THESIS FOR THE DEGREE OF LICENTIATE OF ENGINEERING

# Contribution of stormwater ponds for road runoff to aquatic biodiversity

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Gothenburg, Sweden 2017

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Cover: The stormwater pond Taraldrud Nord in July 2014
Photo: Sondre Meland
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# Abstract

The increased recognition that roads may impair the aquatic environment and ecosystems has led to a shift from conventional drainage systems toward blue-green solutions such as stormwater ponds. Research on blue-green stormwater solutions has until now mainly focused on water quantity and quality. The goals of this study are to evaluate the influence of environmental factors on the biological community composition in stormwater ponds receiving road runoff and to determine to which extent these green infrastructures can promote and maintain pond dwelling organisms.

Biological community composition was investigated in 16 stormwater ponds along the highways E6 and E18 in the counties of Oslo, Akershus and Østfold of Norway. Multivariate statistical methods were used to explore the relationship between the biological community composition and the pollutants in water and sediments, as well as physical factors. Redundancy analysis combined with forward selection showed that the most important water quality and physical variables determining the variation in the biological community composition were pond size, distance to the closest pond from study pond, annual average daily traffic, concentrations of metals, chloride, dissolved oxygen, hydrocarbons and phosphorus. Most taxa were negatively correlated with metals. The results indicate that, compared with smaller ponds, larger ponds are better for supporting aquatic biodiversity due to a more heterogeneous environment and ability to dilute pollutants. Also, the presence of other ponds in the vicinity of the stormwater ponds would facilitate the movement of invertebrates between ponds through increased connectivity. The redundancy analysis showed that different taxa exhibited different responses to pollutants in the sediments, indicating potential differences in pollution tolerance among organisms. Therefore, the factors that may affect pollutant bioavailability in the sediments should be analysed. In addition, since different species, even within one family, responded differently to the same pollutant, it is important to identify organisms to the species level.

Keywords: aquatic biodiversity, metals, pond size, road runoff, road salt, stormwater ponds, sediments, water quality

# List of papers

This thesis is based on the work contained in the following papers.

1. Sun, Z., Brittain, J.E., Sokolova, E., Thygesen, H., Saltveit, S.J., Rauch. S. and Meland S. (2018) Aquatic biodiversity in sedimentation ponds receiving road runoff – What are the key drivers? Science of the Total Environment, 610, 1527-1535.
2. Sun, Z., Brittain, J.E., Sokolova, E., Saltveit, S.J., Rauch. S. and Meland S. The impact of pollutants in sediments on aquatic biodiversity in stormwater ponds receiving road runoff. Manuscript.

Acknowledgements

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My sincere gratitude also goes to all my colleagues at the Division of Water Environment Technology for their help. Special thanks to my licentiate sister Nashita, who is going to defend her licentiate almost at the same time. Thanks for our many late stays in the cold and lonely office and for your encouragement and company.

Last but not least, I would like to thank to my parents for their unlimited support and encouragement throughout my life.

Zhenhua

# Contents

# 1 Introduction

Roads are linear infrastructure, primarily for the use of motor vehicles; roads are widespread in modern landscapes and are important in today's society. However, roads have been reported to have adverse effects on the environment and ecosystems. Aquatic environments have been affected by roads due to the mechanics of sediment and debris transport (Coffin, 2007) and the alteration of hydrological processes (Karlson and Mörtberg, 2015). Road runoff contains large amounts of particles and pollutants, which can cause direct and indirect negative effects on the organisms dwelling in the aquatic habitats. The pollutants include both inorganic and organic chemical substances, originating from vehicles, technical infrastructure on the road, and operation and maintenance (Bohemen and Janssen Van De Laak, 2003). Several studies have demonstrated that chemical pollution may have negative impacts on the aquatic organisms at different biological levels (Wheeler, 2005, Trombulak and Frissell, 2000, Alexander, 1998). In addition, roads also influence the biotic components of aquatic ecosystems by increasing mortality and creating barriers to animal movement (Coffin, 2007). Amphibians may especially suffer from increased mortality caused by vehicle collisions due to their migration across roads (Trombulak and Frissell, 2000).

The recognition of the adverse effects of roads on the environment and ecosystems has led to the development and adoption of sustainable urban drainage systems (SUDS) (Meland, 2015), which are also called green infrastructure. The EU Water Framework Directive pinpoints a necessary trend towards an ecosystem-based approach for water resource management (Wade and McLean, 2014). SUDS provide multiple ecosystem services, such as mitigating runoff volumes and pollution. Recently, the biodiversity service provided by SUDS is getting more and more attention (Hsu et al., 2011, Vermonden et al., 2009).

Several studies have demonstrated the capability of stormwater ponds to promote and maintain aquatic biodiversity (Hassall and Anderson, 2015, Le Viol et al., 2009). Le Viol et al. (2012) found that stormwater ponds could potentially contribute to biodiversity in altered landscapes and even support emblematic rare species. Davies et al. (2008) found that at the regional level, compared with rivers and lakes, ponds had the most species of both wetland plants and macroinvertebrates.

However, not all studies have found that stormwater ponds are able to support a higher aquatic biodiversity. Bäckström et al. (2002) mentioned that stormwater ponds could potentially become ecological traps due to the various pollutants, which result in adverse effects on attracted organisms. In addition, the unintentional or intentional introduction of invasive or non-invasive species of organisms may lead to the potential problem of undesirable species compensating the biodiversity loss caused by human activities (Rooney et al., 2015).

Although the role of stormwater ponds has been expanded from solely stormwater management to a triangle made up by water quantity, water quality and ecology, compared with pollutant removal and flood control, the biodiversity service of stormwater ponds has attracted relatively less attention (Hsu et al., 2011). The overall aim of the project presented in this thesis is to develop a model that can simulate the influences of environmental factors on the aquatic biodiversity in the stormwater ponds receiving road runoff, in order to provie design recommendations for stormwater systems. The developed model will assist in designing stormwater systems that can compensate for the adverse effects of roads on aquatic biodiversity.

The research presented in this thesis has the following objectives:

1) to evaluate the impacts of various environmental factors on aquatic biodiversity in stormwater ponds receiving road runoff, and to identify the factors that have the most contribution to the biological community composition;
2) to disclose the biodiversity in stormwater ponds receiving road runoff and to find out to which extent these green infrastructures can promote and maintain pond dwelling organisms.

The thesis includes two appended papers. In Paper 1, the environmental factors are represented by the physical and water quality data for the stormwater ponds, while in Paper 2, the environmental factors are represented by the data on pollutants in pond sediment.

# 2 Background

## 2.1 Sustainable urban drainage systems

The term SUDS refers to an interconnected network of green spaces and other environmental features that deliver beneficial ecosystem services. In my study, I am focusing on the stormwater ponds (Figure 1), which aim to keep as much stormwater runoff as possible on-site and to improve its water quality. Compared with the traditional drainage system that cannot treat stormwater runoff simultaneously although it can attenuate flooding to a certain extent (Davis, 2005), ponds have the ability to retain and infiltrate runoff, and to control pollution at the source while cleaning it naturally (Jensen, 2008). In addition, stormwater ponds have the potential to promote and maintain aquatic biodiversity (Hsu et al., 2011) because they produce food and provide breeding sites for many organisms.

A) B)

*Figure 1. A) The pond Taraldrud South in August 2014 and B) the pond Enebekkin April 2013. Photos: Henning Pavels.*

Moore and Hunt (2012) have proven that constructed wetlands and man-made ponds are able to support vegetative richness and diversity in temporary inundation zones. Some insect families, especially desirable dragonflies (Odonates), are attracted by emergent vegetation. The emergent vegetation also plays a crucial role in increasing the likelihood of colonisation by a more diverse assemblage of macroinvertebrates (Moore and Hunt, 2012).

## 2.2 Biodiversity

Biodiversity is defined as the variability among living organisms from, e.g., terrestrial, marine, and other aquatic ecosystems, forming the foundation of a large range of ecosystems (Millennium Ecosystem Assessment, 2005). Although there are many tools and data sources, it is still difficult to precisely quantify biodiversity in a given area (Millennium Ecosystem Assessment, 2005). Several indices and methods have been developed for estimation, such as species richness, evenness, Shannon index, and Simpon's index.

Species richness is the most basic metric, which refers to the number of species found in a community or a sample. The methods that can be used to estimate species richness are species accumulation curves, parametric methods, and nonparametric methods (Magurran, 2004).

Evenness is another metric to measure how evenly the individuals in the community are distributed over species (Heip, 1998). Contrary to the evenness, dominance measures the extent to which one or more species dominate the community. Several indices or methods were developed for measuring evenness and dominance, e.g., Simpon's index and Berger-Parker index.

Currently the most widely used diversity index is Shannon-Wiener index, which provides more information about community composition than species richness. Several studies have applied Shannon-Wiener index to interpret the influences of environmental factors on aquatic biodiversity in stormwater ponds (Vermonden et al., 2009, Hsu et al., 2011, Goertzen and Suhling, 2013).

## 2.3 Factors affecting aquatic biodiversity in stormwater ponds

In order to maintain and promote aquatic biodiversity within stormwater ponds, the factors that influence aquatic biodiversity need to be considered in pond design and management (Hsu et al., 2011). However, due to a variety of factors that could potentially affect aquatic biodiversity, there is a lack of comprehensive knowledge on the complex effects resulting from interactions between the different factors; this limits our understanding of ecosystem responses to the factors (Navarro-Ortega et al., 2015). In general, the factors can be classified into abiotic and biotic factors.

### 2.3.1 Abiotic factors

#### *2.3.1.1 Physical characteristics of ponds*

Pond size has been recognized as the most basic factor that potentially influences aquatic biodiversity within ponds. Some studies have demonstrated that species richness increases with pond size (Hsu et al., 2011, Noble and Hassall, 2015); this is in accordance with conventional species-area relationships. However, some studies reported contradictory findings (Oertli et al., 2002, Biggs et al., 2005). For example, Oertli et al. (2002) found that the species-area relationship was apparent for Odonata, but not relevant for Coleoptera and Sphaeriidae.

Pond age was identified as another factor that may influence the variation in the biological community composition. The studies involving pond age have yielded conflicting results. Hart and Horwitz (1991) suggested that older ponds are able to maintain more species due to colonisation, while Williams et al. (2008) found that compared with older ponds, 6-12-year-old ponds can support considerably more species and more uncommon species. Other studies, e.g., Gee et al. (1997), demonstrated that there was no obvious relationship between the number of taxa of macroinvertebrates and pond age.

Pond density and connectivity have been demonstrated to have significant impacts on species richness (Staddon et al., 2010), since higher connectivity could facilitate the mobility of invertebrates between ponds (Gledhill et al., 2008). Hassall (2014) defined such kind of networks as "pondscapes", which constitute a network of distributed discrete habitat patches.

#### *2.3.1.2 Chemical pollutants in the water column*

Stormwater ponds may expose attracted organisms to a wide range of pollutants, which may exert either direct or indirect impacts on organisms, resulting in increased or decreased abundance of associated species (Fleeger et al., 2003).

Many of the organic micropollutants, e.g., hydrocarbons and polycyclic hydrocarbons, potentially have toxic effects on the organisms (Echols, 2009). Water solubility of hydrocarbons plays the crucial role in environmental effects, because dissolved hydrocarbons are responsible for many acute toxic effects on organisms (Trett, 1989). Low-molecular weight hydrocarbons are more toxic to the organisms compared with high-molecular weight hydrocarbons, because the former are more soluble in water (Pettigrove and Hoffmann, 2005b). Polycyclic aromatic hydrocarbons (PAHs) are a group of persistent organic pollutants that often appear at elevated concentrations in road runoff (Meland et al., 2010a); they are highly toxic to aquatic organisms (Greenberg, 2003) and cause mortality in all life stages and decrease in growth (Meland et al., 2010a). The regularly monitored EPA PAH16 only represent a small percentage of the compounds and their toxic potential (Grung et al., 2016). Grung et al. (2017) have found several other compounds present in the particulate phase of road runoff, including polycyclic aromatic compounds (PACs), phthalates, benzothiazoles, organophosphate compounds, musk compounds, and plasticiser.

Metals are especially important pollutants in road runoff. Unlike organic matter or organic pollutants, metals cannot be decomposed or transformed by organisms (Phillips et al., 2015). Metals could have both short-term impacts through acute toxicity and long-term impacts through accumulation (Semadeni-Davies, 2006). The fate and toxicity of metals greatly rely on the partitioning of metals between sediment particles and water (Huang et al., 2017). It has been demonstrated that biodiversity significantly declines with increasing metal concentrations in water bodies due to the higher bioavailability and toxicity of dissolved metals than particulate metals (Phillips et al., 2015).

The widespread use of road salt (NaCl) as a de-icing agent during winter leads to high concentrations of $Cl^-$ in ponds. High concentrations of $Cl^-$ may significantly decrease the species richness and abundance of aquatic organisms through the effects on the osmoregulatory and physiological processes of aquatic invertebrates (Blasius and Merritt, 2002), as well as the indirect effects, e.g., increased concentrations of metals in the aqueous phase (Mayer et al., 2008). Furthermore, the formation of a salt gradient can prevent oxygen transport from the surface water to the bottom, resulting in anoxia and the death of benthic organisms (Nielsen et al., 2003). Salinity is also one of the most important factors that determine the fate (speciation) of metals released from sediments. The increased salinity can also increase the complexation of metals, e.g., Cd, with chlorides (Riba et al., 2003).

Nutrients, e.g., phosphorus and nitrogen, support the growth of algae and aquatic plants, which provide food for smaller organisms. However, too much nutrients can decrease the species richness in the ponds and alter species composition over a short time (Verhoeven et al., 2006). Eutrophication is the problem caused by the excess of nutrients; it has a great impact on small standing water bodies (Menetrey et al., 2005) and causes decrease in aquatic macrophyte communities (Conley, 1999).

#### *2.3.1.3 Chemical pollutants in the sediments*

Some studies have found that sediment pollution can significantly reduce macroinvertebrate diversity and change community composition (Carew et al., 2007, Cox and Clements, 2013). Heavy metals in a stormwater pond tend to deposit onto sediment surfaces and immobilise through various processes, e.g., coagulation and adsorption, and only a small portion of free metal ions is dissolved in water (Zhang et al., 2014). In certain chemical conditions, pollutants from sediments could be released back into the liquid phase and accumulate in plant and animal tissue (Dalu et al., 2017). Therefore, toxic metals in the sediments could pose risks to aquatic organisms, especially to benthic organisms that are in direct contact with the sediments (Beasley and Kneale, 2002). Several factors may influence the bioavailability of metals in the sediments to benthic organisms, such as metals chemical form, sediment geochemical properties, and exposure pathways of the organisms (Zhang et al., 2014). Metals in the sediments may affect the aquatic organisms through long-term adverse impacts or acute toxicity or a combination of these.

Hydrocarbons and PAHs are common anthropogenic pollutants in the sediments of freshwater bodies. Due to the limited solubility of heavier hydrocarbons in water, heavier hydrocarbons are more likely to sorb onto sediments (Pettigrove and Hoffmann, 2005a). Although the presence of heavier hydrocarbons might not have direct effects on aquatic organisms, they could still indirectly influence organisms in several ways. For example, heavier hydrocarbons are able to alter the physical properties of sediments through increasing the sediment oxygen demand from microbial activity, thereby reducing the aerobic layer in sediments (Shin et al., 2000). PAHs are found preferentially to enrich fine particles by surface adsorption, and they pose serious threat to aquatic ecosystems. However, the information about factors that affect the bioavailability of PAHs is deficient.

The impacts of pollutants on aquatic organisms also vary among a wide range of species. For pollutant-tolerant species, stormwater ponds may promote their populations, while for pollutant-intolerant species, stormwater ponds might contribute to population declines (Snodgrass et al., 2008). For example, in a study by Snodgrass et al. (2008), the amphibian *Rana sylvatica* was highly sensitive to exposure to polluted pond sediment and suffered a high level of morality, while *Bufo americanus* was tolerant to polluted sediments and maintained abundant in the ponds.

### 2.3.2 Biotic factors

#### *2.3.2.1 Vegetation*

Vegetation plays an important role in providing food source and habitats for organisms. Several studies have analysed the relationship between richness and abundance of aquatic macroinvertebrates and the coverage of aquatic macrophytes in ponds, and found that aquatic macrophytes can increase richness and abundance of macroinvertebrates due to the more diversified and suitable habitats (Vermonden et al., 2009, Fontanarrosa et al., 2013, Gee et al., 1997). Nevertheless, not all macroinvertebrates prefer to live in the ponds with a high percentage of vegetation cover. The taxa that can stay submerged longer, such as Corixidae and predacious diving beetles, have been found to be negatively correlated with plant cover (De Szalay and Resh, 2000).

#### *2.3.2.2 Biological community interactions*

Competition and predation are the most essential species interactions in ecology, playing an important role in maintenance of biodiversity (Chesson and Kuang, 2008). Predation and resource competition potentially affect biodiversity in the same way, either promoting coexistence or promoting exclusion (Chesson and Kuang, 2008). Furthermore, Chesson and Kuang (2008) found that predation and competition are involved in an interaction, if one is much stronger than the other, the predictions of the stronger dominate. Furthermore, other factors could also affect the biological interactions. For example, body size is an important characteristic of a species, which may influence competitive dominance, predator-prey interactions, physiological rates, and bioconcentration of pollutants in different organisms (Colas et al., 2014). The effects of environmental stressors can also increase competition for resources through, e.g., elimination of prey (Colas et al., 2014). The increased competition resulting from the homogeneous environment could eliminate the most sensitive species and select tolerant species that are less vulnerable to contaminants (Colas et al., 2014).

The establishment of stormwater ponds might introduce invasive alien species, which can be classified into two categories: species that disperse into new areas and the ones that do not disperse beyond the point of introduction (Thomas et al., 2016). The impacts of invasive species that are introduced intentionally or unintentionally by human actions on native organisms are diverse (David et al., 2017). For example, the introduced filter-feeding species are able to deplete planktonic communities, improving water quality and creating substrates that are beneficial for benthic invertebrates and macrophytes (David et al., 2017). However, some studies have demonstrated that species invasion is one of the key factors that lead to biodiversity declines in freshwater (Hassall, 2014, Ricciardi, 2015, Mollot et al., 2017). In addition, invasive species have to consume the resources available in the ecosystem, thereby establishing interspecific and intraspecific interactions with local species.

# 3 Methodology

In Paper 1, twelve stormwater ponds, situated along the highway E6 in Norway were investigated, including Skullerud, Taraldrud north, Taraldrud crossing, Taraldrud south, Nøstvedt, Vassum, Såstad, Fiulstad, Idrettsveien, Karlshusbunn, Nordby, and Enebekk (Figure 2). Three of these ponds, Idrettsveien, Karlshusbunn, Nordby, contain two separate small basins: one receiving runoff from an agricultural area, and the other one receiving road runoff.

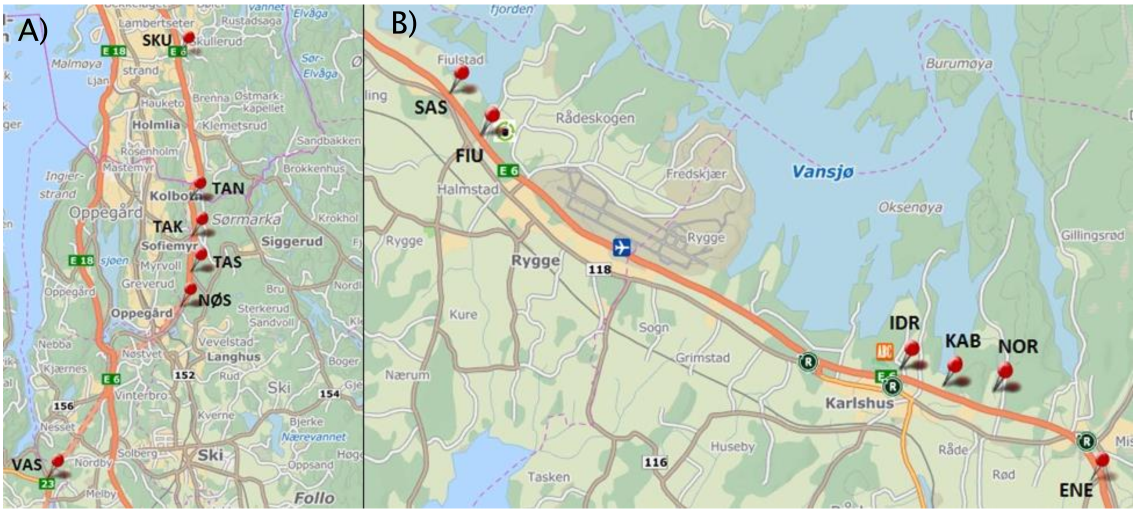



*Figure 2. Location of the studied stormwater ponds (red dots) in Oslo and Akershus county (A) and in Østfold county (B). In A), the ponds are SKU - Skullerud, TAN - Taraldrud north, TAK - Taraldrud crossing, TAS - Taraldrud south, NØS - Nøstvedt, and VAS - Vassum. In B), the ponds are SÅS - Såstad, FIU - Fiulstad, IDR - Idrettsveien, KAB - Karlshusbunn, NOR - Nordby, ENE - Enebekk. The distance between the two farthest ponds (Skullerud and Enebekk) is 71 km.*

Water samples were collected four times (April, June, August and October) in 2012 close to the inlet of the ponds. Twenty-eight water quality variables were analysed, including metals, nutrients and organic pollutants, as well as dissolved oxygen (DO), conductivity, pH and temperature. Physical factors included age and size of ponds, number of neighbouring ponds within a radius of 1 km, distance to the nearest neighbouring pond, and annual average daily traffic (AADT). The physical data were obtained either from digital maps (Norwegian Mapping Authorities) or directly from the Norwegian Public Roads Administration (NPRA).

Aquatic organisms were sampled using either a kick net with an opening of 30 × 30 cm and a mesh size of 0.45 mm or traps made of 1.5 L transparent plastic bottles. Sampling was done once in the inlet basin and twice on either side of the main pond. Biological samples were identified to order, family or species level. Aquatic organisms used in Paper 1 included 91 macroinvertebrates, 2 zooplankton and 3 amphibians.

In Paper 2, apart from the eight ponds (Skullerud, Taraldrud north, Taraldrud crossing, Taraldrud south, Nøstvedt, Vassum, Nordby and Enebekk) that have been studied in Paper 1, four new ponds (Elstadmoen, Hovinmoen, Fornebu, and Tenor) were included, to increase the geographical range of ponds and the range of pond age. Except the pond Fornebu, which is an urban pond, the ponds are located along the major highways E6 and E18 in the counties of Oslo, Akershus and Østfold (Figure 3).

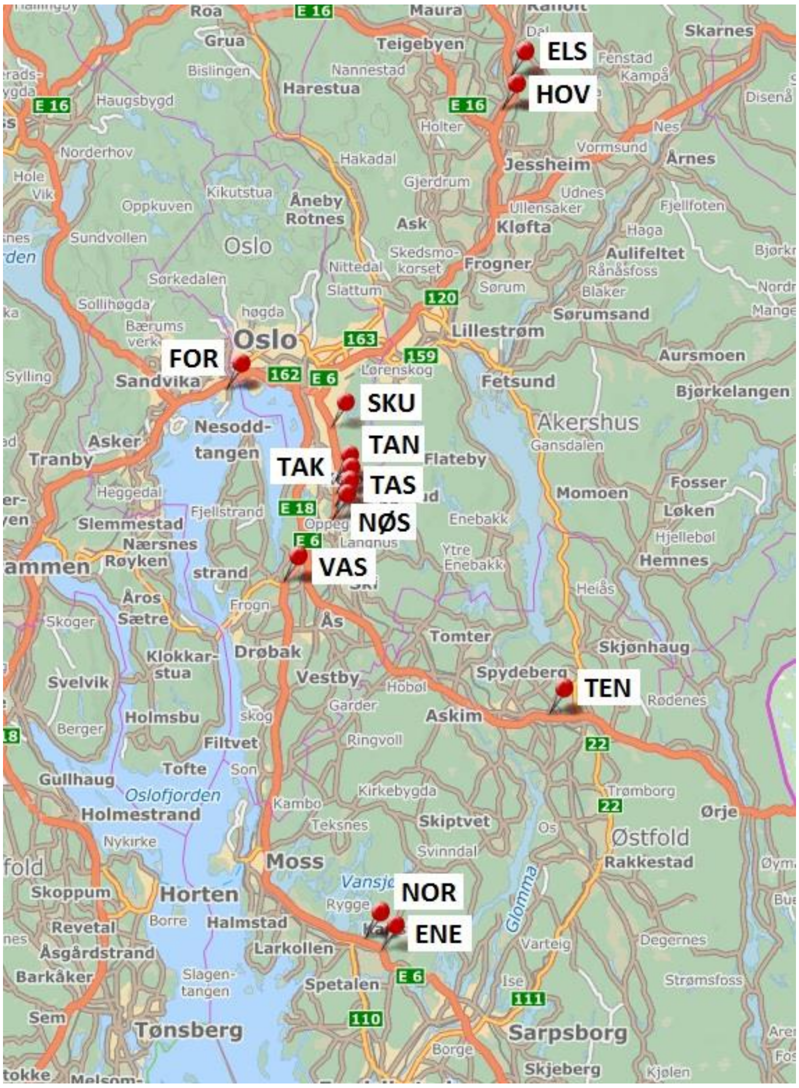



*Figure 3. Overview of the location of all the stormwater ponds (red dots) in the counties of Oslo, Akershus and Østfold. The ponds are ELS – Elstadmoen, HOV – Hovinmoen, FOR – Fornebu, SKU - Skullerud, TAN - Taraldrud north, TAK -Taraldrud crossing, TAS - Taraldrud south, NØS - Nøstvedt, VAS – Vassum, TEN – Tenor, NOR - Nordby, and ENE – Enebekk.*

Sediments were sampled once in 2013 and 2014, respectively, near the inflow with a spade and collected in 1L glass bottles. All sediment analyses were performed by Rambøll Analytics Laboratories in Finland. Eleven variables were analysed, i.e. total organic carbon (TOC), total hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), aluminium (Al), calcium (Ca), chromium (Cr), copper (Cu), iron (Fe), nickel (Ni), lead (Pb), and zinc (Zn). Pyrene was used as a proxy for PAH pollution, because the concentrations of US PAH16 were below the limit of quantification in more than 15% of the total number of samples. Aquatic organisms were sampled four times (April, June, August and October) in 2013 and 2014 respectively using the same methods as in Paper 1; the samples were also collected once close to the inlet and twice on either side of the ponds. Biological samples were identified to order, family or species level. Aquatic organisms used in Paper 2 included 176 macroinvertebrates and 4 amphibians. Compared with Paper 1, there were much more taxa in Paper 2 because the majority of organisms in Paper 2 were identified to species level.

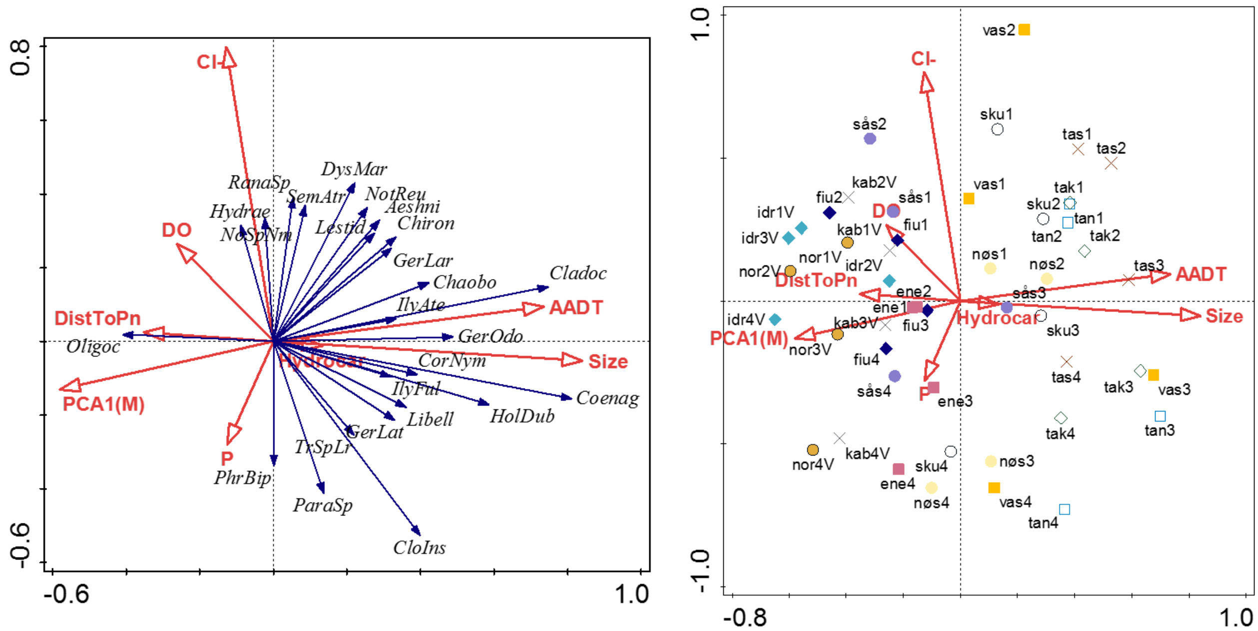
Univariate and multivariate statistical methods were used in both papers to analyse the data.

In Paper 1, principal component analysis (PCA) was used to analyse the general trends in water quality. PCA was also used to narrow down the variables for the subsequent analysis due to the high risk of overfitting the RDA model because of too many explanatory variables. One-way analysis of variance (ANOVA) followed by Tukey post hoc tests were applied in order to see the differences in water quality between different stormwater ponds. In the subsequent analysis, PCA and redundancy analysis (RDA) were applied to exhibit the maximum variation in the biological community, and estimate the relationship between the biological community composition and the environmental data, respectively. Finally, to select the variables that had the most contribution to the variation in the biological community composition, RDA with forward selection was used.

In Paper 2, PCA was used to analyse the general trend in sediments. In order to compare the differences in sediments between twelve ponds, PCA scores for variables extracted from axis 1 were used to create a bar chart. RDA was used to examine the influences of pollutants in the sediments on the biological community composition.

# 4 Results

In Paper 1, 8 out of 14 variables were identified to have the greatest contribution to the explained variation in the biological community composition. These variables were metals (including sulphate ($SO_4^{2-}$)), chloride ($Cl^-$), phosphorus (P), dissolved oxygen (DO), total hydrocarbons, annual average daily traffic (AADT), distance to the nearest neighbouring pond, and pond size (Figure 4). Most taxa were positively correlated with the pond area and AADT, and negatively correlated with metals. In addition, most taxa were negatively correlated with the distance to the nearest neighbouring pond. Some taxa were positively correlated with $Cl^-$, P and DO, while some were negatively correlated. Variation partitioning analysis represented how much of the variation in the biological community composition could be ascribed to the water quality and physical variables. The group of water quality variables (metals (including $SO_4^{2-}$), $Cl^-$, DO, P, and hydrocarbons) explained 48%, and the group of physical variables (pond size, AADT, and distance to the nearest neighbouring pond) explained 41% of the total variation in the biological community composition.

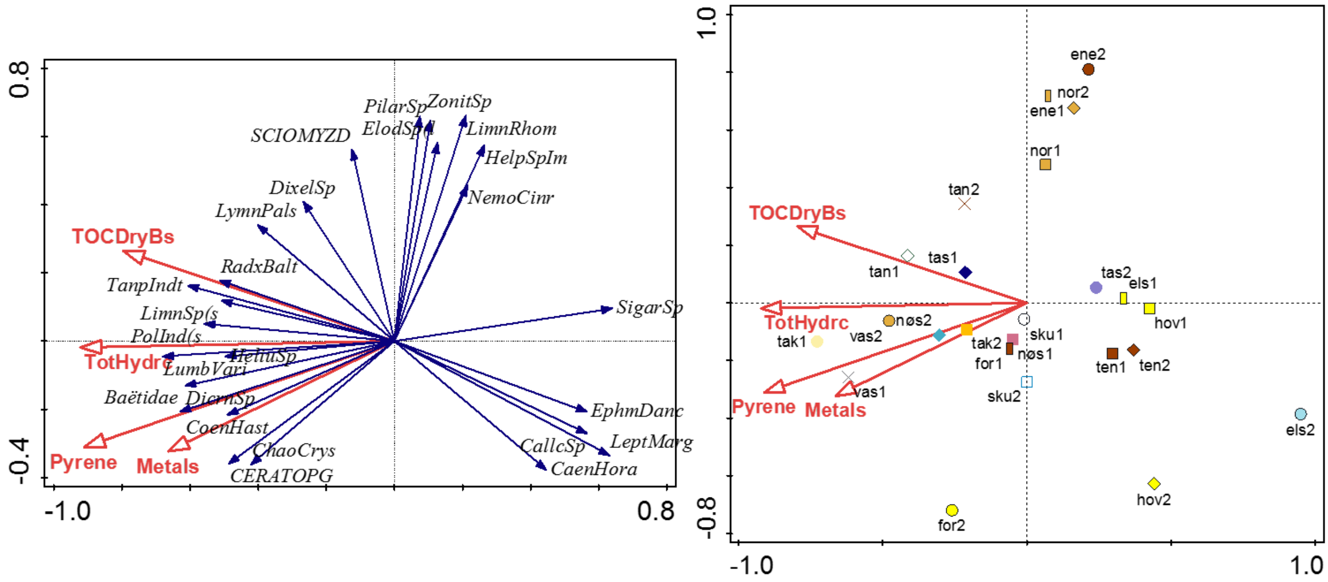



A) B)

*Figure 4. Redundancy analysis (RDA) with forward selection for the relationship between taxa and the water quality as well as physical variables. The effect of covariate "month" was removed. PCA1 (M) represents concentrations of metals (including $SO_4^{2-}$), DO – dissolved oxygen, and P – phosphorus; "DistToPn" represents the distance to the nearest neighbouring pond from each study pond. Blue arrows indicate different taxa. Red arrows indicate explanatory variables. Figure B) describes the relationship between environmental variables and different samples. The same symbol with the same colour indicates that samples were collected from the same pond; the first three letters indicate the name of the pond; "1", "2", "3" and "4" indicate that the samples were collected in April, June, August and October 2012, respectively; "V" used in three ponds indicates the basin receiving road runoff.*

In Paper 2, the sediments in most of the ponds were either slightly polluted or nonpolluted. However, Cu in some ponds was at high concentrations, which can lead to acute toxicity to the organisms.

In Paper 2, the result of the RDA (Figure 5) showed that some mayfly (Ephemeroptera) species were negatively correlated with meals, while some species such as the damselfly *Coenagrion hastulatum* (Odonata) and the snail *Radix balthica* (Gastropoda) were positively correlated. Some taxa, such as the damselfly (Odonata) *Coenagrion hastulatum* and the worm (Oligochatea) *Lumbriculus variegatus* (Lumbriculida) were positively correlated with TOC, while others, such as some species within the mayflies (Ephemeroptera), were negatively correlated TOC. Regarding total hydrocarbons and pyrene, some taxa, e.g., the worm (Oligochatea) *Lumbriculus variegatus* (Lumbriculida) and the damselfly *Coenagrion hastulatum* (Odonata), were positively correlated with elevated total hydrocarbons and pyrene, while other taxa, e.g., the mayfly *Leptophlebia marginata* (Ephemeroptera) and the water boatmen *Sigara* sp. (Hemiptera), were negatively correlated with elevated hydrocarbons and pyrene.




A) B)

*Figure 5. A) Redundancy analysis (RDA) of the relationship between aquatic organisms and pollutants in the sediments. B) RDA of the relationship between pollutants in the sediments and different samples. TOCDryBs represents concentrations of total organic carbon, and TotHydrc represents total hydrocarbons. "1" and "2" indicate that the samples were collected in 2013 and 2014, respectively.*

# 5 Discussion

We examined the potential role of stormwater ponds along the highway as refuges for aquatic biodiversity through analysing the relationship between biological community composition and different environmental variables, including water quality, sediment, and physical variables.

In Paper 1, pond size was the variable that contributed the most to the variation in the biological community composition; large ponds were able to support more species than the smaller ones. However, the results of other studies regarding the pond size are conflicting. Oertli et al. (2002) found that larger ponds can support more Odonata, but not Coleoptera and Sphaeriidae. Biggs et al. (2005) also found that compared with invertebrates, the conventional species-area relationships were more relevant to macrophytes. This is probably due to the larger effect on structuring communities resulting from extrinsic and stochastic processes in ponds (Hassall and Anderson, 2015). AADT acted as the second most important variable, and most taxa were positively correlated with it. This unexpected result in our study can potentially be explained by the fact that AADT was highest for the largest ponds, and dilution could reduce contaminant concentrations in the stormwater ponds. The absence of obvious correlation between AADT and pollutants could be another explanation. For example, Kayhanian et al. (2003) demonstrated that no direct linear correlation between pollutant concentration in road runoff and AADT can be seen. The distance from the study pond to the nearest neighbouring pond was also statistically selected by RDA forward selection, and most taxa appeared to be negatively correlated with it. Some studies have demonstrated the importance of pond density in promoting metapopulations of species, and found that higher connectivity could facilitate the mobility of invertebrates between ponds (Gledhill et al., 2008, Noble and Hassall, 2015, Staddon et al., 2010).

Among the water quality variables, most taxa were negatively correlated with the metal concentrations. In general, biodiversity could decrease with the increase of heavy metal concentrations (Phillips et al., 2015). The concentrations of Zn and Cu in our study were high. Although Zn acts as an essential nutrient for living organisms, it can have negative effects on living organisms when it reaches excessive levels (Weiner, 2008). $Cl^-$ is another water quality variable that greatly influences the biological community composition. Due to the wide use of sodium chloride as a de-icing agent in Norway, $Cl^-$ concentration was quite high in some ponds, ranging from 3.6 to 2090 mg/L. The elevated concentration of $Cl^-$ has several adverse effects on the biological community composition, such as osmotic stress related to overall ionic strength (Elphick et al., 2011), anoxic conditions in bottom waters resulted from the prevention of water circulation, and release of trapped metals from sediment (Van Meter et al., 2011). Compared with other variables selected by RDA forward selection, P, DO, and hydrocarbons exhibited lower importance. The elevated concentration of P could lead to eutrophication that results in episodes of noxious blooms, reduction in aquatic macrophyte communities and the depletion of DO in bottom waters (Conley, 1999). Due to a wide range of structural and behavioural respiratory adaptations among different aquatic organisms, the result exhibited that different taxa had different oxygen requirements. Although DO plays the crucial role in maintaining aquatic life and affects biomobility and toxicity of metals (Rabajczyk, 2010), DO concentrations in the studied ponds do not appear to be a limiting factor, since the concentrations were above the threshold value for aquatic organisms to live.

In Paper 2, the results showed that different species, even within one family, responded differently to each pollutant in the sediments. Some taxa were sensitive to the pollutants in the sediments, while others were tolerant to these pollutants due to the various reasons, e.g., biological characteristics and sensitivity to disturbance of each species. The majority of the taxa that were positively correlated with pollutants were the true flies Diptera that are normally tolerant to pollution (Dalu et al., 2017). Many species of the mayflies Ephemeroptera and the caddisflies Trichoptera have been demonstrated by several studies to be sensitive to pollution in e.g., lakes and streams. Organic enrichment can drastically deplete dissolved oxygen, thereby altering the biological community composition and the abundance of organisms (Hellawell, 1986). Our results showed that family Baetidae (Ephemeroptera) was positively correlated with TOC, and it is in agreement with, e.g., Menetrey et al. (2010) and Ikhlas Alhejoj (2014), who found that Baetidae had the ability to live in waters with moderate organic pollution. In addition, some taxa of caddisflies Trichoptera in our study also exhibited positive correlation with elevated TOC concentrations. This is probably because TOC in the sediments can provide nutrients for many aquatic organisms, particularly filter-feeding macroinvertebrates. Furthermore, some caddisflies (Trichoptera), e.g., Polycentropodidae and Limnephilidae, use a mixture of materials, e.g., plant fragments, to build the case (Linda Kwong, 2011, MOOR, 2003) that may protect them from elevated concentration of TOC.

The RDA results for the response to metals vary within and between different families. The results showed that some Ephemeroptera and Trichoptera taxa, e.g., Baetidae (Ephemeroptera) and Polycentropodidae (Trichoptera), were abundant in the waters with elevated concentrations of metals. Bere et al. (2016) also found that even though most Ephemeroptera, Plecoptera, and Trichoptera taxa are sensitive to organic enrichment, they exhibited high tolerance to metals. However, Beasley and Kneale (2003) had different findings that, except the family Baetidae that was moderately tolerant to metals, the mayflies Ephemeroptera were sensitive to elevated metals. Other taxa in our study, such as *Radix balthica* and *Lymnaea palustris*, were also positively correlated with metals, and Beasley and Kneale (2003) found that the family that they belong to (Lymnaeidae) was tolerant to metals. The families (Leptophlebiidae, Ephemeridae, and Nemouridae) that were demonstrated by Beasley and Kneale (2003) to be sensitive to metals also exhibited negative correlation with metals in our study. The positive correlation between metals and organisms could also be attributed to the relatively low concentrations of most toxic metals, e.g., Pb, Ni, and Cr, in our study. Other factors could also affect the fate/speciation of metals, such as organic matter, salinity, and pH (Riba et al., 2003, Aminayanaba and Lawal, 2017).

Regarding total hydrocarbons and pyrene, some taxa were positively correlated, while some were negatively correlated with the concentration of these pollutants in the sediments. The total hydrocarbons in our study consisted to a large extent of heavy hydrocarbons (C21-40), which are considered less toxic to aquatic organisms than light hydrocarbons due to the lower solubility and mobility in water (Brown et al., 2017). Even though heavy hydrocarbons may not have a direct influence on aquatic organisms, they can have indirect influences through, e.g., alteration of physical properties of sediments and increasing organic enrichment of sediments (Pettigrove and Hoffmann, 2005a). Although pyrene can be toxic to organisms even at low concentrations, some taxa in our study were positively correlated with pyrene. This could be partly explained by the relatively low concentrations of pyrene in our study, and the fact that all measured concentrations were below the value that can lead to acute toxicity to aquatic organisms.

# 6 Conclusions

A wide range of environmental factors was studied in Papers 1 and 2, including physical properties of the ponds, water quality, and pollutants in pond sediments. In Paper 1, several factors that had the greatest influence on the aquatic organisms were statistically selected; among the selected factors, the pond size was the factor that contributed the most to the variation in the biological community composition. The results showed that more species live in bigger ponds due to the “species-area effect” and the dilution of detrimental pollutants. In Paper 2, the responses of different species to pollutants in the sediments were quite different, indicating that it is important to identify organisms to the species level in order to fully understand the influence of environmental factors on the organisms. In addition, due to the fact that a variety of factors may influence the bioavailability of pollutants in the sediments, e.g., pollutant tolerance of organisms and interactions between different variables, as many as possible influential factors should be considered and analysed. Thus, more analyses will be performed for Paper 2. For example, the pond Skullerud was the only pond inhabited by fish, which may have an impact on the macroinvertebrate community. Therefore, the characteristics of organisms and biological interactions between different groups of organisms should be analysed in the next step.

# 7 Perspectives and future work

## 7.1 Metabarcoding

Taxonomic identification in the previous studies was performed via observation of morphologic characteristics. A major challenge involved in this process is identification to the species level. The identification of samples to the species level is difficult for non-specialists; often the specimens are sent to taxonomic specialists of particular groups who may need several weeks to identify the samples just to the genus level (Morinière et al., 2016). Moreover, sometimes even expert taxonomists are unable to identify some freshwater species that lack diagnostic morphological characteristics at the larval and even at the adult stages (Macher et al., 2015). The limitations of the traditional morphological approach are particularly true for the large scale application of macroinvertebrate sampling (Hajibabaei et al., 2011), leading to significantly higher identification error rates for species than family level (Hajibabaei et al., 2012). Even though identification at higher taxonomic levels can work effectively for estimating the impacts at regional or catchment scales, the impacts at smaller scales can be missed (Hajibabaei et al., 2012). In addition, it has been shown that even closely related species within the same families can respond differently to stressors (Macher et al., 2015), leading to the difficulties in identifying specific factors affecting an ecosystem. Therefore, more and more studies tend to use species level of macroinvertebrates in environmental research.

Recently, considerable advances in applying DNA-based diversity methods using high-throughput sequencing have enabled more direct evaluation of biodiversity (Chariton et al., 2015). Metabarcoding allows the rapid and cost-effective assessment and monitoring of biodiversity using massive parallel sequencing of bulk samples or potentially degraded DNA from environmental samples (Cristescu, 2014). Metabarcoding has been successfully applied to samples for which species identification is not practical with traditional methods (Beng et al., 2016). Therefore, in the next study, we are going to apply metabarcoding in our analysis and compare the results with traditional methods.

## 7.2 Process-based modelling

Statistical models have been widely used to estimate variations in the biological community composition caused by anthropogenic activities. However, such kind of models cannot simulate various processes involved in the aquatic ecosystems and provide a direct overview of how different environmental factors influence biodiversity. In addition, most of the present studies related to the stormwater ponds used statistical models instead of process-based models to analyse the influences of environmental factors on the organisms. Therefore, in the next step we are going to use process-based (or mechanistic) models that are established based on a set of equations describing physical, chemical and biological processes taking place, e.g., ecotoxicological models and hydrodynamic models. The combination of statistical and process-based/mechanistic models can also describe the complex interactions between biotic and abiotic elements and the links between the aquatic organisms and their environment.

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