Ancient DNA from soils and sediments from the Lake Krigstjärn area, northern Sweden

Preservation and detection of Holocene mammal sedaDNA

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Abstract

Current knowledge of past vegetation and faunal diversity has been based on pollen and macrofossil analysis from lake sediments. The innovative method of sedimentary ancient DNA (sedaDNA) is a promising, complementary proxy to reconstruct information about past environments. However, to what extent animal DNA can be extracted from old sediments and soils has not been frequently studied. This study explored if ancient DNA of moose (*Alces alces*), reindeer (*Rangifer tarangus*), goat (*Capra aegagrus*) and plants could be extracted from millennia old lake sediments of Lake Krigstjärn and archaeological soil samples in northern Sweden. SedaDNA was successfully extracted and detected from both reindeer and plants DNA, while goats sedaDNA was absent in all sediments. Moose ancient DNA (aDNA) was only detected in the archaeological soils. Yet, there were signs that the applied moose primer set was not optimal for heavily degraded DNA and the validity of this primer needs further research. Earliest detections of reindeer DNA can be dated to ~6500 c. years ago. Oldest sediments contained DNA, indicating sufficient DNA preservation conditions in the sediments of Lake Krigstjärn. Finds of plants DNA in pre-deglacial sediments may indicate the existence of >9500 year old glacial vegetation. Altogether is sedaDNA a highly promising tool to reconstruct diversity, origin and immigration routes of mammals, but technical issues such as primer set specificity and its purpose should be considered and tested carefully in advance.

Key words: ancient DNA, faunal reconstruction, palaeoecology, lake sediments, glacial vegetation
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1 Introduction

Palaeoecological reconstructions are important to understand long-term responses of vegetation and animal composition on changes in climate and environment, as well as the introduction of anthropogenic influences thereon (Seddon et al., 2014). Historical environmental responses recorded in palaeoecological archives help to anticipate on the changes and challenges of the future. Lake sediments constitute one of the most important and frequently used palaeoecological archives (Cohen, 2003). Freshwater lakes are an accumulation point of biological life and material, both terrestrial and aquatic. Its sediments incorporate a valuable source of information on its ecological, geological and climatic historical surrounding. So far, our current knowledge of past vegetation and faunal dynamics has mainly been based on pollen and macrofossil analysis from sediments (Bjune et al., 2004; Krüger et al., 2011; Ghilardi & O’Connell, 2013; Gugerli et al., 2013; Väiliranta et al., 2015). Pollen are mainly wind and water dispersed and may originate from long-distance transport, thus covering a wide spatial scale. Also, palynological analysis is often a liable quantitative interpretation and may give a biased view, with rare species possibly remaining undetected. Macrofossils generally derive from local vegetation and animals (Birks & Birks, 2000; Pansu et al., 2015). Their finds are sporadic but easy to interpret, indicating local presence. Consequently, a combination of both proxies has created a strong analysis profile in past palaeoecological reconstructions. Nevertheless, pollen and plant macrofossils cannot provide information on animal presences in the environment, lowering the ability to reconstruct historical migration dynamics of animals.

During the last two decades, the implementation of the innovative method of sedimentary ancient DNA (sedaDNA) has emerged (Willerslev et al., 2003, 2007; Jørgensen et al., 2012a) and already widened the vision on past environments and ecology (Haile et al., 2007, 2009; Matisoo-Smith et al., 2008; Sønstebø et al., 2010; Anderson-Carpenter et al., 2011; Jørgensen et al., 2012ab; Parducci et al., 2012, 2013; Pedersen et al., 2013; Epp et al., 2015). All organisms contain DNA, at least at the moment they get trapped in the sediments, and although pollen and other kinds of macrofossils may be unrecognizable now when recovered from sediments, DNA might still be present and identifiable (Gugerli et al., 2013). Moreover, exploring studies indicate that DNA of mammals can be extracted from lake sediments (Willerslev et al., 2014; Cooper et al., 2015), revealing that sedaDNA indeed succeeds in giving information on terrestrial animal presences from sediments and other archives.

A major challenge exists as DNA will degrade with time (Willerslev et., 2003; Hansen et al., 2006; Coolen & Gibson, 2009; Thomsen & Willerslev, 2015), and though traces of DNA are present in sediments, amplification might fail. Northern latitudinal areas are beneficial for DNA preservation in sediments, due to lower rates of degradation in cold systems (Willerslev et al., 2003; Pääbo et al., 2004; Hofreiter et al., 2015), as well as the anaerobic conditions in lake sediments during ice cover (Corinaldesi et al., 2011). This makes northern areas the ultimate regions to apply ancient sedaDNA techniques for palaeoecological purposes. Further sediment compounds are working in favour of sedaDNA preservation, that adsorb DNA, such as humics, sand, clay and mineral-bound-organics (Lorenz & Wackernagel, 1987; Pietramellara et al., 2009; Saeki et al., 2011). Thus, tracing this hidden treasure of biological life in sediments largely depends on characteristics originating in its geological past. Another adaptation to get round the problems of DNA degradation, is to design primers targeting only a small region, usually <100 basepairs (bp) (Yoccoz et al., 2012). Recently, scientists seem to turn the disadvantage of degradation into a favour, using the degree of degradation as a rough indication of age, thus being an examiner of modern DNA contamination and eventually even a direct dating technique (Skoglund et al., 2014; Weiss et al., 2015; Linderholm, 2016), due to regular C→T transitions in post-mortem DNA.
For palaeobotany, sedaDNA is found to be a valuable complementary proxy. Its application can both confirm as well as dispute previous theories (Parducci et al., 2012), especially since pollen production may be inhibited in northern regions with cold temperatures (Hicks, 2006; Barnekow et al., 2007), which complicates palynological interpretation in those latitudes (Segerström & Stedingk, 2003; Zazula et al., 2006). SedaDNA is suspected to originate from remains other than wind and water dispersed pollen (Parducci et al., 2013). And thus, just as macrofossils, aDNA (ancient DNA) seems to be of local origin (Haile et al., 2007, 2009; Yoccoz et al., 2012; Jørgensen et al., 2012; Parducci et al., 2013). The chance to detect macrofossils is dependent on the vegetation cover, and subsequently sedaDNA is in the strongest position to detect scarce species (Alsos et al., 2015). Recent studies comparing taxonomic detection of pollen, macrofossils and aDNA from sediments indicate that indeed DNA can be used as a complementary proxy, mostly overlapping in taxonomic finds with macrofossils, but even uncovering previous hidden information of local vegetation (Jørgensen et al., 2012; Parducci et al., 2012; Pedersen et al., 2013; Alsos et al., 2015).

Somehow, reconstructing faunal dynamics has been a minor focus in the field of sedaDNA. Studies mainly focussed on contribution pathways from animal to modern DNA in soils (Andersen et al., 2012) or archaeological purposes, such as human shaping of landscapes and domestication of animals (Hebsgaard et al., 2009; Giguet-Covex et al., 2014). Previously, faunal reconstructions have mainly been based on the less permanent proxy of perceptible fossils, and chemical and isotope analyses thereon (e.g. Hernández Fernández et al., 2007; Britton et al., 2009; Louys & Meijaard, 2010). But also animals deposit DNA, finding its origin in urine, skin, keratin, faeces or hair (Lydolph et al., 2005; Willerslev et al., 2014). Several examples of use of ancient faunal sedimentary DNA have been conducted (Lydolph et al., 2005; Haile et al., 2009; Thomsen et al., 2009; Madeja et al., 2010; Boessenkool et al., 2012; Murray et al., 2012; Haouchar et al., 2014; Willerslev et al., 2014), indicating its utility for palaeoreconstructions (Thomsen & Willerslev, 2015). More and more primers targeting faunal aDNA are being developed (Epp et al., 2012), and the future may bring an abundance of new information on ancient animal dynamics. However, studies applying faunal sedaDNA conducted in Scandinavia, with exception of Greenland, are scarce and more research is convenient to understand and contribute to the future of faunal sedaDNA.

Research goal and description

The general aim of this research project was to explore to what extent mammal ancient DNA could be extracted from millennia old lake sediments and archaeological soil samples in northern Sweden. This study focused mainly on sediments of Lake Krigstjärn, but also on soils from an archaeological Mesolithic cooking area in the close vicinity.

Here, archaeological excavations include moose bones (Johan Linderholm 2016, personal communication), indicating that moose (*Alces alces*) served as an important part of human diet. As game slaughtering and cleaning demands washing, this is likely to have occurred on the lake’s shores. Therefore, I hypothesize that ancient moose DNA can be detected in the sediments of Lake Krigstjärn, as well as in palaeosoils incorporating archaeological evidences for moose meat handling (cooking pit and bone material).

Interestingly, archaeologists have not detected any reindeer remains, although reindeer (*Rangifer tarangus*) have been known to roam northern Scandinavia already for a long time (Bjornstad et al., 2012) and make an excellent and reliable human food source (Geist, 2003). However, the exact arrival and origins of the Scandinavian reindeer is yet unclear and heavily debated. It is unknown if reindeer were absent while nomads hunted moose here, but the reindeer its ecological niche preference of boreal taiga to arctic tundra is food for thought. Even more so when compared to that of moose, which tends to prefer slightly more temperate ecosystems. Ideally, reindeer might be capable of roaming the pristine postglacial tundra, where moose might not, theoretically suggesting that reindeer migrated earlier into northern postglacial Scandinavia. Either way, reindeer are supposed to have migrated into
northern Sweden several thousands of years ago (Ukkonen et al., 2006), and thus traces of reindeer DNA might be able to detect in lake sediments. As a consequence, my second hypothesis predicts that sedaDNA of reindeer can be detected in millennia old sediments from Lake Krigstjärn.

Forest grazing has been known to be a major component in land use of northern Sweden since the 18th century, with traces even indicating grazing already much earlier than this (Larsson, 2012; Hellberg et al., 2003). Goat (*Capra aegagrus*) was a major grazing species in boreal forest. Thus, the third hypothesis includes that traces of goats DNA can be detected in the younger sediments of the core of Lake Krigstjärn.

Furthermore, pollen analyses (Johan Linderholm, personal communication; Berglund, 2008) from the surrounding indicate plant growth already since at latest 9500 years ago, agreeing with flourishing vegetation after deglaciation of the ice caps of the Last Glacial Maximum. My fourth hypothesis therefore suggests that plants aDNA should be present in the sediments of Lake Krigstjärn. Absence-presence patterns can be employed to scrutinize the functioning and success rate of extracting sedaDNA, as well as give possible indications on geological conditions favouring DNA preservation.

Altogether, this study aims to determine...

i) ... if ancient DNA can be successfully extracted from the sediments of Lake Krigstjärn
ii) ... if sedaDNA can be detected from mammals such as moose, reindeer and goat
iii) ... if plants sedaDNA can be detected and can scrutinize DNA degradation
iv) ... the theoretical and methodological implications deriving from this study

## 2 Material & Methods

### 2.1 Study site

Lake Krigstjärn (Figure 1; 250 m.a.s.l.; 63°42′03.6N, 17°46′22.8E) is located in boreal forested area of the province of Ångermanland, northeastern Sweden. The lake is a small, closed kettle pond system (2.2 hectares and 6.6 m maximum water depth) secluded from the river Storån by a steep ridge of glacial till on the southern shore and receives water from precipitation and groundwater. Nowadays, the lake is frozen and covered by ice 5-6 months a year thus creating anaerobic conditions at the lake bottom during this period, favouring DNA preservation (Coolen & Gibson, 2009) on top of the subarctic temperatures (Willerslev et al., 2003; Hofreiter et al., 2015). Normal mean temperatures for January and July based on 1961-1990 are -13°C and 15°C, with a yearly mean of 1°C (SMHI, 2009).

Archaeological findings of a Mesolithic hunting camp have been done on the lakeshore of Lake Krigstjärn, as well as excavations of an approximately 7000 year old settlement at the nearby lakeshores of Lake Lillsjön (Sjölander, 2014). A cooking pit was excavated at a Mesolithic settlement at Lake Lillsjön (ca. 1 km distance) in 2010, including a porous, fragmented bone and teeth (see later Figure 2). It is likely that hunted game was brought to Lake Krigstjärn, where it was cleaned, prepared and consumed, which may have contributed to traces of aDNA of mammals.
2.2 Methodology

2.2.1 Method development

Overall, this study has been dominated by pilot studies exploring the application of methods of DNA extraction, amplification and analysis of millennia old media. Thus the methods were progressively adapted using a combination of modification of existing laboratory protocols and re-iteration of tests based on trial-and-error findings.

In short, DNA was attempted to be extracted from three different matrices. First, I tried to detect moose DNA from four types of soils deriving from a Mesolithic cooking place used for preparing moose. Second, soils encircling unburned bones from next to this cooking place were tested on the ability of detecting moose DNA. Third, the amplification success of the selected primer sets for the targeted DNA were tested on DNA gained from fresh meat of both moose and reindeer, as well as for the most fit general plant primer set. Before starting with the definite DNA analyses of the core, pilot extractions were done and inspected on containing amplified ancient DNA.

2.2.2 Soil sampling

Several soils from the Mesolithic cooking location at the shores of Lake Lillsjön were carefully excavated and stored in the summer of 2010 (Sjölander, 2010). A bulk of soil from next to this pit at a depth of 15-20 cm contained pieces of bone (Figure 2, left) and presumed teeth, which for this study were subjected to X-ray scanning at the University Hospital of Umeå, Department of Odontology. Soils encircling the bone material were sampled for this project. Clear stratigraphic layers originating from the same pit can be observed from a soil bulk sample (Figure 2, right), with distinct soil characteristics. Four samples were obtained from those horizons (Figure 2, right), of which the top soil was brown-reddish sand, the upper middle layer was brown-greyish depleted sand, the lower middle was black and compositied of charcoal and the bottom layer was strongly red, fine sand material.
2.2.3 Sediment coring

A 2.54 m long sediment core was retrieved on the 1st of March 2016 at the deepest point of Lake Krigstjärn (6.6 m below water surface) using a percussion corer attached to a 3.4 m long, 10 cm diameter PVC tube. Both core ends were immediately closed with foam plugs and tape to avoid disturbance of sediments. The core was stored at 4 °C at Umeå University, Department of Ecology and Environmental Sciences, until the opening and subsampling of the core.

2.2.4 Core opening & subsampling

The core was cut lengthwise using a vibration saw, and was split at 2.0 m beneath surface to make it manageable. At depths of 12, 50, 96, 138-142, 174-176 and 228-230 cm from the top sediments were coated with gauze pads drenched with PCR-product (190 bp) of a pCR 4-TOPO® vector (Fisher Scientific) applying M13 Forward and Reverse primers (referred to later as M13-PCR) to identify external contamination. Hereafter, people wore sterile suits, double gloves and masks covering the entire face. Carefully, the core was brought into a room and hood, both thoroughly sterilized with bleach and UV-light for 24 hours. A 50-ml sterile sampling tube was left open in the hood for an environmental blank, as well as more blanks collected from the three sides of the hood, the working bench, suits and gloves. Between 0.5 and 1 cm of outermost sediment was removed using sterile tools to eliminate contamination deriving from smearing and external sources. 2 cm thick slices of sediment were collected in sterile 50 ml tubes, starting at a depth of 100 and 200 cm sampling upwards. This resulted in a total of 100 sediment samples. Encountered macrofossils were collected in separate tubes.

2.2.5 DNA extraction & amplification

DNA was extracted at a dedicated contamination free laboratory at the Department of Chemistry, Umeå University, where no previous DNA research or amplification has been performed (as suggested by Cooper & Poinar (2000)). Here, a total of 20 2-cm-thick sediment slices were extracted of DNA with ranging depths from 4 to 184 cm of which 4 subsamples as replicas per slice were taken. Samples were centrifuged before extractions to remove moisture. Extractions were performed from subsamples of 0.6 ± 0.1 gram of every sampled sediment slice using a PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, USA), following the manufacturer’s instructions. With every extraction procedure for one sediment slice an extraction control was included and treated exactly as the other sediment subsamples (a total of 20 extraction controls), thus being a check on contamination during the extraction process.
DNA from those 4 replicas and the appurtenant extraction control, along with a PCR negative control (without DNA template), was amplified in volumes of 24-25 μl containing 2.5 μl 10x PCR buffer, 0.5 μl 25 mM MgCl₂, 0.05 μl 100 mM of all four dNTPs, 0.5 μl 20 mM of each primer, 0.125 μl 1.25 U Taq DNA polymerase (Applied Biosystems, California, USA), 0.2 μl 10 μg/μl BSA, 20.475 μl sterile water and 1-2 μl DNA template. PCRs were run using different primer pairs (Table 1), targeting moose (Alces alces; Nichols et al., 2012), reindeer (Rangifer tarandus; Bjørnstad and Roed, 2010), goat (Capra aegagrus; Köppel et al., 2009), as well as universal chloroplast trnL g, c and h primers (Taberlet et al., 1991, 2007).

### Table 1. Details of applied primer pairs for DNA amplification, including official identity, sequence (5’ to 3’), expected product size and reference.

<table>
<thead>
<tr>
<th>Species or taxon</th>
<th>Primer name</th>
<th>Official ID</th>
<th>Sequence 5’ to 3’</th>
<th>Product size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Universal chloroplast</td>
<td>Plant-g</td>
<td>trnL-g</td>
<td>GGGCAATCCTGAGCCAA</td>
<td>c – h 250</td>
<td>Taberlet et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Plant-c</td>
<td>trnL-c</td>
<td>CGAAATCGGTAGACGCTACG</td>
<td>g – h 40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plant-h</td>
<td>trnL-h</td>
<td>CACATTAGTCTCTGACCATTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moose</td>
<td>Moose F</td>
<td>Aacytb1044 F</td>
<td>TACCATGGACAACATGGC</td>
<td>74</td>
<td>Nichols et al., 2012</td>
</tr>
<tr>
<td></td>
<td>Moose R</td>
<td>Aacytb1044 R</td>
<td>CGACGGCTGATCTAAGCTGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reindeer</td>
<td>Reindeer F</td>
<td>259B</td>
<td>TGCACCACGTTTTAAGCAAG</td>
<td>271</td>
<td>Bjørnstad and Roed, 2010</td>
</tr>
<tr>
<td></td>
<td>Reindeer R</td>
<td>528R</td>
<td>TAGCTGAGTGGCCTGGAGAAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goat</td>
<td>Goat F</td>
<td>Goat2 F</td>
<td>CACCTTATCCTCCATCTTGCTAC</td>
<td>140</td>
<td>Köppel et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Goat R</td>
<td>Goat2 R</td>
<td>TCTTAATGGTGAGTAGGTTGAAATG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DNA extracts, blanks and negative controls underwent 1) the enzyme activation at 95 °C for 3 min. and were subjected to 2) 10 cycles consisting of touch down PCR at 95 °C for 30 sec., 10 °C above annealing temperature of the primer set (calculated by ThermoFisher) for 30 to 60 sec., and 72 °C for 30-60 sec. (depending on amplicon size), with the annealing temperature being decreased by -1 °C after each cycle. Then, samples underwent 3) 35 cycles of 30 sec. at 95 °C, annealing temperature for 30-60 sec. and 72 °C for 30-60 sec., thereafter, ended with 4) the elongation step at 72 °C for 5 min.

#### 2.2.6 DNA analysis

Amplified DNA (2-5 μl), mixed with 2 μl of 6x DNA Loading Dye, was loaded into a well on 1% agarose gels (p.e. 0.35 gram of agarose powder, 35 mL 1x TBE-buffer and 2 μl GelGreen Nucleic Acid Gel Stain) to determine presence of targeted DNA. Only positive amplification bands of the expected product size (Table 1) in combination with neither positive results of controls or blanks were considered to be genuine.

#### 2.2.7 Radiocarbon dating and chronology

Two macrofossils of Scots pine (Pinus sylvestris) were cleaned and dried for carbon-14 dating (Table 2) using Accelerator Mass Spectrometry (AMS) analysis by Beta Analytic in London, United Kingdom. Dates were calibrated using IntCal13 (Reimer et al., 2013). It should be kept in mind that sediment accumulation rates are assumed to be unrealistic constant over a period of 5500 years, and thus extrapolated dates for sediments give just a rough indication of age (Table 3).
Table 2. Radiocarbon dates based on macrofossils of Lake Krigstjärn, as well as calibrated weighted ranges and mean. Calibration was done using IntCal13 (Reimer et al., 2013) with a probability of 95%.

<table>
<thead>
<tr>
<th>Laboratory identity</th>
<th>Relative depth (cm)</th>
<th>(^{14}\text{C} ) age (BP)</th>
<th>Mean Cal. (^{14}\text{C} ) age (BP)</th>
<th>Cal. (^{14}\text{C} ) age range (BP)</th>
<th>Sample contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>BETA-437179</td>
<td>30 – 32</td>
<td>2300 ± 30</td>
<td>2323 &amp; 2208</td>
<td>2345 – 2300 &amp; 2255 – 2160</td>
<td>Scots pine bark</td>
</tr>
<tr>
<td>BETA-437180</td>
<td>132 - 134</td>
<td>8450 ± 30</td>
<td>9483</td>
<td>9525 – 9440</td>
<td>Scots pine bark and cone scale</td>
</tr>
</tbody>
</table>

Table 3. Sediment sample identities, relative depths (in cm), modelled calibrated radiocarbon dates based on dated macrofossils for all sediment samples of which DNA was extracted (Table 2). Interpretation of modelled calibrated carbon ages should be considered carefully, as modelled ages only give a rough estimation of ages.

<table>
<thead>
<tr>
<th>Sediment sample identity</th>
<th>Relative depth (cm)</th>
<th>Modeled mean (^{14}\text{C} ) age (BP)</th>
<th>Modeled cal. (^{14}\text{C} ) age century (BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 – 6</td>
<td>370</td>
<td>17(^{\text{th}})</td>
</tr>
<tr>
<td>2</td>
<td>14 – 16</td>
<td>1100</td>
<td>4(^{\text{th}})</td>
</tr>
<tr>
<td>3</td>
<td>24 – 26</td>
<td>1820</td>
<td>2nd-3rd</td>
</tr>
<tr>
<td>4</td>
<td>28 – 30</td>
<td>2110</td>
<td>100 BC</td>
</tr>
<tr>
<td>5</td>
<td>38 – 40</td>
<td>2830</td>
<td>800 BC</td>
</tr>
<tr>
<td>6</td>
<td>48 – 50</td>
<td>3550</td>
<td>1600 BC</td>
</tr>
<tr>
<td>7</td>
<td>58 – 60</td>
<td>4280</td>
<td>2300 BC</td>
</tr>
<tr>
<td>8</td>
<td>68 – 70</td>
<td>5000</td>
<td>2500 BC</td>
</tr>
<tr>
<td>9</td>
<td>78 – 80</td>
<td>5720</td>
<td>3700 BC</td>
</tr>
<tr>
<td>10</td>
<td>88 – 90</td>
<td>6450</td>
<td>4500 BC</td>
</tr>
<tr>
<td>11</td>
<td>98 – 100</td>
<td>7170</td>
<td>5300 BC</td>
</tr>
<tr>
<td>12</td>
<td>108 – 110</td>
<td>7890</td>
<td>6000 BC</td>
</tr>
<tr>
<td>13</td>
<td>118 – 120</td>
<td>8620</td>
<td>6600 BC</td>
</tr>
<tr>
<td>14</td>
<td>126 – 128</td>
<td>9190</td>
<td>7200 BC</td>
</tr>
<tr>
<td>15</td>
<td>130 – 132</td>
<td>9480</td>
<td>7500 BC</td>
</tr>
<tr>
<td>16</td>
<td>136 – 138</td>
<td>9920</td>
<td>7900 BC</td>
</tr>
<tr>
<td>17</td>
<td>140 – 142</td>
<td>10210</td>
<td>8200 BC</td>
</tr>
<tr>
<td>18</td>
<td>148 – 150</td>
<td>10780</td>
<td>8800 BC</td>
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<tr>
<td>19</td>
<td>164 – 166</td>
<td>11940</td>
<td>10000 BC</td>
</tr>
<tr>
<td>20</td>
<td>182 – 184</td>
<td>13240</td>
<td>11200 BC</td>
</tr>
</tbody>
</table>

2.2.8 NIR-spectrometry

Additionally, this project utilized near infrared (NIR) spectroscopy as a proxy for major lithostratigraphic changes, potentially imperceptible to the eye. Previous, NIR spectrometry has already been applied to detect the chemical composition of materials, such as C, N, S & P concentrations and the origin and spatial variability of lake sediments (Korsman et al., 1999; Malley et al., 1999; Rosén et al., 2001; Rosén, 2005; Inagaki et al., 2012), and has shown to be a useful quantitative proxy of past climatic conditions (Rosén et al., 2001). To this subjective, measurements were taken every 2 cm on the surface using a JDSU 1700 microNIR spectrometer (908-1676 nm), covering the core of Lake Krigstjärn up to 250 cm depth. Further NIR spectrometry imaging was conducted at the Thermochemical Energy Conversion Laboratory at the Department of Applied Physics and Electronics, Umeå University. The NIR spectrum was subjected to a principal component analysis (PCA). Here, the PCA components were used to identify changing properties and important stratigraphic units of the sediment core. Hereto, it was generally assumed that PC 1 & 2 reflect changes in organic matter and water content (Rosén et al., 2000; Linderholm & Geladi, 2012).
3 Results

3.1 Sediment properties & chronology

Two radiocarbon dated Scots pine (*Pinus sylvestris*) macrofossils originating from depths of 30-32 and 130-132 cm, gained ages ranging from 2300 ± 30 to 8450 ± 30 BP, corresponding with calibrated mean ages of 2265 and 9485 cal. BP (Table 2). Age-depth extrapolations were done applying those calibrated ages (Table 3), but direct application of those integrated ages should be considered with care, due to lack of detail (n=2). Three major stratigraphic subdivisions (Zone 1 - 4) can be made based on sediment properties indicating geological stages (Figure 3).

**Zone 1 – Pre-deglaciation stage (>154 cm depth).** Sediments were mainly consisting of sands, with coarser materials downwards from 249 cm. Upwards, the core consisted of glacial till with unsorted sandy material and was reddish, gradually shifting into shades of grey, even sporadically consisting silt inclusions between 222 – 168 and a stone (⌀ 5 cm). From a depth of 168 cm sediments contain grey glacial sands with rounded gravels with an abrupt end at 154 cm depth.

**Zone 2 – Marine stage (154 – 134 cm).** An abrupt transition occurs into sorted material deposited in water at 154 cm. This consists of grey marine clay, gradually becoming lighter unto 139 cm, where it shifts into green marine clay probably originating from a first cut-off-from-the-sea-stage up to 134 cm deep. During this time-zone those sediments experienced a transition from long glacial ice coverage to their melt and retreat, and the successive post-glacial rise of sea level.

**Zone 3 – Initial lake stage (134 – 130 cm depth, 9800 – 9400 cal. BP).** This small lithological zone is composed of olive green, minerogenic marine clay. After deglaciation, the system became more-and-more cut-off from the sea, but was still subjected to marine influences. Thus, the conditions were brackish. Sediments were rich with aquatic mosses, indicating shallow water levels allowing aquatic plants to thrive at the brackish lake bottom.

**Zone 4 – Lacustrine stage (130 – 0 cm depth, 9400 – 100 cal. BP).** In this zone sediments originate from a freshwater lake system. Overall, sediments were moist and soft of texture. Just above the brackish zone, sediments are rich of plant materials. From 130 to 104 cm depth there is a gradual shift from brown to poorer grey material. Abruptly, at 104 cm depth there is a layer rich in organics again and shift from brown organic rich to grey, compact materials lower in water content up to 37 cm depth. From 37 cm to the top of the core, sediments were composed of gyttja, were dark brown and high in organics, with two plant material hot-spots at 37-32 cm and 3-0 cm.

3.2 NIR spectrometry analyses

The result of NIR spectroscopic scanning used to describe the properties of the Lake Krigstjärn core is shown in Figure 4, and contributed to the stratigraphic core description of Figure 3. NIR spectrometry indicated two principal components (PC’s) explaining variance for 85.5 % for t[1] and 10.5 % for t[2] (Figure 4 & 5).

In short, PC 1 was used as a proxy for substantial variations in organic matter content and increased gradually from the base of the core to a depth of 154 cm, where an abrupt decrease occurred (Figure 4). This observation seemed to coincide with the interpretation of the transition from glacial till to lacustrine sediments at 154 cm depth. Notable, PC 1 also detected strong changes at 200 cm depth and several above 154 cm.
Figure 3. Core description including integrated age (cal. BP), depth (cm), lithology, origin of samples used for DNA extraction (see also Table 3), as well as zonation. Arrows indicate depth of carbon dated pine macrofossils.
PC2 decreases slightly from the base upwards but experiences a sudden negative peak at 200 cm and then increases abruptly at the transition from glacial deposits to marine sediments, coinciding with the determination of this stage transition (Figure 4). Hereafter, PC2 shows small changes but overall keeps constant in the lacustrine sediments.

Remarkable is the behaviour of PC1 and PC2 with respect to each other in the differing sediment zones (Figure 4). Both principal components show no statistical correlation whatsoever in brackish and freshwater lake systems (zones 3 & 4), yet in the pre-lacustrine sediments (zone 1 & 2) t[1] and t[2] show a strong negative relationship (R² = -0.81; p < 0.0001), which is also reflected in the linear formation represented by the principal component class model (Figure 5). It can be deduced that the variances covered by both PC1 and PC2 in the zone 1 & 2 are induced by chemical composition characteristics with an inverse relationship. With the possibility of variances of PC1 being caused greatly by organic matter content, together with the fact that marine sediments contained glacial sands in contrast to the lake sediments, variances covered by PC2 might be induced by sandy material.

Hence, if NIR spectrometry indeed maps organic matter contents in detail, this is a helpful proxy to track down biological activity and enhanced DNA preservation and thus indicating eligible sediments horizons for DNA extraction.

![Figure 4](image1.png) **Figure 4.** Principal components t[1] and t[2] plot against core depth (cm), including the transition.

![Figure 5](image2.png) **Figure 5.** Principal component (PC) class model of NIR series of 250 cm of the core of Lake Kriptjärn, categorized into lake, transition and marine sediments. Variances are explained by 85.5% of t[1] and 10.5% of t[2].
3.3 Ancient DNA

3.3.1 Primer validation

Selected primer sets for moose and reindeer were validated on amplification success and both primers gave positive indications of its targeted modern DNA. Furthermore, amplification of plants DNA was conducted with trnL primer sets c & h, as well as g & h, but results were more distinct for trnL c –h analyses, and further plant detecting has thus been conducted using this primer set.

3.3.2 Soil samples

DNA extracts encircling bones and presumed teeth from the excavation did not gain any positives applying the moose primer set. However, the several X-ray scans (Figure 6) revealed that the bone was not a jaw as assumed previously, but a piece of pelvis bone. Hence, it can also be stated that there were no teeth in the soils.

However, moose DNA was extractable and amplifiable from the stratigraphic ordered soil layers from the Mesolithic cooking pit (Figure 2, right). Here, moose DNA was found in three out of four layers, including the charcoal rich layer. From the top layer no DNA detection was indicated.

Figure 6. One of the many X-ray images for identification of bones, encircling soils and pieces presumed to be teeth found next to the Mesolithic cooking put with Lake Lillsjön.
3.3.3 Sediment samples

3.3.3.1 Mammalian

Reindeer DNA was successfully detected in the sediments (Figure 7) for both the first and second run, reaching to a depth of 90 cm. DNA detection is expressed as the amount of positive DNA finds from the four replicates deriving from a single sediment sample (Figure 7). A clear decline in those detections per sediment segment can be observed from the upper, younger strata (3 or 4 finds out of 4) to the deeper, older layers (1 or 2 out of 4). Thus, earliest detection of DNA amplified by the reindeer primer set can be estimated to an age of 6000-7000 years old. One blank contamination occurred in the second series executed on the sediment at 14-16 cm depth.

Importantly, besides the presence of the expected amplicon (271 bp; Table 1), several bands with deviating sizes were observed (Figure 8). To ensure the primer set was specific to reindeer, and reindeer only, it was tested on modern reindeer DNA, as well as on moose DNA, both derived separately from freshly defrosted meat. Reindeer meat yielded the expected single product size of 271 bp, but reindeer primers showed also positive bands repetitively when tested on moose (Figure 9). In addition to expected size of around 271 bp of moose, additional bands were always observed with sizes of 700-800 bp (Figure 9).

Fragments similar to the mysterious ones (700 - 800 bp) observed with moose DNA appear repetitive and already early in the sediments (30 - 80 cm depth, ± 2000 - 6000 years BP), afterwards not appearing any further (Figure 9). The shorter amplicon (± 200 bp) starts to occur in the core sediments much later in the two highest layers (Figure 8; <16 cm, 1000 - 1500 years BP). Importantly, moose primers didn't detect any DNA.

3.3.3.2 Plant DNA

Chloroplast DNA was successfully detected applying universal chloroplast primers trnL c & h (Figure 7). The first amplification round resulted in only three sediments with positive detections of plant DNA. Two of those sediments (4 - 6 & 98 – 100 cm) are located in layers highly concentrated with plants, with the third sediment (48 – 50 cm) containing a lot of organics. In contrast, the second series indicates chloroplast DNA down to 184 cm (one contaminated blank at 108 – 110 cm) with exclusion of several sediments (14-16, 28-30, 58-60, 118-120 and 140-142 cm; Figure 7). This means chloroplast DNA is already observed preceding deglaciation, exceeding ages of ca. 9.5 kyr. In the depth range of 14 – 120 cm almost all sediments yield no or barely a single positive out of four replicas, applying to both series (Figure 7).
Figure 7. DNA from reindeer (a) and plants (b) recorded in Lake Krigsjärn sediments. DNA finds are indicated by the number of positives out of the 4 replicates for two separate PCR amplification series 1 and 2 for reindeer (271 bp) and universal chloroplast products (250 bp). Stars (*) show sediment samples without finds of DNA sequences. Dots (•) indicate positive of blanks.
Discussion

4.1 Mammalian DNA

4.1.1 Moose

Moose sedaDNA was recorded in none of the sediments by the targeting primer sets. Nonetheless, moose existed in the area as bones have been excavated in the close vicinity by archaeologists dating back to 7000 years (personal communication Linderholm). These archaeological finds are supported by the presence of moose aDNA from the cooking pit soils, although not in all soils, as no moose DNA was detected in the soils encircling moose bones. Thus, my first hypothesis that moose DNA can be detected in the sediments of Lake Krigstjärn is invalid. Previous studies highlight the need for a relatively large amount of DNA accumulated in lake sediments to be detected (Giguet-Covex et al., 2014) and it seems likely that genetic material preserved in the lake sediments was too little. Moose roam the landscape solitary and populations might not have been dense, thus DNA deposits could have been minimal and therefore remain undetected. This demonstrates that undetected DNA in sediments does not rule out the appearance of targeted species, and negative results in ancient sedaDNA are not sufficient to exclude occurrence of species in past ecosystems.

An alternative, supplementary explanation for the absence of moose aDNA in the sediments is the intended function of the designed moose primer set used, which is ecological monitoring based on modern DNA (Nichols et al., 2012). Five criteria exist for the development of an ideal primer set, with different significances depending on the

Figure 8. Resulting DNA sequences based on reindeer primers from sediment samples 1 – 4, including 4 replicas, negative controls (N) and blanks (B) for each corresponding sediment layer, together with 100 bp ladders. The arrow indicates the expected amplicon size of reindeer DNA.

Figure 9. Resulting DNA sequences based on reindeer primers tested on fresh moose meat. Arrow indicates expected reindeer amplicon size. A negative control (N) is included.
The researcher's purpose (Chase et al., 2005). The primer set should be i) discriminative among all species, ii) a standardized DNA region, iii) with sufficient phylogenetic information to determine its taxonomy, iv) with robust, conserved priming sites and v) a region short enough for amplification of degraded DNA (Taberlet et al., 2007). However, such perfect primer sets are limited and design demands consideration of the objective. The characteristics of the applied primer enable to amplify modern DNA, and therefore might be unsuited for degraded, ancient DNA, explaining the inability to detect ancient moose DNA.

However, the soils deriving directly below the cooking pit are indicating presence of moose DNA. In short, this might be ascribed to three major factors: i) DNA signal strength, ii) DNA leakage direction and iii) DNA preservation conditions in soil. Importantly, the source of this DNA must have been the repetitive cooking of moose meat, releasing a strong signal of moose DNA behind, with possible influences of leakage in downwards direction. The chances of an intact targeted sequence is bigger with high amounts of DNA, and even primers designed for only minor degraded DNA, as might be the case with this moose primer set, might be able to detect aDNA. Furthermore, depth together with the charcoal generates a relative high soil pH, thus favouring DNA preservation conditions in those soils (Herrman et al., 1999; Lindahl, 1993). Also, the soils contain a reddish colour (Figure 2, right), indicating the presence of iron-hydroxides, promoting organic matter contents in soils, which helps to preserve DNA (Ladoande et al., 2012). Altogether, favouring conditions seems to prevail in the soils from the Mesolithic cooking pit of Lake Krigstjärn.

Then again, the absence of aDNA from the shallow soils encircling the moose pelvis bone can be explained by similar principles, although, generating a divergent effect. The moose bone is just a single DNA source, probably totally cleaned from meat, and thus generates a weak DNA signal. Yet, DNA might have been present in collagen of the bone, but the DNA extraction method is developed on soil- and sediments, and thus this DNA signal may be bypassed. Additionally, samples were taken surrounding the bone, and thus DNA leakage might not have been into surrounding soils, but downwards. Also, the chemical environment from those soils might not have been supportive, as the bone is located in weathered grey soils, containing little supporting organic matter content or iron-hydroxides as seen in the previous soils. Also, the bone was excavated from a shallow site, indicating that DNA has been exposed to aerobic conditions, which enhances degradation. This considered, positive DNA detections in the cooking pit soils cannot originate from leaking modern moose DNA, for then it should have been detected in the bone encircling soils as well.

4.1.2 Goat

No goat sedaDNA has been detected. Although only expected to occur in the youngest sediment, its absence indicates that forest grazing by goats did not occur in the surrounding until the c. 17th century. However, the age of the youngest sediment layers analysed equals or even predates the 17th century, and thus it is logical that no ancient goat DNA can be found in the sediments of Lake Krigstjärn. To evaluate the hypothesis, younger sediment layers should be analysed on sedaDNA of goat in the future. The hypothesis that ancient DNA of goat can be detected in the sediments of Lake Krigstjärn is neither approved nor rejected.

4.1.3 Reindeer

Although not all positive detections within the reindeer analyses are straightforward, it can be stated that millennia old reindeer sedaDNA was found. Thus, my second hypothesis that reindeer sedaDNA can be detected in the sediments of Lake Krigstjärn can be confirmed. Nowadays, reindeer roam the surroundings still, and as the landscape was not objected to major changes during some millennia, it is indeed likely that reindeer is the genetic source of the detected DNA.

The positive signals and additional fragments gained with moose DNA amplified with the reindeer primer set implies that the primers are not specific to reindeer as apprehended
Consultation of NCBI Genbank with Primer-BLAST (KM506758) confirms this, as DNA of many other cervid- and bovid species turn out to be targeted, albeit that those species are mostly not native to Scandinavia. There are some exceptions: roe deer (Capreolus capreolus), moose (Alces alces) and human-introduced livestock such as goat (Capra hircus), cattle (Bos taurus) and sheep (Ovis aries); the last three known to arrive recent in northern Sweden, and unlikely to be present at all early in the surroundings of Lake Krigstjärn. Nowadays, roe deer and moose roam through northern Sweden, yet roe deer are assumed to have colonized northern Fennoscandia very late in the Holocene (Sommer et al., 2009; and references therein). Peculiarly, no targeted DNA amplicon with 700-800 bp (Figure 9) does exist for moose based on GenBank. Nonetheless, the pilot on fresh reindeer meat implies that reindeer DNA does not generate such an amplicon, and moose does. This mysterious amplicon is only observed in sediments corresponding of ages between ca. 6000 – 2000 years, thus excluding the double bands to derive from roe deer DNA. Thus, it could indicate presence of moose, although amplicon origin remains unclear. Future amplicon sequencing will indicate the species identities.

Inspection of small amplicons of 200 bp generated by the reindeer primers indicates that no products match this size (GenBank). However, the arrangement of those amplicons together in two adjacent sediments is not random and must find its origin in type of DNA. Noteworthy, these 200 bp fragments only show up in the youngest sediments (<1500 cal. years), and might indicate a species previously not existent in the area.

Encouraging is the continuity of positives amplified by the reindeer primers (Figure 7), confirming non-random emergences and narrowing down contamination of sediment samples considerably. The single contaminated blank (Figure 7; series 2, 14-16 cm depth) seems to find its origin in the high amount of DNA in all four replica’s, as the extraction has been done separately ruling out cross-contamination. Furthermore, the first manifestation of reindeer DNA is in sediments approximately 6000 - 7000 years of age.

The colonization pattern and timing of the early wild tundra reindeer into northern Scandinavia is still largely unknown, because of the scarcity of fossil remains (Ukkonen, 1993). So far, four optional migration routes have been suggested for reindeer moving into Fennoscandia: from central Europe via i) Denmark into Sweden (Björck et al., 1996), ii) the North Sea land into coastal Norway (Rankama & Ukkonen, 2001), iii) the Baltic into Finland (Ukkonen et al., 2006) and iv) an alternative route from Russia via Kola Peninsula into northern Fennoscandia (Rankama & Ukkonen, 2001). Oldest finds of reindeer fossils of c. 12000 – 11500 years are done at the Norwegian coast, from where wild mountain reindeer are thought to have migrated into northern Lapland some 9000 years ago. Thus, the oldest reindeer sedaDNA of c. 6500 calibrated years seems a very acceptable date for reindeer presences in the surrounding. Another subspecies of reindeer, of which DNA is also targeted, is the forest reindeer (Rangifer tarandus fennicus), thought to have migrated into Finland from Siberia at earliest some 5000 years ago (Rankama & Ukkonen, 2001). Thus, it is most likely that the detected sedaDNA derives from Eurasian tundra reindeer. However, future sequencing will scrutinize this.

A combination of previous interpretations on the absence of moose and irregular positives for reindeer primers emerges into a complementary proposition. If indeed the applied moose primer set is unsuitable to detect heavily degraded DNA, the mysterious fragments amplified with the reindeer primer set could indicate moose presences from 6000 to 2000 cal. years ago. This also agrees with the archaeological findings of moose remains from humans hunting approximately 7000 to 5500 years ago at Lake Krigstjärn and Lillsjön.
4.2 Vegetation

4.2.1 Plant DNA

Chloroplast DNA was successfully detected confirming the expectation. Hence, my fourth hypothesis stating that plants aDNA can be detected in the sediments of Lake Krigstjärn can also be accepted. Multiple positives were even found in the deepest sediments analysed in this project, which indicates that preservation was sufficient enough to detect aDNA. However, in five out of twenty examined sediment fragments, no DNA has been encountered. As pollen from Lake Lillsjön (data unpublished, 2015) indicate presences of local vegetation communities at least 9500 years ago, absence of DNA finds in those sediments does point at poor DNA preservation or difficulties in extracting DNA. Here the former option cannot be appointed directly to low organic matter contents as both sediments observations as NIR spectrometric component PC 1 indicate very divergent organic contents within those sediments. Furthermore, organic hot spots, densely packed with visible plant material, do not necessarily mean generous DNA detections, as substantiated by sediment fragments 5 and 11 (38-40 & 98-100 cm respectively). This can be assigned to inhibiting function of organics with the DNA extraction procedure in organic rich samples (Harry et al., 1999), as seen more often with high concentrations of humic acids (Hebda et al., 2015).

Analyses from sediments between 14 and 120 cm mostly yielded none or just one positive out of four replica’s, often even applying for only one of the two PCR-series. This highlights that DNA detection is somewhat random, and absence of detection, again, cannot be interpreted as absence of species.

An intriguing finding is the early presence of chloroplast DNA. Clearly, DNA was not only detected from the post-glacial landscape, when ice sheets had retreated and left behind pristine land to colonize by plants, but even DNA originating earlier has been detected in glacial and marine sediments. Fundamentally, the trnL intron targeted by the applied primers set is specific to chloroplasts and besides targeting DNA of Angiosperms, Bryophytes and Gymnosperms, some algal are responsive as well (Taberlet et al., 2007). Positive DNA detections, such as in marine sediments, might thus possibly represent algae, but the sandy sediments in which the deepest DNA detections were done are of pre-deglacial origin. This indicates most likely that not algae, but plants might be represented in the oldest detections.

Yet, the question rises where the plants sedaDNA from pre-deglacial sediments has its origin. Northern Sweden has been fully glaciated by thick ice sheets during the Last Glacial Maximum and the Younger Dryas (Helmens, 2014; Böse et al., 2012), and is assumed to make vegetation growth impossible. Therefore, speculation on the origin of the sedaDNA finds of pre-deglacial sediments gives several scenarios explaining vegetation presences. First, the expanding of ice sheets is a dynamic, vigorous process, likely accompanied by pushing and lifting of soils and vegetation. Consequently, plant material can be enclosed in or deposited on glaciers, afterwards with deglaciation being deposited together with glacial sediments. In such a case, plants derive from just before intrusion and expansion of the Scandinavian Ice Sheet. Another scenario follows similar principles as ice sheets might have lifted up packages of soils or debris, where it can accommodate plant growth and form into patches of vegetation on ice. Nowadays, such debris-covered glacial vegetation exists, ranging from sparse pioneer vegetation to abundant conifer forests (Fickert et al., 2007), supporting such a possibility. A third scenario includes the existence of ice free areas within the ice sheets, where vegetation might have thrived. However, such a scenario draws an image of vegetation patches very sporadic in the landscape, and the likelihood of an encounter with remains is small. In this study both pre-deglacial sediments detected plants, suggesting a more permanent source of plants DNA. A last option might be that those sediments have older origins than discussed above, preceeding the Younger Dryas and the Last Glacial
Maximum, and have been mixed by moving ice. Sequencing of the detected DNA up to species-level would help to understand its context.

4.2.2 Pine macrofossils

Based on pollen finds, it has long been assumed that plants such as Scots pine were wiped out of Fennoscandia by the ice-sheets of the Last Glacial period and the Younger Dryas, whereafter re-colonization took place in a process taking decennia (Birks et al., 2005). In this way, Scots pine is thought to arrive between 8000 and 9000 years ago in northern Sweden (Cheddadi et al., 2006). However, the carbon dating of a pine macrofossil of this project gained a calibrated age of 9525-9440 years, which is in contradiction with the previous assumed arrival based on pollen. Probably, this fossil might even indicate extensive growth of pine, as the chance of detecting a single individual is small. Yet, it supports other studies also based on fossilized pine (Kullman, 2008) and ancient DNA (Parducci et al., 2012) pointing towards earlier presence just after deglaciation of Scots pine throughout Scandinavia (Van Woerkom, 2016). Even pollen analysis from Lake Lillsjön starting 9500 cal. years ago (personal communication Johan Linderholm, 2016) indicates the highest percentages recorded in the Holocene of Scots pine pollen already directly after deglaciation (9.5 kyr), which strongly contradicts former palynological studies (Birks et al., 2005)

5 Implications

The overall aim of this study was to explore whether ancient DNA could be detected from lake sediments and to evaluate its capability to trace down aspects of past ecosystems, specifically the presence of moose, reindeer and plants.

DNA was present in almost all sampled sediments, even the oldest, suggesting sufficient conditions for preservation and detection of sedaDNA in Lake Krigstjärn. SedaDNA is well capable to trace down ancient presences of mammals and plants. However, this study identified some methodological challenges and theoretical implications for future sedaDNA studies.

First, it is crucial that a primer set has the ability to amplify heavily degraded DNA. In this study, this is demonstrated by undetected moose although there are signs that moose DNA is present in the sediments from archaeological excavations. The characteristics of the applied primer enable to amplify modern DNA, and therefore might be unsuited for degraded, ancient DNA, explaining the inability to detect ancient moose DNA. Furthermore, selection of a species-specific primer set can be challenging, especially in combination with the optimal yielding for heavily degraded DNA. The reindeer primer set seemed besides reindeer, also to target species such as moose and roe deer. Yet, sequencing of the detected sedaDNA can resolve its origin.

Second, sedaDNA is a promising proxy to reconstruct vegetation, but especially for mammal dynamics, otherwise difficult to construct due to the discontinuous, random proxy of fossils. Thus so, genetic techniques enable us to reveal a rich treasure of untouched information still hidden in lake sediments and soils. Yet, sedaDNA only becomes a strong proxy when put in context, and it is therefore a powerful complementary technique in palaeoeological reconstructions together with other independent archives providing validation of lake sedaDNA finds.

That sedaDNA can gain new insights is demonstrated by the successful detection of reindeer aDNA already in sediments of 6500 cal. years old. Although this is not a controversial find, as Ukkonen and colleagues (2006) put it, “new dates and DNA analyses from ancient Scandinavian and Baltic specimens would considerably help in the reconstruction of the
origin and immigration routes of the extant wild reindeer populations in northern Europe". Besides the implications for mammal dynamics, genetic analysis on sediments of Lake Krigstjärn has promising finds of glacial plants, opening up new research objectives, as well as some positive evidence for the highly debated early presence of pine in the postglacial landscape.

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