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Chronicling the rise and fall of the invasive species *Elodea canadensis* in a eutrophic lake using paleolimnological methods

(Beskrivelse av invasjon og tilbakegang for den uønskede arten *Elodea canadensis* i en eutrof, belastet innsjø ved bruk av paleolimnologiske metoder)

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Abstract
The history of the introduction and disappearance of the invasive aquatic plant *Elodea canadensis* (*E. canadensis*) in lake Årungen, a eutrophic, disturbed lake, was studied using paleolimnological techniques to investigate how the species was introduced, and why it disappeared. A variety of field, lab and numerical methods were used to pursue this investigation.

The paleolimnological methods included the removal of two sediment cores from the lake and spectroscopically analyzed for absorbance and for concentrations of the metals cadmium, copper, zinc, chromium, lead and manganese to determine if the disappearance was associated with the presence of metals. High levels of metals associated with road runoff from a nearby highway had been measured in another study, leading to the hypothesis that road runoff could have caused the demise of *E. canadensis* in lake Årungen. The sediment cores were also analyzed for percent water, dry matter and organic matter. A numerical analysis was conducted on the absorbance data obtained from the spectroscopic analysis to separate individual pigment data from the absorbance data, using methods developed by Prof. Hendrik Küpper (Küpper et al., 2007)

Field surveys were conducted in lake Årungen and in the streams contributing to the location where the sediment cores were removed. Streams and overflow pipes were sampled during low flows and high flows including locations receiving discharge from road and urban runoff and combined sewer and stormwater discharge. Stream and sediment samples and samples of E. canadensis were analyzed for cadmium, copper and zinc. Historical documents were also searched for information about changes in the landscape that could have contributed to the introduction and disappearance of *E. canadensis.*

The search of historical records show that *E. canadensis* was very likely introduced during the construction of a rowing pier in the lake. The pier construction occurred during the same time period in which a new highway was constructed, resulting in a change in traffic patterns that greatly reduced traffic on a road adjacent to the lake, and reduced the amount of metals being introduced into the lake. An investigation of the installation of municipal and highway stormwater drainage systems revealed that runoff from several drainage systems was entering the lake at one location. Over time, the levels of metals in the lake increased concurrently with increasing traffic on the highway, which is attributable to levels of metals measured in road and urban runoff discharging into this location.

The continuous inputs of metals and perhaps other pollutants from urban and road runoff very likely caused an initial decline of *E. canadensis*. Multiple sources of pollutants discharging at high concentrations during extreme rainfall and rain-on-snow events in 2006 and 2007 probably caused the final die-off of the plant. If this is the case, this has larger ecological implications for both lake Årungen and other lakes receiving multiple inputs of urban and road runoff in the form of peak flows.

Norsk Sammendrag

Introduksjon, oppblomstring og tilbakegang for Vasspest, *Elodea canadensis* (*E. canadensis*) i Årungen, som er en eutrof, belastet innsjø, ble studert med paleolimnologiske metoder. En kombinasjon av feltstudier, laboratorieundersøkelser og numeriske metoder ble benyttet til å studere hvordan arten ble introdusert, og hvorfor den forsvant.

De paleolimnologiske metodene omfattet innhenting av to sedimentprøver, og spektroskopisk analyse av disse. Videre ble prøvene analysert med hensyn på konsentrasjon av kadmium, kobber, sink, krom, bly og mangan, for å se om artens tilbakegang kan være forbundet med tilstedeværelsen av metaller. Høye forekomster av metaller i forbindelse med avrenning fra en motorvei i nærheten har blitt målt i en annen studie. Dette ledet til hypotesen om at avrenning fra vei kan ha forårsaket tilbakegangen av *E. canadensis* i Årungen. Sedimentprøvene ble også analysert for vanninnhold, tørrstoff og organisk materiale. En numerisk analyse ble utført på resultatene fra den spektroskopiske undersøkelsen, for å skille ut individuelle pigmenter, med en metode utviklet av Prof. Hendrik Küpper (Küpper et al., 2007)

Det ble foretatt feltundersøkelser i Årungen og bekkene med tilførsel til området hvor sediment-prøvene ble tatt. Det ble tatt prøver i bekker og overvannsrør ved både lav og høy vannføring, også for steder med tilførsel fra vei og avrenning fra bebygde områder, overvann og kloakk overløp. Prøver fra bekkene, sedimentene og vasspestplanter ble analysert for kadmium, kobber og sink. Historiske kilder ble undersøkt for å finne endringer i landskapet som kunne ha påvirket forholdene for *E. canadensis.*

De historiske kildene viser at E*. canadensis* sannsynligvis ble introdusert ved byggingen av rostadion i Årungen. Byggingen av bryggene skjedde samtidig med byggingen av den nye motorveien, noe som reduserte trafikken på den eksisterende veien nær Årungen, som på kort sikt reduserte tilførselen av metaller. Dreneringssystemet for den nye motorveien, og økningen av trafikken førte over tid til en økning av tilførselen av metaller til Årungen.

Den kontinuerlige tilførselen av metaller og kanskje andre forurensninger fra vei og bebygde områder første sannsynligvis til den første tilbakegangen for E. *canadensis*. Flere forurensningskilder tilført i høye doser ved ekstrem nedbør og regn-på-snø episoder i 2006 og 2007 førte antakeligvis til den endelige utryddelsen av vasspesten. Hvis dette viser seg å være tilfellet, har dette større økologiske implikasjoner for både Årungen og andre innsjøer som utsettes for kombinasjoner av spisslaster fra veier og bebygde områder.

Contents

Introduction

Elodea canadensis: problem invasive species

The physical, chemical and biological integrity of freshwater systems are increasingly being degraded from a variety of threats including climate change, habitat degradation and flow modification, changes in land-use, pollution, and the introduction of aquatic invasive species (Carpenter et al., 2011; Dudgeon et al., 2006) Invasive aquatic plants can negatively impact ecosystems and economies (Oreska and Aldridge, 2011). An understanding of the ecology of invasions can therefore contribute to the control of established populations and prevent the establishment new invasive species (Mack et al., 2000; Parker et al., 1999; Vitousek, 1990).

One of the more notorious invasive submergent macrophytes, *Elodea canadensis,* (*E. canadensis*), can within one to two growing seasons cover large areas with dense stands of biomass, completely choking the entire water column. The rapid and dense growth of this species gives it the ability to outcompete other submergent macrophytes, causing a cascade of ecosystem impacts including changes in shading, circulation, sedimentation, nutrient cycling, primary production, trophic status, biomass and pH (Bowmer et al., 1995; Simpson, 1984) as well as negatively impacting fisheries and out-competing native and or endangered species (Bazarova and Pronin, 2010; Mjelde et al., 2012; Kelly and Hawes, 2005). Impacts of *E. canadensis* on human activities include clogged waterways that can reduce or eliminate boat traffic and fisheries, clog the water intakes and outflow from power plants (Bazarova and Pronin, 2010; Oreska and Aldridge, 2011). A large scale analysis of the economic costs of invasive species management in Great Britain by Oreska and Aldridge (2011) estimated the annual cost of managing *E. canadensis* at £ 11,640,579 (approximately 1.2 billion Norwegian kroner (NOK)). In Norway, the costs of removing *E. canadensis* from the Skas-Heigre Canal were estimated at 3.4 million NOK (Elnan, 2008). Negative impacts from invasive aquatic plants in Europe has been estimated to cost 2.2 billion Euros annually (approximately 18.15 billion NOK) (Keller et al., 2011).

The release of ornamental aquatic plants such as *E. canadensis* into water bodies via hobby aquarists, commercial aquarium outlets, garden ponds, and botanical gardens has been a primary mode of introduction for aquatic invasive plants around the world via unintentional and intentional, or deliberate, pathways since at least the 19th century (Bazarova and Pronin, 2010; Bowmer et al., 1995; Brundu, 2014; Hulme, 2009; Simpson, 1984). Examples of unintentional pathways include fragments of plants tangled in boating, fishing or other types of equipment that 'stowaway' or 'hitchhike' to new locations; unaided dispersal and spread within watersheds along natural waterways or between watersheds via canals, ditches and other types of diversions. Intentional pathways are often created through negligence or ignorance such as dumping aquarium contents into water bodies, mislabeled or contaminated containers of live fish or aquatic plants, and the release of species into garden or botanical ponds that then escape into nearby water bodies. (Brundu, 2014; Hulme, 2009). Once released, fragments can colonize new habitats within and between watersheds via unintentional pathways such as hitchhiking and unaided dispersion (Barrat-Segretain and Bornette, 2000; Brandrud and Mjelde, 1999; Riis et al., 2009). Illegal trade is a major intentional pathway for the introduction of invasive aquatic plants and animals (Champion et al., 2014). A poorly regulated internet trade continues to provide a supply of aquatic species that have been outright banned (Kay and Hoyle, 2001; Padilla and Williams, 2004).

<u>Taxonomy and Ecology</u>

E. canadensis is a member of the *Hydrocharitaceae* family which includes approximately 80 freshwater and marine aquatic monocots from around the world. A significant percentage of species from this family have been labeled invasive including sixteen of the 96 aquatic plant species currently known to be established in Europe. (Hussner, 2012) Of these, five are considered among the most invasive and damaging freshwater aquatic invasive plants globally including *E. canadensis* and its relatives *E. nuttallii, L. major, E. densa,* and *H. verticillata.* They are morphologically similar and easy to misidentify (Ghahramanzadeh et al., 2013). The latter three species *(L. major, E. densa, and H. verticillata)* are all present in central Europe but have thus far not been detected in Norway. The distribution of *E. nuttallii* in Norway has so far been limited to a few locations in on the southwest coast (reference?). Reproduction in invaded habitats for all five species is non-sexual reproduction, a characteristic that greatly increases their ability to spread (Barrat-Segretain and Bornette, 2000; Riis et al., 2010; Santamaría, 2002).

E. canadensis is native to the temperate central region of North America. Its native range stretches from the west to the east coast (Bowmer et al., 1995) and from approximately 32 to 55 degrees north latitude (Nichols and Shaw, 1986). It has been categorized as an aggressive nuisance weed in most of the industrialized world and has been introduced to all continents except Antarctica (Hussner, 2012). *E. canadensis* was first observed in Europe in Ireland in 1836 (Bazarova and Pronin, 2010; Simpson, 1984) and was apparently intentionally introduced by hobby aquarists (Simpson, 1984). It subsequently spread throughout the British Isles and into continental Europe. It was introduced into the Berlin Botanical Gardens in Germany in 1852 and spread eastward to Poland in 1877, Finland in 1884, and into Russia and Siberia. (Bazarova and Pronin, 2010).

The combined native and invasive range of *E. canadensis* covers an extensive area that encompasses enormous variations in topography, elevation, and climate, indicating that it is able to survive in a broad range of temperatures (Riis et al., 2012) and under ice cover as well as in ice for short periods (Bowmer et al., 1995). Both the native and non-native habitat of *E. canadensis* can be characterized as sluggish or slow moving water bodies such as lakes, ponds, and low-gradient streams, canals and ditches (Bowmer et al., 1995) with a preference for water bodies that are cation rich (Spicer and Catling, 1988). In its native range, *E. canadensis* is more often found in mesotrophic to eutrophic waters (Pagano and Titus, 2004) with a pH that ranges from 6.5 to 10, although it has also been observed in oligotrophic waters in both its native and non-native range (Spicer and Catling, 1988). Invaded water bodies tend to be eutrophic (Bowmer et al., 1995; Simpson, 1984; Spicer and Catling, 1988). *E. canadensis* has been categorized as a generalist species due to its ability to survive in a wide variety of conditions (Riis et al., 2012).

It is a long-lived perennial that thrives in depths of 4 to 8 meters. In some locations it has been observed at depths of 12 meters (Nichols and Shaw, 1986) and in Lake Baikal up to 37 meters (Kravtsova et al., 2010). It does not seem to flourish in depths less than 0.5 meters (Nichols and Shaw, 1986). Stems can be long and reach up to 3 meters in low light conditions, while in shallower depths the stems are more likely to branch out from nodes (Riis et al., 2009). They are brittle and susceptible to breaking off if exposed to waves or strong currents (Barrat-Segretain et al., 2002), which contributes to passive dispersal. The root system of *E. canadensis* is fragile, poorly developed and shallow (Maberly and Madsen, 2002). These two characteristics, stem breakage and poor rooting ability, may account for its preferences for silty substrate and slow moving water as it can more easily anchor in fine sediments where there are minimal currents (Bowmer et al., 1995), allowing the plant to accumulate significantly more biomass in silty rather than in gravelly and sandy substrates (Madsen and Adams, 1989).

Aquatic photosynthesis

The availability of carbon appears to be one of the primary factors limiting growth and photosynthesis for submergent macrophytes (Maberly and Madsen, 2002; Madsen and Sand-Jensen, 1994; Madsen et al., 1996). The majority of terrestrial plants have a more or less continuous supply of carbon dioxide available for photosynthesis from the air where there is little resistance in the boundary layer surrounding plant tissues (Lambers et al., 2008). The high viscosity of water compared to air decreases the rate of diffusion of dissolved gases, which occurs at a rate that is approximately 10^4 times slower in water than in air (Lambers et al., 2008), and increased rates of resistance in the aqueous boundary layer surrounding the plant epidermis (Madsen and Sand-Jensen, 1994), thereby limiting the amount of carbon dioxide (CO_2) available for photosynthesis (Lambers et al., 2008). High rates of primary production and CO_2 uptake in aquatic ecosystems can increase CO_2 limitation. Carbon dioxide limitation caused by high rates of photosynthetic metabolism fluctuates diurnally and seasonally with light availability. To overcome CO_2 limitation in the aquatic environment, submergent macrophytes have evolved mechanisms that allow them to concentrate carbon, hereafter referred to as carbon concentrating mechanisms (CCM) (Lambers et al., 2008). *E. canadensis* can use bicarbonate (HCO_3^-) in the form of dissolved inorganic carbon (DIC) for photosynthesis (Madsen and Sand-Jensen, 1987). Bicarbonate availability increases with increasing pH and alkalinity. The ability to utilize HCO_3^- is therefore common among freshwater and marine plants with preferences for high pH and alkalinity. Many HCO_3^--users can also utilize CO_2, and are therefore able to adapt to a wider range of aquatic conditions and outcompete plants that exclusively use CO_2 as a carbon source (Maberly and Madsen, 2002). It is therefore not surprising that many of these species are members of the *Hydrocharitaceae* family and are classified as invasive species (Hussner, 2012).

Metals accumulation

E. canadensis is known to be an efficient accumulator of heavy metals (Basile et al., 2012). While some heavy metals are essential nutrients at lower concentrations, iron, manganese, copper and zinc, (hereafter Fe, Mn, Cu, Zn), concentrations of both essential and non-essential metals such as cadmium, lead, mercury, chromium, aluminum, and silver, (hereafter Cd, Pb, Hg, Cr, Al, Ag) can be toxic to plants; toxicity can vary greatly depending on environmental factors and the metals present (Malec et al., 2011). Metals accumulate preferentially in roots, shoots or leaves, or they are mobile in plants tissues (Kähkönen et al., 1997; Fritioff and Greger, 2007; Nyquist and Greger, 2007). The main variables affecting the uptake of metals include pH, microbial activity, organic matter present in sediments, available nutrients, redox potential, water hardness and alkalinity, and light (Guilizzoni, 1991). At low pH and alkalinity, heavy metal cations are more soluble and bioavailable, and thus more toxic for both aquatic plants and animals. There seems to be a relationship between pH, alkalinity, carbon utilization, and metal uptake that has yet to be more fully researched.

The toxicity of metals varies with the concentration and duration of exposure and with the protective responses elicited by individual species (Bertrand and Poirier, 2005). Küpper et al. (1996) established the order of heavy metals toxicity in *E. canadensis* as follows: $Hg^{2+} > Cu^{2+} > Cd^{2+} > Zn^{2+} > Ni^{2+} > Pb^{2+}$. In this study, I will focus primarily on Cu, Cd and Zn, and to a lesser degree on Pb, Cr and Mn.

Inexplicable decline and die-out

After becoming well established in a new territory and altering the local ecology, some populations of invasive species suddenly and inexplicably decline and die out, often after scarring or permanently altering the local ecosystem. *E. canadensis* is an example of this phenomenon, exhibiting a pattern of rapid colonization, stability, decline, and die-out, often followed by a resurgence that is not as dense or widespread as the initial colonization. (Simberloff and Gibbons, 2004; Strayer, 2012). Simpson's documentation (1984) of the rise, fall, complete die-out and resurgence of *E. canadensis* populations in various locations in the United Kingdom is probably the most often cited example of this phenomenon. This pattern has also been reported in Czeckoslovakia (Pyšek et al., 2002), Germany (Hilt et al., 2006), Lake Mälaren in Sweden (Josefsson and Andersson, 2001), and in Siberia in Kotokel'skoe Lake and Lake

Baikal (Bazarova and Pronin, 2010). Simpson's (1984) description of the pattern of introduction, establishment, decline and disappearance seems to be accurate compared to the previously mentioned accounts, where after becoming established in two to four growing season and maintaining “pest proportions” for three to 10 years, *E. canadensis* goes into decline and is present for another seven to 10 years, reduced to small relic populations or completely dying out.

The two hypotheses most often cited to account for the decline and die-out of *E canadensis* seem to have originated from a paper by Bowmer et al. (1995) include: 1) micronutrient limitation (Sculthorpe, 1967), possibly the lack of Fe in a reduced form in sediments (Olsen, 1954)*;* 2) the assumption that *E. canadensis* is a relatively light-demanding plant (Bowmer et al., 1995). There are some discrepancies between the first hypotheses and research conducted since the Sculthorpe's and Olsen's papers were published. Some lakes where E. canadensis has established itself and later disappeared from have high levels of reduced Fe in the sediments and are eutrophic and thus rich in sediments. The basis of the second hypothesis has since been proved to be inaccurate in that it is well documented that *E. canadensis* can thrive and indeed is more likely to be found in greater depths in low light conditions, and can thrive as well in low light conditions caused by eutrophication (Vestergaard and Sand-Jensen, 2000). That *E. canadensis* can thrive and out-compete other species for many years before declining and dying out implies that ecological conditions must deteriorate, and that there must be a factor or factors causing the deterioration.

E. canadensis in Norway

The introduction and spread of E. canadensis in Norway seems to have followed the same pattern of invasion as in other European countries. It was first observed in Norway in 1925 in Østensjøvatn, a lake located in Oslo municipality, the most populated region of Norway where it is believed to have been introduced by aquarists (Myrmæl, 2012). According to the Norwegian species database that catalogs and maps changes in the distribution of native and non-native species, (Norwegian Biodiversity Information Centre) after the initial introduction of *E. canadensis* in 1925, an average of nine new observations per year were reported until 1969, when observations increased to an average of 42 new sites per year. In 2009 and 2010, 100 and 82 new sites respectively per year had been reported. (See Figure 1.) Despite being placed on the Norwegian Black list of invasive species (Gederaas et al., 2007, 2012) and a subsequent 2009 ban on import, sale and release of *E. canadensis, (Klima og Miljødepartement, 2009)* new observations of *E. canadensis* continue to be reported in Norway.

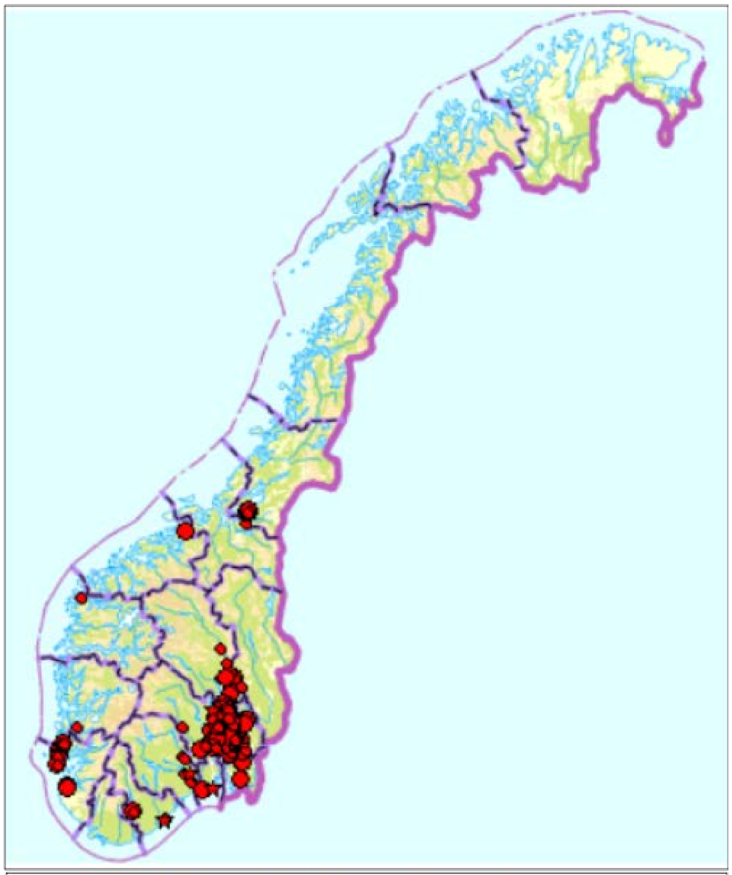


Figure 1: Distribution of *Elodea canadensis* in Norway.
Source: artskart.artsdatabanken.no Date accessed: 27.June, 2014

Initial observations of *E. canadensis* within water bodies have often been near boat docks, fishing areas, and recently disturbed areas such as dredged channels indicating that hitchhiking is an important pathway of *E. canadensis* from infected to uninfected sites in Norway (Elnan, 2008; Brandrud and Mjelde, 1999; Mjelde et al., 2012). How much of the recent increase in observations has been due to actual spread or to better monitoring is unclear, although regular monitoring since the 1990's implies *E. canadensis* is spreading passively downstream in some watersheds (Myrmæl, 2012).

E. canadensis is most prevalent in water bodies located in southern and eastern Norway in coastal areas and sections of rivers such as the Glomma, Gudbrandsdalslågen and Numedalslågen that lie below the marine boundary (below approximately 200 meters above sea level) and thus are rich in deposits of marine clays and sediments. These deposits, which make up a small percentage of Norway's total area and represent some of Norway's most productive agricultural soils, are alkaline and high in cations. The water quality of lakes, ponds and dams overlying these deposits tend to be cation-rich, and have naturally high rates of sedimentation. (Rørslett and Skulberg, 1968). Glacial and alluvial sediments supplied by rivers running from northern mountainous regions south to Oslofjord and the Skagerrak and agricultural runoff from adjacent fields contribute additional nutrient and sediment loads to these water bodies.

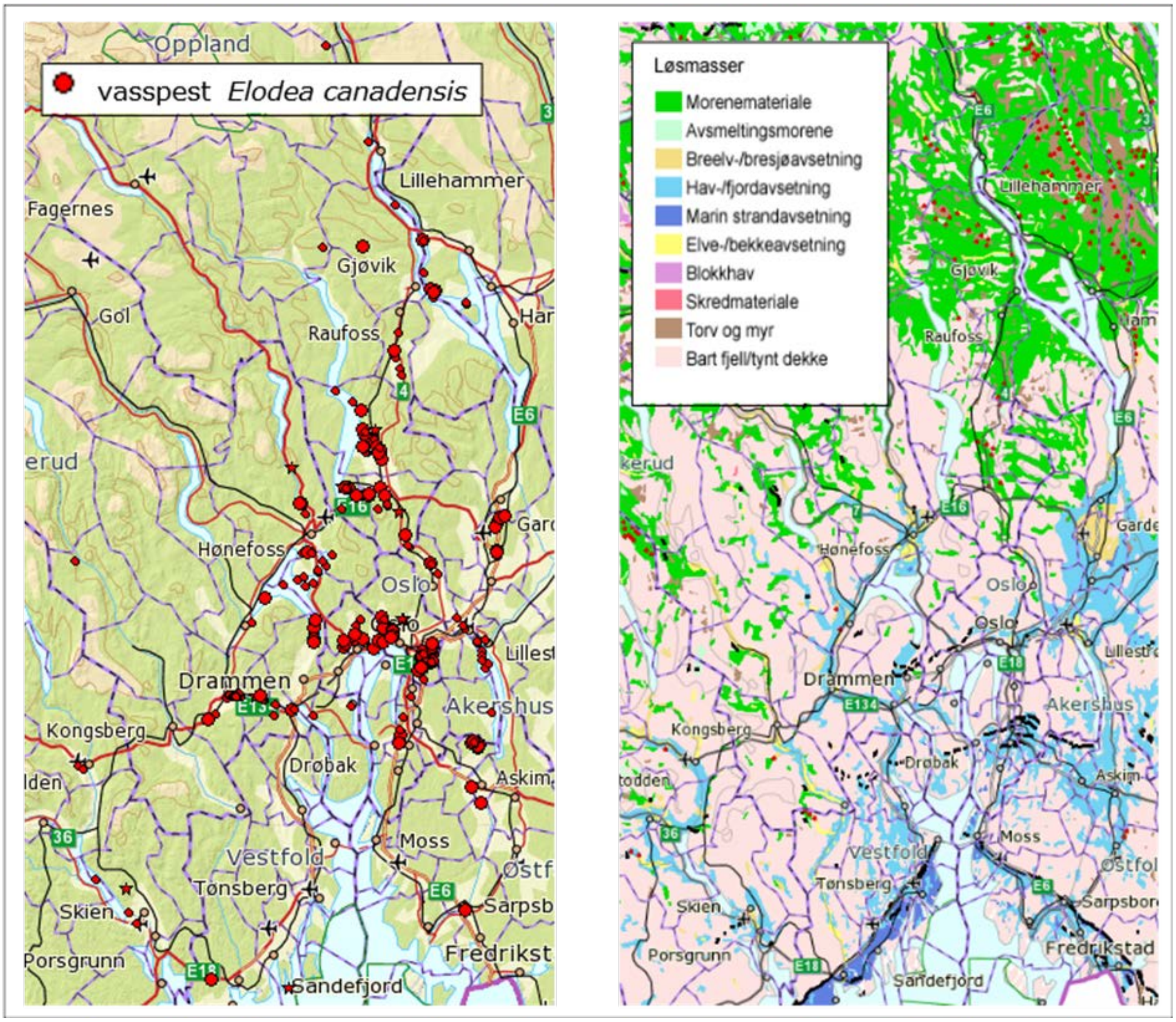


Figure 2: Maps showing:1) on the left, the distribution of E. canadensis along river valleys and major roads in the extended Oslo-Akershsus region, and 2) on the right, depositions of marine sediments.

Human settlements and transportation routes developed along Norwegian river valleys and lakes due to the rich agricultural soils and flat terrain. Today, the main highways heading out of the Oslofjord region run along these river valleys which are populated by small to medium sized towns. Thus the river valleys and low lying coastal areas in these regions provide two important factors promoting the spread of E. canadensis in Norway: 1) efficient intentional and unintentional pathways of introduction and spread, i.e. human presence and transportation directly adjacent to water bodies; 2) water bodies providing high quality habitat and unaided dispersion routes. *E. canadensis* is now well established throughout the extended Oslofjord region including Oslo, Akershus, Vestfold and Buskerud.

Patterns of *E. canadensis* decline and die-out have also been reported in Norway. In a 2012 report on *E. canadensis* in Oslo and Akershus counties, it was present in only nine out of 26 sites where it had previously been observed. Surveys were conducted by walking along the water's edge rather than by boat, using an underwater viewer (vannkikkert) and a sampling rake (kasterive). Given the preference of *E. canadensis* for depths greater than 0.5 meters, the lack of monitoring in deeper water raises the question of whether it was present at more sites than reported and standardized surveying protocols are necessary. The report also added six new sites where *E. canadensis* had not been previously observed. *E. canadensis* was first observed in Lake Årungen (hereafter Årungen), the location used for this study, in 1992, and was last observed in Årungen in 2008. (Myrmæl, 2012).

<u>Choice of study site</u>

Norway became signatory to the European Union Water Framework Directive (EUWFD) in 2008. The primary goals of the EUWFD are to attain good ecological, chemical and hydrological status for all groundwater and surface water bodies within a set deadline, and mandates regular assessments of water bodies to determine the status and subsequent restoration measures of water bodies. In Norway, the responsibility for implementing the EUWFD falls to counties and designated regional water authorities who oversee implementation within municipalities. Local municipalities determine which local water issues should be prioritized.

The largest watershed in Norway, the Glomma, was chosen as the first watershed in which to begin implementing the EUWFD. The focus of this study, Årungen, is located in the Bunnefjorden watershed, a sub-basin of the Glomma watershed. The Bunnefjorden watershed has been designated as an administrative watershed management district which encompasses portions of the municipalities of Frogn, Nesodden, Oppegård, Oslo, Ski and Ås. In order to manage the implementation of the EUWFD, these municipalities formed a watershed organization which has taken the name PURA. The overall status of Årungen has been assessed and categorized according to EUWFD criteria as 'moderate'. Eutrophication began to occur in the1960's, in large part due to a variety of anthropogenic inputs such as nutrients and suspended sediments in agricultural runoff and untreated waste from rural residences (Rørslett and Skulberg, 1968; Skogheim and Erlandsen, 1984).

Since the onset of eutrophication, there have been ongoing efforts to restore Årungen that have focused on reducing the inputs of nutrients, especially nitrogen and phosphorous, by providing incentives to farmers to increase the use of sustainable agricultural and best management practices, and requiring rural homes and businesses install decentralized wastewater treatment facilities (Borge et al., 2012). In a report published in 2012 by the Norwegian Road Authority (Bækken and Åstebøl, 2012) high levels of Cu were reported in a number of lakes adjacent to European highway six (E6), including Årungen. The input of road and urban runoff into Årungen constitutes a heretofore unconsidered impact on the lake, although high levels of some metals in sediments have been measured in several previous studies (Skogheim, 1979; Zambon, 2010). Despite ongoing efforts begun in the 1960's, the overall environmental health of Årungen has only slightly improved in recent years (Borge et al., 2012). The clarity of the lake increased in 2012 and 2013 (Romarheim, 2012), which might be interpreted as a sign that the ecological health of the lake is improving although the clarity as measured by Secchi-depth has fluctuated for several decades (Borge et al., 2012).

The historical use of the lake for recreational and sports activities continues today, ensuring that the restoration of Årungen remains a priority for local communities. The responsibility for coordinating restoration efforts has been delegated to PURA, which expressed interest in investigating the invasion and disappearance of *E. canadensis*. This study is a result of PURA's interest. With naturally high alkalinity, hardness, and pH, Årungen should have provided high quality habitat for *E. canadensis.* Årungen is therefore an ideal location for the study of the invasion and disappearance of *E. canadensis*, providing an opportunity to test other variables that may account for its decline and disappearance as well as explain changes that have occurred in the lake ecosystem.

Paleolimnology

While most contemporary studies of invasive species involve direct sampling of both the species of interest and concurrent ecological variables, in this study an aquatic freshwater species is being investigated that is no longer present. Paleolimnological methods, the study of lake sediments, lend themselves to such a study, particularly in a lake such as Årungen which has a relatively high rate of sedimentation due to its geomorphological history.

Lake sediment samples can be dated by analyzing the decay of radioactive isotopes such as Cesium-137 (^{137}Cs) (Avery, 1996). Sediment cores can be analyzed for a variety of biotic and abiotic parameters such as and pigments, pollen, organic matter, sediment size, pH, ions, and pollutants (Vinebrooke and Leavitt, 1999). These techniques are not without error and uncertainty. Photo- and chemical oxidation can alter the chemical characteristics of sampled sediments and lead to errors and uncertainties in the analysis and interpretation of results. Error and uncertainty tend to increase with the chronological age and depth of the sediments. Fortunately, degradation occurs primarily in the water column as pigments settle, and are more likely to be preserved in anoxic sediments. (Leavitt, 1993). The use of pigments as biomarkers to elucidate recent ecological changes in the ecology of a water body is a common paleolimnological method (Reuss, 2005) which I have utilized in order to study changes in the *E. canadensis* population in the southern end of Årungen.

Objectives and hypotheses

The two primary objectives of this study are the investigation of: 1) the introduction of *E. canadensis* into Årungen and likely vectors, and 2) the decline and disappearance of *E. canadensis.* In addition to searching scientific literature on *E. canadensis* using several search engines including ISI Web of Science, Google Scholar, NORART, BIBSYS and BRAGE, a history of Årungen was conducted using documents such as permits, reports, and theses from a variety of local, regional, national and academic sources such as Ås and Frogn municipalities, the Norwegian State Road Authority, (Statens Vegvesen and Vegdirektorat), the Norwegian Environment Agency, the Norwegian Institute for Water Research (NIVA), and Bioforsk. Based on literature searches and results from the analysis of sediment pigments and other variables, I developed the following hypotheses:

1) *E. canadensis* was introduced into Årungen during construction on either the rowing facilities in the lake, or on construction on E6, both of which occurred in the early 1990's.

2) *E. canadensis* began to decline as a result of the combined exposure from metals flowing into the lake, and that accumulated in lake sediments. *E canadensis* was probably extirpated due to the synergistic effects of metals, deicing agents and high intensity runoff events carrying relatively large concentrations of metals and deicing agents. The primary source for metals and deicing agents flowing into Årungen appears to be road runoff from E6. Urban storm runoff as well as historical, unidentified sources of metals may also contribute to the total load of metals flowing into Årungen.

Description of Årungen watershed and the sediment core sampling site

The following descriptions of the watershed and the sediment core sampling site are based on examinations of historic maps aerial photos, online mapping tools and satellite photos, and traditional references, which are cited below, as well as field observations made during field surveys (see the Methods section).

Overview

The Årungen watershed is located in the southeastern region of Østlandet approximately 30 km south of Oslo and 2 km away from Oslofjord. It is relatively small (50.4 km^2) with low-gradient streams (1 %), and a low drainage density (0.21). The outlet of Årungen, Årungselva, flows into Bunnefjord, a small sub-fjord on the east side of Oslofjord. Bunnefjord has a high sill at the outlet to Oslofjord that causes poor circulation and water quality. The elevation in the watershed ranges from 34 m at the water surface of Årungen to a maximum of 162 m above sea level. The predominant land surface types are agricultural fields (49 %) and managed forests (35 %). Other predominant surfaces include urban (5 %), surface water (3.1 %) and wetlands (0.1 %) (Norwegian Water Resources and Energy Directorate, 2014b).

Geomorphology

The watershed is roughly rectangular in shape with the longest axis running east-west. The drainage pattern can also be described as rectangular or trellis, with tributaries running almost perpendicular to each other approximately in east-west and north-south directions. Rectangular and trellis channel networks are indicative of shallow, weathering-resistant lithology such as schist and gneiss that are interspersed with bedrock joints or fractures composed of softer material which erodes and form stream channels, which is consistent with the exposed Pre-Cambrian geology of the eastern side of Oslo fjord (Bargel, 2005).

Årungen itself exemplifies this rectangular pattern, having a long, narrow rectangular shape with the long axis running north-south and roughly bisecting the surrounding watershed. Årungen and Bunnefjord were both formed by a graben fault that runs from Oslofjord south into the Follo region (Bargel, 2005; Abrahamsen et al., 1995). As the last ice age ended approximately 10,000 years ago, glacial debris were deposited along the receding ice edge forming end moraines running east-west. Two prominent moraines form the topographical northern and southern borders of the Årungen watershed. The Ski moraine forms the northern border, running east-west at Vassum. The formation of the Ski moraine dammed the underlying graben fault, resulting in the development of lake Årungen. The hydrological southern boundary is formed by the moraine at Korsegården, upon which road 152 runs, i.e. Drøbakveien (Bargel, 2005). The intersection of road 152 and highway E6 at Korsegården was excavated, lowering the elevation to accommodate the highway under an overpassfor road 152. This excavation extended the southern hydrological boundary approximately 2 km south in a narrow strip of land approximately 300 m wide that encompasses E6 (Follo Kommunene).

As mentioned previously, this is a productive agricultural region due to the deposition of marine sediments during the last ice age, when sea level was approximately 200 m higher than today due to glacial depression of the land masses. In an undisturbed state, these clay-dominated soils are unsuitable for agriculture as they are poorly drained and often saturated. This has necessitated the installation of agricultural drainage systems including surface ditches and subsurface piping. Agricultural drainage systems can significantly alter the geomorphology and hydrology of a watershed (Blann et al., 2009). In southern Norway, the spacing of subsurface drainage pipes tends to be closer and the depth shallower than in some other European countries and as a result may contribute the majority of runoff and sediments to recipient water bodies (Deelstra et al., 2010).

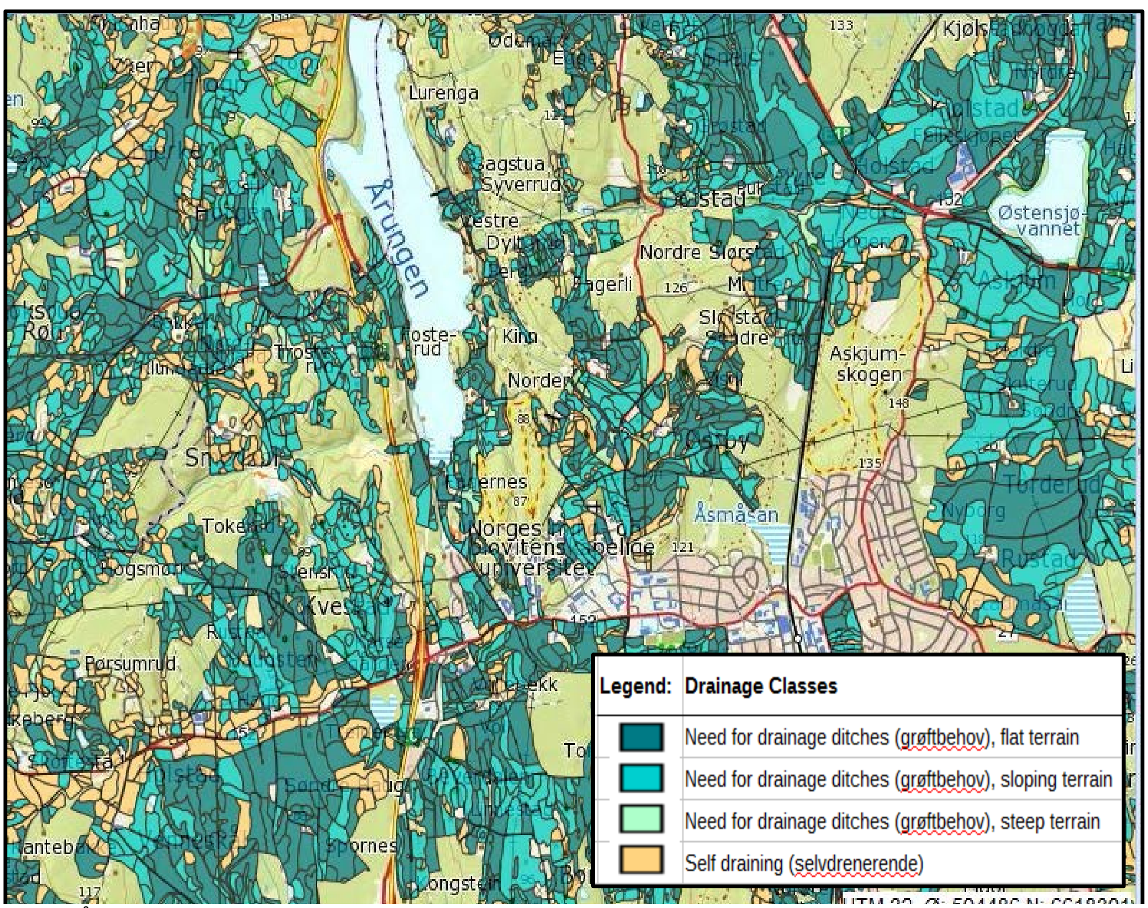


Figure 3: Map of Årungen watershed showing poorly drained agricultural soils according to the need for drainage systems. Slope is combined with soil characteristics to categorize the potential for soil saturation and surface ponding and thus need for installation of agricultural drainage systems. Source: Skog og Landskap, online tool for mapping land use: http://kilden.skogoglandskap.no/map/kilden/index.jsp? theme=JORDSMONN&mapLayer=DRENERINGSFORHOLD. Accessed July 15,

Climate and hydrology

Despite a latitude of approximately 60 degrees north, the climate in the Oslo-Akershus region is temperate due to the influence of the North Atlantic current. The 30-year return period from 1961-1990 is the current reference period established by the World Meteorological Organization for calculating hydrological and meteorological variables. Based on this return period and data from the meteorological station located at NMBU in Ås, Norway, which is located within the Årungen watershed approximately 2.4 km from the lake, the average annual temperature is 5.3 ° C, with the average high and low temperatures occurring in July (16.1 ° C) and in January and February (- 4.8 ° C). The average total annual precipitation is 785 mm, with October receiving the highest average monthly precipitation (100 mm) and February receiving the lowest average monthly precipitation (35 mm). (Hansen and Grimenes, 2014)

Analysis of regional data for the southeastern region of Østlandet comparing the reference return period 1961-1990 and 1979-2008 show significant changes. The average annual temperature has increased 0.63 ° C while average winter temperature has increased 1.34 ° C. Winter runoff has increased 51 % in low-lying areas, the largest regional increase for the winter season across Norway, while snow season and accumulation, which is strongly correlated with elevation and distance from the coast, has decreased. This increase is attributed to an increased number of periods of warmer weather occurring during the winter which results in high runoff rain-on-snow events. The frequency and intensity of short-term (< 1 hour) rainfall events is increasing in Østlandet. (Hanssen-Bauer et al., 2009) These regional trends are reflected in analysis of FAGKLIM data for the return period 1990-2013 showing that the average annual temperature in the Årungen watershed was higher in 22 out of 24 years, average annual precipitation was greater in 18 out of 24 years. In December 2013, the average temperature was 2.3 °C, the third highest average temperature for December ever recorded, and the total precipitation was 167 mm, which was 215 % over normal and the greatest amount of precipitation ever recorded at FAGKLIM since its establishment in 1859. (Hansen and Grimenes, 2014)

Hydrology

Six perennial streams enter Årungen, including Brønnerudbekken, Bølstadbekken, Norderåsbekken, Smedbøllbekken, Vollebekken, and Storgrava. Of these, Smedbøllbekken, Brønnerudbekken and Vollebekken flow into the southwest corner of the lake. From its headwaters in the vicinity of Drøbak City shopping center, Storgrava flows into the northwest side of the lake. Norderåsbekken, which has headwaters in the Åsmåsen area near Ås city center, flows into the east side of the lake. There is one other lake in the watershed, Østensjøvann, located on the eastern side of the watershed. Østensjøvann receives discharge from Ski city center near the Ski train station and from the Skuterud-

Rustad residential area in Ås municipality. Østensjøvann flows into Årungen via Bølstadbekken. Outflow from Østensjøvann passes the local landfill before flowing towards Årungen. At 25.5 km^2, Bølstadbekken forms the largest sub-basin in the Årungen watershed.

According to the Norwegian Water Resources and Energy Directorate's (Norges vassdrag- og energidirektorate, NVE) online hydrology tool (Norwegian Water Resources and Energy Directorate, 2014b), modeled stream discharge for all streams in the Årungen watershed ranges from 1.0 $l/s/km^2$ for minimum low discharge to 15.7 $l/s/km^2$ for average low flow discharge. The lavvannskart results do not include estimates for peak flows, i.e. flows resulting from high intensity rainfall-runoff events. Peak flow data for the Årungen watershed are sparse.

A useful example of a peak flow event in Årungen was given in Gunnarsson, (2007). Discharge was measured at various stages on all streams in the watershed in the spring of 2006. Peak flows were measured on the 14th of April. Peak flows on this date would have occurred as a classic example of a rain-on-snow event. Snowfall for the winter of 2005-2006 was above normal; temperatures were below normal through March. Snow depth began to rapidly decrease from 52 cm at the end of March as a result of increasing maximum daily air and soil temperatures and rain-on-snow precipitation beginning on the 27th of March (Gunnarsson, 2007; Hansen and Grimenes, 2007). The snow had disappeared completely by the 14th of April. Between the 28th of March and the 14th of April, 55.3 mm of rain fell (Gunnarsson, 2007), of which 8.4 and 10 mm of precipitation fell on the 12th and 13th of April (Hansen and Grimenes, 2007). Stream discharge measured on the 14th of April ranged from 311 l/s in Brønnerudbekken, to 7,391 l/s in Bølstadbekken, compared to 7.9 l/s and 315 l/s measured in May of 2006 (Gunnarsson, 2007).

Description of lake Årungen

Skogheim and Abrahamsen (1979) mapped the morphology and bathymetry of the lake, which according to their measurements is 0.63 km at its widest point by 3.03 km long, with a total surface area of 1.18 km^2, a maximum depths of 13.1 m, and a volume of 9.1×10^6 m^3. The residence time of the lake has been estimated at approximately 4 to 4.5 months (Hexum, 1963). The shorelines running north-south are generally steep as is the bathymetry, resulting in a narrow littoral zone a few meters wide along the majority of the north-south shores, with the exception of the bay (Årungsbukta) into which Storgrava flows. The northern and southern ends of the lake have lower gradient shorelines and bathymetry and the most developed littoral zones in the lake. The only outlet of the lake, Årungselva, is located on the northwestern corner of the lake. The littoral zone in the southern end is located between Smedbøllbekken and Morteberget, and extends out from the wetland area surrounding the SCB. More developed littoral zones are also found at the inlets of Norderås, Bølstadbekken and Storgrava streams. The cold winters, warm summers, northern exposure to winds blowing south from

Oslofjord along the long axis of the lake combined with the shallow bathymetry of the lake result in a dimictic pattern of spring turnover of the water column, followed by thermal stratification in late summer, and a second period of turnover in the fall as temperatures cool (Hexum, 1963).

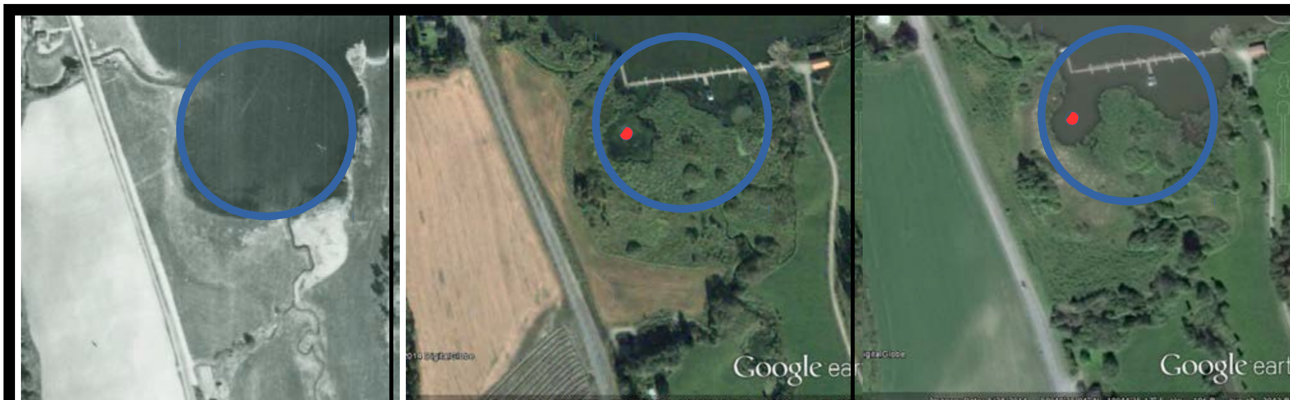
Sediment core sampling site

The site where the sediment core was sampled is located approximately in the center of a small bay (sediment core bay, hereafter SCB), in the southwestern corner of Årungen. The bay is surrounded by a wetland on the west, south and east sides, and flanked on the north by a pier used for sculling practices and competitions. The pier was first installed in the 1970's. A new pier was constructed beginning in 1989 and completed in 1992 (Strengelsrud and Heien-Bjonge, 2014), overlapping with the period in which E6 was being expanded and the new Korsegården overpass was completed. It is anchored to Morteberget, a rocky outcrop on the east side of the southern end of Årungen. The pier cuts across the south end of the lake from east to west, leaving a narrow open channel on the west side of the lake near the outlet at Smedbølbeken.

The watershed area contributing to the SCB encompasses approximately 3.3 km^2 of the southern portion of the Årungen watershed including the tributaries Brønnerudbekken and Vollebekken. The predominant surface types are agricultural fields (40 %), forest (23.5 %), urban (6.6 %) and wetland (0.4 %) (Norwegian Water Resources and Energy Directorate, 2014a). Topographically, the headwaters of Brønnerudbekken begin in the vicinity of the intersection of Korsegården, E6, and road 152. The headwaters of Vollebekken begin approximately 600 hundred meters east of this intersection near road 152. (Norwegian Water Resources and Energy Directorate, 2014a). The topographical headwaters of both streams are located approximately 1.5 km south of Årungen, (Norwegian Water Resources and Energy Directorate, 2014a) although both streams have been channeled into underground culverts in the upper reaches of the watershed.

A comparison of aerial and satellite photos from 1956 (Widerøe, 1956) and 2013-2014 (Follo Kommunene; Google, 2013) show that the entire southern end of Årungen was an open bay in 1956, and the wetland surrounding the SCB has developed since that time. It appears that the SCB formed as a result of the patterns of sediment deposition and subsequent development of the wetland, which started as an alluvial fan at the Brønnerud-Vollebekken stream outlet and has been filling in with sediments. The construction of the rowing pier very likely changed circulation patterns, slowed incoming water velocity, and increased the rate of sediment deposition in the southern end of Årungen, thereby increasing the rate at which the wetland has increased in size. The percentage of open surface water in the south end of the lake between the Smedbøllbekken inlet and Morteberget has decreased by approximately 60 - 75 % due to the high sedimentation rate. The stream channel has maintained its historical location from 1956 as it entered the bay, now wetland. This is consistent with previous estimates showing a dramatic increase in the sedimentation rate after 1954 (Skogheim and Erlandsen, 1984). In the not-so-distant future, the entire area south of the pier will probably be filled with sediments if current sediment deposition rates continue.

Prior to entering the wetland, the Brønnerud-Vollebekken stream channel meanders through a short valley approximately 200 m long where the stream channel is unconfined with low gradient stream banks and a low gradient stream profile prior to entering the wetland area. Upon entering the wetland area, stream channel sinuosity increases and smaller channels branch off from the main stream channel. The main stream channel is approximately 200 m in length and crosses a distance of approximately 120 m across the wetland, resulting in a sinuosity index of 1.67 (Norwegian Water Resources and Energy Directorate, 2014a). The high sinuosity index value, multiple channels and areas of open surface water, and low-gradient stream banks within the wetland area indicate that there is significant hydrological exchange between the Brønnerud-Vollebekken stream channel and the wetland. Although Smedbølbekken is topographically not part of the SCB watershed, it may contribute flow and sediments during peak flow events when it can overtop its banks and flow across the wetland and into the SCB. When low-flow conditions prevail, Smedbølbekken is confined to an incised stream channel and therefore cannot contribute flow or sediment to the SCB. The transport of dissolved solids in soil-, pore- and groundwater is a possibility.



1956, source: Widerøe, used With permission from Ås municipal library.

May 2002, source: NASA satellite, Google Earth. Accessed 11.October.2014

May 2014, source: NASA satellite, Google Earth. Accessed 11.October.2014.

Figure 4: Increasing wetland area and decreasing open surface water in the southern end of Årungen between 1956-2014 resulting from high rates of sediment transport and deposition. The approximate location where the sediment cores were removed is shown by red circles in the 2002 and 2014 aerial photos, located in the center of the SCB, the small bay surrounding the red circle. The blue circles show the approximate area of open surface water in 1956 compared to the changes between 2002

and 2014. The differences in wetland area between 2002 and 2014 may be explained by differences in rainfall and temperature.

During spring, summer and fall when wetland vegetative biomass is at a maximum and water level at a minimum, the surface roughness of the wetland would increase, thereby decreasing flow velocity through the wetland, increasing the ability of larger sediments to fall out of the water column and accumulate in the wetland area. During fall, winter and spring conditions when vegetative biomass and surface roughness of the wetland are at minimum levels and water level is at maximum levels, high intensity rain and rain-on-snow events combined with saturated soils and or frozen surfaces could produce large amounts of runoff and stream discharge and generate large loads of suspended particles and dissolved solids from both Brønnerud-Vollebekken and Smedbølbekken. The wetland area probably plays an important role in controlling the flow and distribution of water and sediments and therefore pollutants into the SCB. (personal observations)

Anthropogenic Influences

Although humans have been living in the area around Årungen for thousands of years, anthropogenic influence on the lake and surrounding watershed seems to have been relatively insignificant until post World War II. The low-density population and agricultural economy of pre-WWII Norway very likely precluded significant negative impacts from urbanization and industrial development. The combination of technological and industrial development during and after World War II, the reconstruction of Europe and Norway in Post-World War II, the discovery of oil and the development of the oil industry since the 1970's and the effects of globalization have increased the rates of population growth, urban development and consumption in Norway, especially in historically more populated regions such as Oslo and Akershus.

Not least of these are the expansion of agricultural and the so-called green revolution, which was supported by the Marshall Plan after World War II. The economy including the agricultural sector was severely impacted by WWII. One result of the Marshall plan in Norway was the supply of seed, fertilizer and agricultural machinery. (Hertzberg Erichsen and Halvorsen, 1998). There were significant changes in agricultural methods post-WWII, including a decrease in spring plowing and sowing, and an increase in fall plowing, which has been shown to significantly increase erosion and the transport of suspended sediments compared to spring plowing (Lundekvam and Skøien, 1998; Skøien et al., 2012), a shift from using land for raising livestock to instead grain production, (Statistisk Sentralbyrå, 1953, 1954) and a significant increase in the use of commercial fertilizers (nitrogen, phosphorous and potassium), the use of which increased from 29,039 metric tons in 1939 to 92,206 metric tons by 1953. (Statistisk Sentralbyrå, 1954). The post-WWII agricultural expansion also increased the construction of agricultural drainage ditches from 37,000 km dug between 1939 and

1949, to 87,000 km dug between 1949 and 1959 (Statistisk Sentralbyrå, 1959). Årungen had been categorized as a mesotrophic lake until the 1950's (Skogheim and Erlandsen, 1984).

The level of eutrophication increased during the late 1950's and 1960's as a result of increasing inputs of untreated sewage as well as agricultural trends that mirrored the rest of the nation, as described above, with decreasing amounts of land used for foraging of livestock, increasing amounts of land used for growing grain requiring regular plowing, more frequent plowing in the fall, and the increasing usage of fertilizers (Borgstrom J.A. Eie, O. Skogheim, 1980). This resulted in the lake becoming highly eutrophic, frequent fish kills (Borgstrom J.A. Eie, O. Skogheim, 1980) and regular algal and toxic cyanobacterial blooms (Borgstrøm et al., 1980; Romarheim et al., 2012). Although the use of phosphorous fertilizer has decreased significantly in recent years, the lake is still eutrophic and experiences regular algal and toxic cyanobacterial blooms. It is unclear how much of an effect agricultural chemicals currently have on water quality in Årungen (Romarheim et al., 2012). The water quality classification in Årungen has been raised from 'bad' in 2008 (PURA, 2011) to 'moderate' in 2013 (PURA, 2013).

There are a number of other known diffuse and point pollutant sources in the Årungen watershed that are capable of delivering a wide range of chemicals to the lake including inorganic ions and ionic compounds such as metals and salts, organic pollutants and additional nutrients and pesticides. Other diffuse sources include road runoff from major highways and roads such as E6 and E18, local roads 152 and 56, and urban runoff from Ski, Drøbak and Ås. Potential known point sources include the Bølstad landfill, an experimental mink farm operated by NMBU on the eastern side of Årungen, a site south of Årungen near Fagernes on NMBU property that is registered by the Norwegian Department for the Environment (Miljødepartement) as suspected of having metals-contaminated soil (Norwegian Ministry for Climate and Environment, 2014), a muck tank south of Årungen near Fagernes next to the NMBU summer barn, two currently operating gas stations (Esso near Ås city center, Shell at Korsegården), a gas station located at Smedbølbekken next to Årungen that operated until the opening of the new highway E6 in the 1990's operates as a truck depot, and a potato chip factory (S.K. Huseby A/S) that operated until sometime in the late 1980's whose waste was deposited directly into the lake (Rosland, 1979).

Many of these sources ceased production and the input of pollutants into Årungen decades ago. Lake sediments can function as a sink of historic pollutants. Stored pollutants can be re-released into the water column by physical disturbances and bioturbation as well as chemical changes. The availability of metals in porewater is a complex interaction controlled by a number of parameters such as pH, salinity, reduction potential, the importance of which varies with the individual metal (Eggleton and

Thomas, 2004; Hong et al., 2011). For example, increasing salinity is an important factor in the mobilization of Cd and Mn, whereas decreasing pH is the most important factor in the mobilization of Zn (Hong et al., 2011).

The rate of growth south of Oslo has been increasing over the last decades, which also contributes to pollutant loads in Årungen. Until the 1990's, a major portion of highway E6 in Ås municipality consisted of the small 2-lane road that runs along the western shore of Årungen (Osloveien). As part of a major expansion of E6 from Oslo south to the Swedish border, E6 was relocated and expanded to its current location. Construction on the Vestby-Korsegården-Vassum section of E6 was started in 1990 and completed in 1995. In conjunction with the E6 project, sections of roads 152, 156 and E18 were also upgraded. In 1991, the E6 overpass at 152-Korsegården was completed. Infrastructure that was built as part of these upgrades included the installation of stormwater drainage systems. (Skari, 2002) Sections of the stormwater drainage systems underlying these roads discharge into Årungen watershed. In addition to road runoff, Årungen receives discharge from the stormwater drainage systems from all three local municipalities (Ski, Frogn, Ås).

The naturally high inputs of suspended particles from the surrounding clay soils, a form of pollution in itself to which cations can bind, has been exacerbated by agricultural runoff including the discharge of nutrients and other agricultural chemicals. Ironically, the high levels of suspended sediments, cations, alkalinity, hardness, and pH associated with the high sediment loads in Årungen may have buffered the lake from acid rain and possibly from exposure to other pollutants such as metals and road salt in urban and road runoff. Although historically pollutants associated with urban and road runoff very likely occurred at low levels due to the low level of development in the area, the types, amounts and proportions of pollutants discharging into Årungen are without a doubt increasing and changing with anthropogenic changes.

This is reflected in the growing population, number of vehicles, increasing traffic on roads such as E6 and 152, and the concurrent increase in the use of road salt (Strøm, 2012). A survey of lakes near major highways conducted in September of 2011 reported the development of a weak chloride-induced chemocline in Årungen, presumably caused by the accumulation of road salt, i.e. sodium chloride (Bækken and Åstebøl, 2012). With climate change and the predicted increasing intensity in precipitation and runoff that have already begun to occur, it should be expected that the synergistic effects of increasing runoff and pollutant loads will have negative impacts on water bodies such as Årungen.

Table 1: Increases in population, vehicle ownership, traffic; and the estimated amount of salt used as a deicing agent in Ås municipality and in the Brønnerud-Vollebekk watershed in 20, the use of salt as a deicing agent in the winter of 2012-2013. Sources are listed at the bottom of the table.

Year	1972 (1)	2013 (2)	% Change
Total Population in Ås Municipality	9624 (2)	14746 (2)	65.00%
Total number of vehicles (personal and commercial, not including tractors)	3008 (1)	14807 (1)	492.0%
Persons per vehicle	3.2	1.0	320.0%
Annual Average Daily Traffic (AADT) on major local roads:			
E6 going north from Korsegården	6900 (1)	38500 (2)	558.0%
Riksvei 152 (Drøbakveien) between E6 and NMBU	3600 (1)	10350 (2)	287.5%
Riksvei 152 (Drøbakveien) between NMBU and Ås city center	4500 (1)	11700 (2)	260.0%
Riksvei 56 (Kongeveien)	1750 (1)	1100 (2)	63.0%
Sum–AADT contributing to SCB	16750	61650	368.0%
Sum kilometers contributing to SCB (E6, 152)		11.3	
Salt use (tons/km), average for Follo region in 2012/2013		11.5 (4)	
Salt applied in tons on E6 & 152 within Brønnerud-Vollebekken drainages, 2012/2013		129.5	

Sources:

(1) Ås Vegplanutvalg, 1974. Used with permission by Ås Municipality.

(2) Statistics Norway (www.ssb.no)

(3) Statens Vegvesen Vegkart (www.vegvesen.no/vegkart) (Inneholder data under norsk lisens for offentlige data (NLOD) tilgjengeliggjort av Statens vegvesen.)

(4) Sivertsen, Å and I. Ofstad Skolmli. Mengderapportering vinteren 2013/2014. Statens Vegvesens Rapport Nr. 321. Statens Vegvesen.

Effects of drainage systems on Årungen Watershed

Hydrological alteration, or the modification of the natural flow regime in a watershed, is considered one of the top threats to freshwater ecosystems (Carpenter et al., 2011; Dudgeon et al., 2006). Two of the most important factors changing the natural flow regime in a watershed are the increase in the amount of impermeable surface area, and the channelization of flow including drainage systems. In undeveloped watersheds, whose surfaces are rocky, vegetated, sinuous, permeable, varying in slope and roughness, and absorb, intercept and decrease the volume, velocity and transport of water, the time to peak flow is longer and the volume of peak flows is smaller. In developed, urban watersheds, flat, impermeable surfaces, channelized streams and piped flows increase discharge volume by decreasing infiltration and increase velocity by reducing friction and sinuosity, resulting in larger peak flows of shorter duration. Peak flows in developed watersheds are more likely to cause flooding, have greater erosive power and are therefore more likely to deliver larger loads of pollutants occurring as dissolved or soluble substances, or that have been sorbed to suspended particles.

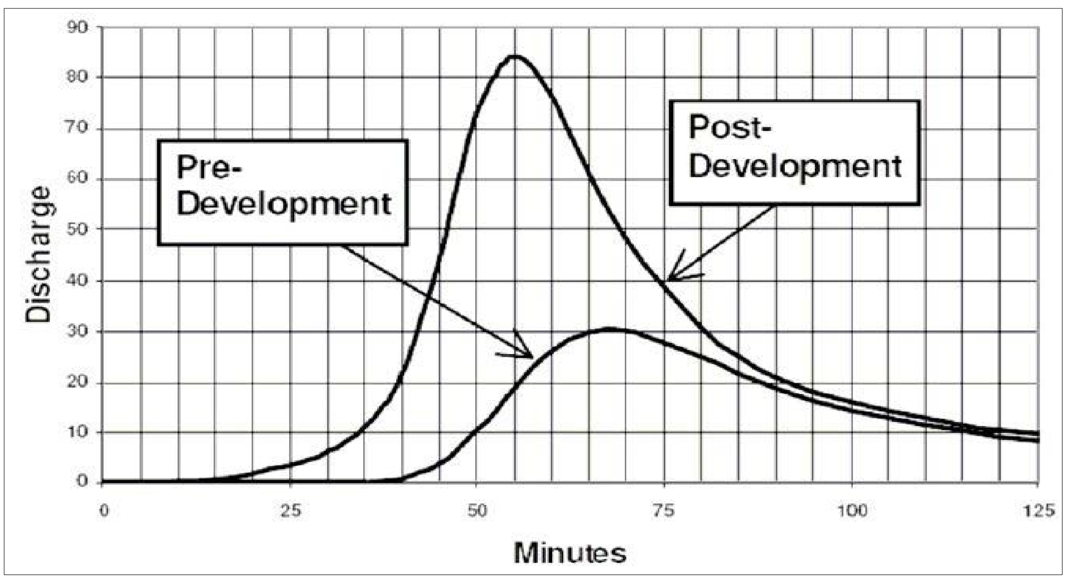


Figure 5: Hydrograph showing differences in discharge volume and time to peak flow between developed watersheds with larger percentage of impermeable surfaces, channelized flow and drainage systems, and undeveloped watersheds with permeable surfaces and undisturbed stream channels. Source: Ramachandra T. V. and Pradeep P. Mujumdar. 2009. Urban Floods: Case Study of Bangalore, Journal of the National Institute of Disaster Management, Vol. 3, No. 2. pp. 1 – 98.

The occurrence and fate of pollutants entrained in flow is dependent on a large number of factors such as the size, chemical species, and charge of pollutants, and is also a function of characteristics such as the pH and salinity of the solution, all of which can change during transport as a result of the dynamic, stochastic conditions caused by turbulence and chemical interactions. Cations, for example many metals and phosphorous, tend to adsorb to negatively charged particles which, when large enough, can fall out of solution. Nitrogen and phosphorous can occur in various forms, including dissolved and particulate, and can be transformed during transport. However, significant proportions of some pollutants tend to occur as dissolved substances, including organic pollutants such as PAH's and notably Cu, Ni, and especially Zn (Camponelli et al., 2010; Roger et al, 1998; Sansalone and Buchberger, 1997, reviewed in Kayhanian et al., 2012; Zuo et al., 2012).

Sediment loads are categorized by size according to particle diameter. Total suspended solids (TSS) are defined as the concentration of particles >0.45 um. The concentration of total dissolved solids (TDS) is defined as the concentration <0.45 um. The entrainment, transport and deposition of particles are a function of particle size and flow velocity. In slow moving waters, larger particles fall out of suspension, leaving smaller particles and a larger proportion of TDS entrained in the flow. Increasing the distance water travels increases the amount of time larger particles have to fall out of suspension, thereby decreasing the load of TSS.

The conventional treatment for removing pollutants in runoff prior to entering a water body is the use of sedimentation ponds. Sediment ponds have historically been designed to remove suspended solids (> 0.45 um) which can fall out of solution if the velocity is very slow and the length of a pond is long enough to allow particles to fall out of solution. The velocity-distance relationships are based on Stoke's law, which can be used to calculate the time required for a particle of a given size to fall a given depth. In combination with the total surface area contributing runoff to a sedimentation pond, the dimensions of a sedimentation pond can be calculated based on an assumed volume of water entering the pond over time, i.e. a design rainfall-runoff event. The size of treatment ponds required to remove dissolved constituents would be enormous and is simply not feasible. It is a design characteristic of most sediment ponds that a certain proportion of dissolved constituents will be released into a recipient water body. This is also based on the assumption that the water quality characteristics will be conducive to the aggregation of particles in solution, which generally requires high pH and low salinity.

Because drainage systems significantly alter the transportation of both the quantity and the quality of water to recipient water bodies, they play a major role in altering the geomorphology and hydrology of watersheds and the water quality of recipient water bodies, especially lakes. Each type of drainage system delivers different types and quantities of pollutants to a recipient water body, the quantity of which is a function of the dimensions of a drainage system and temporal and spatial variations in discharge, as well as the sum total of discharge produced by all contributing drainage systems.

Although the amount of impermeable surface area in the Årungen watershed has not increased significantly in the last decades, the number of drainage systems and subsequently the volume of discharge transported by drainage systems have increased significantly. As presented in the previous sections, there are four types of drainage systems in the Årungen watershed, including: 1) agricultural drainage systems; 2) rural decentralized sewage treatment facilities; 3) separate stormwater drainage systems; and 4) combined sewage and stormwater drainage systems. A description of the function, impacts and type of pollutants transported by each type of systems and the location relative to Årungen follows.

Agricultural Drainage Systems

The main purpose of agricultural drainage systems is to decrease the amount of water stored in soil. Surface and subsurface agricultural drainage systems have different impacts. Surface agricultural systems are composed primarily of ditches, and alter the surface geomorphology, reducing roughness, sinuosity, and surface drainage density. (Blann et al., 2009) They can increase the amount of surface runoff and erosion, and can therefore increase peak flows, loads of suspended sediments, sorbed and dissolved pollutants (Kayhanian et al., 2012) including phosphorous (Blann et al., 2009). Water infiltrating through the soil into subsurface piping can transport soluble chemicals such as nitrate and phosphorous; remove water that would otherwise be stored in soil is discharged, thus increasing stream baseflow. Subsurface drainage systems have also been shown to increase drainage density and reduce peak flows. (Blann et al., 2009) This is however based on the premise that a drainage system is hydrologically isolated and does not receive contributions from other drainage systems.

In Norway, it is common practice to connect other types of drainage systems, in particular decentralized rural sewage treatment systems, to subsurface agricultural drainage systems (Ensby, 1984). This is an artificial extension of the hydrological catchment area of a drainage system. While there are some local regulations requiring that a map documenting the approximate location of a connection be submitted to local authorities, there are otherwise no requirements regulating the amount or quality of discharge entering a subsurface system. This results in unknown amounts of discharge entering a recipient that can increase stream baseflow. With regard to water quality, unregulated connections can result in pollutants being directly discharged to a recipient, and potentially infiltrating into soils if a drainage system leaks.

Because there are no reporting requirements, it is unclear how prevalent agricultural drainage systems are in the Årungen watershed, and it is therefore not possible to quantify discharge in terms of either volume or water quality. It can be surmised that they are quite common if not ubiquitous (Deelstra et al., 2010; Ensby, 1984) and are found in the majority of agricultural fields making up 49% of the surface area of the watershed. Approximately one third of the perimeter of lake Årungen is ringed with agricultural fields in close vicinity to the edge of the lake whose drainage systems presumably discharge directly into the lake.

Rural Waste systems

Decentralized waste systems are common in rural areas where it is not logistically or economically feasible to connect the sewage system from rural homes and businesses to a conventional municipal sewage system, i.e. piping system. In recent years, there has been increased emphasis placed on requiring property owners to upgrade to authorized prefabricated decentralized treatment units which treat waste biologically, chemically or using a combination of treatments. Other types of treatment include septic tanks, leach fields, and constructed treatment wetlands. The main disadvantages of small rural treatment systems include water quality requirements for treated wastewater are more relaxed than for larger wastewater treatment plants; poor reliability due to sensitivity to changes in pH, such as large inputs of household chemicals, sodas or urine which disrupt the ability of biological or chemical systems to break down waste; and a lack of regular water quality monitoring, which occurs every second or third year. Advantages of rural waste systems with regard to the transport of pollutants include that they tend to be isolated, often discharge into infiltration or leach field, which act as secondary treatment, and unless they are directly connected to agricultural drainage systems, they are discharged into streams. Pollutants are similar to those found in conventional sewage systems. In the Årungen watershed, they are most prevalent in the Storgrava watershed.

Separate and combined stormwater and sewage systems

A separate system refers to two separate piping systems. A separate stormwater drain system receives only runoff generated from urban and or road surfaces, and discharges untreated runoff directly into a recipient. A separate sewage system receives exclusively sewage that is piped to a wastewater treatment facility, treated and then discharged into a recipient. Combined sewage stormwater systems are designed to receive sewage 100 % of the time, and in addition receive runoff generated from urban and or road surfaces under the assumption that the most prevalent flow regime will be no or low flow, i.e. that the amounts of rainfall-runoff entering the system will be so low they will not overwhelm the system. During high intensity rainfall and rain-on-snow events, large volumes of runoff can be generated, especially on impermeable urban and road surfaces. The volume of discharge from such events can overload the capacity of a combined sewage stormwater system, and subsequently cause flooding and exceed the volumetric and chemical treatment capacity of wastewater treatment facilities. To prevent flooding, excess volume in a combined system is piped or pumped into an overflow unit that usually filters out large solids and then diverts the discharge into a recipient water body. Combined sewage and stormwater that discharge untreated into a recipient is referred to as a combined sewer overflow (CSO).

Because they are less costly to install, requiring one piping system instead of two, combined systems were historically very common installations. Separate sewage and stormwater drainage systems are clearly preferable with regard to reducing flooding and inputs of pollutants into recipient water bodies. Sewage contains pollutants such as bacteria and viruses; nutrients; residues from household cleaning products, personal care products, pharmaceuticals, and illegal narcotics, among other things. In an effort to reduce pollutant loads and flooding, there has been increasing emphasis in recent years on replacing combined systems with separate systems, and on requiring new construction projects to install separate systems. Runoff from roads and urban surfaces contains automotive fluids, road dust containing particles from vehicle exhaust, the weathering of asphalt, concrete and vehicles, solvents used in cleaning and maintaining road surfaces, deicing agents such as road salt, as well as garbage and animal excrement. Pollutants range from heavy metals to organic pollutants and nutrients. It is therefore also highly polluted and is discharged untreated into recipient water bodies.

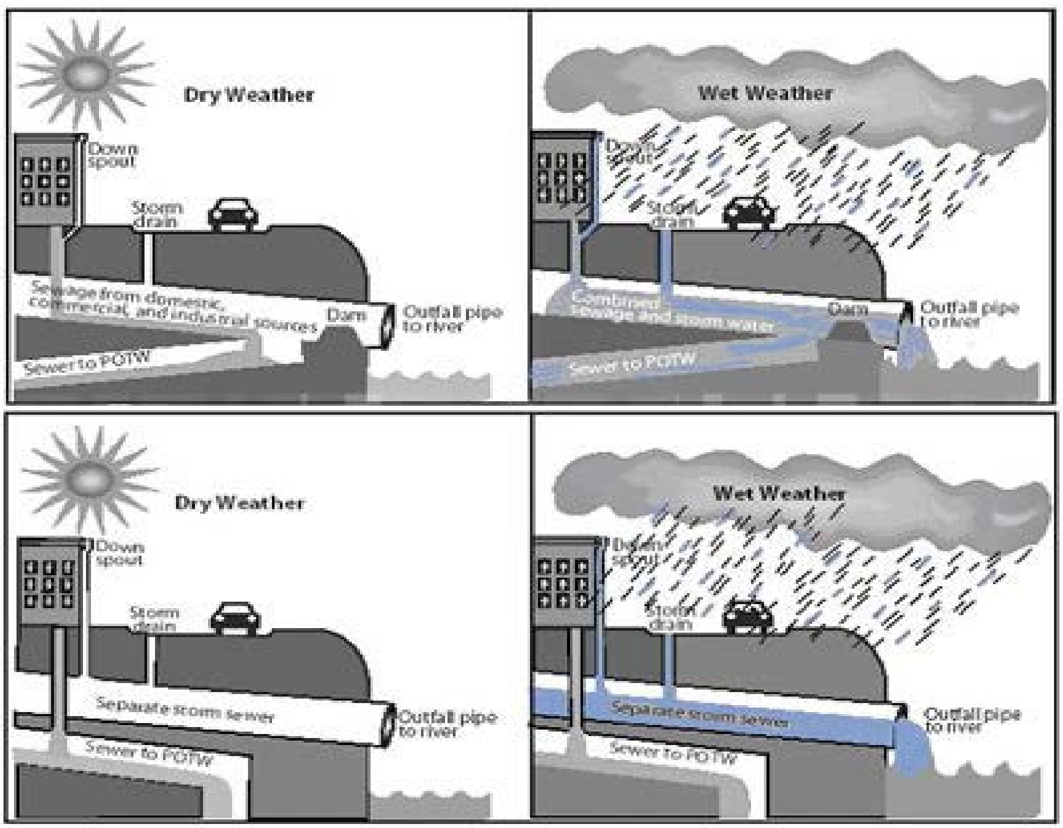


Figure 6: Graphic showing the differences between combined (top) and separate (bottom) systems, during low flow conditions with no runoff (left) and high flow conditions occurring with runoff generated by intense rainfall.
Source: http://water.ky.gov. Accessed 30.November.2014.

Inputs to Årungen from drainage systems

Urban stormwater systems in Ski have not been investigated in this study. Any runoff originating from Ski city center would be discharged into streams in the headwaters of Bølstadbekken and travel 2.5 km first to Østensjøvann, and then another 4 km via Bølstadbekken to Årungen. All stormwater and sewage systems in Ski municipality are separate systems, precluding the occurrence of CSO events that could affect water quality in Årungen (Statistisk Sentralbyrå, 2014). The stormwater drainage systems in Frogn located in the vicinity of Drøbak City shopping center discharge into the headwaters of Storgrava and travel about 5 km before reaching Årungen. These systems are also supposedly separate systems (personal knowledge). Urban runoff from the western and central part of the town of Ås discharges into Vollebekken (Ås municipality, 2014) and travels about 0.7 km before discharging into Årungen. Although Ås municipality has not yet managed to replace all of the combined sewage systems yet, only 2 % or 2.6 km out of a total of 129 km of sewage pipelines are combined stormwater sewage systems. The municipality also has two overflow units which divert excess volumes of discharge from sewage systems into a recipient. (Statistisk Sentralbyrå, 2014) Both overflow units are connected to stormwater systems that discharge into Vollebekken. One of these, which is located next to Vollebekken on NMBU property near the animal husbandry facilities, receives runoff from a large area of NMBU. (Ås municipality, 2014). The NMBU combined sewage and stormwater system is apparently in poor condition and a major contributor to CSO discharge (Buhler, 2014) Discharge from the NMBU combined systems is pumped, via a pumping station located next to Vollebekken (Ås municipality, 2014), into a centrifugal unit to remove large solids (personal observation), and then into the municipal stormwater system, which then discharges the combined flows into Vollebekken (Ås municipality, 2014). In 2012, a total of 14,364 m^3 of sewage were discharged from the pumping station at NMBU as CSO discharges into Vollebekken during high rainfall-runoff events. With the expansion of NMBU currently underway as part of the merger of NMBU and the Norwegian Veterinary College, there are plans to upgrade NMBU's combined sewage and stormwater system. (Buhler, 2014)

In addition to stormwater from urban systems, Årungen receives stormwater from drainage systems associated with E6, 152, 56 and E18. Of these, the E6 system probably delivers the largest volume and total load of pollutants simply because of its larger size and higher AADT, as well as proximity. Approximately 6 km of E6 lie within the Årungen watershed, beginning 2 km south of Korsegården at Revjatopp bridge (Revjatoppbrua), north to Vassum and the entrance to Nordby tunnel. Based on an average width of 40 m including shoulders, this is equal to approximately 240 decares. Road runoff along this section of E6 is piped to two sedimentation dams completed in 2000, as well as directly to Brønnerudbekken. The northernmost 700 m from the bridge at Fosterud north to the southern entrance of Nordby tunnel lies within Storgrava watershed. Runoff from this section, which is 56 decares (Snilsberg and Roseth, 2001) is channeled into a sedimentation pond located in the center divide of E6 near the entrance to Nordby tunnel. Treated discharge from this pond is piped 85 m downhill to Årungen and is discharged into Årungen at the northern end of Årungsbukta.

From the high point at Fosterud bridge south to Fagernes is piped into a second sedimentation pond located at Fagernes (Fagernes sedimentation pond), which receives runoff from a 37 decare section of

E6 (Snilsberg and Roseth, 2001). Construction drawings provided by the Statens Vegvesen detailing the stormwater drainage system in this vicinity also show that there are two pipes discharging stormwater from E6 into Brønnerudbekken upstream from the Fagernes sedimentation pond, one immediately upstream from Osloveien, and a second further upstream. The total area of E6 that generates runoff that is discharged into the Årungsbukta and Fagernes sedimentation ponds is equal to 93 decares or amount of runoff from total number of decares. Based on the topography of E6 south of Korsegården, and an examination of data in Statens Vegan's online mapping tool Vegkart (Statens Vegvesen, 2014) it seems likely that the remaining 147 decares of E6 within Årungen watershed is discharged untreated into Brønnerudbekken or alternatively into the section of municipal stormwater drainage system located in the vicinity of Korsegården. Statens Vegvesen has an internal mandate to maintain the environmental quality of water bodies affected by the roads it maintains, and as such has implemented the installation of treatment ponds and other facilities to treat road runoff throughout Norway. There are no requirements regarding the treatment of road runoff in Norway or for that matter in most countries.

Without in-depth research and continuous monitoring of flows, it is difficult to ascertain which streams discharging into Årungen receive larger inputs of pollutants. However, there appear to be a larger number of inputs from urban and road runoff into Brønnerud-Vollebekken, including CSO discharge. Another significant difference between Storgrava and Bølstadbekken vs Brønnerud-Vollebekken is the combined effect of distance, time and velocity; here again Stoke's law applies. Most pollutant sources flowing into Storgrava and Bølstadbekken are located several kilometers away from the recipient Årungen, and travel in stream channels which, albeit being altered, will slow the velocity due to their increased roughness, relatively unconstrained flow in open channels with perhaps wider stream profiles, and increased sinuosity. The pollutant sources entering Brønnerud-Vollebekken are all located a short distance from Årungen, and travel via piped flow which will increase the velocity significantly, and once discharged into Brønnerudbekken and Vollebekken, travel a very short distance to Årungen. In Storgrava and Bølstadbekken, the larger distances, slower velocities and therefore increased transport time will allow a larger proportion of particles to fall out of solution. In addition, natural stream channels provide sediments, vegetation and other in-stream materials which will increase the entrapment of particles including dissolved solids, thereby reducing the total load of pollutants entering the recipient.

The effect of pipeflow from multiple sources at short distance very likely increases the frequency and the magnitude of peak flows as well as the concentration of pollutants delivered by Brønnerud-Vollebekken to the southern end of Årungen including the SCB. This effect in turn will be exacerbated by high intensity rainfall and rain-on-snow runoff events that are predicted to occur with climate change. The addition of road salt to the mixture of pollutants will increase the solubility of pollutants as well as the bioavailability, potentially making spring flows, during which first flush events deliver large volumes of pollutants stored in snow depots during the winter, particularly toxic.

Field Methods

Sediment sampling – March 7, 2014

Sediment sampling took place on March 7, 2014 while Årungen was still covered in ice. A Rapala ice drill with a diameter of 155 mm was used to drill through the ice at a point approximately in the center of the SCB. Two sediment cores of different lengths were sampled adjacent to each other at different depths. A Uwitec corer was used to collect sediment down to 45 cm below the water-sediment interface in an acrylic tube measuring 60 cm long with an inside diameter of by 59.5 mm (hereafter referred to as the short core). The short core was removed from the tube and processed in the lab. A Russian corer modified after Jowsey (1966) with a sampling length of 100 cm and a diameter of 10 cm was used to collect sediments down to 118 cm below the water-sediment interface (hereafter referred to as the long core). The long core was immediately removed from the Russian core sampler while still at the field site, carefully cut in half longwise, wrapped in plastic, and placed in 100 cm long pieces of 10 cm diameter plastic pipe that were pre-cut lengthwise.

E. canadensis survey: Steinsfjorden, 2013

On the 3rd of September, 2013 I was participated in a survey of lake Steinsfjorden (Steinsfjorden) led by Marit Mjelde, an aquatic botanist at NIVA with expertise in aquatic invasive species. Steinsfjorden is a branch of Tyrifjorden, a large freshwater lake that drains into Oslofjord located approximately 40 kilometers northwest of Oslo. This survey was conducted to monitor the current status of *E. canadensis*, and was a follow-up to previous surveys conducted since the species was first reported in Steinsfjorden in 1981. Participating in this survey provided me with valuable experience identifying *E. canadensis* and observing its ecology and distribution in a lake where it had been thriving for XX decades.

The length of Steinsfjorden from its northern end to its narrow outlet into Tyrifjorden is approximately 8 km; it is approximately 2.5 km wide. The survey was conducted by boat. We re-visited sites that had been monitored in earlier surveys and used a bathyscope to view the lake bottom and determine whether E. canadensis was present or absent. Visibility was approximately 3 m. Deeper locations where the visibility was reduced were sampled using an aquatic plant sampling rake (rake). We also sampled randomly by trawling with the rake while traveling between surveying sites.

E. canadensis survey: Årungen, September 2013

Employing the techniques learned on the Steinsfjorden survey, I surveyed Årungen by boat to confirm that *E. canadensis* was no longer present. On the 16th of September, 2013, I surveyed the western shoreline including Årungsbukta and the center of the lake south to Smedbølbekken using a bathyscope to view the lake bottom, and an aquatic plant sampling rake to sample deeper sections of the lake down to approximately 3 m where visibility was reduced. On the 26th of September, 2013 I sampled the southwestern, southern and southeastern portions of the lake, including inlets from Smedbølbekken, Brønnerud-Vollebekken, Syverud and Norderås streams, repeating the methods used on the previous survey. Visibility during both surveys was approximately 2.6 m. Due to time constraints; the northern end of the lake was not surveyed. These surveys were completed with the assistance of Jonny Kristensen and Prof. Gunnhild Rii**se.**

Stream and sediment sampling: Årungen watershed, April and November 2014

Flows from Smedbølbekken, Brønnerud and Vollebekken were sampled during low and high flows on the 5th of April and on the 9th and 10th of November in 2014. Sediments and samples of *E. canadensis* were sampled from Fagernes sedimentation pond. The purpose of these surveys was to compare water quantity and quality between high and low flows resulting from rainfall-runoff events in streams that contribute flows to the SCB watershed. All water quality samples were grab samples. Grab samples cannot replace frequent, composite or continuous (i.e. using data loggers) water quality monitoring methods, which are more able to capture changes in pollutant concentrations over time especially between low and high flows. Combined with measurements of discharge (volume/time) during rainfall-runoff events, the total pollutant loads can be calculated. Single grab samples, which are usually taken during low flow conditions, given no indication of concentrations occurring during high flows and without discharge measurements, no indication of total pollutant loads. However, grab samples taken during low and high flows have value in that they provide a comparison of conditions and point to potential pollutant sources that are not always present during low flow conditions.

Sample containers and lids were made of polypropylene and polyethylene terephthalate plastic and were clean prior to use. Immediately prior to sampling, containers and lids were rinsed three times with the sample flow from a location downstream from the sampling location and were carefully handled to prevent samples from being contaminated. Where flows were easily accessible, sample containers were filled directly from the stream. When direct access was difficult due to steep stream banks or dense vegetation, a 2-liter sampling bottle or bucket was lowered into the discharge and filled. Locations of sampling sites are described below and shown in a map at the end of this section.

April 5th, 2014,

On Sunday April 5th, 2014, I surveyed the area around the southwestern side of the SCB at the beginning and height of a rainfall event. In addition to sampling and observing changes in stream flow in Smedbølbekken, Brønnerudbekken and Vollebekken, I was interested in sampling and observing discharge into the Fagernes sedimentation pond. I chose to conduct the survey during this rainfall event because it was one of the first spring storms of the year and there had been little precipitation in the weeks prior to the storm.

Smedbølbekken, 5.April.2014

Two locations along Smedbølbekken were sampled at high flow. One site is located immediately upstream of the Gamle Mossveien road (S1), and the other is located approximately 7 meters from the outlet of Smedbølbekken into Årungen (S2).

Brønnerudbekken, 5.April.2014

Three locations were sampled in Brønnerudbekken. The point at which the stream flows under the road at Fagernes (B1) was sampled during low flow and high flow. Downstream from the road, Brønnerudbekken flows out of a culvert towards Årungen. One sample taken at this culvert (B2) during low flow. A sample was taken from high flow discharge from a culvert (diameter = 140 cm) that intersects Brønnerud downstream from the road and from the inlet from Fagernes sedimentation pond (B3).

Fagernes sedimentation pond, 5.April.2014

The discharge entering Fagernes sedimentation pond (F) was sampled during low and high flows.

Vollebekken, 5.April.2014

Discharge was sampled where Vollebekken enters a culvert near the muck tank at the NMBU summer barn (V1) during low and high flows. During low flow, a sample was taken from a pipe discharging into the stream at the culvert (V2). The pipe carries discharge from a nearby manhole located across the road and approximately 4 m from the muck tank. Samples taken at location (V1) were taken upstream from location (V2).

November 9th and 10th, 2014

On Sunday the 9th of November, 2014 I took grab samples during low flow conditions from Vollebekken where a combined sewer overflow (CSO) enters the stream near a pumphouse operated by Ås municipality. At this point, the stream receives inputs from two CSO systems, one entering the stream at the sampling location and another located upstream located across from the main entrance to NMBU on the south side of road at 52 Drøbakveien that enters Vollebekken via piped flow. I returned on Monday the 10th of November during high flow conditions when the pump controlling CSO flow from the municipal combined sewage and stormwater system was pumping overflowing sewage into Vollebekken stream.

Samples were taken approximately 3 m upstream of the CSO (V6) during low and high flows, in the CSO pipe where it discharges into the stream (V5) during high flow, and approximately 4 m downstream from the CSO during high flow (V4). A sample was also taken during low flow directly from an agricultural drainage pipe on the west side of the stream, located approximately 100 m downstream from the CSO (V3). Sediments were sampled from Fagernes sedimentation pond and below the agricultural drainage pipe discharging into Vollebekken (V3). Samples of *E. canadensis* including roots, shoots, stems and leaves, were taken from Fagernes sedimentation pond, after which sediments were rinsed off in the pond and shaken to remove excess water. Roots were separated from shoots, stems and leaves. Sediments and separated *E. canadensis* samples were dried at 55 ° C for 24 hours. All samples were stored at 4 ° C until they were submitted for analysis.

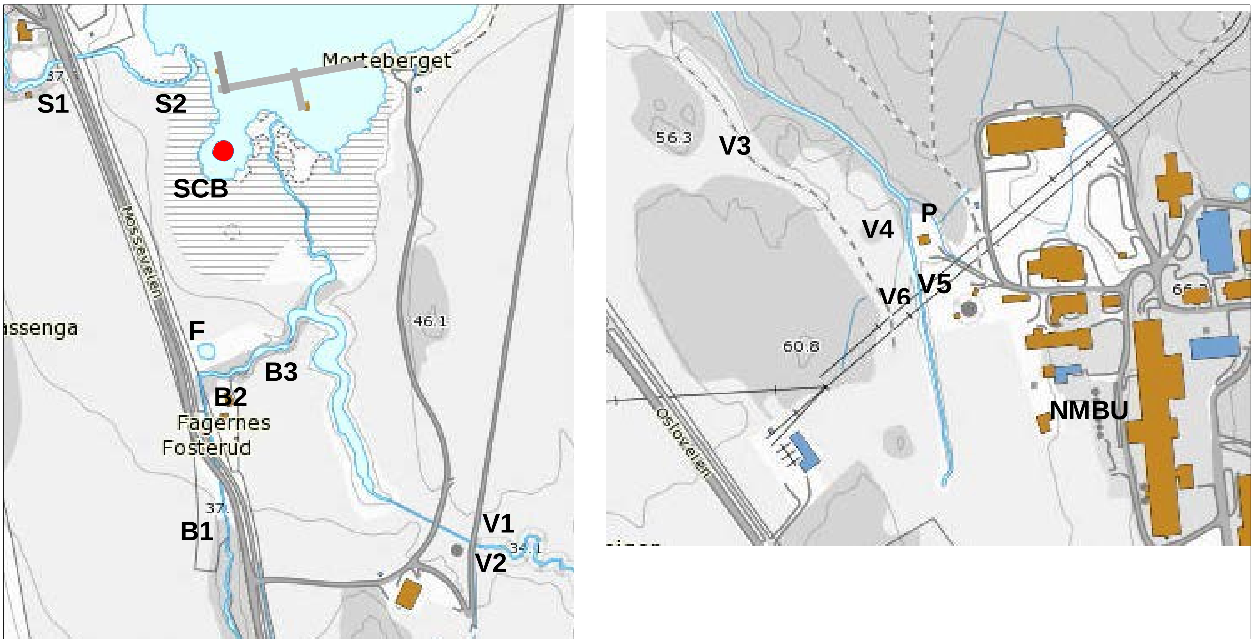


Figure 7: Map showing sediment core sampling site (red dot) and SCB, and sampling locations from stream, sediment and E. canadensis sampling on 5. April and 9 – 10. November. Smedbølbekken sampling sites: S1, S2. Fagernes: F. Brønnerudbekken: B1, B2, B3. Vollebekken: V1, V2, V3, V4, V5, V6. P=sewer overflow pumphouse. The agricultural pipe at V3 and the CSO pipe are not shown here.

Laboratory Methods

Sediment core - Processing

Processing the cores began approximately one hour after field sampling was completed. The short core was cut into 1 cm layers and the long core was cut into 3 cm layers along the horizontal axis, resulting in an initial total of 79 samples. Each layer was mixed to homogenize the sample and divided into three approximately equal portions that were later analyzed for the following variables: 1) percent water, dry matter and loss-on-ignition which was converted into percent organic matter (OM); 2) ^{137}Cs dating and metals (Cd, Cr, Cu, Mn, Pb, Zn); 3) absorbance. Plastic containers including lids were weighed prior to and after the addition of sample material in order to calculate sample weight and labeled, after which sample material was added and the sample plus the container were weighed again.

Sediment core - Water content and percent organic matter

The gravimetric water content (Θ_g), which is the ratio of the mass of water per mass of the total sample weight, and the percent organic matter (% OM) were calculated for each sample following methods in (Jury et al., 1991). The container weight was subtracted from the total weight of the wet sample and container weight to obtain wet sample weight. To remove all water vapor, samples were oven-dried for a minimum of 48 hours at a temperature of 55 °C. After drying, samples were weighed again, and container weights were subtracted to obtain the dry weight. In order to determine the amount of organic matter in each sample, ceramic cups were weighed, a sub-sample of between 2 and 5 g of sediment was placed in each cup, and the cups plus sample material were weighed again, subtracting the difference to obtain sample weight. Cups with sample material were then placed in an oven at a temperature of 550 °C for a minimum of 24 hours in order to remove all organic matter. To prevent water vapor from being absorbed into the samples and ensure accurate measurements, cups plus sample material were weighed immediately after removal from the oven, after which cup weights were subtracted from the total weight to obtain sample weight. The second sample weight was subtracted from the initial sample weight to obtain the loss-on-ignition, i.e. the amount of organic matter removed during combustion. The amounts and percentages of water, dry matter, and organic matter were calculated according to the following formulas:

$$\Theta_g = \frac{mass\ of\ water}{(mass\ of\ dry\ sediment + mass\ of\ water)}$$

$$LOI = (mass\ of\ dried\ sediment) - (mass\ of\ burned\ sediment)$$

$$\%OM = \frac{LOI}{mass\,of\,dried\,sediment}$$

Sediment core - ^{137}Cs Dating

Cesium 137 is a product of nuclear fission; because it is not naturally occurring, is non-exchangeable and binds to soil particles, it functions well as a tracer for measuring erosion and sedimentation. Testing of nuclear weapons testing in the 1950's and 1960's and the 1986 Chernobyl nuclear reactor accident released ^{137}Cs into the atmosphere. Peak measurements of ^{137}Cs can be correlated with the year in which specific fallout events occurred and used to determine sedimentation rates in recently deposited sediments and soils. (Ritchie and McHenry, 1990) While fallout from weapons testing can be found around the world, the ^{137}Cs fallout from the Chernobyl event was deposited primarily in Europe and parts of Asia. Particles of ^{137}Cs continue to be eroded and deposited in sediments, and can migrate downward through sediments layers, changing the vertical distribution and obscuring the record of less significant fallout events. As a result, the larger peak associated with the more recent Chernobyl event makes it a more useful geochronological marker in Europe and Asia than ^{137}Cs fallout from older events. Measuring the ^{137}Cs DPM in layers deposited after 1986 is probably not necessary if a reliable estimate of the depth at which the Chernobyl event occurred can be estimated. (Klaminder et al., 2012)

The sediment rate in Årungen for the period 1954-1978 was estimated at 8.9 mm/year, based on ^{137}Cs dating of a sediment core sampled in 1978 from the deepest point of the lake (13 m), and was estimated at 3.4 mm/year for the period 1900-1950 based on ^{210}Pb dating (Skogheim and Erlandsen, 1984). These figures are consistent with history. Stable rates of development and land-use prior to World War II, followed post-World War II by a period of rapid development and agricultural expansion that would have increased erosion rates including increasing the amount of arable land, the installation of agricultural drainage systems, and the increased use of agricultural chemicals and machinery. Based on comparisons of an aerial photo from 1956 (Widerøe, 1956), online mapping tools with aerial photos (Follo Kommunene; Google, 2013) and the percentages of agricultural and forested land in 1984 (approximately 50 % and 40 %) cited in Skogheim and Erlandsen (1984), the proportions of agricultural landscape types seems relatively constant since 1956.

Based on estimates of the sedimentation rate from the 1978 sediment core and on the historical trends outlined above, I made the following assumptions: 1) the 1986 Chernobyl event would occur within a 45 cm depth, i.e. the length of the short core, and could be used to calculate the sedimentation rate from the period 1986-2012; 2) increases in sedimentation rates in the Østlandet region are primarily a function of agricultural expansion; 3) the annual rate of sediment deposition in

Årungen was constant prior to 1953, increased dramatically immediately thereafter, and stabilized after a short period of development; 4) the sedimentation rate in Årungen was constant from 1954 to 2012; 5) the sedimentation rates for both time periods (1900-1953) over the entire lake were proportional to the sedimentation rates cited in Skogheim and Erlandsen (1984).

Samples from each 1 cm layer from the short core were analyzed at the NMBU isotope laboratory using a Perkin-Elmer Wallac Wizard 3 inch sodium iodide (NaI) detector for the number of disintegration per minute (DPM) of the radionuclide ^{137}Cs. The peak DPM value was assumed to have been deposited in 1986. The difference in years between the sampling year and 1986 divided by the depth at which the maximum DPM value (DPMmax) occurred represented the number of years (Y) during which 1 cm of sediment was deposited, i.e. sediment depth (SD):

$$\mathbf{(2013 - 1986)/SD_{DPM\text{-}max} = YP/SD_{1\,cm}}$$

The value for $YP/SD_{1\,cm}$ was subtracted from the initial year (2013) and each subsequently calculated year thereafter. To obtain the year associated with each sample in the long core for the post-1953 period, $YP/SD_{1\,cm}$ was multiplied by 3, i.e. 3 cm, the depth of each sample:

$$\mathbf{(YP/SD_{1\,cm})*3 = YP/SD_{3\,cm,\,long\,core}}$$

This value was subtracted from the last calculated year in the short core, and for each layer thereafter in the long core, until the period prior to 1954 was reached. To calculate years for the period prior to 1953, the ratio 3.4:8.9 was used as a proportionality constant that was multiplied with $YP/SD_{3\,cm,\,long\,core}$ where 3.4 mm/year and 8.9 mm/year are the rates of sedimentation estimated by Skogheim and Erlandsen:

$$\mathbf{(YP/SD_{1\,cm})* 3 * (3.4/8.9) = YP/SD_{3\,cm,\,long\,core,\,pre\text{-}1954}}$$

This value was subtracted from the last calculated year occurring before 1953 until the end of the sediment core was reached.

<u>Sediment core - The use of pigments as biomarkers in the sediment recorded</u>

In order to determine which taxonomic groups of photosynthesizing organisms were present in the sediment record, I used pigments as biomarkers by first measuring the absorbance of sediment samples. The manual measurement of individual pigments is labor-intensive and costly, as it requires first separating the pigments within a sample using chromatography, and then analyzing each pigment separately on a spectrometer. A numerical method was therefore used to calculate values for selected individual pigments after initial absorbance values were measured for each sample.

Photosynthetic pigments are chemical compounds that absorb photosynthetically active radiation (PAR) between the wavelengths of 400 to 700 nm that include the chlorophylls, the carotenoids and the phycobilins. The distribution of pigment wavelengths is normal, or Gaussian, around maximum absorbance values. Most pigments have multiple peaks, with peak values corresponding to different colors in the visible light spectrum. For example, chlorophyll a has two peaks, an absorbance maxima for violet-blue light between 400 – 440 nm, and an absorbance maxima for red light between 640- 680 nm. Absorption values are normally distributed, also referred to as a Gaussian distribution, around peak wavelengths. An example is shown below in Figure 6.

Although not as widely distributed as the chlorophylls, the carotenoids are also found in plants, algae and cyanobacteria, whereas the phycobilins are found exclusively in cyanobacteria and algae. Chlorophyll molecules contain a magnesium ion that plays an indispensable role in photosynthesis by exciting electrons which are then passed on in pairs to an electron receptor. These reactions occur in both photosystem I and II. Other heavy metals have been shown to be incapable of raising the excitation level of electrons to a high enough state thereby causing a complete disruption of photosynthesis (Küpper et al., 1996, 1998).

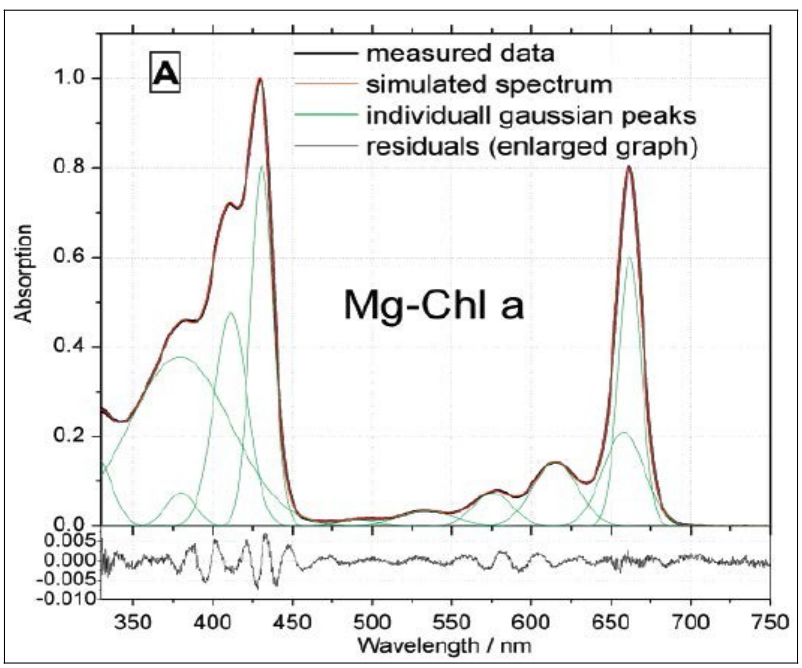


Figure 8: Plot of absorbance vs wavelength for chlorophyll a showing the Gaussian distribution of wavelengths distributed around peak absorbance values. Source: Kupper et al., 2007.

The environmental conditions in a water body can affect the rate of pigment degradation and are an important consideration in sampling and analyzing sediments. Pigments degrade more rapidly in the presence of light and oxygen; herbivory by zooplankton and algal decomposition are also important factors. Pigment degradation occurs primarily in the water column during the sedimentation process. After deposition at the sediment-water interface, lower exposure to light, oxygen and biotic factors decreases the degradation rate. As pigments are buried by layers of sediments, the rate of degradation decreases significantly; rapid sedimentation rates, anoxic conditions, and low light conditions can increase the degree of preservation. (Leavitt, 1993) The sedimentation rate varies with bathymetry and location within a lake (Reuss, 2005). This implies that the sediment record of pigments in locations with higher sedimentation rates, such as stream inlets, deltas and alluvial fans, and in particular wetlands, which are also often anoxic, would be more intact than at locations with slower deposition rates. The sediments analyzed in this study are relatively recent; this combined with a rapid sedimentation rate, a history of eutrophication, and anoxic sediments lead to the assumption that the relative rate of pigment degradation at the sampling site in Årungen is slow.

The wide distribution of pigments among aquatic photosynthesizing organisms, specificity with regard to taxonomic group, and resistance to degradation particularly in sediments, make pigments useful as biomarkers of ecological status and change. Pigments can be statistically analyzed with variables such as organic matter, chemical characteristics, fossil assemblages (Reuss, 2005) and in more recent sediments, pollutants that can help tease out the effects of anthropogenic change on the surrounding watershed and subsequently within a water body. The identification of pigments requires that they be extracted using organic solvents such as acetone and methanol which break down water soluble compounds to free up non-water soluble compounds. The phicobilins are water soluble, making them impracticable for use as biomarkers, whereas chlorophylls, carotenoids and pigment degradation products such as pheophytin a and b are more lipophilic and are readily extracted using organic solvents. (Leavitt, 1993) Once extracted, pigment solutions are ready to be spectroscopically analyzed.

<u>Sediment core - Spectroscopic analysis of pigments</u>

Spectroscopy is the study of the interaction between matter and the entire spectrum of electromagnetic radiation, which spans wavelengths ranging from 10^{8} to 10^{-18} meters. A wide variety of spectroscopic technologies have been developed that are used in fields as disparate as astronomy and paleolimnology. For this analysis, ultraviolet (UV) spectroscopy was used, where a beam of light of a known wavelength in units of nanometers (nm) is projected onto a cuvette of known width (i.e. pathlength) containing a known concentration of sample material in order to measure the absorbance of a material, be it plant, sediment, water, food or other media. This is a common method used to determine which pigments are present in a sample.

Absorbance is the measurement of wavelengths of light absorbed by a material. Mathematically, absorbance is described by the Beer-Lambert law as a dimensionless logarithmic ratio which can also be expressed as being directly proportional to the concentration of a substance and the distance light travels through the substance, referred to as pathlength:

$$A_\lambda = \log 10 \frac{I_o}{I} = \epsilon c L$$

Absorbance can also be expressed as the negative logarithmic inverse of transmittance *T,* a dimensionless measurement of the wavelengths of light transmitted through a material:

$$A_\lambda = -\log 10 T \quad \text{and} \quad T_\lambda = \frac{I}{I_o}$$

where:

$A = absorbance\,(dimensionless)$
$T = transmittance\,(dimensionless)$
$\lambda = a\ given\ wavelength\ of\ light$
$I_o = intensity\ of\ incident\ light$
$I = intensity\ of\ transmitted\ light$
$\epsilon = absorption\ coefficient$
$c = concentration$
$L = pathlength$

Measurements of the intensity of the incident and transmitted light can be used to calculate transmittance which is then used to calculate absorbance. The terms absorption coefficient, extinction coefficient and molar absorptivity are often used synonymously and refer to the intensity of light absorbed by a known substance at a known wavelength. This constant is determined in lab experiments for specific substances such as pigment molecules and food additives. Larger attenuation values indicate opaque materials that absorb and scatter light, whereas smaller values indicate a more transparent material through which light travels without being absorbed or scattered. (Campbell and Reece, 2007; Crowe and Bradshaw, 2010) The type and concentration of solvent used in extracting pigments and the choice of absorption coefficient can cause the calculation of wavelength and absorption to vary (Küpper et al., 2007).

<u>Sediment core - Absorbance measurements</u>

The absorbance of sediments was measured at 1 cm layers from 1 to 41 cm (i.e. samples from the short core), and at every 3 cm from 43 to 118 cm (i.e. samples from the long core). Absorbance samples were frozen immediately after the sediment cores were processed in the lab at NMBU. Plant material was also frozen after being collected. Prior to measuring absorbance, all samples were freeze dried for 24 hours and placed in freezer storage. Only leaves, shoots and stems from the *E. canadensis* sample were freeze dried and measured for absorbance; roots were not included.

Unless notes, methods for preparation and analysis followed recommendations in Küpper et al.(2007). One day prior to conducting absorbance measurements, plant pigments were extracted from the samples. Between 0.05 and 0.20 g of sample was weighed and placed in test tubes, after which 5 ml 100 % acetone (J.T.Baker®) was added. Samples were then immediately covered with a dark cloth to prevent photodegradation of pigments.

After 24 hours, samples were centrifuged for 15 minutes at 3,400 revolutions per minute (RPM) in a Hettiche Rotanta AP centrifuge. From each centrifuged sample, 4 ml of extracted pigment solution was pipetted into clean test tubes; pipettes were not re-used. A Perkin-Elmer Lambda 40 Spectrometer was used to measure absorbance. The spectral band-width (slit-width) was set to 1 nm and maximum absorbance to 1.5. The range for recording wavelengths was set to 350 to 900 nm (note: Küpper et al.(2007) recommends a setting of 400 to 700 nm). The spectrometer was calibrated using a 1 cm cuvette filled with a minimum of 4 ml 100 % acetone as a standard. A second 1 cm cuvette was used for analyzing samples, which was rinsed with 100 % acetone prior to, between and upon completion of each set of absorbance measurements.

Results from absorbance measurements included plots of absorbance versus wavelength that were displayed in real time as a sample was analyzed. If the concentration of pigment was very high, the absorbance peaks exceeded the maximum recommended value of 1.5 (Küpper et al., 2007) and flattened out. When this occurred, the sample was diluted by adding a known amount of 100 % acetone to the sample, and absorbance was measured again. If necessary, dilution was repeated until peak values of absorbance were reduced to less than 1.5. The results included data for dimensionless values of absorbance and wavelength in units of nm. Absorbance data was analyzed using Perkin-Elmer's UV- WinLab software program. Data were saved as ASCII delimited text files.

Processing of samples after field sampling, initial weighing, freezing and storage of samples was conducted at the soils laboratory in the Institute for Environmental Sciences (IES) at NMBU. Freeze drying, extraction preparations, extraction and absorbance measurements were conducted in the spectrometry section of the laboratory at the Norwegian Institute for Water Research (NIVA) in Oslo, Norway.

<u>Sediment core - Metals analysis</u>

Based on research showing the toxicity of heavy metals on *E. canadensis* (Dalla Vecchia et al., 2005; Küpper et al., 1996; Mal et al., 2002; Maleva et al., 2004; Nyquist and Greger, 2007; Thiebaut et al., 2010) and several earlier studies showing moderate to high levels of metals, in particular copper, in the water column and in sediments, in Årungen, a decision was made to analyze the sediment core for heavy metals. Positive results would support the hypothesis that *E. canadensis* disappeared from Årungen as a result of heavy metals toxicity, while negative results would imply that the hypothesis is false. Previously dried and frozen sub-samples of sediments from 1 to 40 cm depth from the short core were analyzed for total Cd, Cu, Zn, Ni, Mn and Pb by personnel at the soils laboratory at IES NMBU using inductively coupled plasma mass spectrometry (ICP-MS). Samples were prepared by digesting an aliquot of 0.25 g in 5 ml nitric acid at 260 ° C in a Milestone Ultraclave. Samples were then analyzed as fluids on an Agilent 8800 ICP-MS. ICP-MS analysis is, as the name implies, a two-part analysis where a fluid sample is heated, vaporized and constituent elements ionized. Ions are then induced into the mass spectrometer where they are separated, identified based on mass and charge, and the concentration is calculated.

<u>Stream, Stream sediments, *E. canadensis:* metals analysis</u>

In order to compare the concentrations of metals during low and high flow conditions and investigate potential pollutant sources contributing to the SCB watershed, flows from Smedbølbekken, Brønnerudbekken and Vollebekken were sampled during low and high flows on the 5th of April and on the 9th and 10th of November in 2014. All samples were stored at 4 ° C until they were submitted for analysis. Both sets of samples were submitted to Eurofins Environmental Testing located in Moss, Norway for analysis of total Cu, Cd and Zn. Analysis was conducted according to Norwegian Standard NS-EN ISO 17294-2:2004, an internationally accredited method of ICP-MS analysis.

Numerical analysis of sediment absorbance values

Given the history of eutrophication and periodic blooms of cyanobacteria, the dense mats of biomass that occurred when E. canadensis was present, particularly in the southern end of the lake in the SCB, and the presence of other higher plants in addition to *E. canadensis,* several assumptions were made. It was assumed that several taxonomic groups of photosynthetic organisms could have been present during the period of analysis determined by ^{137}Cs dating. Results from the absorbance measurements also indicated adequate quantities of pigment and numerous wavelength peaks, making this likely.

Based on these assumptions and results from spectrometric analysis, it seemed feasible to conduct a numerical analysis of the absorbance data that would mathematically separate individual pigments from the absorbance data. The numerically obtained pigments could then serve as biomarkers of the taxonomic groups of photosynthesizing organisms that had been present during the period of analysis represented by the sediment core. The numerical analysis was conducted following methods developed by Prof. Hendrik Küpper and colleagues (Küpper et al., 2007, 2000). The method is based on the premise that the area under the curve of a plot of the data obtained from the spectroscopic analysis (data-based curve) is equal to the sum of the areas under the curves representing individual pigments that are present in solution. In addition, each pigment has multiple peaks which can also be represented by a series of linear functions.

The Küpper method and R-program

The Küpper method was developed in order to quantify the most abundant chlorophyll and carotenoid pigments. In addition to quantifying normal chlorophyll, it can also quantify chlorophyll in which the central Mg^{+} ion has been exchanged with Cu, Cd or Zn. The method utilizes known extinction coefficients for specific peaks for each pigment and known dimensions of Gaussian distributions of absorption values at peak wavelengths such as peak width, wavelength and the weighted relative value of absorption, or Gaussian peak spectrum values, (GPS values). The GPS values and the absorption data obtained from the spectroscopic analysis are used to solve the linear equations adapted for the individual peaks of the pigments which are assumed to be present. The Küpper method solves these functions for each individual pigment of interest by iteratively plugging in possible absorption values and summing the individual curves until a solution is found that most closely fits the curve from the actual data, where the sum total of area under each modeled curve most closely matches the area under the data-based curve.

Using extinction coefficients in units of mass, modeled absorbance values, and pathway length, the concentration of individual pigments in each sediment depth can be calculated by solving a transformation of the Beer-Lambert law:

$$A_\lambda = \log 10 \frac{I_o}{I} = \epsilon c L$$

$$A_{\lambda,modeled} = \epsilon \, c_{calculated} \, L$$

$$c_{calculated} = \frac{A_{\lambda,modeled\ pigment}}{\epsilon L}$$

where:

$A_{\lambda,modeled} = modeled\ absorbance\ for\ a\ specific\ pigment\ at\ a\ given\ wavelength(dimensionless)$ $c_{calculated} = concentration\ of\ an\ individual(modeled)\ pigment\ ,(units\ of\ ug/ml)$ $\epsilon = extinction\ coefficient\ of\ an\ individual\ pigment\ (units\ liter/(g*cm))$ $L = pathlength(1cm)$

This concentration can then be converted into mass concentration in units of ug/g can by multiplying the calculated concentration with the volume of extracted solution, and dividing through by the mass of dry sediment used in the extraction.

Küpper et al. used the computer program Sigma plot to model pigments using their method. For this study, the functions presented in Küpper et al. (2007) were coded into scripts using the non-negative linear least squares package (nnls) in the statistical program R. Text files GPS values presented in Küpper et al. (2007) and absorption coefficients were input into the R program. Prof. Tom Anderson of the University of Oslo programmed the scripts and input files, and graciously allowed the use of his in this thesis. In this study, I used the molar absorption coefficients from Table 1 in Küpper et al. (2007) and from other sources. I transformed molar coefficients into units of mass, which were included as input in R or used cited values when necessary. The molar and mass absorptivity (absorption coefficients) are listed below in Table 2. Output includes absorbance and wavelength data and concentration (ug pigment/g dry sediment) for each pigment at each sample depth, a scatter-plot of observed versus fitted absorbance, and plots of absorbance versus wavelength and pigment concentration vs sediment depth.

Choosing pigments

To develop a complete model of pigments, another optimization process involved choosing a group of pigments which could represent the taxonomic groups known to be present. As the goal was the use of pigments as taxonomic biomarkers, criteria for choosing an effective biomarker were considered including: 1) given the history of water quality in Årungen and subsequent likelihood of degradation of pigments, which pigments that were likely to have been present in the water column were also stable and resistant to degradation and therefore likely to be present in the sedimentary record; 2) which pigments could represent as few taxonomic groups as possible thereby being as specific as possible in indicating the presence and magnitude of each taxonomic group; 3) which combination of pigments would produce as few zero values as possible. Initially, a number of pigments were chosen that fulfilled criteria 1) and 2) above, based primarily on two papers discussing the use of pigments in sediments as biomarkers (Leavitt, 1993; Reuss, 2005); see especially Table 1 in Leavitt (1993) and Table 1 in Reuss, (2005). The number of pigments that could possibly be present in the sediment record was relatively large. However, fewer variables reduces uncertainty and error in a numerical or statistical model. To fulfill criterion 3) I conducted an iterative optimization process where each combination of pigment GPS values and the spectroscopic data were run in R-program, where all possible combinations of pigments that fulfilled criteria 1) and 2) were calculated that were in accordance with restrictions and recommendations in applying the methods, as discussed by Küpper et al. (2000, 2007). I used a backwards stepwise selection process, where all initially chosen pigments were entered into the model and removed or replaced depending on results. All results were compared to determine the optimal combination of pigments.

Table 2: Molar and mass absorptivity (absorption coefficients) for pigments used in the numerically analysis.

Pigment	molar absorptivity (cm-mM)-1 (3)	molecular weight (g/mol)	mass absorptivity (L/g*cm) *	Maximum wavelength (nm) associated with absorptivity
Mg-Chl a	82	893.5	91.8	662.1 (1)
Mg-Chl b	49	907.49	54.0	645.5 (1)
pheophytin.a	46.5	871.21	53.4	665.5 (1)
pheophytin.b	31	885.2	35.0	653.5 (1)
b.carotene	134	536.9	249.6	454 (1)
canthanxin	NA	564.84	220 (2)	469 (2)
diatoxanthin	119	272	437.5	453 (2)
echinenone	116	550.86	210.6	472 (1)
fucoxanthin	69.7	658.91	166 (2)	443 (2)
lutein	145	568.871	254.9	474 (1)
myxoxanthophyll	158	731	216.1	NA

* Mass absorptivity values are calculated unless otherwise noted.

Sources: (1) Jeffrey et al., 2007; (2) Roy et al, 2011; (3) Table 1, Kupper et al., 2007

Results

Sediment core

Sampling and processing

There was approximately 15 cm of ice covering the lake. Two holes were drilled approximately 50 cm apart from each other from which sediment cores were taken where the depth of water was 72 cm. Photos of the long core show clearly distinguishable alternating grey and black varves that vary in texture and depth. The grey varves had a silty texture, whereas the black varves had a muddier texture and stronger odor, presumably because these layers had higher levels of organic matter, were more anoxic and had higher rates of methanogenesis. A systematic examination of the changes in color at specific depths was unfortunately not undertaken before the cores were processed.

The short core was sectioned into 1 cm layers resulting in a total of 45 samples. Sub-samples were taken to be used submitted for ^{137}Cs analysis from all 45 samples. The bottom 4 cm were slightly damaged and were therefore discarded leaving 41 samples that were analyzed for absorbance, pigment analysis and heavy metals. As the short core provided greater resolution at 1 cm sample intervals, and the events of interest were assumed to have occurred during the period in which the short core was deposited, it was decided that only the 41 samples from the short core would be analyzed for metals. The 100 cm long core was sectioned into 3 cm layers resulting in 33 samples. The top 1 cm of the long core was discarded. A total of 74 samples were obtained from the two cores which were analyzed for % OM, % water content, and absorbance. The two sediment cores overlapped between 22 and 41 cm depth. In order to assemble the results from the two overlapping cores into a composite record of changes over the entire depth, some of the overlapping samples would have to be discarded. Of the overlapping samples, 19 were from the short core (22-41 cm) and seven were from the long core (22-40 cm). To provide better resolution, and utilize as much sample material as possible, the seven samples from the long core were not used in the analysis of the composite core, leaving a total of 67 samples that make up the composite sediment core.

A number of miscellaneous observations were made while samples were being processed. During sample processing, pieces of plants and insects were observed in the sediment materials including stems ranging in size from approximately 1-2 mm in diameter by up to 20 mm long, leaf fragments ranging in area from approximately 4-10 $mm^{2,}$ and what appeared to be beetle wings approximately 5 mm long by 2 mm wide at 76 cm depth. Unfortunately these observations were not systematic.

Percent mass water and dry and organic matter

The percent of water and dry matter were relatively constant and evenly split between the bottom of the core to 18 cm depth, varying between 40 and 60 % around an average of 50 %. From 17 cm depth the percent of water increased 88.5 % at 1 cm below the sediment-water interface. The % OM varied with depth, showing three obvious trends over depth. From 6 % OM at 118 cm, OM increased gradually to 11.1 % at a depth of 49 cm. The % OM decreased to 8.4 % at 46 cm and remained relatively stable with moderate fluctuations around 8 % OM up to a depth of 13 cm. At 12 cm depth, the % OM increases rapidly to 12.8 % at a depth of 2 cm, and decreases to 9.3 % OM in the top 1 cm of sediment.

<u>Cesium dating</u>

The peak ^{137}Cs DPM value of 170 occurred at 31 cm depth. Setting this depth to the year 1986 when the Chernobyl event occurred, there is a difference of 27 years between 2013 and 1986, making Y/SD_{1cm} equal to 0.871 years/cm, almost equal to the 0.89 mm calculated by Skogheim and Erlandsen (1984). The short core ended at 41 cm which, after subtracting 0.871 from 2013 and each calculated year thereafter, is equivalent to the year 1977. The long core began at 43 cm. Multiplying 0.871 by 2 cm, the difference between 41 and 43 cm, sets 43 cm depth equivalent to the year 1976. From there, each sample layer was 3 cm in depth. Multiplying $Y/SD_{1\,cm}$ by 3 results in a $YP/SD_{3\,cm,\,long\,core}$ of 2.613. Subtracting 2.613 from 1976 and each calculated year thereafter puts the year 1953 between 67 cm and 70 cm depth. From 70 cm depth, the YP/SD was adjusted to take into account the decreased sedimentation rate estimated by Skogheim and Erlandsen (1984) of 3.4 mm/year occurring prior to 1954 by multiplying $YP/SD_{3\,cm,\,long\,core}$ by the proportionality constant of 0.38, resulting in $YP/SD_{3\,cm,\,long\,core,\,pre\text{-}1954}$ equal to 1.00. This was subtracted from each year beginning from 1953, putting the final depth of 118 cm equal to the year 1938

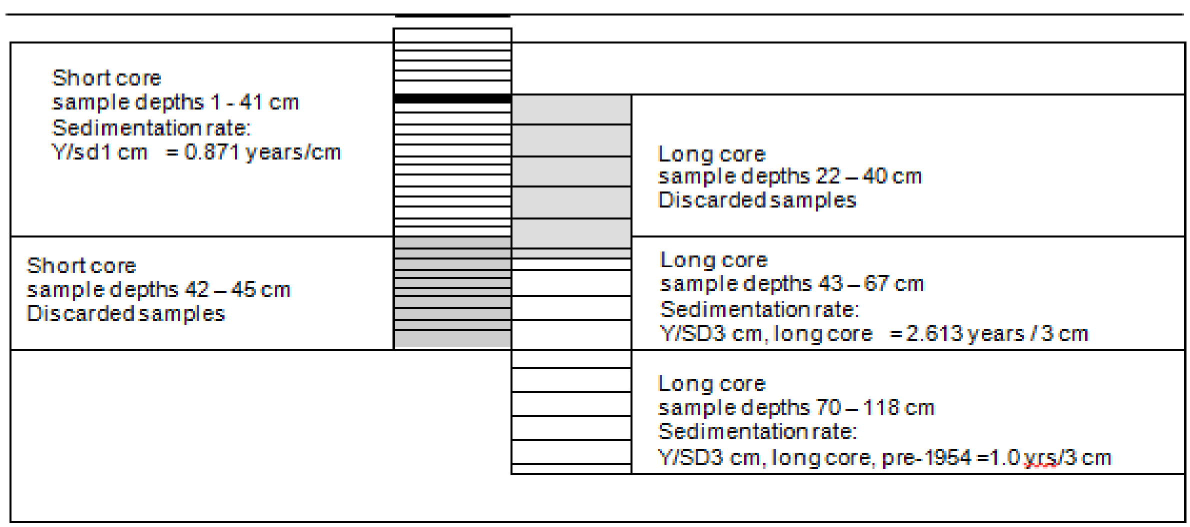


Figure 9: Schematic of short and long cores showing calculations for sedimentation rates and discarded samples.

Absorbance

Absorbance values for each wavelength between 350 and 900 nm for each of the 74 samples were obtained from the spectroscopic analysis. There were two primary absorbance peaks. The largest peak occurred between 403 and 424 nm with λ ranging between 0.378 and 1.6489. As λ declined in value, numerous smaller peaks occurred between approximately 425 and 535 nm. Values for λ decreased between 540 nm and 640 nm, forming a trough between the two major absorbance peaks. The second peak occurred between 600 and 666 nm, with absorbance values ranging from 0.0797 and 0.4118. Numerous smaller peaks occurred between approximately 425 and 535 nm in nearly all 74 samples. A small but distinct peak is visible at sediment depths between 5 - 8 cm and 12 - 13 cm, occurring between wavelengths ranging from 363 to 378 nm with absorbance ranging from 0.872 to 1.1906. The absorbance data is presented below in two formats: 1) the average, minimum and maximum values of absorbance for each sediment depth were calculated and plotted against wavelength; 2) the average, minimum and maximum values of absorbance for each wavelength were calculated and plotted against sediment depth.

A total of 16 samples required dilution. Even after dilution, the absorbance values for some samples peaked at > 1.5. As long as the peak was visible on the plot and therefore below the maximum detection level and absorbance was < 2.0, the results were considered acceptable and the sample was not further diluted.

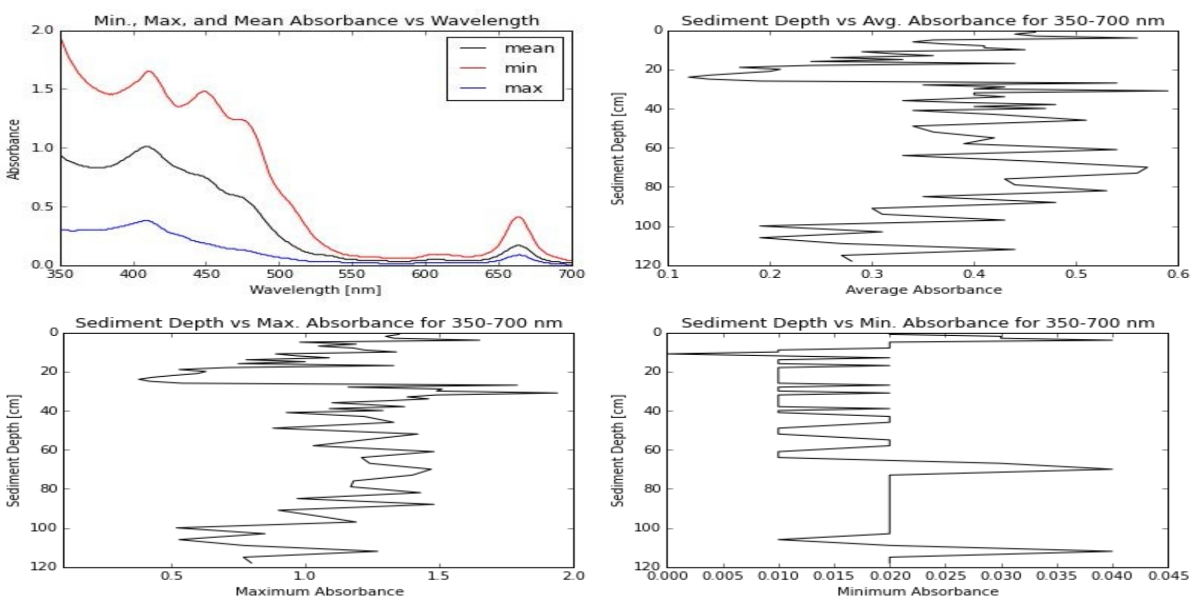


Figure 10: Upper left: the average, minimum and maximum values of absorbance for each sediment depth vs wavelength; upper right: average absorbance/wavelength vs sediment depth; lower left: maximum absorbance/wavelength vs sediment depth; lower right: minimum absorbance/wavelength vs sediment depth.

The initial group of pigments chosen for modeling included 11 pigments representing a variety of aquatic organisms. After numerous iterations, the final model included seven pigments. Both the initial and final groups of pigments are shown below.

Table 3: Pigments included in the initial model and final model of the numerical analysis using the Kupper method. Modified after Table 1 from Reuss (2005) after Leavitt and Hodgsen (2001) and Jeffrey et al. (1997).

Initial model	Taxonomic Representation	Stability	Final model
chlorophyll.a	all photosynthetic algae, higher plants	3	chlorophyll.a
chlorophyll.b	green algae, euglenophytes, higher plants	2	chlorophyll.b
pheophytin.a	chl a derivative	1	pheophytin.a
pheophytin.b	chl b derivative	2	pheophytin.b
echinenone	cyanobacteria	1	echinenone
fucoxanthin	diatoms, prymnesiophytes, chrysophytes, raphidophytes, some dinoflagellates	2	fucoxanthin
lutein	green algae, euglenophytes, higher plants	1	lutein
b.carotene	most algae and plants	1	
diatoxanthin	diatoms, dinoflagellates, chrysophytes	2	
canthaxanthin	colonial bacteria	1	
myxoxanthophyll	colonial bacteria	NA	

After summing results for chlorophyll a and b and pheophytin a and b (chl.a + pheo.a, chl.b + pheo.b), which reduces the number of pigments from seven to five, the results from the final model of pigments show similar trends. All of the groups have relatively low concentrations and between the bottom depth of 118 cm up to the 22 cm, although echinenone and lutein values increase significantly between 88 and 43 cm depth and then rapidly decrease back to the previously low levels. All pigment values increase significantly from approximately 19 cm depth and reach a maximum level between from 18 cm depth until 7 cm, at which point all the pigment values decrease rapidly at approximately 6 cm depth, after which there is another increase and slight decrease at the sediment-water interface at 1 cm depth.

Heavy Metals – Cu, Cd, Zn, Pb, Cr, Mn

The results from ICP analysis of metals in the top 40 cm of the sediment core show several interesting trends. Between 40 and 27 cm depth, the changes in concentrations of Cd, Cu, Zn and Pb over time are very similar and show variability but no significant change in concentration until 28 cm depth.

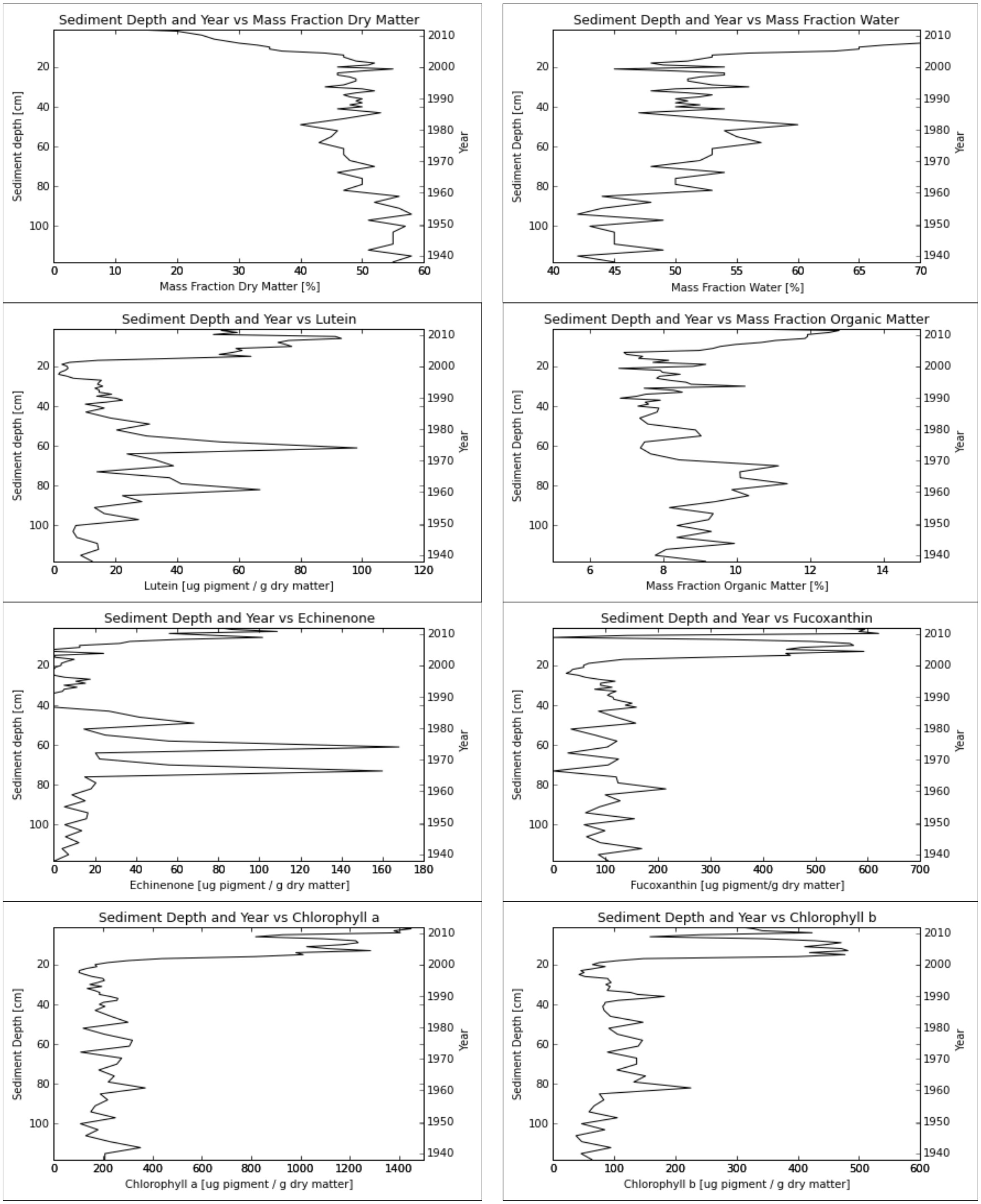


Figure 11: Plots of sediment depth (cm) on the left y-axis and year on the right y-axis vs % dry matter, water, and organic matter and the pigments lutein, echinenone, fucoxanthin, chlorophyll a + pheophytin a, and chlorophyll b + pheophytin b in ug pigment/g dry sediment.

Table 4: Pollution classes for sediments for total Cd, Cu and Zn. Sources: 1) Attachment 14 in Lund, Johansen and Thygesen, 2013; 2) Norwegian Ministry for Climate and Environment, 2012.

Metal	Class I	Class II	Class III	Class IV	Class V
(mg/kg)	Background	Good	Moderate	Bad	Very Bad
Copper	20	84	84	147	>147
Cadmium (hard water)	0.2	2.5	16	160	>160
Cadmium (soft water)	0.2	1.5	16	160	>160
Zinc	90	139	340	2600	>2600
Lead	25	66	NA	NA	NA
Chrome	60	90	NA	NA	NA

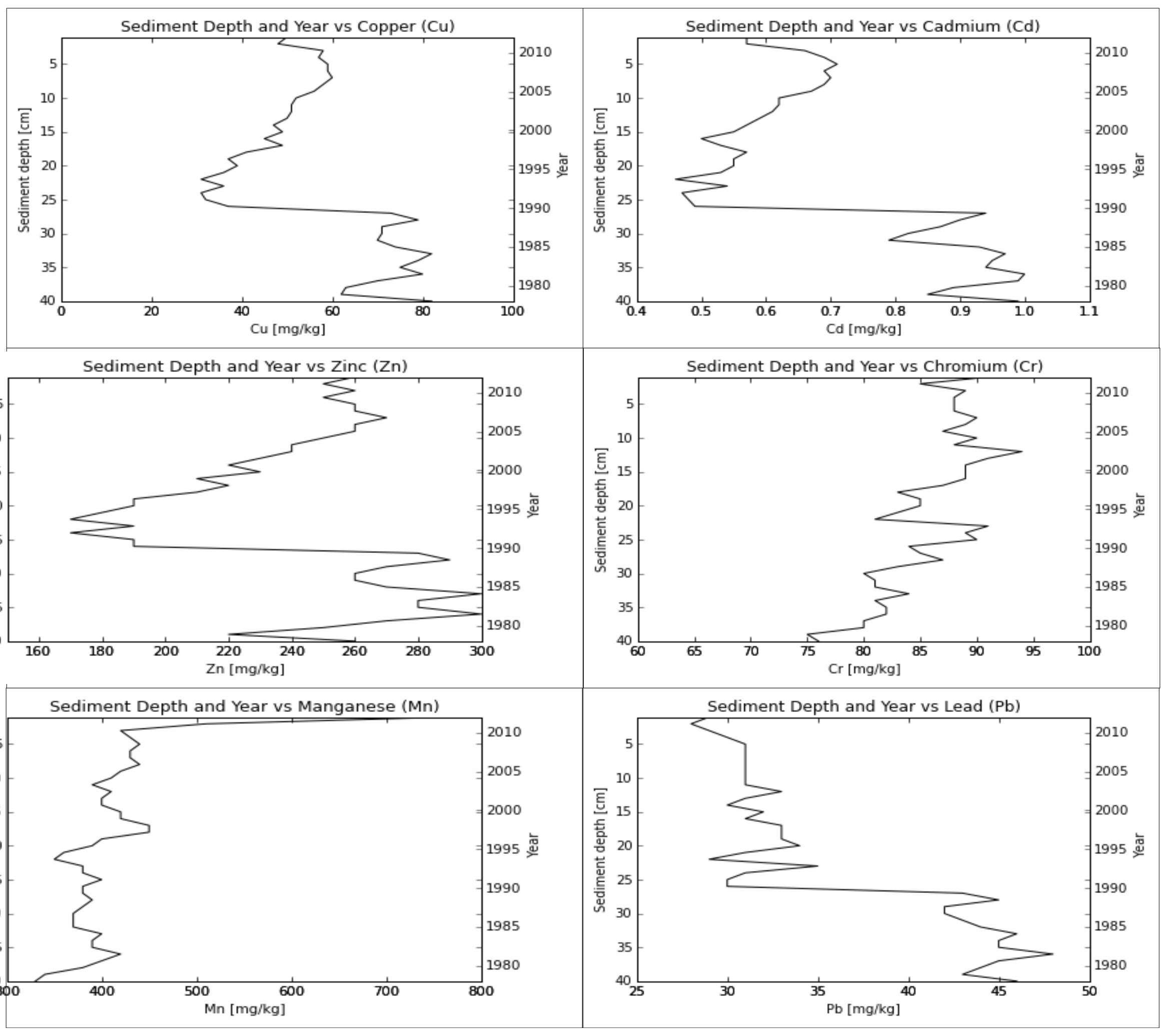


Figure 12: Plots of sediment depth (cm) on the left y-axis, and year on the right axis vs total Cu, Cd, Zn, Pb, Cr, and Mn in (mg/kg).

Between 28 and 27 cm, the concentrations of Cd, Cu, Zn and Pb decrease by approximately 30 %. From 27 to 7 cm depth the concentrations of Cu, Cd and Zn increase 40 to 60 % until 7 cm depth, after which they are stable for approximately 4 cm of depth, and begin to decline again. The concentration of Pb varies noticeably between 26 and 19 cm depth, after which it steadily declines. Chromium and Mn show somewhat different trends, with Cr showing variable but constantly increasing concentration over time from 40 cm depth until the concentration decreases noticeably between 23 cm and 22 cm depth, after which it increases again until 10 cm depth, when it again begins to slowly decline.

E. canadensis surveys

Steinsfjorden, September, 2013

The results from the Steinsfjorden survey are observational and were made during the survey. According to Marit Mjelde, the distribution of E. canadensis in the lake had changed. In the past it had been present in dense mats in some of the shallower areas, particularly along the southwestern shoreline (Viksvik, Steinsvik) and along the eastern shore (Elvika, Torsrud). Aside from small fragments it was no longer present in these locations. It was still found in profuse amounts in deeper areas (3 to 4 m) such as the west side of Herøya. The endangered aquatic plant *Najas flexilis,* which had disappeared from shallower areas after been outcompeted by *E. canadensis* in shallower areas, was again present albeit in small numbers.

Årungen, September 2013

The results from the Årungen survey are observational and were made during the survey. I saw no sign of *E. canadensis* in the parts of Årungen that were surveyed. Most of the lake bottom that was observed was completely free of vegetation, with the exception of those areas directly adjacent to wetland areas and in the littoral zones, where there were patchy areas of *Potamogetan natans* (broad-leafed pondweed), *Nymphaea species* (water lily) and *Polygonum amphibium* (knotweed). Mats of algae on the lake bottom were frequently observed, as were freshwater mussel shells (dead) in depths greater than approximately 1.5 m.

Stream and sediment sampling: Årungen watershed, April and November 2014

The results of the analysis of Cu, Cd and Zn from stream, sediment and *E. canadensis* samples taken from locations in Smedbølbekken, Brønnerudbekken, Vollebekken, Fagernes sedimentation pond during spring and fall rainfall events provide an interesting comparison of potential sources of metals between low and high flows, and upstream and downstream locations. Low levels of Cd were measured in all samples. Copper levels were significantly higher in the majority of samples. Zinc levels were high or very high in many of the samples. There were noticeable differences in levels of metals between streams, between low and high flows, and between upstream and downstream measurements. The highest level reported occurred in the CSO high flow for Zn.

On 5.April, 1.2 mm of rain fell between 16:00 and 18:00. Soils were saturated prior to rainfall, which would have decreased infiltration and increased runoff. Stream discharge in all streams prior to rainfall beginning was low to moderate. After rainfall began, discharge increased somewhat significantly in Smedbølbekken, and Vollebekken, Flows in Brønnerudbekken were much higher after rainfall. At the culvert below Osloveien (V2), the depth of pipe-flow in the culvert at low flow was approximately 10% of the diameter and velocity was low. After rainfall began, the depth of pipe-flow was approximately 60% and water was gushing out of the pipe, i.e. high velocity flow. Conditions were similar at Fagernes sedimentation pond, although to a much lesser degree. The level of pipe-flow was lower and the apparent velocity much lower. The culvert at Brønnerud is much larger in diameter than the pipe outlet at Fagernes sedimentation pond, allowing much larger volumes of water to be transported.

On 9.November, there was very little precipitation which took place in the morning. Soils were saturated from previous rainfall. Streamflow was low to moderate. The depth of pipeflow in the culvert carrying stormwater discharge was approximately 0.1% of the diameter. On 10.November 8.2 mm of rain fell between 10:00 and 20:00. The resulting streamflow was very signficant. The depth of pipeflow in the drainge pipe had increased to approximately 10%, of the diameter, and was flowing at very high velocity. The depth of flow in Vollebekken had increased by approximately 20 cm. The high flow discharge from the stormwater pipe into Vollebekken could definitely be classified as a CSO discharge, given that pumps in the nearby pumphouse were running, pipes exiting from the pumphouse were discharging at high velocity, and the odor of sewage was omnipresent.

Table 5: Pollution classes for freshwater for total Cd, Cu and Zn. Sources: 1) Attachment 14 in Lund Johansen and Thygesen, 2013; 2) Norwegian Ministry for Climate and Environment, 2012.

Metal	Class I	Class II	Class III	Class IV	Class V
(ug/l)	Background	Good	Moderate	Bad	Very Bad
Copper	0.3	0.3 – 7.8	7.8	7.8 – 78	>78
Cadmium (hard water)	0.03	0.03 - 0.19	0.19 – 1.5	1.5 – 15	>15
Cadmium (soft water)	0.03	0.03 – 0.08	0.08 – 0.45	0.45 – 4.5	>4.5
Zinc	1.5	1.5 – 11	11	11 – 60	>60

Smedbølbekken, 5.April 2014

There were low levels of all three metals in samples taken during low and high flows from the two locations along Smedbølbekken.

Brønnerudbekken, 5.April 2014

Values of Cu and Zn from Site (B1) during low flow and high flow were nearly identical. Copper levels at low and high flows were 15 ug/l and 13 ug/l respectively (class IV); Zn levels at low and high flows were 74 ug/l and 73 ug/l respectively. At Site (B2) the Cu concentration during low flow 6.9 ug/l and the Zn concentration was 18 ug/l (class IV). A sample was taken from low flow discharge from a culvert (diameter = 140 cm) that intersects Brønnerudbekken downstream from the road and from the inlet from Fagernes sedimentation pond (B3). At this location there were 3.9 ug/l Cu and 2.1 ug/l Zn.

Fagernes sedimentation pond, 5.April 2014

The discharge entering Fagernes sedimentation pond (F) was sampled during low and high flows. During low flow, 8.6 ug/l Cu and 17 ug/l Zn were measured, and during high flow 11 ug/l Cu and 28 ug/l Zn were measured. All four measurements fall into class IV.

Vollebekken, 5.April 2014

Discharge was sampled where Vollebekken enters a culvert near the muck tank at the NMBU summer barn (V1). During low flow, 12 ug/l Cu (class IV) and 91 ug/l Zn (class V) were measured. The concentrations were lower during high flow, with 8.1 ug/l Cu and 50 ug/l Zn, both of which fall into class IV. A sample was taken during low flow from a pipe discharging into the stream at the culvert (V2). The pipe carries discharge from a nearby manhole into the stream. Concentrations were almost identical to the low flow concentration, with 12 ug/l Cu and 90 ug/l Zn.

Vollebekken 9-10 November

On Sunday the 9^{th} of November, 2014 I took grab samples from Vollebekken where a combined sewer overflow (CSO) enters the stream near a pump-house operated by Ås municipality. At this point, the stream receives inputs from two CSO systems, one entering the stream at the sampling location and another located upstream located across from the main entrance to NMBU on the south side of road at 52 Drøbakveien that enters Vollebekken via piped flow. All Cd values were low except during high flow from the CSO (V5), which was 0.4 ug/l (class III). All low flow Cu values were low (3.1 – 6.7 ug/l) while all high flow Cu values were 'bad' (class IV) and ranged from 11 – 38 ug/l. Low flow values for Zn were 11 and 16 ug/l, or 'bad' (class IV). High flow values for Zn included one 'bad' class IV value of 30 ug/l, and two class V values, 75 and 210 ug/l. The last and highest Zn value was measured in the CSO.

Table 6: Total Cd, Cu and Zn concentrations in ug/l taken on April 5 and November 9 – 10 along with location codes, date and time of sampling, and flow regime. The results from the analysis of sediments from Fagernes sedimentation pond and the agricultural drainage pipe; and from the roots, and leaves, shoots and stems of *E. canadensis* are also shown, where classes are based on standards for sediments, which are significantly higher than for water. See also the table of classes for sediments presented earlier with sediment metals.

Stream Name	Location Code	Date and Time	Low Flow = (0) High Flow = (1)	Cd (ug/l)	Cu (ug/l)	Zn (ug/l)
Smedbøllbekken	S1	5.April 1840	0	0.01	4.5	4
	S2	5.April 1824	0	0.01	2.6	2.4
Brønnerudbekken	B1	5.April 1545	0	0.087	13	73
	B1	5.April 1850	1	0.024	15	74
	B2	5.April 1530	0	0.059	6.9	18
Fagernes sedimentation	F	5.April 1445	0	0.035	8.6	17
	F	5.April 1745	1	0.01	11	28
Sedi ment	***F***	***9.Nov 1730***	***NA***	***0.73***	***50***	***230***
leaves, shoots,	***F***	***5.April 1400***	***NA***	***3.3***	***53***	***520***
roots	***F***	***5.April 1400***	***NA***	***2***	***57***	***210***
Vollebekken	V1	5.April 1500	0	0.12	12	91
	V1	5.April 1915	1	0.07	8.1	50
	V2	5.April 1500	0	0.041	12	90
	V3	9.Nov 1545	0	0.084	3.1	27
Sedi ment	***V3***	***9.Nov 1545***	***NA***	***0.18***	***5.1***	***62***
	V4	10.Nov 2145	1	0.042	11	30
	V5	9.Nov 1630	0	< 0.01	5.4	11
	V5	10.Nov 2220	1	0.4	38	210
	V6	9.Nov 1715	0	0.031	6.7	16
	V6	10.Nov 2250	1	0.097	35	75

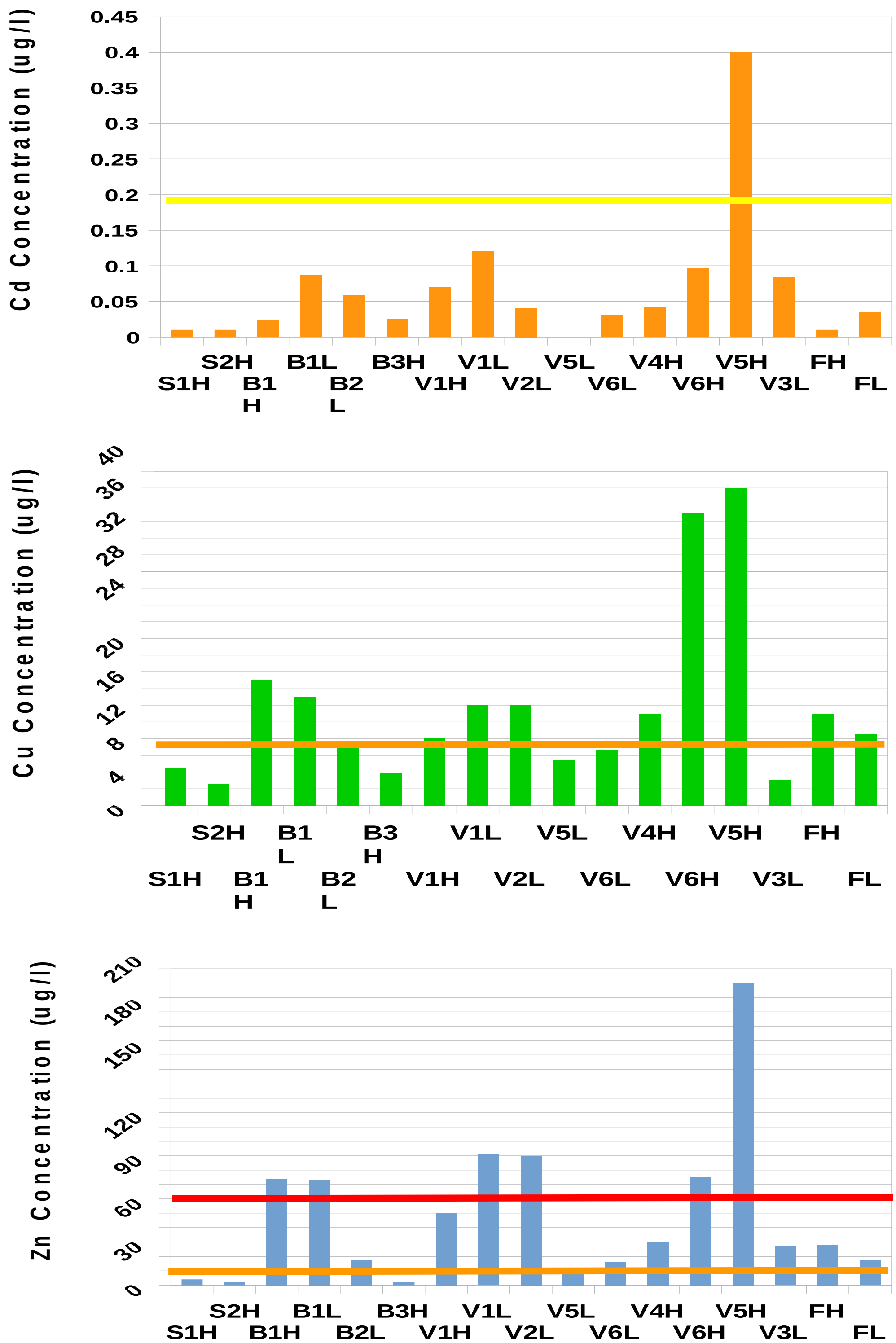


Figure 13: Bar graphs showing total Cd, Cu and Zn concentrations in ug/l taken on April 5 and November 9 – 10. Location codes are shown on the x-axis. Lower limits for classes III, IV and V for the different metals are shown as horizontal lines in corresponding colors (yellow=class III, orange=class IV, and red=class V).

Discussion

Introduction and establishment of *E. canadensis* in Årungen

Values of pigments and metals measured in the sediment core show changes that can be associated with the introduction of *E. canadensis*. Between 1989 and 1990, there were sharp declines in the concentrations of Cu, Cd, Zn and Pb and noticeable decline in concentrations of all sediment pigments. The decrease in all these variables continued with some small variation until 1994, when levels of the chlorophylls and pheophytins began to increase, followed by fucoxanthin in 1995, echinenone in 1996 and lutein in 1998. Metals concentrations began to increase beginning with Cd in 1995, followed by increases in Mn in 1996, and Cu and Zn increases in 1997.
Lead continued to decline over time with some year to year variation. The general trend for Cr appears to be a general albeit slow increase over time with much year to year variation. These changes in concentrations occurred at the same time as the E6 expansion (1989 – 1995) and the construction of the new rowing pier at the south end of Årungen (1989 - 1992). In 1991, *E. canadensis* was first reported present in Årungen. Given the small size of the Årungen watershed, and the fact that *E. canadensis* has not been observed elsewhere in the watershed prior to 1991, it seems very probable that it was introduced as a hitchhiker on construction equipment into Årungen during the construction of the rowing pier.

Prior to 1989 and the start of construction on E6, the concentrations of metals reach the following maximum levels: 1.00 mg/kg Cd in 1982, and 82 mg/kg Cu and 300 mg/kg Zn in 1984, all of which are higher than concentrations from 2012. The traffic on the original E6, now Osloveien, which runs next to Årungen, was likely the primary source of these metals. After the start of construction, concentrations dropped between 1989 and 1990 as follows: 0.94 to 0.49 mg/kg Cd, 73 to 37 mg/kg Cu, and 280 to 190 mg/kg Zn. With the construction of E6, once the south bound lanes were completed early in the 1990's, traffic was rerouted from old E6 (Osloveien) to the southbound lanes while construction continued on the northbound lanes (Skari, 2002). This decreased the AADT to such a degree that the levels of Cd, Cu, Zn and Pb entering Årungen dramatically declined. The completion of the entire section of new E6 from Vestby to Vassum including Nordbytunnel was completed in 1995. This corresponds to a second drop in the concentrations of all metals except Cd, which actually increased slightly. In 1993 there were increases in the concentrations of Cu, Zn, Pb and especially Cr for reasons that are unexplained.

The construction of the new pier at Molteberget combined with construction activities and runoff from soil depots at Fosterud may have disrupted the lifecycles of plankton and algae and, to a lesser degree, higher plants. During the period 1989 – 1998 the decrease in pigments was most pronounced for echinenone, which decreased from 17.7 ug/g in 1989 to 5.3 ug/g in 1990, and was not detectable between 1992 and 1994; and lutein, which decreased from 15.4 ug/g in 1989 to 6.3 ug/g in 1990, and reached a low in 1992 of 1.5 ug/g. Echinenone is a biomarkers for cyanobacteria, and lutein is a biomarker for green algae, euglenophytes, and higher plants. Fucoxanthin, a biomarker for many groups including diatoms, prymnesiophytes, chrysophytes, raphidophytes, and some dinoflagellates, declined from 85.9 ug/g 1989 to 61.6 ug/g in 1990, and reached a minimum concentration of 24.97 ug/g in 1992. The chlorophylls and pheophytins were the least affected groups. Chlorophyll a + pheophytin a, a biomarker for all photosynthetic algae, and higher plants, declined from 199 ug/g in 1989 to 155 ug/g in 1990, and reached a low of 101.9 ug/g in 1992. Chlorophyll b + pheophytin b, a biomarker for green algae, euglenophytes, and higher plants, declined from 89.3 ug/g in 1989 to 51.8 ug/g in 1990, and reached a low of 42.4 ug/g in 1991. It would seem that cyanobacteria and other forms of photosynthetic plankton suffered the most during the construction of E6, and higher plants were least affected. A decline in the percentage of organic matter occurs during this same period, which would be consistent with a decline in plant and plankton populations. The introduction of a highly invasive plant such as *E. canadensis* during this time period would have provided an excellent opportunity, when competition from other plants would have been at a low. That the levels of metals during this period were also at a low must have been an advantage as well.

After *E. canadensis* was introduced, it rapidly spread around the perimeter of the lake up to a depth of approximately 2 m. It was never observed in the middle of the lake or in deeper areas. The areas of the lake with the densest growth and largest surface area of biomass were in the vicinity of the inlets from the perennial streams: Brønnerud-Vollebekken, Smedbølbekken, Storgrava, Norderås, Syverud, and Bølstadbekken. The northern shore in the vicinity of the outlet of the lake, Årungselva, was also infested. The density and surface area waxed and waned over the years, with noticeable increases in 1993, 1996 and 1999, and declines in 1995 and from 2002 onward until it disappeared. All pigments except echinenone begin to increase rapidly from approximately 1996, the time period when *E. canadensis* was at its peak density in biomass. The concentration of echinenone begins a rapid increase in 2001. From approximately 1997 onward, Cu and Zn show significant increases. In 1998, Cd also begins to increase.

Chromium and Mn and to a great degree Pb are for the most part stable despite year to year variation and do not follow the trends of Cd, Cu and Zn. Chromium concentrations seems to be increasing over time. The concentrations of Mn are the most stable of all the metals, showing few increases or decreases until 2011 and 2012, when concentrations rapidly increase. That Cr and Mn concentrations did not decrease dramatically with other metals from 1989 to 1990 implies that the sources of these metals are independent of traffic and road runoff. The steady overall decline in Pb concentrations is a result of the ban on leaded gasoline. From approximately 2004 onward, Pb appears to have reached a stable minimum, although it shows a slight decrease in 2011 and 2012. The concentration of Pb in 1978 is 46 mg/kg, which is already at class II levels ('good'). Background sediment concentrations of Pb are 25 mg/kg. As of 2012, the concentration had dropped to 29 mg/kg.

The disappearance of *E. canadensis*

E. canadensis was last officially observed Årungen in 2008. The levels of Cd, Cu and Zn had been increasing since 1995. These metals reached maximum levels between 2006 and 2007 of 0.7 mg/kg Cd, 60 mg/kg Cu and 270 mg/kg Zn. Prior to the relocation of E6, the levels of Cd, Cu and Zn reached maximums of 1.0 mg/ kg Cd in 1982, and 82 mg/kg Cu and 300 mg/kg Zn in 1984. That the levels of these metals have increased since 1995 despite the fact that E6 was relocated away from Årungen has presumably been caused by the steady increase in traffic on E6, which in turn increases the amounts of metals and other pollutants on the road surface and on surfaces adjacent to the road. Pollutants including metals are then entrained in road runoff and discharged into Brønnerudbekken and Vollebekken, as described earlier.

The levels of Cd, Cu and Zn in Fagernes sedimentation pond, where *E. canadensis* is also established and thriving, are 0.73 mg/kg Cd, 50 mg/kg Cu and 230 mg/kg Zn, similar to the concentrations measured in the sediment core from 2006 and 2007. Therefore, the concentrations of Cd, Cu and Zn from 2006 and 2007 do not seem adequately high to have killed off the entire population of *E. canadensis* in Årungen, although the population of *E. canadensis* was likely weakened by the continual exposure to high levels of metals and possibly other pollutants, which would have made it more susceptible.

A comparison of the concentrations of metals in the runoff entering Fagernes sedimentation pond vs the concentrations of runoff in Brønnerudbekken and Vollebekken show clear differences by orders of magnitude in the concentrations of metals. Because Fagernes sedimentation pond receives runoff from a relatively small area, and receives discharge from only one source, E6, the levels of Cd, Cu and Zn are less concentrated, and very likely there are fewer types of pollutants than there are in the discharge entering the SCB from Brønnerud-Vollebekken. Therefore, the combined discharge from multiple sources traveling at rapid velocities, i.e. in peak flows, greatly contributed to the disappearance of E. canadensis.

The story is not yet complete however. Repeated inputs of concentrated pollutants from multiple sources delivered in peak flows from 1995 and onward, as shown in the increase concentrations of Cd, Cu and Zn, very likely weakened the population of *E. canadensis* over time and contributed to its observed decline (Magnusson and Jacobsen, 2012). The extreme rainfall and rain-on-snow runoff events that occurred in 2006 (Hansen and Grimenes, 2007) , were nearly repeated again in 2007, when precipitation during the months of January, June, and July were nearly twice normal values for the return period of 1961-1990, contributing to an annual precipitation total that was 117 % of normal (Hansen and Grimenes, 2008), must have produced a large number of highly polluted extreme peak flows, as described by Gunnarsson (2007), that caused the final downfall of *E. canadensis* in Årungen.

Pigment concentrations decreased in 2008 and 2009 for chlorophyll a + pheophytin a, chlorophyll b + pheophytin b, and fucoxanthin, which is not a biomarker for higher plants, decreased to zero. The remains of dying and dead biomass probably took some time to be completely flushed from the system before a difference could be seen in pigment concentrations. In addition, *E. canadensis* can appear to be healthy and green for some time after senescence if it has taken up high concentrations of Cu (Küpper et al., 1998).

There is also the question of how the entire population of *E. canadensis* in Årungen disappeared. This can be answered by considering several factors. Lake circulation in Årungen can be assumed to flow from the south to the north, where the outlet is located. As peak flows from Brønnerud-Vollebekken and Smedbøllbekken enter the lake and flow north, other streams would be contributing to the total pollution load, albeit to a lesser degree. The turnover time of 4.5 months would allow pollutants time to be taken up by organisms. If flows were carrying high concentrations of road salts, pollutants would be more bioavailable. In addition, the growth of *E. canadensis* around the perimeter of the lake at the inlets of the streams would change the hydrodynamics of flow, essentially blocking flow from spreading throughout the lake, and in effect maintaining high concentrations of pollutants within the stands of *E. canadensis.* Flow would then spread laterally behind the stands of *E. canadensis* and parallel to the shoreline, exposing the perimeter of the lake to higher concentrations. The presence of submergent macrophytes is a significant variable affecting the hydrodynamics of lake flow, particularly when thick stands are located at stream inlets (Teeter et al., 2001).

Larger implications

Although the removal of an unwanted, invasive species from an ecosystem may initially seem positive, the causes are far from being a good thing, and have larger implications. It implies that higher plants and other organisms in Årungen, even plants such as *E. canadensis* that are relatively resilient to metals toxicity, are being severely impacted by urban and road runoff. That fucoxanthin concentrations also decreased in 2008 and 2009, which is a biomarker for diatoms, prymnesiophytes, chrysophytes, raphidophytes, and some dinoflagellates, implies that other taxonomic groups may also be affected. Likewise, other taxonomic groups may not be affected or be affected to a lesser degree. This appears to be the case in 2008 and 2009, when the levels of lutein increased significantly. After 2009, all pigment levels increased. Chlorophyll a + pheophytin a showed the most signficant increase which was sustained from 2010 to 2012, while levels of the other pigments decreased.

The influence of urban and road runoff on Årungen may be having significant ecological impacts that may be affecting competition between different groups by inferring a competitive advantage to species resilient to pollutants contained in runoff. The number of species of cyanobacteria in Årungen has increased in recent years (Romarheim G. Riise, 2009). This might be a result of the complex interplay between multiple sources of urban and road runoff being discharged during extreme peak events.

If this is occurring in Årungen, it is certainly occurring in other lakes. Results from the survey of *E. canadensis* in Oslo and Akershus in 2012 (Myrmæl, 2012) imply that it is. An examination of the results from the survey show that *E. canadensis* appears to have died out more often in those lakes located in urban areas and near large roads than in lakes located in isolated locations at a greater distance to urban areas and roads. This could be partially an artifact of surveying techniques at larger lakes which were not surveyed by boat but rather by walking the perimeter of the lake. There are also locations which regularly receive inputs of urban and road runoff with thriving populations of *E. canadensis,* such as Fagernes sedimentation pond which also has high levels of metals in both the leaves and roots. However, given time and a few peak flow events, these populations may disappear as well.

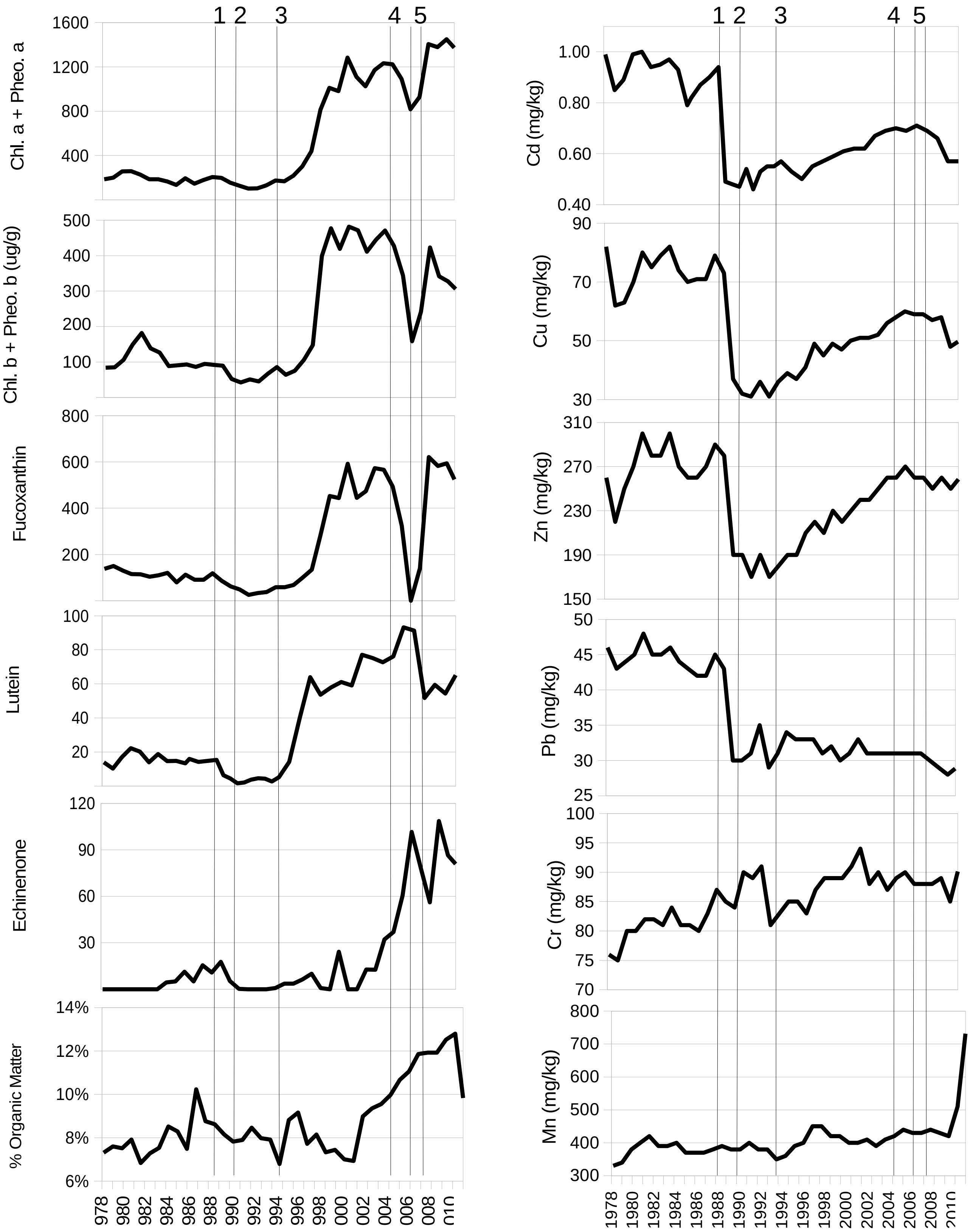


Figure 14: Plots of pigment concentrations in units of ug pigment/g dry sediment, and % organic matter on the left; sediment metal concentration on the right in mg metal/kg dry sediment. The number lines correspond to events: 1) 1989, beginning of construction on E6; 2) 1991, first observation of *E. canadensis*; 3) 1995, completion of entire E6 expansion; 4) 2006, extreme rainfall-runoff event; 5) disappearance of E. canadensis.

Conclusion and Recommendations for management

The introduction of *E. canadensis* into lake Årungen almost certainly occurred during the construction of a rowing pier, perhaps by a fragment of the plant accidently released from a piece of equipment used in an infested lake. The disappearance of *E. canadensis* appears to have occurred as a result of the combination of long term, continuous exposure to high levels of metals and perhaps other pollutants discharging into the lake from several urban and road stormwater drainge systems, as well as one combined stormwater and sewage system, with extreme rainfall and rain-on-snow runoff events causing extreme peak flows. The die out of *E. canadensis* was probably due to the synergistic combination of pollutants, as opposed to a single metal or other pollutant. The die out of *E. canadensis* to urban and road runoff and peak flows has larger ecological implications for lake Årungen as well as other lakes receiving urban and road runoff, including the potential to infer a competitive advantage to species that are more resistant to pollutants in urban and road runoff.

Once established, aquatic invasive species are virtually impossible to control without damaging the surrounding ecosystem. Therefore the most effective type of measure is preventive. I would therefore like to recommend the following courses of action:

1) More stringent restrictions regarding sterilizing boating, fishing, construction equipment etc. should be put in place and enforced in order to prevent introductions of both already established and not yet established invasive species. Such restrictions should be implemented at ports of entry such as border crossings, airports, marine boat harbors to prevent the introduction of costly invasive species that have not yet arrived in Norway.

2) Standard survey protocols should be developed for all five *Hydrocharitaceae* species that are similar in appearance: *E. canadensis, E. nuttalii, L. major, E. densa, and H. verticillata*. Some of these species are already present in Europe. They are all able to utilize a broad range of non-native habitats, and all have had considerable impacts in their invasive ranges (Hussner, 2012). They tolerate wide ranges of pH, nutrient and oxygen levels, and can rapidly establish dense mats of biomass that can fill the entire water column up in a wide range of depths up to depths of 12 meters (Nichols and Shaw, 1986), effectively out-competing other species (Mjelde et al., 2012; Kelly and Hawes, 2005). Surveying protocols should require surveying by boat in shallow and deep areas using a rake sampler and a bathyscope.

In the long term, the use of metabarcoding techniques to analyze water and sediment samples for the DNA of related species such as the invasive *Hydrocharitaceae* species would be very beneficial. It is conceivable that some of these species are already in Norway. Without large scale DNA tests, finding aquatic invasive species is difficult.

3) Freshwater and sedimentation classes for pollutants should be developed that consider the cumulative effects of multiple pollutants, i.e. synergistic effects.

4) Water quality surveying protocols should require regular monitoring of peak flows, especially resulting from intense spring rainfall and rain-on-snow events and after fall ploughing.

5) The discharges from agricultural drainage systems should be mapped and monitored. Locations and connections from other systems as well as discharge points into recipient water bodies should be mapped. Ideally, this information would be accessible in an online, GIS database, similar to Lavvannskart and Vegkart, which are excellent resources.

<u>How this paper could have been improved and future research</u>

There are many topics that did not get or could have received better coverage. The complete list is too long to present; here is the short list of things I would like to have done, addressed or improved on:

1) Use of statistics: This paper was a qualitative analysis of quantitative data, primarily because the sample size was one, that is one sediment core. To do a statistical analysis of the variables measured would have been an example of pseudoreplication (Hurlbert, 1984). Statistically correct or not, very similar work on a sample size of one has been statistically analyzed and published, see for example (Deshpande and Tremblay, 2014). It would have been interesting but expensive and logistically challenging to obtain an adequate sample size. A very interesting statistical project would be a landscape scale analysis of comparing lakes geographically isolated to urban lakes that are infested with *E. canadensis.*

2) Long distance atmospheric deposition plays a significant role in water quality. Addressing that issue in the context of the topic was beyond the scope of this study.

3) I would like to have measured absorbance data from samples of *E. canadensis* taken from Fagernes sedimentation pond and other lakes, and numerically analyze them for both pigments and metals, but there was not enough time.

4) This thesis would have been more complete with a discussion about the preference of *E. canadensis* for deeper waters, why E. canadensis never became established in the deeper areas of Årungen, and why the distribution in Steinsfjorden changed.

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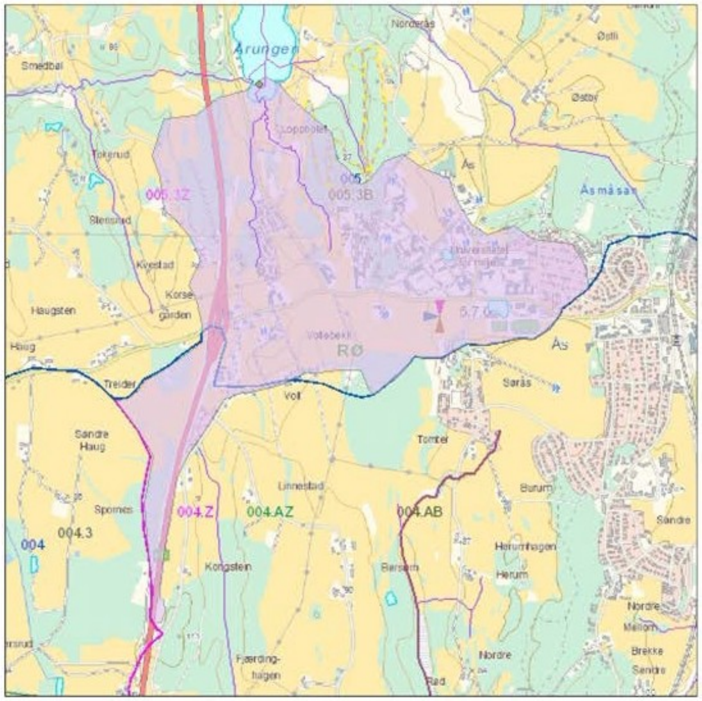
Appendix A

Watershed Map, Årungen (NVE)



Norges vassdrags- og energidirektorat

Kartbakgrunn: Statens Kartverk

Kartdatum: EUREF89 WGS84

Projeksjon: UTM 33N

Nedb0rfeltgrenser, feltparametere og vannf0ringsindekser er automatisk generert og kan inneholde feil. Resultatene ma kvalitetssikres.

Lawannskart

Vassdragsnr.: 005.3B

Kommune: As

Fylke: Akershus

Vassdrag: ARUNGELVA

Vannforingsindeks. se merknader

Middelvannforing (61-90)	15,6	lls/km'
Alminnelig lavvannforing	0.7	l/s/km'
5-persentil (hele Arel)	0,8	llslkm'
5-pcrscntil (1/5-30/9)	0,4	l/s/km'
5-persentil (1/10-30/4)	1,5	llslkm'
Base Oow	6,1	l/s/km'
BF!	0.4	

Klima

Klimnregion	Ost	
Arsnedbor	807	mm
Sonunernedoor	383	11\lll
Vintemedbor	424	mm
A rstemperatur	5,4	°C
Somrncrtcmpcralur	13,4	°C
Vintertemperatur	-0,4	°C
Tcmpcratur Juli	16,0	°C
Tempcratur August	14,9	°C

Feltparamecere

Areal (A)	3.3 km²
Effc 1iv sjo (S<1u	-999.0%
Elvclengde (El.)	1.4 km
Elvegradient <Eo>	18.Sm/km
Elvcgradic111 1095 (G1095)	23,7 m/km
Feltlengde(FJ	3,3 km
Hmin	34 moh.
11,0	55 moh.
l"20	63 moh.
Hw	73 moh.
H40	77 moh.
	80 moh.
!fro	83 moh.
1-l,0	86 moli.
!fro	90 moh.
Hoo	99 moh.
11,,,,,.	120 moh.
Bre	0,0%
Dyrket mark	40.3%
Myr	0,4%
Sjo	1,0%
Skog	23.5%
Snaufjcll	0,0%
Urban	6,6%

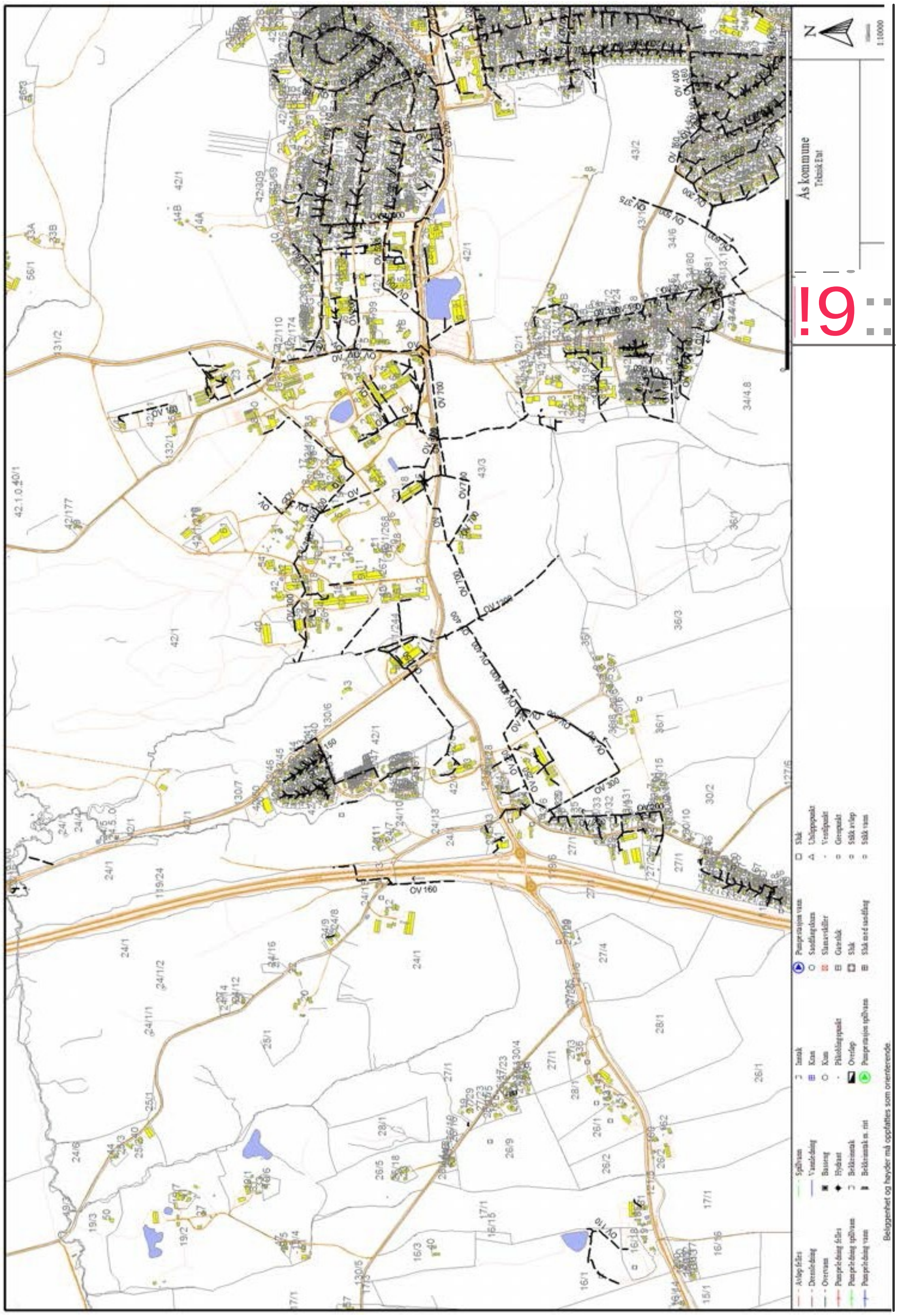
Det er generelt stor usikkerhet i beregninger av lavvannsindekser. Resultatene b0r verifiseres mot egne observasjoner eller sammenlignbare malestasjoner.

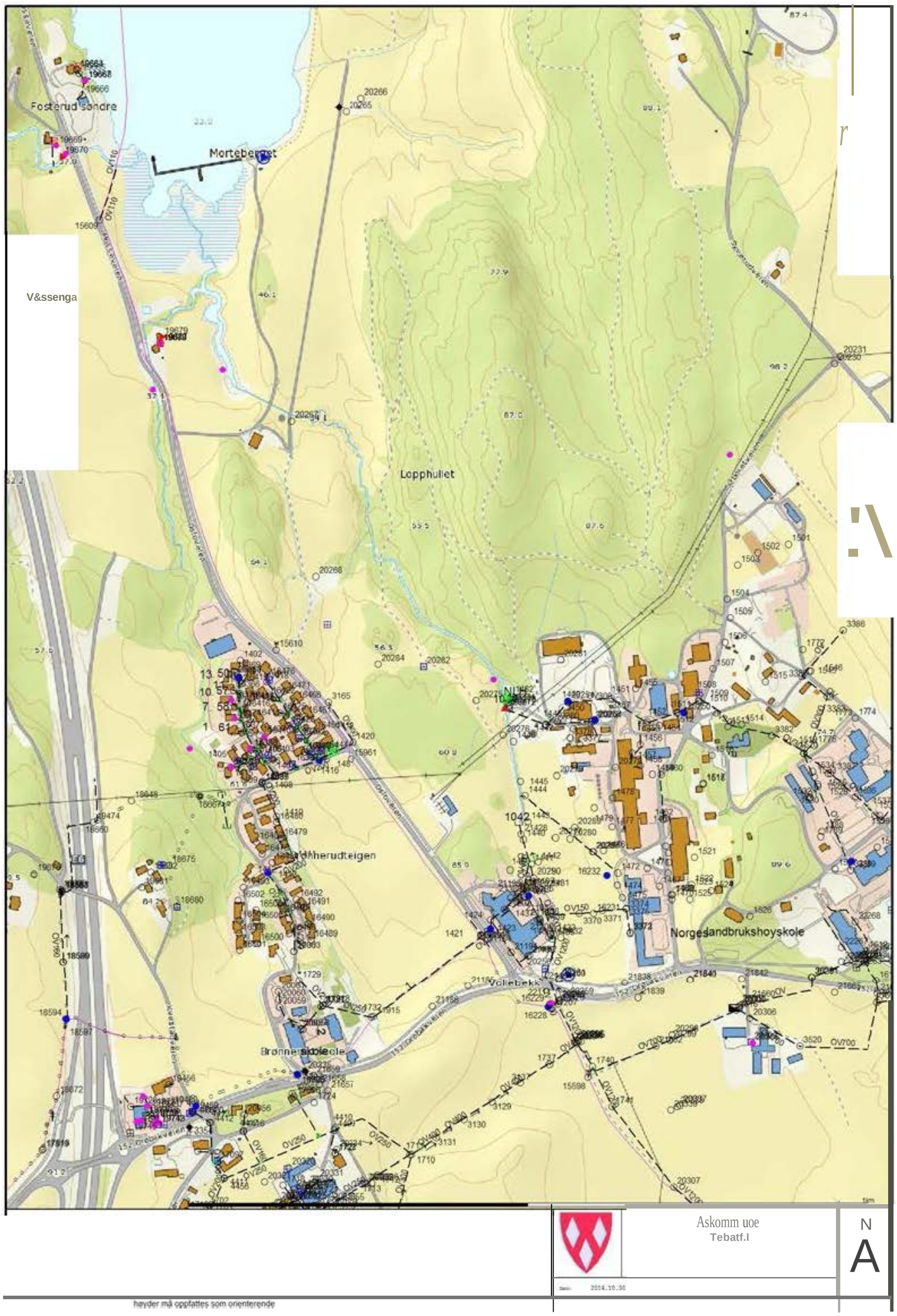
I nedb0rfelt med h0y breprosent eller stor innsj0prosent vil 0rrvrersavrenning (baseflow) ha store bidrag fra disse lagringsmagasinene.

De estimerte lavvannsindeksene i denne regionen er usikre. Spesielt gjelder dette 5-persentil (vinter) nar sj0prosenten er h0y.

Appendix B

Municipal Stormwater and
Combined Sewage Overflow
Ås Municipality





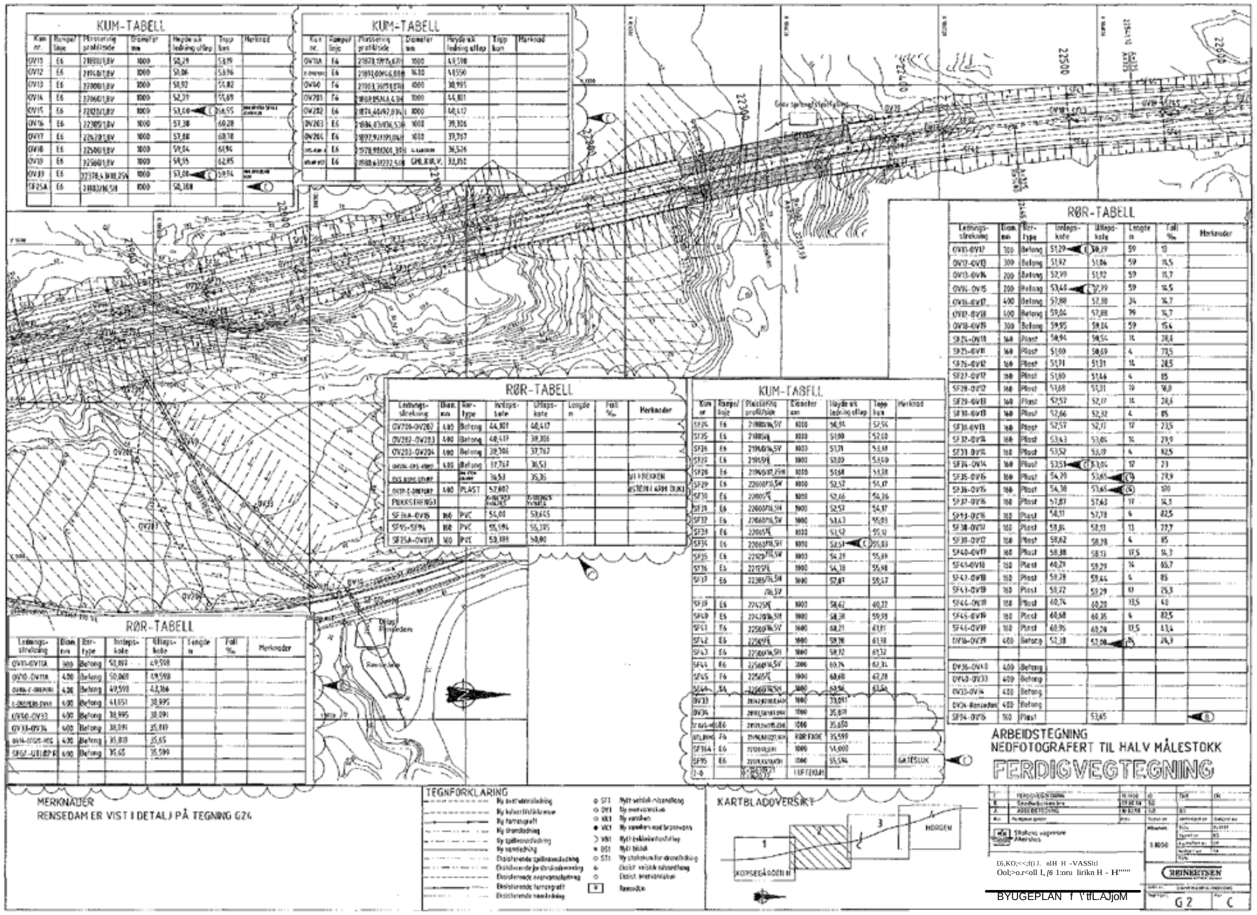
Fosterud søndre
Mortebergset
V&ssenga
Lopphullet
Norgesandbrukshoyskole
Vollebekk
Brønnerud skole
Askomm uoe
Tebatf.l
N
A
høyder må oppfattes som orienterende

Appendix C

Surface Water System, Årungen
Fagernes Sedimentation Pond Drawings

(Used with the permission of the Norwegian Public Roads Administration)


Statens vegvesen
VEGKART
BETA
Norderas
Fagernes
Ostby
Tokerud
Stensrud
Universitetet
for miljø-
og biovitenskap
Kvestad
Rustad
Korse-
gården
Haugsten
Treider
Voll
Ås
Landås
Beverdalen
Tomter
Haug
Linnestad
Burum
Kum
423 treff
Greift,apen
271 treff 36545 meter
Lukket rørgrøft
2 treff 60meter
Reirledning
Ingen treff
Pumpe
Pumpestasjon
1treff
Stikkrenne/Kulvert
58 treff
Klingenbi
BjGrnebekk


KUM-TABELL
KUM-TABELL
RØR-TABELL
RØR-TABELL
KUM-TABELL
RØR-TABELL
MERKNADER
RENSEDAM ER VIST I DETALJ PÅ TEGNING G24
TEGNFORKLARING
KARTBLADOVERSIKT
ARBEIDSTEGNING
NEDFOTOGRAFERT TIL HALV MÅLESTOKK
FERDIGVEGTEGNING
G 2
C

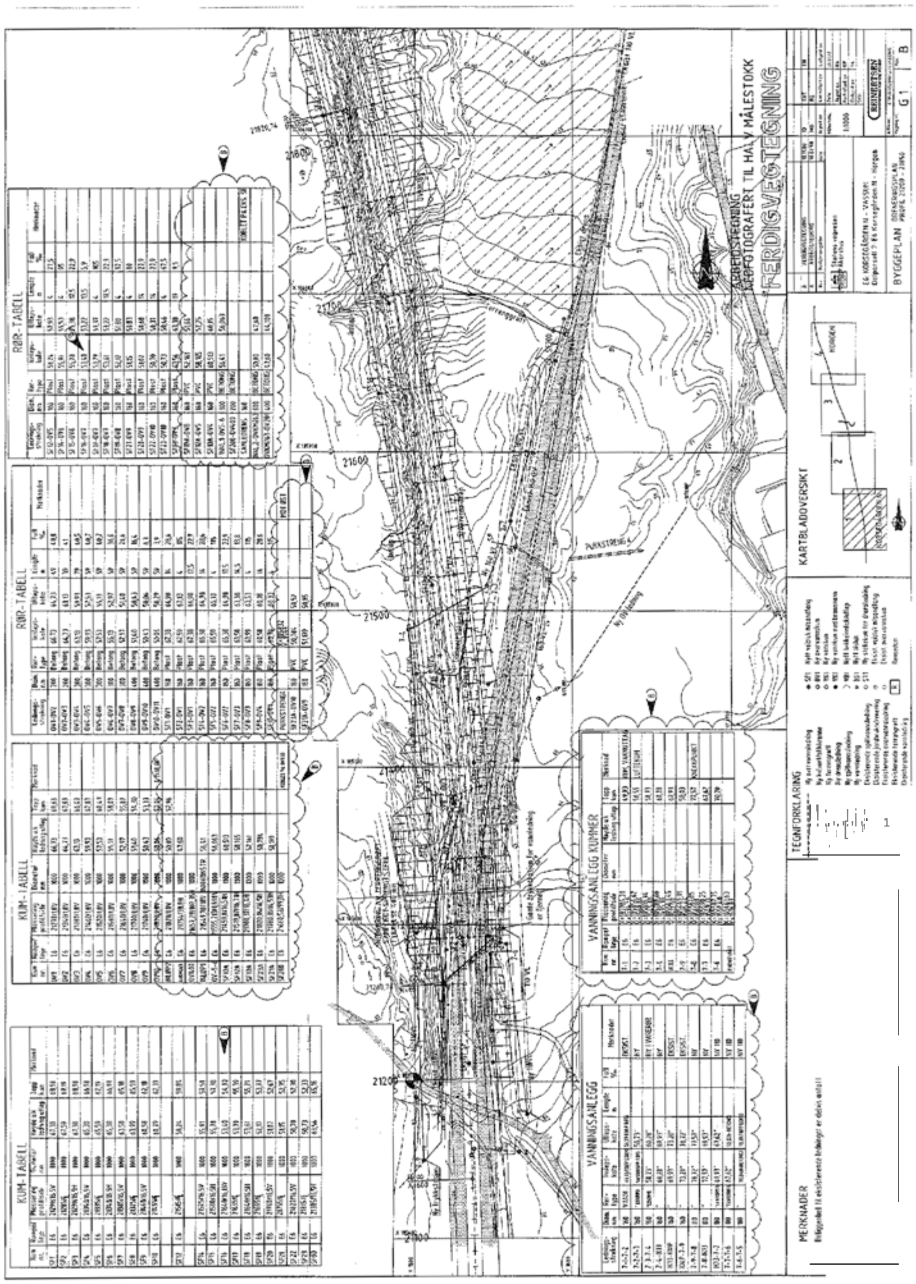
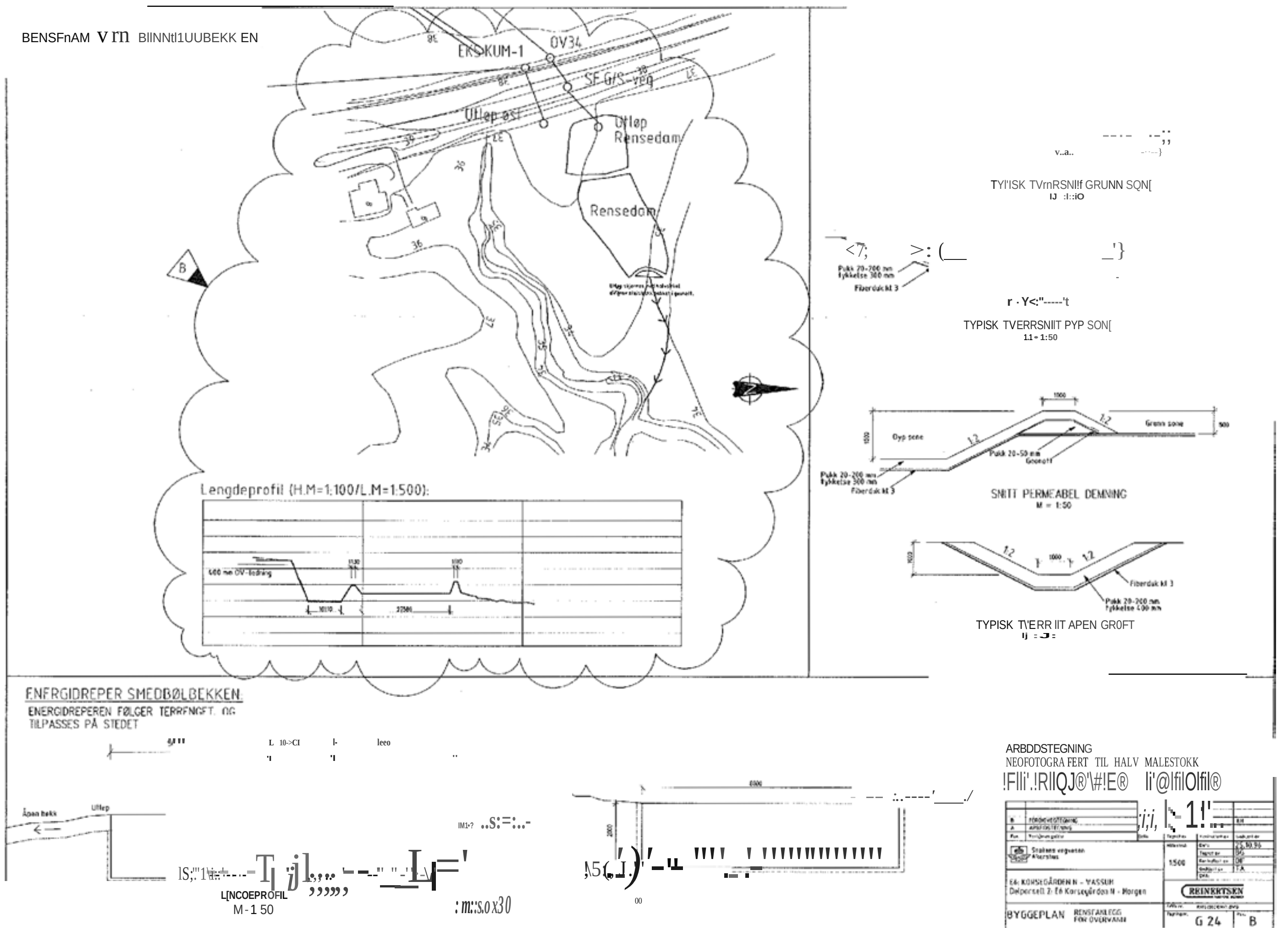

KUM-TABELL
RØR-TABELL
VANNINGSANLEGG
VANNINGSANLEGG KUMMER
TEGNFORKLARING
KARTBLADOVERSIKT
MERKNADER
FERDIGVEGTEGNING
BYGGEPLAN

Figure 2: Maps of the Oslo-Akershus metropolitan area



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