	16.060	0.711	0.732	0.002	0.002	5.397	0.003	0.055	0.016	0.023	2.499	22.387	1.520	1.425	0.483	0.117	0.070	2.897	0.000	10933.346	5.948
16_12	0.015	5.279	12.967	13.150	0.537	0.615	0.001	0.001	4.817	0.002	0.063	0.034	0.060	1.394	11.082	0.248	0.759	0.247	0.067	0.000	2.108	0.000	12050.715	4.466
18_1	0.161	6.811	8.242	9.825	0.477	0.658	0.002	0.002	4.501	0.003	0.051	0.196	0.029	2.682	23.197	1.326	3.273	0.000	0.154	0.078	3.067	0.000	31015.981	7.638
18_3	0.033	5.526	18.947	18.430	0.602	0.598	0.017	0.017	3.362	0.006	0.112	0.152	0.048	1.962	18.066	1.028	1.065	0.295	0.000	0.000	1.069	0.000	5434.430	0.688
19_10	0.030	5.814	8.856	9.693	0.773	0.926	0.006	0.006	3.335	0.006	0.255	0.141	0.049	3.251	24.797	1.517	1.921	0.000	0.136	0.083	4.265	0.000	12107.037	6.193
19_11	0.032	5.589	11.310	19.490	1.227	1.858	0.003	0.003	5.715	0.005	0.641	0.122	0.039	2.941	24.149	1.970	1.753	0.254	0.000	0.000	3.330	0.445	5664.136	8.300
19_15	0.056	5.753	18.490	18.770	1.019	1.019	0.001	0.001	6.598	0.006	0.262	0.089	0.044	2.711	26.086	2.534	1.302	0.454	0.127	0.080	2.090	0.000	16857.475	1.720
19_17	0.101	5.645	35.973	42.900	1.963	2.264	0.004	0.004	9.016	0.006	0.620	0.143	0.126	3.268	27.763	4.026	1.638	2.142	0.000	0.000	1.597	0.000	8555.255	9.452
19_2	0.188	7.597	8.016	7.965	0.928	0.881	0.007	0.007	4.491	0.005	0.261	0.051	0.072	2.100	18.940	1.329	1.711	0.000	0.072	0.000	2.872	0.000	24641.962	16.234
19_4	0.047	5.654	12.417	14.090	0.435	0.543	0.005	0.005	8.102	0.003	0.031	0.028	0.025	3.111	27.431	1.869	2.004	0.434	0.000	0.000	1.510	0.000	11749.681	3.148
19_5	0.034	6.032	6.952	7.628	0.346	0.412	0.002	0.002	3.261	0.004	0.028	0.018	0.015	2.934	24.468	0.674	1.690	0.127	0.109	0.000	2.568	0.000	11749.681	3.148
2_28	0.349	7.091	6.900	7.615	0.423	0.509	0.006	0.006	3.319	0.004	0.074	0.066	0.024	2.041	18.848	0.883	8.486	0.000	0.000	0.000	2.388	1.108	120319.109	10.012
2_55	0.024	5.306	9.761	11.430	0.531	0.565	0.001	0.001	4.777	0.002	0.049	0.028	0.025	2.230	17.767	0.661	10.663	0.000	0.000	0.000	1.668	0.000	9392841.623	0.025
2_68	0.046	5.556	8.776	10.250	0.490	0.573	0.009	0.009	4.524	0.005	0.057	0.070	0.030	3.019	25.270	1.212	1.983	0.245	0.000	0.000	2.492	0.000	8337.321	4.145
2_8	0.046	5.793	10.021	16.090	1.002	1.380	0.000	0.000	6.713	0.008	0.222	0.304	0.046	2.646	24.494	1.173	1.736	0.394	0.073	0.000	1.803	0.000	19687.625	1.015
20_1	0.465	6.779	17.850	19.610	0.957	1.125	0.001	0.001	8.611	0.010	0.223	0.017	0.092	3.078	28.908	2.085	9.570	0.494	0.000	0.000	0.621	3.787	3209.348	5.161
3_113	0.015	5.425	7.554	10.940	1.073	1.242	0.011	0.011	4.236	0.004	0.232	0.339	0.068	2.095	19.483	1.411	2.412	0.000	0.144	0.085	2.337	0.000	156457.359	2.178
3_62	0.029	5.617	9.709	17.790	0.777	1.394	0.005	0.005	6.708	0.006	0.179	0.155	0.086	2.763	26.363	1.845	1.873	0.313	0.240	0.000	1.700	0.000	89069.317	3.563
3_8	0.393	6.611	6.448	6.768	0.875	0.950	0.006	0.006	5.030	0.005	0.345	0.013	0.051	1.962	17.000	1.362	6.739	0.178	0.133	0.000	0.504	0.000	9591.553	56.315
3_91	0.042	5.484	16.323	23.810	0.717	0.962	0.002	0.002	7.986	0.006	0.163	0.045	0.034	2.793	25.038	1.817	2.149	0.705	0.000	0.000	1.030	0.000	2309.896	15.344
4_10	0.013	5.136	11.590	12.700	1.158	1.176	0.002	0.002	5.582	0.012	0.478	0.093	0.022	1.607	16.696	1.129	1.968	0.256	0.000	0.000	1.500	0.000	12377.469	3.200
4_9	0.024	5.406	9.035	10.750	0.518	0.681	0.007	0.007	5.051	0.007	0.087	0.057	0.041	1.642	16.154	1.379	1.046	0.227	0.000	0.000	1.585	0.000	50381.120	2.706
5_38	0.002	4.921	9.198	13.220	0.656	0.900	0.002	0.002	5.128	0.004	0.105	0.062	0.025	2.288	21.622	1.380	1.331	0.342	0.000	0.000	1.456	0.000	10832.701	9.809
5_40	0.029	5.685	13.117	19.190	1.072	1.481	0.007	0.007	6.595	0.005	0.132	0.328	0.089	1.746	15.375	0.793	1.536	1.256	0.000	0.000	1.587	0.000	15056.793	14.047
6_3	0.677	7.129	11.553	11.210	0.283	0.323	0.003	0.003	4.838	0.004	0.037	0.011	0.024	7.184	55.395	2.664	13.231	0.331	0.154	0.088	3.802	0.000	4237.539	5.944
6_5	0.228	6.452	9.523	9.004	0.269	0.274	0.002	0.002	4.127	0.003	0.024	0.010	0.030	2.670	41.434	1.011	5.427	0.196	0.000	0.000	3.205	1.213	3052.453	7.915
6_6	0.032	5.581	10.580	10.370	0.412	0.480	0.002	0.002	3.986	0.002	0.047	0.019	0.016	2.372	18.562	1.035	1.415	0.201	0.000	0.000	2.773	0.000	5361.749	2.956
7_1	0.016	5.416	10.413	10.160	0.398	0.433	0.002	0.002	4.315	0.004	0.029	0.013	0.020	2.630	23.416	1.613	1.587	0.161	0.087	0.000	2.219	0.000	4159.597	1.414
7m_1	0.044	5.911	10.139	11.410	0.830	0.954	0.004	0.004	5.491	0.008	0.221	0.066	0.045	2.999	22.569	0.943	3.069	0.187	0.000	0.000	4.115	0.000	7063.749	13.026
7m_2	0.186	6.457	16.743	17.490	0.773	0.964	0.002	0.002	5.624	0.006	0.078	0.036	0.043	2.257	31.332	1.729	6.353	0.312	0.089	0.069	2.584	0.723	4143.776	8.330
7m_3	0.035	5.411	12.600	13.860	0.542	0.699	0.002	0.002	4.807	0.003	0.056	0.102	0.017	2.312	20.903	1.598	2.446	0.299	0.220	0.093	2.553	0.000	13513.198	9.229
7s_5	0.016	5.532	7.034	8.147	0.407	0.452	0.005	0.005	3.409	0.004	0.048	0.030	0.026	2.585	18.165	0.740	1.434	0.055	0.000	0.000	3.335	0.000	7034.565	11.588
8_1	0.035	6.035	6.835	8.328	0.277	0.452	0.005	0.005	3.009	0.002	0.024	0.010	0.029	3.892	33.011	1.027	3.382	0.000	0.000	0.000	5.501	0.000	19489.404	3.976
A_108	0.126	6.567	9.209	9.140	0.375	0.452	0.009	0.009	5.589	0.004	0.025	0.022	0.049	2.188	17.633	1.124	2.289	0.272	0.000	0.000	1.274	0.000	37930.350	0.310
Ny svr Avc	0.157	6.380	20.860	22.700	0.563	0.750	0.017	0.017	5.433	0.005	0.048	0.040	0.061	3.828	27.098	1.111	2.763	0.348	0.000	0.000	1.759	0.000	1495.470	0.133
Stenbjerg	0.020	5.439	8.113	9.506	0.284	0.494	0.002	0.002	5.432	0.003	0.039	0.033	0.035	1.341	13.425	0.800	1.179	0.236	0.000	0.000	1.818	0.555	1580.145	0.589
Stenbjerg																								



Appendix 3: Spearman's Rank Correlations Coefficients of 22 variables in 20 lakes in August, 2017.

	Alk	pH	DOC	TOC	TN	Chl-a	CDOM	PO ₄ ³⁻	NH ₄ ⁺	NO ₃	Na	Fe	S	Area	FBOM	%Fens	%Heath	%Pasture	%Plantation	%Bog	%Agriculture	
pH	0,955***																					
DOC	-0,049	-0,006																				
TOC	-0,023	-0,008	0,608**																			
TN	-0,269	-0,235	0,409	0,581**																		
Chl-a	-0,094	-0,156	0,26	0,376	0,244																	
CDOM	0,595**	0,589**	0,155	0,038	0,029	-0,301																
PO ₄ ³⁻	0,144	0,083	0,241	0,265	0,565**	0,049	0,344															
NH ₄ ⁺	-0,064	0,014	-0,025	-0,117	0,184	-0,495*	0,26	0,309														
NO ₃	-0,167	-0,042	0,149	-0,11	-0,089	-0,245	0,258	0,205	0,615**													
Na	0,102	0,203	0,346	0,25	0,248	-0,487*	0,347	0,099	0,128	-0,042												
Fe	0,383	0,372	0,214	-0,005	-0,055	-0,235	0,572**	0,354	0,147	0,275	0,324											
S	-0,042	0,078	0,18	0,292	0,427	-0,206	0,15	0,183	0,102	-0,115	0,728**	-0,033										
Area	-0,203	-0,135	-0,069	-0,164	0,269	0,341	-0,165	0,094	-0,186	-0,081	-0,197	-0,378	0,31									
FBOM	0,068	0,011	-0,395	-0,266	-0,277	-0,135	0,313	0,052	0,029	0,139	0,042	0,256	-0,164	-0,232								
%Fens	0,125	0,212	0,03	-0,054	-0,189	-0,209	0,072	0,174	0,235	-0,016	0,19	0,236	0,026	-0,447*	-0,007							
%Heath	-0,038	-0,139	-0,072	0,109	-0,004	-0,305	0,044	0,028	-0,119	-0,057	0,432	0,33	0,186	-0,322	0,25	-0,401						
%Pasture	0,169	0,21	-0,121	-0,362	-0,02	-0,239	0,12	0,325	0,527*	0,374	-0,154	0,01	-0,079	0,104	-0,288	0,14	-0,282					
%Plantation	0,392	0,450*	-0,02	-0,01	-0,319	-0,008	0,348	0,139	0,088	0,062	-0,165	-0,021	-0,138	-0,116	0,062	0,570**	0,712**	0,099				
%Bog	-0,139	-0,196	0,075	-0,017	0,352	-0,001	-0,158	0,25	-0,096	-0,093	0,09	-0,129	0,044	0,292	-0,108	-0,742**	0,493*	-0,08	-0,817***			
%Agriculture	0,278	0,339	-0,048	-0,151	-0,419	-0,179	0,13	0,131	0,19	0,13	-0,03	0,188	-0,2	-0,497*	-0,025	0,852***	-0,443	0,382	0,657**	0,808***		
Dist.plant.	-0,163	-0,223	0,155	0,206	0,442	0,063	-0,167	0,012	-0,136	-0,318	0,376	0,062	0,315	-0,045	-0,229	-0,308	0,561*	-0,263	-0,723**	0,578**	-0,466*	



Appendix 4: Spearman's Rank Correlations Coefficients of 22 variables in 50 lakes in November, 2017.

	Alk	pH	DOC	TOC	TN	Chl-a	CDOM	PO ₄ ³⁻	NH ₄ ⁺	NO ₃ ⁻	TP	Na	Fe	S	Area	FBOM	%Fens	%Heath	%Pasture	%Plantation	%Bog	%Agriculture	
pH	0,901***																						
DOC	0,108	0,166																					
TOC	0,036	-0,199	0,893***																				
TN	-0,061	-0,131	0,397**	0,615***																			
Chl-a	0,015	0,096	-0,114	0,03	0,215																		
CDOM	0,137	-0,047	0,542***	0,705***	0,669***	0,003																	
PO ₄ ³⁻	0,191	0,176	0,214	0,321*	0,573***	0,168	0,430**																
NH ₄ ⁺	-0,018	-0,077	0,22	0,353*	0,861***	0,11	0,440**	0,568***															
NO ₃ ⁻	-0,07	-0,042	0,075	0,242	0,619***	0,134	0,317*	0,480**	0,653***														
TP	0,047	-0,004	0,323*	0,474**	0,623***	0,399**	0,612***	0,368**	0,459**	0,335*													
Na	0,339*	0,253	0,208	0,199	0,125	-0,092	0,138	0,217	0,064	-0,052	0,162												
Fe	0,048	-0,199	0,747***	0,796***	0,504**	-0,155	0,789***	0,266	0,297*	0,204	0,435**	0,227											
S	-0,056	0,084	-0,281*	-0,345*	-0,222	-0,063	-0,491**	-0,210	-0,141	-0,135	0,395**	0,361*	-0,460**										
Area	-0,008	0,120	-0,420**	-0,315*	0,033	0,262	-0,145	0,027	0,080	0,284*	0,003	-0,056	-0,271	0,067									
FBOM	0,086	0,059	-0,157	-0,080	0,097	-0,016	0,067	0,018	0,143	-0,016	-0,085	0,129	-0,020	0,243	0,063								
%Fens	0,123	0,033	0,157	-0,045	-0,098	-0,232	-0,014	-0,131	-0,044	-0,136	-0,112	-0,079	0,093	-0,043	-0,061	0,036							
%Heath	-0,215	-0,239	0,128	0,120	-0,002	-0,255	0,058	-0,039	0,017	-0,100	-0,096	0,402**	0,205	0,327*	-0,223	0,164	-0,258						
%Pasture	0,059	0,072	-0,122	-0,081	0,166	-0,044	-0,020	0,127	0,165	0,037	0,148	-0,119	-0,114	0,301*	0,284	0,170	0,205	-0,371**					
%Plantation	0,309*	0,383**	-0,165	-0,289*	-0,356*	0,076	-0,242	-0,115	-0,337**	-0,239	-0,177	-0,272	-0,412**	0,026	-0,061	0,146	0,224	-0,675**	0,1661				
%Bog	-0,1267	-0,1761	0,1352	0,2753	0,402**	0,1036	0,1682	0,1412	0,410**	0,312*	0,2448	0,2526	0,309*	0,0326	0,300*	0,130	-0,382**	0,314*	-0,098	-0,771**			
%Agriculture	0,13	0,045	0,099	-0,063	-0,213	-0,228	-0,103	-0,091	-0,202	-0,213	-0,232	-0,188	-0,017	-0,145	-0,041	0,032	0,821***	-0,360*	0,405**	0,415**	-	0,535***	
Dist.plant.	-0,253	-0,237	-0,004	0,096	0,17	-0,046	0,002	0,163	0,18	0,156	-0,015	0,345*	0,23	0,229	0,056	0,159	-0,109	0,619***	-0,286*	-0,808***	0,599***	-0,285*	



Appendix 5: Isotopic fingerprints from $\delta^{15}N$ and $\delta^{13}C$ of lakes 12_6, 12_28 and 12_21.

12_6	Normalised $\delta^{12}C/^{13}C$	Normalised $\delta^{14}N/^{15}N$	12_28	Normalised $\delta^{12}C/^{13}C$	Normalised $\delta^{14}N/^{15}N$	12_21	Normalised $\delta^{12}C/^{13}C$	Normalised $\delta^{14}N/^{15}N$
Agrostis stolonifera (1m)	-30.5341,47	-1.3242,29	Agrostis stolonifera (1m)	-32.0560,27	-1.9441,42	Agrostis stolonifera (10m)	-31.0541,24	-5.5740,79
Byrophyte (10m)	-29.8740,66	-8.1340,65	Carex sp. (1m)	-29.4141,28	-2.0541,19	Agrostis stolonifera (1m)	-31.0140,16	-3.6341,47
Carex sp. (10m)	-28.0340,77	0.3740,50	Eleocharis multicaulis (1m)	-29.7741,58	2.3240,20	Agrostis stolonifera (lake)	-31.2240,43	-3.0340,47
Eleocharis multicaulis (10m)	-27.50	0.37	Eleocharis multicaulis (lake)	-29.1540,46	-0.0741,35	Carex sp. (10m)	-31.6240,56	-3.1240,43
Eleocharis multicaulis (1m)	-27.9641,07	0.6541,90	Eleocharis palustris ssp. vulgaris (1m)	-30.04	0.64	Carex sp. (1m)	-29.7140,73	-0.8941,10
Eleocharis multicaulis (lake)	-28.7140,70	1.9740,47	Eleocharis palustris ssp. vulgaris (lake)	-28.00	-0.98	Eleocharis multicaulis (10m)	-30.54	-0.86
Empetrum nigrum (10m)	-29.2340,23	-10.9540,24	Erica tetralix (10m)	-29.66	-6.20	Eleocharis multicaulis (1m)	-29.2240,56	-0.2540,94
Erica tetralix (10m)	-27.8640,47	-9.4240,83	FBOM	-28.3840,56	-1.1140,18	Eleocharis multicaulis (lake)	-29.4240,61	0.8941,11
FBOM	-27.9340,18	-2.0340,38	Glyceria fluitans (1m)	-28.9741,66	2.2840,31	FBOM	-29.5640,67	-2.3640,41
Hydrocotyle vulgaris (1m)	-30.3441,66	-1.0641,63	Glyceria fluitans (lake)	-29.5140,30	3.9642,20	Glyceria fluitans (lake)	-29.4341,66	1.0040,27
Juncus bulbosus (1m)	-28.6240,97	1.1243,18	Juncus bulbosus (10m)	-29.0940,84	0.0743,75	Hydrocotyle vulgaris (10m)	-31.3040,21	-6.2840,67
Juncus bulbosus (lake)	-28.1740,62	1.0640,63	Juncus bulbosus (lake)	-29.1441,03	0.7741,30	Hydrocotyle vulgaris (1m)	-31.3240,84	-2.6440,91
Littorella uniflora (lake)	-30.0040,53	-1.5540,88	Juncus bulbosus (lake)	-24.3341,38	1.7041,19	Hydrocotyle vulgaris (lake)	-31.8140,82	-2.1141,88
Lobelia dortmanna (lake)	-27.5541,12	0.4542,10	Lobelia dortmanna (lake)	-25.14	-0.77	Juncus bulbosus (10m)	-30.0040,94	-3.9742,05
Molinia caerulea (10m)	-27.7240,04	-4.1241,94	Molinia caerulea (10m)	-28.9341,83	-4.6742,65	Juncus bulbosus (1m)	-28.3841,35	-0.6740,92
Myrica gale (10m)	-29.9140,92	-1.8740,25	Molinia caerulea (1m)	-29.2540,69	-4.3743,90	Juncus bulbosus (lake)	-29.2841,12	-0.7241,58
Myrica gale (1m)	-30.75	-0.80	Myrica gale (10m)	-30.2940,75	-0.2141,02	Littorella uniflora (lake)	-30.9840,01	-2.9540,07
Salix repens ssp. repens var. argentea (10m)	-29.2541,16	-7.0040,26	Myrica gale (1m)	-30.5041,05	0.3640,88	Molinia caerulea (lake)	-29.69	-1.13
Salix repens ssp. repens var. argentea (1m)	-31.24	-1.06	Salix repens ssp. repens var. argentea (1m)	-29.13	2.39	Myrica gale (10m)	-31.8141,18	-1.3040,43
Sphagnum sp. (1m)	-26.8740,51	-1.1040,57	Sphagnum sp. (10m)	-28.7840,74	-2.3541,90	Myrica gale (1m)	-31.2040,93	-0.9840,37
Sphagnum sp. (lake)	-26.4240,12	0.4340,46	Sphagnum sp. (1m)	-29.82	-1.49	Salix repens ssp. repens var. argentea (10m)	-32.03	-3.25
Terrestrial substrate (10m)	-28.2440,53	-3.2741,45	Terrestrial substrate (10m)	-29.3840,33	-2.4340,83	Sphagnum sp. (10m)	-29.4841,36	-2.2941,14
Terrestrial substrate (1m)	-28.2440,19	-2.0940,34	Terrestrial substrate (1m)	-28.7640,52	-1.6140,46	Sphagnum sp. (lake)	-27.78	-0.18
						Terrestrial substrate (10m)	-29.5340,57	-2.4840,64
						Terrestrial substrate (1m)		-1.7440,66



Appendix 6: Species richness, evenness and diversity indices of 50 lakes in Thy National Park on aquatic macrophytes.

Lake	Species richness	Evenness	Shannon Diversity	Simpson Diversity
12_12	14	0,543	1,433	0,6047
12_16	7	0,833	1,622	0,7881
12_17	10	0,78	1,796	0,7798
12_21	12	0,799	1,986	0,8343
12_28	8	0,907	1,887	0,8233
12_29	8	0,747	1,552	0,7152
12_6	9	0,76	1,670	0,7753
12_7	8	0,898	1,866	0,8269
12_8	14	0,703	1,855	0,7674
14_2	17	0,812	2,301	0,8722
16_1	12	0,839	2,086	0,8561
16_12	9	0,812	1,784	0,7975
18_1	11	0,765	1,834	0,7994
18_3	7	0,844	1,643	0,7689
19_10	15	0,784	2,123	0,8542
19_11	15	0,842	2,279	0,8795
19_15	12	0,834	2,072	0,8476
19_17	7	0,797	1,551	0,7539
19_2	8	0,648	1,347	0,6248
19_4	21	0,832	2,534	0,893
19_5	10	0,89	2,050	0,8568
2_28	26	0,847	2,760	0,9099
2_29	23	0,759	2,380	0,8819
2_55	10	0,683	1,572	0,6854
2_56	13	0,671	1,720	0,7619
2_68	7	0,687	1,337	0,6712
20_1	14	0,759	2,003	0,8055
3_113	13	0,881	2,261	0,8721
3_62	11	0,836	2,004	0,8258
3_8	17	0,823	2,333	0,8795
3_91	6	0,536	0,961	0,525
4_1	10	0,493	1,136	0,4749
4_10	9	0,853	1,875	0,825
4_9	12	0,85	2,112	0,8529
5_38	5	0,542	0,872	0,403
5_40	13	0,768	1,970	0,8043
6_3	20	0,744	2,228	0,845
6_5	12	0,703	1,747	0,7798
6_6	11	0,837	2,007	0,8305
6_7	10	0,791	1,822	0,7939
7m_1	10	0,734	1,690	0,7797
7m_2	16	0,709	1,965	0,7693
7m_3	10	0,879	2,023	0,8531
7s_5	15	0,778	2,106	0,8331
8_1	14	0,68	1,795	0,754
A_108	13	0,79	2,028	0,8025
St_n_l_e	8	0,67	1,394	0,6633
St_n_l_s	9	0,599	1,317	0,569
St_n_l_w	10	0,686	1,581	0,6824
Ål_n_l	4	0,617	0,855	0,4756

