The Ecological Role of Roadside Stormwater Ponds
Potential to Support Biodiversity

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Department of Architecture and Civil Engineering
Water Environment Technology
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden 2020
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Cover: The stormwater pond Elstadmoen in June 2016
Photo: Henning Pavels
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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 aim of this study was to explore the ability of highway stormwater ponds to provide suitable habitats and support, especially macroinvertebrate biodiversity, and to identify the key environmental variables that affect biological community composition and the number of taxa, utilizing data at regional and global scales. Ultimately, this research informs design recommendations for stormwater systems that simultaneously provide multiple ecosystem services.

The results of this thesis indicate that larger ponds are better for supporting aquatic biodiversity due to a more heterogeneous environment and the ability to dilute pollutants. Also, the presence of other ponds in the vicinity of the stormwater ponds can facilitate the movement of invertebrates between ponds through increased connectivity. An apparent negative effect of pollution levels on the macroinvertebrate community composition was observed, but not on the biodiversity measured as the number of taxa or Shannon index. The analyses based on the datasets identified using both morphology and DNA metabarcoding demonstrated that DNA metabarcoding captured and identified more than twice the number of taxa compared to morphological identification. Application of DNA metabarcoding greatly increases the number of species identified at each sampling site, thereby providing more accurate information regarding the way the ponds function and how they are affected by management. Subsequently, the differences in the macroinvertebrate community composition between different types of ponds were compared at the regional and global scale. The results indicated that environmental characteristics, especially conductivity and pH, were different between different types of ponds. Alpha and gamma diversity were similar or even higher in manmade ponds compared to natural ponds due to very different macroinvertebrate communities. Moreover, generally ponds exhibited high levels of spatial heterogeneity, which subsequently enhances gamma diversity.

In summary, stormwater ponds have the potential to provide suitable habitats to foster biodiversity. When such systems are created, larger ponds should be built to provide more heterogeneous habitat and dilute harmful pollutants. Additional ponds should also be created in the vicinity of the ponds, thereby promoting aquatic biodiversity through higher connectivity. Although stormwater ponds accumulate pollutants due to their primary functions, this pollution retention process creates a unique environment within the stormwater ponds, which are more suitable for taxa that are moderately to strongly tolerant to pollutants and that may not be found in natural ponds. In this way, stormwater ponds constitute an option in the areas along the highway so that they could combine water treatment properties with providing a suitable habitat for aquatic organisms.

Keywords: aquatic biodiversity, DNA metabarcoding, high throughput sequencing, macroinvertebrates, road runoff, stormwater ponds, sediments, water quality
LIST OF PUBLICATIONS

This thesis is based on research accomplished at the Division of Water Environment Technology (Chalmers University of Technology) between May 2015 and April 2020 under the supervision of Sebastien Rauch, Ekaterina Sokolova (Chalmers University of Technology), and Sondre Meland (Norwegian Institute for Water Research, NIVA). The research was funded by the Norwegian Public Roads Administration. The papers are referred to by their numbers and are appended at the end of the thesis:


The author of this thesis made the following contributions to the papers.

Paper I: Contributed to the conception and design of the study, carried out the data analysis and interpretation, wrote the first draft of the paper and critically revised the manuscript until publication.

Paper II: Contributed to the conception and design of the study, carried out the data analysis and interpretation, wrote the first draft of the paper and critically revised the manuscript until publication.

Paper III: Contributed to the conception and design of the study, conducted the bioinformatic processing for DNA metabarcoding data, carried out the data analysis and interpretation, wrote the first draft of the paper and critically revised the manuscript until publication.

Paper IV: Carried out the data interpretation, wrote the first draft of the paper, except methodology part, and critically revised the manuscript until publication.

Paper V: Contributed to the conception and design of the study, collected datasets from the different countries, carried out the data analysis and interpretation, wrote the first draft of the paper, and critically revised the manuscript until publication.
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<tr>
<td>AADT</td>
<td>Annual average daily traffic</td>
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<td>ANOVA</td>
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<td>CoCA</td>
<td>Co-correspondence analysis</td>
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<td>db-RDA</td>
<td>Distance-based redundancy analysis</td>
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Gothenburg, April 2020

Zhenhua Sun
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1. Introduction

Roses 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 aquatic habitats (Klöckner et al., 2019, Haake and Knouft, 2019, Niu et al., 2019). 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, Balmori and Skelly, 2012). Amphibians, which migrate across roads, may especially suffer from increased mortality caused by vehicle collisions (Trombulak and Frissell, 2000).

The recognition of the adverse effects of roads on the environment and ecosystems by most roads administrations 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). Sustainable Development Goal (SDG) 15 “Life on land” also aims to protect, restore and promote the conservation and sustainable use of ecosystems as well as their services, ensuring the integration of land-based ecosystems and biodiversity values into national and local planning (United Nations, 2019). 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). Constructed wetlands and man-made ponds are examples of such systems.

Constructed wetlands and ponds can be used as stormwater management installations to attenuate flood impacts, to remove the pollutants at the source through sedimentation, to encourage infiltration of stormwater into the ground or discharge to a water body (Hoang and Fenner, 2015). Nowadays increasing number of studies focus on the aquatic biodiversity conservation of wetlands and ponds (Hsu et al., 2011, Vermonden et al., 2009, Meland et al., 2019). Wetlands and ponds may be effective in creating suitable habitats for hosting a number of organisms, including amphibians and invertebrates, and vegetation, because such systems provide food and provide breeding sites. The role of stormwater ponds and wetlands can be expanded from solely stormwater management to a triangle made up of water quality, water quantity and ecology. 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 odonates, are attracted by emergent vegetation. The emergent vegetation also plays the crucial role in increasing the likelihood of colonization by a more diverse assemblage of macroinvertebrates (Moore and Hunt, 2012). Hsu et al. (2011) also found that along with treating wastewater, the constructed wetlands have ability of supporting various wildlife and enhancing biodiversity. It has been proven that there is a positive relationship between the richness of aquatic macroinvertebrates and the cover of aquatic macrophytes (Hsu et al., 2011).

Such anthropogenic systems have been viewed as new aquatic habitats maximizing biodiversity opportunities and providing new opportunities for species to resist disturbances (Chester and Robson, 2013). Wetlands and ponds are a valuable resource for maintaining aquatic biodiversity, supporting many organisms, e.g. insects, amphibians and macroinvertebrates (Le Viol et al., 2012, Scheffers and Paszkowski, 2013, Hassall and Anderson, 2015). However, multiple ecosystem services interact in complex ways, and they can be affected negatively or positively as one service increases (Angeler et al., 2014). The interactions among different ecosystem services should be highlighted to discourage interventions enhancing single ecosystem service without considering other services (Queiroz et al., 2015). Due to the complex interactions among these ecosystem services, it is questioned to which extent SUDS can support biodiversity (Briers, 2014a, Tixier et al., 2012). For example, given the significant role of constructed wetlands and ponds in storing and treating road runoff, wetlands and ponds may contain high concentration of chemical pollutants. The diversity and composition of invertebrate communities may be negatively affected by road runoff due to exposure to pollutants and bioavailability of pollutants accumulating in sediments and exposure to pollutants (Tixier et al., 2012, Briers, 2014b). Another problem may occur from the intentional introduction of invasive species of animals and plants that are used to aid treatment function or unintentional introduction of invasive species (Briers, 2014b). As there are many factors affecting aquatic biodiversity in wetlands and ponds, a detailed analysis of these is needed to provide solid basis for biodiversity protection. Research is needed to identify and quantify the impact of the most relevant factors that regulate aquatic biodiversity, and to make a comprehensive plan of designing sustainable stormwater systems that integrate different aspects of sustainable development.
2. Aim and objectives

The overall aim of the research presented in this thesis is to provide a better understanding of the potential of stormwater ponds to support biodiversity. This research should lead to design recommendations for stormwater systems that would simultaneously provide multiple ecosystem services, including pollution retention, flood control, and promotion of biodiversity. This research focuses on the promotion of biodiversity in consideration of pollution retention.

The research presented in this thesis was mainly based on the roadside stormwater ponds, while other kinds of ponds and wetlands were included for comparison purposes. The objectives can be summarized as:

1) to identify the key environmental factors shaping the biological community composition in roadside stormwater ponds (papers I and II);
2) to compare the power of DNA metabarcoding and morphological identification in identifying macroinvertebrates to species level and analyzing impacts of environmental factors (paper III);
3) to assess differences in biological community composition, particularly macroinvertebrates, and environmental factors between roadside stormwater ponds and natural ponds at the regional spatial scale (paper IV);
4) to disclose differences in macroinvertebrate community composition and water chemistry parameters between stormwater ponds and natural ponds at the global spatial scale (paper V).
3. Background

3.1 Stormwater and sustainable urban drainage systems

3.1.1 Stormwater runoff pollution

Stormwater runoff is recognized as a major nonpoint source of pollutants released in the urban environment, thereby significantly contributing to the deterioration of receiving waters as well as ecosystems (Muthusamy et al., 2018). The pollutants come from a mix of activities and land uses, e.g. highway, construction sites, commercial and industrial activities (Müller et al., 2020, Lundy et al., 2012).

Roads and traffic are a major source of pollutants and contribute to stormwater pollution primarily through mechanical wear of pavement surface and vehicle tires and by release of chemicals into water running off the pavement surface (Müller et al., 2020). The road and traffic related pollutants mainly include road salt, suspended solids, polycyclic aromatic hydrocarbons (PAHs), metals (e.g. copper, lead, nickel and zinc) and nutrients. Vehicle wear, such as tires and brakes, contributes to total suspended solids (TSS), Cu, Zn, Cd, Pb, and PAHs (Councell et al., 2004, McKenzie et al., 2009, Hjortenkrans et al., 2007, Kose et al., 2008); road abrasion contains such pollutants as TSS and PAHs (van Duin et al., 2008, Horton et al., 2017, Markiewicz et al., 2017); vehicle washing is a source of Pb, Cd, Cr, and Zn (Sörme et al., 2001). Road salts are normally used during the winter and spring as de-icing agent, resulting in high concentrations of chloride discharged into receiving waters (Sun et al., 2018). The application of sand and grit for snow management leads to the higher concentration of TSS (Galfi et al., 2016) and pollutants, such as metals and PAHs, tend to partition onto such solids, in which smaller particles hold higher concentrations (Lau and Stenstrom, 2005).

The first flush effect is also important to consider in the context of managing urban nonpoint source pollution. The “first flush” effect refers to the higher concentration of pollutants during initial storm runoff (Acharya et al., 2010), and the magnitude of the first flush effect is higher in case of impervious areas compared with the pervious areas (Zeng et al., 2019). Therefore, highway runoff is most toxic in the early part of a storm (Kayhanian et al., 2008). Pollutant first flush can be affected by a variety of variables, such as rainfall depth and intensity, types of pollutants, and size of runoff peak (Perera et al., 2019, Lee et al., 2002).

3.1.2 Sustainable urban drainage systems

Traditional urban drainage system refers to the human-engineered centralized approaches, e.g. grey buried pipework and ancillary structures, to water management (Kapetas, 2020). Sustainable urban drainage systems are the stormwater facilities that have been built in cities and along highways due to the awareness of environmental impacts caused by the alteration of hydrological processes (Karlson and Mörtberg, 2015) and large amounts of particles and pollutants in the stormwater and road runoff (Bohemen and Janssen Van De Laak, 2003). These systems use green spaces and other environmental features, such as water bodies, to store the
stormwater and to reduce the first flush pollution within the catchment area. SUDS can reduce the negative impact of effluents from the conventional drainage systems on the water quality and biological integrity of receiving water bodies (Chocat et al., 2001). In addition to the ability to retain and treat runoff, SUDS have the potential to promote and maintain aquatic biodiversity (Hsu et al., 2011).

The adoption of SUDS is also in compliance with the main goal of the EU Water Framework Directive (WFD, 2000/60/EC), which is to achieve “good ecological and chemical status” for all of Europe’s surface waters and groundwater (Meland, 2015). Engineered sedimentation ponds and wetlands are two examples of SUDS commonly applied by many national road administrations. Designed and operated properly, these SUDS have proven to mitigate peak runoff volumes and protect waterbodies from road related pollution (Jato-Espino et al., 2016, Allen et al., 2019, Charlesworth et al., 2003). Stormwater ponds and wetlands are the main focus in this thesis (Figure 1).

3.2 Ecology basics and biodiversity

3.2.1 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 ecosystem services (Millennium Ecosystem Assessment, 2005). A unit of biodiversity is species, which is the lowest taxonomic rank of an organism. Biodiversity plays an important role in driving various ecosystem functions. For example, higher diversity of primary producers could produce more biomass (Cardinale et al., 2012) that can be used as biofuel; a high biodiversity of host species could reduce pathogen or herbivore damage on humans and animals through a “dilution effect” hampering transmission of pathogen or herbivore (Hambäck et al., 2014, van der Plas, 2019).
simultaneous performance of multiple ecosystem functions strongly relies on the biodiversity (Truchy et al., 2015), and ecosystem multifunctionality is more susceptible to species loss compared with single functions (Gamfeldt et al., 2008). Although biodiversity is strongly related to the ecosystem functioning, various anthropogenic activities, such as agricultural and urban expansion (Young et al., 2005, Feng et al., 2018), have resulted in the global biodiversity loss. Compared with any other ecosystem, the degradation and loss of aquatic ecosystems and their biodiversity is occurring faster (Aznar-Sánchez et al., 2019). Therefore, in face of the accelerated rate at which biodiversity is declining and the consequences for the ecosystem functioning, there is a need to protect biodiversity and the services biodiversity delivers to society and humans.

Several indices and methods have been developed for quantifying biodiversity, such as species richness, evenness, Shannon index, and Simpson’s index. Species richness is the most basic metric, which refers to the number of different 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., Simpson’s index and Berger-Parker index. Shannon-Wiener index, which provides more information about community composition than species richness, is one of the most widely used diversity indices. 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).

3.2.2 DNA metabarcoding and morphological identification

Identifications of taxonomy is usually performed through observation of morphological characteristics. A major challenge involved in this process is identification of organisms to the species level, and it is especially difficult for non-specialists. Even for taxonomic specialists, it may take several weeks to identify organisms just to the genus level (Morinière et al., 2016). Moreover, sometimes even experts are unable to identify some species that lack morphological diagnostic characteristics at the larval as well as at the adult stage (Macher et al., 2015). The limitations of morphological identification are particularly obvious for the large scale application of macroinvertebrate sampling (Hajibabaei et al., 2011), resulting in significantly higher identification error rates for species than family level (Hajibabaei et al., 2012). Regarding the responses of organisms to anthropogenic stressors, even closely related species within the same families could have quite different responses (Macher et al., 2015), therefore accurate identification of organisms to the species level is required to better understand how biodiversity responds to the anthropogenic stressors.

DNA-based identification is a method to identify specimens based on DNA sequence that provides massive amounts of reproducible and robust genetic data, e.g. cytochrome c oxidase I (COI) (Shokralla et al., 2012). Recently, DNA-based identification, e.g. DNA metabarcoding,
has gained popularity in overcoming the problems related to the morphological identification. DNA metabarcoding allows the rapid and cost-effective assessment and monitoring of biodiversity with massive parallel sequencing of bulk samples (Cristescu, 2014), and it has been successfully applied to samples for which species identification using traditional methods is impractical (Beng et al., 2016).

3.3 Factors affecting aquatic biodiversity in stormwater ponds

Although several studies have demonstrated and confirmed that stormwater ponds have the ability to promote and to maintain aquatic biodiversity, at least for certain species (Hassall and Anderson, 2015, Le Viol et al., 2009, Stephansen et al., 2016), there are also studies questioning whether stormwater ponds can support biodiversity taking into account the fate of pollutants accumulated in sediments (Hale et al., 2015, Bäckström et al., 2002). Therefore, understanding the factors that could potentially affect aquatic biodiversity plays the crucial role in better understanding to which extent stormwater ponds/wetlands are able to support and maintain aquatic biodiversity. In general, these factors can be classified into abiotic and biotic factors.

3.3.1 Abiotic factors

3.3.1.1 Physical characteristics of ponds

Pond size has been identified as one of the most important factors determining aquatic biodiversity within ponds. Several studies have shown that large ponds tend to support more species than small ones due to the lower vulnerability to disturbance (Gotelli and Graves, 1996, Hsu et al., 2011, Noble and Hassall, 2015). However, some studies found that this relationship had limitations; for example, Biggs et al. (2005) found that larger ponds were able to support more species for macrophytes, but not for invertebrates. Another observation is that a set of small ponds is likely to have more species than a single large pond with similar total area (Gee et al., 1997).

The density and connectivity of ponds within the landscape also affect species richness. Hassall (2014) defined these networks of distributed discrete habitat patches as “pondscapes”. The assemblages of species for both aquatic invertebrates and macrophytes are more homogenous in the areas with higher connectivity between ponds, because in such areas species are able to spread more easily between ponds (Gledhill et al., 2008). In contrast, fragmentation and isolation of pond habitats cause decline in species richness (Gledhill et al., 2008).

Pond age is another factor that may affect the variation in the biological community composition. The physicochemical environment of new ponds is quite different from that of older ponds. New ponds are dominated by inorganic substrates and lack vegetation cover, in which a variety of taxa thrive (Williams et al., 2008). However, the results from different studies were quite diverse. For example, Scher and Thiéry (2005) suggested that older ponds support
greater species richness, while Gee et al. (1997) demonstrated that there was no significant correlation between the number of taxa of macroinvertebrates and pond age.

### 3.3.1.2 Pollution levels in the water column and sediments

Several groups of pollutants such as trace metals, polycyclic aromatic hydrocarbons and nutrients are likely to absorb onto the surface of particle surfaces (Casey et al., 2007). Since in sedimentation ponds particles settle and accumulate at the bottom, these ponds act as pollution traps with high pollution levels in the sediments (Bäckström et al., 2002, Anderson et al., 2004). Thus, pond sediments are likely to store and convey toxic metals and organic micropollutants, thereby increasing the potential risks to aquatic ecosystems (Stephansen et al., 2016). Carew et al. (2007) mentioned that sediment pollution can reduce macroinvertebrate diversity and change community composition. Cox and Clements (2013) also found that concentrations of PAHs in the burrowing mayfly *Hexagenia* were significantly correlated with concentrations in sediments. Heavy metals that deposit onto sediment surfaces normally immobilize through, e.g., coagulation and adsorption, and only a small portion of free metal ions is dissolved in water (Zhang et al., 2014). The accumulated heavy metals in the sediments are removed through food chain, which may result in toxic effects for higher trophic level, e.g. nematodes and snails, through digestion (Crowder, 1991). Pollutants from sediments could also be released back into the water column under certain conditions and accumulate in plant and animal tissue (Dalu et al., 2017). Bioavailability and toxicity of heavy metals to aquatic organisms can vary with changes in e.g. pH, metal concentrations, salinity, and nutrients (Zhang et al., 2014). For example, Karouna-Renier and Sparling (2001) found that all macroinvertebrate groups in their study had much lower level of Pb accumulation than that in the sediments owing to the alkaline pH of the ponds.

In addition to the pollutants attached onto the sediments, the pollutants in the water column can also pose risks to aquatic organisms. For example, dissolved hydrocarbons are responsible for many acute toxic effects on organisms (Trett, 1989), especially low-molecular weight hydrocarbons, which are more toxic to the organisms due to the higher solubility in water (Pettigrove and Hoffmann, 2005). PAHs have been demonstrated to be highly toxic to aquatic organisms (Greenberg, 2003) and cause mortality in all life stages and decrease in growth (Barron et al., 2004). High concentrations of chloride (Cl\(^{-}\)) resulted from the use of road salt (NaCl) as a de-icing agent during winter could significantly decrease the species richness and abundance of aquatic organisms through negative effects on the osmoregulatory and physiological processes of aquatic invertebrates (Blasius and Merritt, 2002) and other indirect effects, e.g. increased concentrations of metals in the aqueous phase (Mayer et al., 2008). Furthermore, although nutrients, e.g. phosphorus and nitrogen, support the growth of algae and aquatic plants, excessive nutrients could limit vegetative productivity and alter species composition (Helfield and Diamond, 1997). It has been proven that eutrophication has a great impact on small standing water bodies (Menetrey et al., 2005) and leads to decrease in aquatic macrophyte communities (Conley, 1999).
The impacts of pollutants on aquatic organisms vary between species. Stormwater ponds may promote populations of pollutant-tolerant species and cause decline of pollutant-intolerant species. Snodgrass et al. (2008) found that *Rana sylvatica* was highly sensitive to exposure to polluted pond sediments, suffering a high level of mortality, while *B. americanus* were tolerant to polluted sediments and remained abundant in the ponds.

### 3.3.2 Biotic factors

#### 3.3.2.1 Vegetation

Several studies have showed that the diversity and abundance of aquatic macroinvertebrates are positively correlated with the coverage of macrophytes, because macrophytes provide more diversified and suitable habitats (Gee et al., 1997, Vermonden et al., 2009, Fontanarrosa et al., 2013a). In addition, greater amounts of vegetation provide more opportunities for amphibian presence, greater species richness and breeding success for amphibians (Scheffers and Paszkowski, 2013). However, the presence of dense vegetation may lead to problems associated with low dissolved oxygen and with increased nutrient loads from decomposition of plants (Sartoris et al., 1999), thereby negatively influencing aquatic invertebrate. Furthermore, some taxa that can stay submerged longer, e.g. Corixidae and predacious diving beetles, have been demonstrated to be negatively correlated with plant cover (De Szalay and Resh, 2000).

#### 3.3.2.2 Biological community interactions

Competition and predation are the most essential biological interactions in ecology and play a crucial role in maintenance of biodiversity (Chesson and Kuang, 2008). These interactions exist within native species as well as between invasive and native species.

The establishment of constructed ponds might introduce invasive alien species. Invasive species, which are introduced intentionally or unintentionally by human actions, may have negative interactions with native organisms (Shulse and Semlitsch, 2013). The vulnerability of freshwater ecosystems to being invaded and transformed by alien invertebrates is high (Ricciardi, 2015). This transformation process is also called biotic homogenization, in which a small number of expanding species that thrive in human-altered environments increasingly dominate species assemblages, leading to further losses in biodiversity and extinction of native species (Millennium Ecosystem Assessment, 2005).

Furthermore, predators in lotic water bodies have top-down effects on their prey through direct consumption of prey populations (Baum and Worm, 2009). Fish serving as vertebrate predator plays a crucial role in affecting invertebrate biodiversity, especially in small waterbodies (Wellborn et al., 1996, Chester and Robson, 2013). However, Sih and Wooster (1994) found that compared with fish, invertebrate predators appear to have a greater effect on benthic prey due to different behavioral responses of prey. Fish reduces the movement rate of invertebrate prey and makes them to seek refuge in the substrate, while invertebrate predators increase prey
4. Methodology

4.1 Study area

In paper I, 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 SAS – Såstad, FIU – Fiulstad, IDR – Idrettsveien, KAB – Karlshusbunn, NOR – Nordby, ENE – Enebekk. In B), the ponds are SKU – Skullerud, TAN – Taraldrud north, TAK – Taraldrud crossing, TAS – Taraldrud south, NØS – Nøstvedt, and VAS – Vassum. The distance between the two farthest ponds (Skullerud and Enebekk) is 71 km (Sun et al., 2018).](image)

In papers II and III, apart from the eight ponds (Skullerud, Taraldrud north, Taraldrud crossing, Taraldrud south, Nøstvedt, Vassum, Nordby and Enebekk) that have been studied in paper I, 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).
In paper IV, in addition to the ponds analyzed in papers II and III, 19 ponds located in the Akershus and Østfold counties in south-east of Norway were studied (Figure 4), including Arnestad, Ekeberg, Elingård_K, Elingård_M, Elingård_MV, Elingård_N, Glenge, Hyllibråten, Meum, Moer, Mørksand, Narvestad, Nordre Ugjestråd, Skoestrad-E, Skoestrad-S, Sutterhol, Svenke-Rånås_E, Svenke-Rånås_S, and Øvre Bjerketvedt. Since these 19 ponds were located within or in vicinity of cultivated landscape, they were categorized as forest ponds, meadow ponds and farmyard ponds. In this paper, all of them were considered as natural ponds.
In paper V, 1352 ponds from nine countries, including Argentina, Canada (Waterloo), Ireland, Norway, Slovakia, Sweden, Switzerland, the UK, and the United States (California), were studied. The detailed information for each pond was presented in Table 1.

### 4.2 Data collection

The data collection, except bioinformatics processing for the DNA metabarcoding, was carried out in separate studies. The author did not participate in sampling or data collection but obtained the data to perform statistical analysis.

In paper I, 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 analyzed, 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 neighboring ponds within a radius of 1 km, distance to the nearest neighboring pond, and annual average daily traffic (AADT). 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. Kick sampling with five sweeps was used when there were small stones on the bottom. When the bottom material
was not stony, five sweeps were taken through the water and aquatic vegetation when present at approximately 50 cm depth. Biological samples were identified to order, family or species level.

In paper II, sediment samples were collected in April 2013 and April 2014 close to the inlet with a spade and stored in 1 L glass bottles. Eleven sediment variables were analyzed in this paper, including total organic carbon (TOC), total hydrocarbons, US EPA 16 PAHs, and metals. Pyrene was used as proxy for PAH pollution, because it was quantified in all samples. Water samples were collected once in 2013 (April) and three times in 2014 (April, June, and August) close to the inlet of the ponds. The analyzed parameters included metals, chloride (Cl⁻), total nitrogen (TN), total phosphorus (TP) and sulphate (SO₄²⁻). Macroinvertebrates and amphibian samples were collected four times (April, June, August, and October) in both 2013 and 2014, respectively, and the sampling methods were the same as in paper I. Zooplankton was analyzed once in 2013 in the kick and sweep net samples, as well as separate plankton net hauls (mesh 90 µm).

In paper III, DNA metabarcoding was applied to the macroinvertebrates collected in 2013 (the sampling methods are the same as in paper II). The samples were preserved in 70% ethanol and stored at +5°C for the DNA extraction. Two fragments of the mitochondrial COI gene were amplified three times from each three DNA extraction subsamples. The polymerase chain reaction (PCR) and bioinformatic processing were carried out subsequently to identify the taxa. Parts of the COI gene from bulk samples were sequenced in parallel and the resulting reads were compared with a reference library to identify the taxa present in the samples.

In paper IV, the same data regarding the highway stormwater ponds as in paper II were used. The data obtained for the natural ponds were collected in May, July and September 2002. The water samples were random samples collected in 250ml plastic bottles, representing a picture of the situation at the time of sampling. The water quality parameters included conductivity and pH value, and the physical variables included pond size and the distance to the closest neighboring pond. A long shaft with 25×25 cm opening and 200 µm mesh width was used for the biological collection. The entire sample was sieved through a screen with a mesh size of 900 µm in the lab.

For papers I-IV, the physical data were obtained either from digital maps (Norwegian Mapping Authorities) or directly from the Norwegian Public Roads Administration (NPRA).

The information of environmental variables and methods that were used to collected macroinvertebrates in paper V are presented in Table I.
Table 1. Summary of the geographic scale, type of water bodies, macroinvertebrate sampling methodology, and environmental variables of included studies. Number of ponds is indicated by n.

<table>
<thead>
<tr>
<th>Location</th>
<th>Type</th>
<th>Macroinvertebrate sampling method</th>
<th>Environmental variables</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argentina, Buenos Aires, n=5</td>
<td>5 manmade permanent ponds</td>
<td>Monthly Oct 2003-Sep 2004 using rectangular nets (10 × 7.5 cm; 350 µm mesh)</td>
<td>NA</td>
<td>Fontanarrosa et al. (2013b)</td>
</tr>
<tr>
<td>Canada, Waterloo, n=10</td>
<td>5 stormwater ponds and 5 natural wetlands</td>
<td>Two-week intervals between May 2 and Aug 22, 2008 using Ekman grab (0.0225 m²)</td>
<td>Area, pH, conductivity, and nitrate (NO₃⁻)</td>
<td>Woodcock et al. (2010)</td>
</tr>
<tr>
<td>Ireland, Annestown River Catchment, Co. Waterford, n=10</td>
<td>5 integrated constructed wetlands and 5 natural ponds</td>
<td>Mar-Apr and Jul-Aug 2006 using 1-mm pond net (frame size 20 × 25 cm) and sweep</td>
<td>Area, pH, NO₃⁻, phosphate (PO₄³⁻), conductivity, and chloride (Cl⁻)</td>
<td>Becerra Jurado et al. (2009)</td>
</tr>
<tr>
<td>Norway, Oslo, Akershus and Østfold, n=43</td>
<td>12 highway stormwater ponds, 19 natural ponds (i.e. 8 meadow, 7 farm, 4 forest ponds)</td>
<td>Stormwater ponds: Apr, Jun, Aug and Oct 2013 and 2014 using kick net (opening 30 × 30 cm, mesh size 0.45 mm) and traps; Natural ponds: May, Jul and Sep 2002 using kick net</td>
<td>Area and conductivity</td>
<td>Sun et al. (2019b), Meland et al. (submitted)</td>
</tr>
<tr>
<td>Sweden, Stockholm, n=51</td>
<td>3 natural ponds and 48 urban ponds</td>
<td>May or Jun 2013 and 2014 using a bottom scoop net (diameter 20 cm, mesh size of 1.5 mm)</td>
<td>Area, total phosphorus (TP), and total nitrogen (TN)</td>
<td>Heino et al. (2017)</td>
</tr>
<tr>
<td>Switzerland, n=81</td>
<td>23 natural ponds and 58 manmade ponds</td>
<td>2012-2013 using PLOCH with original ploch sampler</td>
<td>Area, pH, conductivity, and Chl_a</td>
<td>Hepia (2014)</td>
</tr>
<tr>
<td>Slovakia, n=88</td>
<td>36 natural ponds and 52 manmade ponds</td>
<td>Summers 2012 and 2013 using PLOCH with original ploch sampler</td>
<td>Area, phosphorus (P), NO₃⁻, PO₄³⁻, pH, conductivity, and Cl⁻</td>
<td>Ladislav Hamerlik (Unpubl.)</td>
</tr>
<tr>
<td>UK, n=1022</td>
<td>230 urban and 607 non-urban ponds (environmental variables and invertebrates) and 10 extra urban and 175 extra non-urban ponds (environmental variables)</td>
<td>Across two or three seasons (spring/summer/autumn) using 3-min sweep</td>
<td>Area</td>
<td>Hill et al. (2017)</td>
</tr>
<tr>
<td>USA, California, n=42</td>
<td>9 natural ponds and 33 manmade ponds</td>
<td>Spring and summer (May 10-Jul 13) using 20 active net sweeps (500 µm D-frame dipnet)</td>
<td>Area, pH, conductivity, total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP)</td>
<td>Lunde and Resh (2012)</td>
</tr>
</tbody>
</table>
4.3 Data analysis

Multivariate statistical analysis was the main method used in this thesis to group different environmental variables, to examine variation of biological communities across a range of environmental conditions, and to explore the relations between different biological community compositions. Principal component analysis (PCA) and principal coordinates analysis (PCoA) were used to do indirect gradient analysis, summarizing the distributional properties and similarities of different environmental variables and biological communities. Redundancy analysis (RDA) and distance-based redundancy analysis (db-RDA) were used to do direct gradient analysis, examining the relationship between biological community composition and environmental variables. The relationship between different biological communities was investigated using co-correspondence analysis (CoCA). In addition, Chao 2, Mann-Whitney and Kruskal Wallis were used to examine differences in the number of taxa, while permutational analysis of variance (PERMANOVA) and betadisper function in R environment were used to examine difference in community composition between different types of ponds. Mann-Whitney and Kruskal Wallis were also used to compare environmental characteristics between different types of ponds. The detailed information for each analysis method is presented in the following sections. Further details are provided in the appended papers.

4.3.1 Key factors shaping the biodiversity in stormwater ponds

In papers I and II, PCA was applied to analyze the general trends in pollution levels in the water column and sediments, as well as to narrow down the variables for the subsequent constrained analysis.

In paper I, one-way analysis variance (ANOVA) followed by Tukey post hoc tests were used to examine the differences in water quality between different stormwater ponds. PCA and RDA were subsequently applied to exhibit the maximum variation in the biological community and to check the relationship between the biological community composition and the environmental factors, respectively. RDA with forward selection was applied to select the variables that had the most contribution to the variation in the biological community composition.

In paper II, RDA was used to evaluate the relationship between the variation in the macroinvertebrate community and the environmental variables. In order to check whether the biodiversity was linked to the environmental variables, a non-parametric multiple linear regression by RDA was applied, and the biodiversity was measured as Shannon index and the number of taxa. In addition, two separate RDAs were applied to explore the differences in number of taxa and Shannon index between the ponds; ponds were used as categorical explanatory variable. The ordination method symmetric CoCA was used to compare different biotic communities and explore the similarities between them. The compared communities were macroinvertebrates, zooplankton, and plant communities; the plant community was separated into plants within the ponds and plants along the edge of the ponds.
4.3.2 DNA metabarcoding

In paper III, PCoA was applied to compare the (dis)similarity of macroinvertebrate community composition in different ponds based on the datasets obtained using both DNA metabarcoding and morphological identification. Also, db-RDA was applied to examine the influences of environmental variables on the macroinvertebrate groups in common of the two methods. In addition, similar to papers I and II, PCA was used on both the sediment and water quality data to reduce the skewness and improve the normality of the data for the db-RDA.

4.3.3 Comparison of different types of ponds at a regional spatial scale

In paper IV, since two biological datasets were from different sources, which had different levels of taxonomic determination, detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA) were used to determine which taxonomic levels should be used in the subsequent analysis. Similar to paper III, PCoA was used to explore differences in community composition between highway stormwater ponds and natural ponds, while db-RDA was used to check relationships between the variation in the biological community composition and the explanatory variables. In addition, RDA was used to compare various explanatory variables and taxa richness between highway stormwater ponds and natural ponds, as well as to examine any relationship between taxa richness and explanatory variables.

4.3.4 Comparison of different types of ponds at a global spatial scale

All ponds were classified into two different ways. The first classification was based on the K-means value, which groups sampling ponds based on the similarity in land uses around each pond in certain areas (Table 2), and the second classification was based on the origin of ponds, i.e. natural and manmade ponds.

Table 2. Proportion of different land uses in each K-means cluster. The numbers in the “Motorway” column represent presence (1) and absence (0) of motorway.

<table>
<thead>
<tr>
<th>K-means cluster</th>
<th>Agricultural area</th>
<th>Natural area</th>
<th>Developed area</th>
<th>Motorway</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 “Motorway”</td>
<td>15%</td>
<td>64%</td>
<td>21%</td>
<td>1</td>
</tr>
<tr>
<td>2 “Natural”</td>
<td>6%</td>
<td>90%</td>
<td>4%</td>
<td>0</td>
</tr>
<tr>
<td>3 “Developed”</td>
<td>3%</td>
<td>51%</td>
<td>46%</td>
<td>0</td>
</tr>
<tr>
<td>4 “Agricultural”</td>
<td>51%</td>
<td>44%</td>
<td>5%</td>
<td>0</td>
</tr>
</tbody>
</table>

Chao 2, Mann-Whitney and Kruskal Wallis were used to examine differences in the number of taxa, while permutational analysis of variance (PERMANOVA) and betadisper were used to examine differences in community composition among K-means clusters and between pond types. Mann-Whitney and Kruskal Wallis were also used to compare environmental data among
K-means clusters and between pond types. Bray-Curtis similarity was used to analyze the macroinvertebrate datasets, while Euclidean distance was used for the environmental datasets.
5. Results

In paper I, 8 out of 14 variables were identified to have the significant contribution to the explained variation in the biological community composition. These variables were metals, Cl\(^-\), phosphorus (P), DO, total hydrocarbons, AADT, distance to the nearest neighboring 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 neighboring 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, Cl\(^-\), DO, P, and hydrocarbons) explained 48%, and the group of physical variables (pond size, AADT, and distance to the nearest neighboring pond) explained 41% of the total variation in the biological community composition.
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 neighboring pond from each study pond. Blue arrows indicate different taxa. Red arrows indicate explanatory variables. Figure A) describe the relationship between environmental variables and aquatic organisms. Figure B) describes the relationship between environmental variables and different samples. The same symbol with the same color 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. (Sun et al., 2018)

In paper II, the results (Figure 5) showed that pollution levels in the water column and sediments explained most of the variation in the macroinvertebrate community composition, and most of the displayed taxa were negatively correlated with them. The number of neighboring ponds within a radius of 1 km also had a considerable contribution to the variation, and some taxa were positively correlated with the number of neighboring ponds, while some were negatively correlated. Moreover, the results for pond size are in agreement with paper I, in which most displayed taxa were positively correlated with pond size. In addition to the community composition, the relationships between various environmental variables and biodiversity were also explored. The results showed that the explanatory variables, including the physical characteristics of the ponds, the pollution levels in the water column and sediments, and the presence of amphibians, were not significantly related to the biodiversity measured as the number of taxa and the Shannon index.
The results of relationship between different biological communities revealed that the similarity between macroinvertebrate and zooplankton taxa as well as macroinvertebrates and the plants within and along the edge of the ponds was not high. Furthermore, there was a non-significant positive correlation between the number of plant taxa and the number of macroinvertebrate taxa as well as between the number of macroinvertebrate taxa and the number of zooplankton taxa.

Figure 5. (A) Redundancy analysis (RDA) of the relationship between macroinvertebrates and environmental variables as well as amphibians. TheClsPn represents the distance to the nearest pond from the study pond; NumbOfPo represents the number of ponds/water bodies within 1 km; WatPoll and SedPoll represent the pollution levels in the water column and sediments, respectively. FroPrese and FroAbsen represent presence and absence of frogs; SalPrese and SalAbsen represent presence and absence of salamander. (B) RDA of the relationship between Shannon indices and environmental variables as well as amphibians. The circles represent Shannon indices. (Sun et al., 2019b)

In paper III, a total of 156 macroinvertebrates were identified using morphological identification, while 599 operational taxonomic units (OTUs) were identified to invertebrate taxa using DNA metabarcoding, and most of which were species. All identified groups of macroinvertebrates were compared between morphological identification and DNA metabarcoding. Diptera species identified with DNA metabarcoding were significantly higher than that identified with morphology (Figure 6).
Figure 6. Comparison of the number of species detected by DNA metabarcoding and morphology in the taxonomic groups in common. The abbreviations along the x-axis represent names of ponds: els-Elstadmoen, ene-Enebekk, for-Fornebu, hov-Hovinmoen, nor-Nordby, nos-Nostvedt, sku-Skullerud, tak-Taraldrud crossing, tan-Taraldrud north, tas-Taraldrud south, ten-Tenor, and vas-Vassum. (Sun et al., 2019a)

The results of db-RDA showed that pollution levels in the water column and sediments, as well as pond size explained the most of variances in the macroinvertebrate community composition for the morphological dataset based on abundance, followed by presence/absence dataset based on morphological identification. The environmental variables explained the least variation in the biological community composition identified by DNA metabarcoding due to the increased complexity resulting from the increased number of species. Regarding the responses of macroinvertebrates to the environmental stressors between different methods, the difference was quite obvious within the dipteran group (Figure 7). For the DNA metabarcoding dataset, 12 of 26 displayed dipteran species were positively correlated with the pollution level in the sediments, while 14 were negatively correlated. Similarly, 13 dipteran species were positively correlated with pollution level in the water column, while 13 were negatively correlated. For the morphological dataset, most of the displayed dipteran taxa (seven out of nine) were positively correlated with pollution levels in the water column and sediment. The other groups exhibited similar response to the pollution levels in the water column and sediments between
morphological identification and DNA metabarcoding. For example, most taxa from groups Ephemeroptera, Plecoptera, and Trichoptera were negatively correlated with the pollution level in the sediments.

Figure 7. The relationship between macroinvertebrate community composition and pollution levels in the water column and sediments, as well as pond size. (A) Results obtained from db-RDA for the DNA metabarcoding dataset. (B) Results obtained from db-RDA for the morphological dataset based on presence/absence. (C) Results obtained from db-RDA for the morphological dataset based on abundance. The purple arrows represent different species; the red arrows represent environmental variables, in which WaterPol and Sediment represent pollution levels in the water column and sediments, respectively. (Sun et al., 2019a)

In paper IV, the distribution of the number of taxa in highway stormwater ponds and natural ponds was significantly different (pseudo-F=9.1, p=0.006) (Figure 8). Number of taxa
determined to genus level appeared slightly higher in highway stormwater ponds than that in natural ponds.

Figure 8. Taxa richness depicted in boxplot showing number of taxa determined to genus level present in natural ponds (n=19) and in highway stormwater ponds (n=24). Natural ponds and highway stormwater ponds are colored green and grey, respectively.

The db-RDA with forward selection selected three variables, which significantly contributed to the observed variation in the biological community composition within two types of ponds, including conductivity (explained variation=12 %, p=0.0005), surface area (explained variation=4 %, p=0.006) and number of ponds within 1 km (explained variation=3 %, p=0.0325). The exhibited organisms dominating in the highway stormwater ponds were positively correlated with the pond surface area and conductivity, while most of the exhibited organisms dominating in the natural ponds, except Dytiscidae, were negatively correlated with pond surface area and conductivity (Figure 9).
Figure 9. A) Principal coordinate analysis (PCoA, Hellinger distance) and B) distance-based redundancy analysis (dbRDA, Hellinger distance) using forward selection on data with taxonomic resolution at Genus level (n=43 cases and 98 taxa). The natural ponds are depicted by green circles and highway stormwater ponds are depicted by grey squares. Explanatory variables that were statistically significant (p<0.05) are depicted by red arrows and taxa are depicted by mauve arrows.

In paper V, there was no statistically significant difference in the alpha diversity among K-means clusters in Argentina, Canada (Waterloo), Slovakia and Switzerland, while in Norway, Sweden, the USA (California), and the UK, alpha diversity was significantly different among K-means clusters, and ponds in cluster 1 generally supported significantly greater number of taxa compared to ponds in cluster 3. Regarding gamma diversity, it was similar among K-means clusters in Canada (Waterloo), Sweden, and Switzerland (Figure 10) and significantly different in the rest countries. Ponds in cluster 3 supported significantly greater number of taxa than cluster 1 in Slovakia, the USA (California) and the UK, while in Norway it was opposite. Furthermore, alpha and gamma diversity was similar between natural and manmade ponds in most countries, except Norway and Sweden. In Norway, ponds in cluster 1 supported significantly greater alpha and gamma diversity than cluster 3, and in Sweden alpha diversity was significantly higher in cluster 1 compared to cluster 3. In addition, manmade ponds in Norway and Sweden supported significantly greater gamma diversity compared to natural ponds (Figure 11).

PERMANOVA based on the Bray-Curtis dissimilarity matrix confirmed that K-means clusters were not significant drivers of macroinvertebrate community composition in Argentina, Canada (Waterloo), Switzerland, and the UK, while in Norway, Slovakia, Sweden, and USA (California), the variation in the community composition was significantly different between K-means clusters. Nevertheless, all K-means clusters had quite high heterogeneity, and heterogeneity in cluster 3 was significantly higher than that in cluster 1 and 4 (Figure 12).
Regarding the type of ponds, except Switzerland, there was a significant effect of pond types on the variation in the community composition in the rest countries, and natural ponds generally had a slightly or considerably greater heterogeneity compared to manmade pond (Figure 13).

Figure 10. Median macroinvertebrate taxa richness for K-means clusters based on land use: cluster 1 “motorway”, cluster 2 “natural”, cluster 3 “developed”, and cluster 4 “agricultural”. Boxes show 25th, 50th and 75th percentiles.
Figure 11. Median macroinvertebrate taxa richness for natural (N) and manmade (M) ponds. Boxes show 25th, 50th and 75th percentiles.
Figure 12. Boxplot of heterogeneity among K-means clusters based on land use: cluster 1 “motorway”, cluster 2 “natural”, cluster 3 “developed”, and cluster 4 “agricultural”. Boxes show 25th, 50th and 75th percentiles.
Figure 13. Boxplot of heterogeneity between types of ponds. “N” represents natural ponds, and “M” represents “manmade ponds”. Boxes show 25th, 50th and 75th percentiles.
6. Discussion

The potential role of stormwater ponds along the highway as refuges for aquatic biodiversity was examined through analyzing the relationship between biological community composition and different environmental variables, including water quality, pollution in sediments, and physical variables. It should be noted that there is a limitation in the water quality datasets from 2013 and 2014, because the water samples were collected only once in 2013 and three times in 2014. Concentrations of pollutants in stormwater ponds vary a lot because of the episodic nature of road runoff. It is beneficial to have several samples within one year. Therefore, sediments samples are more suitable for assessing the general pollution level in ponds.

6.1 Key factors shaping the biodiversity in stormwater ponds

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). Furthermore, in our study, due to the higher connectivity between ponds, facilitating mobility of invertebrates between ponds, most displayed taxa exhibited positive correlation with the number of ponds; this was demonstrated by Gledhill et al. (2008). Several studies had the same finding with us, proving the importance of nearby ponds in the maintenance and promotion of aquatic biodiversity (Staddon et al., 2010, Noble and Hassall, 2015). The relationship between organisms and AADT was unclear in our study. In paper I, most taxa were positively correlated with AADT, while in paper II, most taxa were negatively correlated with AADT. Kayhanian et al. (2003) demonstrated that no direct linear correlation between pollutant concentration in road runoff and AADT can be seen. Therefore, there may be other factors playing the dominant role in affecting aquatic biodiversity in the ponds instead of AADT.

The pollution level in the water column had negative effects on many taxa in our study. 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, the Cl⁻ concentration was quite high in some ponds. 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 caused by lack of water circulation due to the higher density of saline water, and release of trapped metals from sediment (Van Meter et al., 2011). 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 behavioral 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 did not appear to be a limiting factor, since the concentrations were above the threshold value for aquatic organisms to live. Some of the taxa that were positively correlated with the pollution level in the water column are known to be very tolerant to pollution, and some of them were air-breathing organisms. The oxygen levels in the highway stormwater ponds may be hypoxic and even anoxic (Meland, 2010), and such conditions may support the presence of air-breathing taxa.

Most of the displayed taxa in ordination plots showed negative correlation with the pollution level in the sediments. Various biological and ecological characteristics of each species determine the nature of species exposure and sensitivity to disturbance, as well as species ability to deal with environmental change (Colas et al., 2014). Among the taxa that were positively correlated with the pollution level in the sediments, the midges are normally considered pollution tolerant taxa (Dalu et al., 2017). In addition, the toxicity of pollution level in the sediments to the midges may be reduced due to the relatively high abundance of *Lumbriculus variegatus* in some ponds. It has been proven that *Lumbriculus variegatus* was able to change sediment geochemistry through digging burrows and depositing a layer of fecal pellets, thereby decreasing zinc concentration in pore water that is the main exposure pathway to the midges (Colombo et al., 2016). Many taxa that were negatively correlated with the pollution levels in the sediments and water column belong to Ephemeroptera and Hemiptera. Many Ephemeroptera species have been demonstrated to be sensitive to organic pollution and metals (Ab Hamid and Md Rawi, 2017, Clements et al., 2002). In our study, the concentrations of most toxic metals in the sediments, e.g. lead and nickel, were relatively low. Pyrene that was used as a proxy for polycyclic aromatic hydrocarbons in the analysis, can lead to acute and chronic toxicity, and six sediment samples in our study were categorized as “poor quality” regarding pyrene.

Regarding the biodiversity measured as the number of taxa and Shannon index, the explanatory variables did not have a significant impact on it. Therefore, more studies are still needed.

The similarities in patterns between macroinvertebrates and zooplankton as well as macroinvertebrates and plants within and along the edge of the ponds were not high. In addition, the number of all macroinvertebrates and the number of odonates, which are predatory macroinvertebrates, exhibited a non-significant correlation with the number of zooplankton. Tolonen et al. (2005) also found there was no congruence existing in the species richness or evenness between macroinvertebrates and zooplankton.
6.2 DNA metabarcoding

Due to practical reasons, existing freshwater bioassessment programs normally use higher-level taxonomic groups (Carlson et al., 2018, Pandey et al., 2017), e.g. genus or family, and this causes uncertainty in determining species-specific responses of pond ecosystem to environmental stressors. In this thesis, it was investigated whether DNA metabarcoding can provide better results than morphological methods in identifying organisms to species level and examining effects of environmental stressors.

DNA metabarcoding identified significantly more Diptera species than morphological identification in all ponds. Due to the lack of diagnostic characters, it is very difficult to identify Diptera larvae, especially Chironomidae, using morphological identification (Dobson, 2013, Baloglu et al., 2018). Moreover, the damage of important morphological features resulting from improper specimen handling makes morphological identification a difficult task (Chan et al., 2014). The findings of this thesis are in agreement with several other studies (Ekrem et al., 2010, Silva and Wiedenbrug, 2014).

However, for some groups, e.g. Odonata, the morphological method identified more species in some samples than DNA metabarcoding. This may be attributed to the “barcode gap”, which determines the accuracy of DNA metabarcoding. Species-level identification becomes ambiguous for species complexes where maximum intraspecific distance exceeds the distance to the nearest neighbor (Jinbo et al., 2011, Sun et al., 2016). If high intraspecific divergence represents cryptic species, it can also influence identification success. In addition, the failure of DNA metabarcoding could also be caused by the lack of reference sequences in established reference databases (Chan et al., 2014). Several reasons could be behind the missing Oligochaeta, Odonata, and Gastropoda species in our DNA metabarcoding results, e.g. very low abundance of oligochaetes, poorly preserved samples, and not good matching between our primers with the species.

Results of the (dis)similarity of macroinvertebrate community composition from morphological identification using abundance explained the highest variation of macroinvertebrates. However, the dimensionality of the DNA metabarcoding data is more complex compared with the morphological data due to a much higher number of detected taxa, which caused a decrease in the explained variation.

The result of db-RDA for the morphological method showed that most dipteran taxa were positively correlated with the pollution levels in the water column and sediments. This is in accordance with findings of several studies, which considered dipteran taxa as pollution tolerant taxa and dominating in polluted waters (Tchakonté et al., 2015, Arimoro et al., 2015). The results for DNA metabarcoding, however, indicated that dipteran species exhibited a broad variation in their tolerance to pollution. This is because Diptera were only identified to genus, subfamily, or family level using the morphological method, while using DNA metabarcoding Diptera were identified to species level. Raunio et al. (2007) suggested that chironomid genera
should be classified as pollution intolerant when they are identified to the species level, because congeneric species may be different in their tolerance.

For some groups, the results of db-RDA for the morphological identification and DNA metabarcoding exhibited similar results. For example, the species *Libellula quadrimaculata* in the Odonata group showed the same response to environmental variables based on both DNA metabarcoding and morphological identification, i.e. it was positively correlated with the pollution levels in the sediments and water column. *Libellula quadrimaculata* belongs to the family Libellulidae, which contains many tolerant species (Villalobos-Jiménez et al., 2016). Another common species from Odonata group, *Aeshna juncea*, was positively correlated with pollution level in the sediments and pond size, while negatively correlated with the pollution level in the water column. *Aeshna juncea* has been demonstrated to be highly tolerant of manganese and nickel (Girgin et al., 2010).

### 6.3 Comparison of different types of ponds at the regional spatial scale

The biological community composition in the highway stormwater ponds was quite different from that in the natural ponds. Most of the dominating taxa in the highway stormwater ponds were pollution tolerant. Moreover, unique taxa in the stormwater ponds, such as Veliidae, do not completely rely on water due to their habits of utilizing, e.g. oxygen (Barman and Gupta, 2015). The differences in the physical and chemical characteristics, except conductivity, were small between natural and highway stormwater ponds. Conductivity in the highway stormwater ponds was significantly higher than that in the natural ponds due to the use of sodium chloride as a de-icing agent on roads in Norway (Sun et al., 2018).

The result of db-RDA with forward selection showed that ponds within a radius of 1 km, pond surface area and conductivity had the greatest effects on the biological community composition, and the latter two explanatory variables were significantly different between natural and highway stormwater ponds. The taxa that were dominating in the highway stormwater ponds were positively correlated with these three variables, while most of the taxa that were dominating in the natural ponds responded in the opposite way. Within the taxa that were positively correlated with the conductivity, Baetidae appears to be the taxa that was more tolerant of salinity than other mayflies (Ephemeroptera); this is in agreement with the findings of Timpano et al. (2018) and Pond (2010). Dytiscidae is another taxon that exhibited positive correlation with conductivity; it has been previously shown that the taxa belonging to the order Coleoptera were positively correlated with conductivity (Mereta et al., 2012).
6.4 Comparison of different types of ponds at the global spatial scale

Differences in environmental characteristics were investigated. However, it should be noted that it is not possible to unambiguously confirm the influence of landscape on environmental characteristics, because each cluster was characterized by a combination of land uses.

Biological datasets showed that landscape has certain influences on the alpha and gamma diversity in some countries, while alpha and gamma diversity were similar or even higher in manmade ponds compared to natural ponds. Le Viol et al. (2009) and Hill et al. (2017) also found that stormwater or urban ponds are able to support similar richness and community compositions as natural or non-urban ponds. In our study, manmade ponds from Norway and Sweden supported significantly higher gamma diversity of macroinvertebrates than natural ponds, and manmade ponds from Norway also supported significantly higher alpha diversity than natural ponds. This is probably because due to the different functions of manmade and natural ponds, they support very different macroinvertebrate communities. It has been demonstrated by our previous study (Meland et al., submitted) that more pollution tolerant taxa, e.g. Coenagrionidae and Libellulidae, and unique taxa, e.g. Veliidae that are present on the water surface, were found in stormwater ponds, while in natural ponds there was a higher prevalence of less tolerant taxa. This finding is consistent with study of Hill et al. (2017), who found that tolerant taxa were the dominant taxa in urban ponds. Regarding spatial heterogeneity, K-means clusters and pond types, in our study, generally had quite high heterogeneity, and natural ponds had slightly or considerably greater heterogeneity than manmade ponds. Landscape heterogeneity plays a crucial role in promoting biodiversity, and several studies have confirmed that due to the greater heterogeneity in species assemblages small water bodies can contribute to biodiversity (Davies et al., 2008, Thiere et al., 2009). Therefore, the ponds in our study are able to effectively enhance gamma diversity due to the high levels of spatial heterogeneity.

6.5 Design recommendations

Stormwater ponds have been developed for the primary purpose of stormwater management, and only limited consideration has been given to the design components that have been shown to affect the biodiversity potential of these systems. In this thesis, the following considerations are given focusing on aspects of the design that yield the best results from a biodiversity perspective. As discussed in the previous sections, pond surface area is considered a key factor in the presence and colonization of aquatic macroinvertebrates. Based on the results, it is highly recommended to establish bigger ponds due to the lower vulnerability to disturbance, e.g. water pollution, thereby supporting high species richness. Larger ponds could dilute concentrations of pollutants and reduce the negative effects resulting from pollution on aquatic organisms, and larger ponds could also provide a more heterogeneous environment, which is better for supporting aquatic biodiversity. Another important design component that is worth considering is connectivity. High connectivity between different ponds or with other water bodies provide
continuous habitat for highly dispersive aerial macroinvertebrate. Therefore, it is highly recommended that several ponds should be established within one catchment to connect isolated areas in the landscape. However, larger ponds and additional ponds are not easily implemented due to the increased costs during construction and operation phase. Even though water and sediment pollution seem like a major issue for the conservation of biodiversity in stormwater ponds, many aquatic organisms in stormwater ponds have broad tolerances to pollution. It is worth noting that this thesis has limitation in providing the specific numbers for the optimal pond size and optimum number of ponds within one catchment. Further research is needed to provide such information.
7. Conclusions

The general aim of this thesis was to determine to which extent stormwater ponds can support and promote aquatic biodiversity based on a comprehensive understanding of environmental factors and to provide design recommendations for stormwater systems. The main conclusions for the objectives listed in chapter 2 are provided below.

1. Identification of the key environmental factors shaping the biodiversity in roadside stormwater ponds
   - One of the main factors controlling the aquatic organisms is the pond size, and most taxa were positively correlated with the pond size due to the “species-area effect” and the dilution of harmful pollutants.
   - The number of neighboring ponds is another physical factor that had a great contribution to the biological community composition, and most taxa were positively correlated with it.
   - Pollution levels in the water column and sediments are also important factors governing the biological community composition. Most of the taxa were negatively correlated with pollution levels in the water column and sediments. However, there was no statistically significant impacts of environmental variables on biodiversity, measured as the number of taxa and Shannon index.

2. Comparison of the power of DNA metabarcoding and morphological identification in identifying macroinvertebrates to species level and analyzing impacts of environmental stressors
   - DNA metabarcoding is powerful in identifying invertebrates, especially for dipteran species. DNA metabarcoding identifies much more species than morphological identification, thereby providing a more accurate estimate on how aquatic organisms respond to environmental stressors. The reliance on morphological methods has limited our perception of the aquatic biodiversity in response to environmental stressors. Although most dipteran families have been considered pollution tolerant, most dipteran species vary with respect to pollution tolerance.

3. Assessment of differences in biological community composition, in particular macroinvertebrates, and environmental factors between roadside stormwater ponds and natural ponds at the regional spatial scale
   - The biological community composition appeared to be very different between the natural and roadside stormwater ponds. Most of the dominating taxa in the roadside stormwater ponds were pollution tolerant.
   - Conductivity in the roadside stormwater ponds was significantly higher than that in the natural ponds. However, there was no adverse effect of conductivity on the exhibited taxa dominating in the stormwater ponds due to the high pollution tolerance.
4. Assessment of differences in macroinvertebrate community composition and water chemistry parameters between roadside stormwater ponds and natural ponds at the global spatial scale
   - Landscape type had no influence on pH in most countries, while manmade ponds generally had slightly or significantly higher pH than natural ponds. Conductivity and chloride concentrations were affected by landscape type in some countries, and conductivity in K-means cluster 1 “Motorway” was higher than cluster 3 “Developed” due to the presence of a motorway. In addition, no significant difference in nutrient concentrations was observed among K-means clusters due to the combination of land uses in each cluster.
   - Alpha and gamma were similar or even higher in manmade ponds compared to natural ponds. All ponds based on either K-means value or the types of ponds showed high levels of spatial heterogeneity, which enhances gamma diversity.

This research shows that roadside stormwater ponds have the potential to provide suitable habitats for taxa that are moderately to strongly tolerant to pollutants, but for taxa that are not tolerant to pollutants, stormwater ponds may not provide additional habitat. Habitat characteristics, such as pond size and connectivity, also act as important factors in shaping the assemblages and compensating the negative effects of pollution. Therefore, when such kind of systems are established, larger ponds should be built to promote aquatic organisms while considering the functions of pollutant removal. In addition, creating some additional ponds in the vicinity of the ponds would assist in promoting aquatic biodiversity through higher connectivity. What is more, the pollution retention processes would create a unique environment within the stormwater ponds, presumably characterized by higher pollutant concentrations, which would be suitable for species not typically found in natural ponds. All in all, stormwater ponds constitute a valid option in the areas along the highway, since they have the potential to combine water treatment properties with providing a suitable habitat for aquatic organisms.
8. Perspectives for future research

At present, engineers and planners lack the comprehensive knowledge and tools to be able to establish stormwater ponds that can provide multiple ecosystem services simultaneously. This research is a foundation for addressing this need. There was an apparent negative effect of pollution levels on the macroinvertebrate community composition, but not on the biodiversity measured as the number of taxa or Shannon index. Further analyses offer the potential to assist engineers and managers in optimizing stormwater systems. A larger number of ponds could be included in the future research to capture a broader range of conditions in terms of water quality and pollution levels. Furthermore, a systemic assessment of environmental change requires a large amount of data with sufficient temporal span and spatial extent (Angeler et al., 2014). Consequently, datasets from long-term and more frequent monitoring programs may allow for a better insight of biodiversity responses to pollution level. For example, more frequent monitoring could reveal that there were changes at a shorter temporal scale due to polluted runoff episodes.

Additionally, experiments with different pond designs could be carried out in combination with the application of current findings. It has been demonstrated in this research that ponds characterized by relatively large surface area and high connectivity are more likely to yield higher invertebrate biodiversity. In future research, designs with different sizes and numbers of ponds could be set up for experimental manipulation, facilitating hypothesis testing for the optimal pond size and optimum number of ponds within one catchment. In addition, different types of ponds receiving different amounts of runoff from the same road stretch could be built in the same area, thereby allowing monitoring of the ecological succession and colonization in these ponds. This could give new insight into how pond design impacts biodiversity while still enabling effective pollution treatment and flood mitigation.

In further research, ephemeral and permanent ponds could be compared. Ephemeral ponds may provide seasonal differences in water quality parameters, and such differences affect which organisms can live within these different habitats. All ponds studied in this project were permanent ponds.

Another potential avenue to pursue in use of process-based modelling. Statistical models have been widely used to estimate variations in the biological community composition caused by anthropogenic activities. However, this type of models cannot simulate the various processes involved in aquatic ecosystems or provide a direct overview of how different environmental factors influence biodiversity. In addition, most of existing studies relating to stormwater ponds used statistical models instead of process-based models to analyze the influences of environmental factors on the organisms. The next step would therefore be 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 water quality 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 aquatic organisms and their environment.
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