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Aquatic biodiversity in sedimentation ponds receiving road runoff – What are the key drivers?

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Abstract

Recently, increased attention has been paid to biodiversity conservation provided by blue-green solutions such as engineered ponds that are primarily established for water treatment and flood control. However, little research has been done to analyse the factors that affect biodiversity in such ponds. The purpose of this study was to evaluate the influence of environmental factors on aquatic biodiversity, mainly macroinvertebrate communities, in road sedimentation ponds in order to provide a foundation for recommendations on aquatic biodiversity conservation. Multivariate statistical methods, including unconstrained and constrained analysis, were applied to examine the relationships between organisms and the water quality as well as physical factors (including plant cover). Stepwise multiple regressions indicated that the most important variables governing the variation in the biological community composition were pond size, average annual daily traffic, metals, chloride, distance to the closest pond from study pond, dissolved oxygen, hydrocarbons, and phosphorus. The presence of most taxa was positively correlated with pond size and negatively correlated with metals. Small ponds with high pollutant loadings were associated with a low diversity and dominated by a few pollution tolerant taxa such as oligochaetes. A comprehensive understanding of impacts of various environmental factors on aquatic biodiversity is important to effectively promote and conserve aquatic biodiversity in such sedimentation ponds. Our results indicate that road sedimentation ponds should be designed large enough, because large ponds are likely to provide a more heterogeneous habitat and thus contain a species rich fauna. In addition, larger ponds seem to be less contaminated due to dilution compared to smaller ponds, thereby maintaining a higher biodiversity. Finally, creating some additional ponds in the vicinity of the sedimentation ponds in areas with few water bodies would increase the connectivity that facilitates the movement of invertebrates between ponds.

Keywords: aquatic biodiversity; pond size; road runoff; road salt; sedimentation ponds; water quality
1. **Introduction**

It is widely accepted that roads have major environmental impacts on aquatic ecosystems. For example, habitat quality can be altered through sediment loading (Angermeier et al., 2004) and pollutants released from transportation (Le Viol et al., 2009, Scher and Thiéry, 2005). Runoff from roads contains a plethora of pollutants and is considered a major source of diffuse pollution (Bohemen and Janssen Van De Laak, 2003), causing negative impacts on the receiving water bodies (Meland et al., 2010a, Jensen et al., 2014). Therefore, the national road administrations as well as the environmental authorities consider pollution reduction to be important. In most countries, blue-green solutions such as engineered sedimentation ponds and wetlands are the preferred mitigation measure for protecting receiving waters both from peak runoff volumes and elevated pollution loadings and concentrations (Meland, 2016). In addition to pollution, roads and the construction of them may disturb or even destroy aquatic habitats physically. Disruption of connectivity by roads may also negatively affect the movement of animals (Forman et al., 2003). In comparison to terrestrial habitats, freshwater habitats suffer greater biodiversity decline due to various stressors dominated by anthropogenic variables, such as habitat loss and degradation, and pollution (Hassall, 2014, Burroni et al., 2011).

Ponds are defined as engineered and natural water bodies between 1 m² and 2 ha in area, that may be permanent or temporary (Biggs et al., 2005). A highway sedimentation pond, which reduces the peak flow during major storm events and prevents water from either chronic or acute contamination reaching streams and lakes (Scher et al., 2004), functions as a part of an urban drainage system. Sedimentation ponds have also recently gained interest in an ecological context due to their potential capacity to conserve and promote aquatic biodiversity e.g. Le Viol et al. (2009) and Chester and Robson (2013). Only in Europe, thousands of ponds and other blue-green solutions are built along major roads (Meland, 2016). The high number may in fact underline their importance and relevance in an ecological context. Compared with other freshwater habitats, natural ponds can support significantly more species, especially rare, endemic and/or Red List species (Céréghino et al., 2007). These
multiple ecosystem services provided by ponds make them excellent candidates to be incorporated into road construction.

Some studies found that pond density is a major variable that determines aquatic macroinvertebrate richness (Gledhill et al., 2008, Staddon et al., 2010, Hassall, 2014). Gledhill et al. (2008) indicated that species richness for macroinvertebrates was higher in an area with more ponds, potentially due to higher connectivity between ponds. Hassall (2014) suggested maximizing the habitat connectivity between ponds to enhance and protect biodiversity. Plant cover is another factor that influences the distribution of aquatic invertebrates by, for instance, affecting predation and food availability (De Szalay and Resh, 2000). The richness and density of aquatic macroinvertebrates in ponds with vegetated areas are significantly greater than in ponds lacking vegetation (Hsu et al., 2011). Pond size is also likely to affect aquatic biodiversity; larger ponds tend to contain more species. However, Oertli et al. (2002) demonstrated that this biogeographic principle has limitations when it is applied to ponds; they found that it was only relevant for a few taxa, such as Odonata. Regarding pond age, Williams et al. (2008) found that compared with older ponds, 6-12-year-old ponds were able to support significantly more species and more uncommon species, while Gee et al. (1997) demonstrated that the number of taxa of macroinvertebrates was not significantly related to pond age. In addition, owing to the pollutant retention function, ponds normally contain high levels of pollution (Karlsson et al., 2010, Vollertsen et al., 2007), and may become sink-habitats and ecological traps. Chemical pollutants have lethal and sublethal effects on aquatic organisms via physiological and behavioural processes (Foltz and Dodson, 2009). Even if the concentration of a pollutant is low, chemical accumulation in roadside ponds can be an issue, as in the case of metals (Chester and Robson, 2013). Accumulation of metals and organic pollutants in the sediments may have long-term adverse effects on aquatic organisms (Grung et al., 2016), and it has been shown that metals and PAHs are easily accumulated in aquatic organisms (Meland et al., 2013, Grung et al., 2016).

There is still a lack of comprehensive understanding of factors that influence aquatic biodiversity (Hsu et al., 2011). Although some studies have examined the effects of
certain factors on biodiversity in ponds, few of them have combined water quality and physical factors into a single comprehensive analysis. Moreover, those few studies that have combined various factors, included only a very limited number of chemicals such as nutrients. Without this information, biodiversity conservation is likely to be impeded or even impossible. It has been questioned whether ponds that are designed for treating stormwater runoff are also able to enhance or maintain regional biodiversity (Hassall and Anderson, 2015). Therefore, it is necessary to examine the relationship between different factors and aquatic biodiversity.

The objectives of this study were to (1) assess the impact of a number of environmental factors on aquatic organisms, mainly macroinvertebrate communities, in road sedimentation ponds and (2) identify the factors that contribute the most to their abundance and diversity. Given the lack of comprehensive knowledge about the relationship between environmental factors and biological communities, both water quality and physical variables (including plant cover) were investigated in this study. The water quality variables included nutrients, metals, organic pollutants (such as PAHs and hydrocarbons), pH, dissolved oxygen (DO), total organic carbon (TOC), and conductivity. The physical variables recognized in this study as drivers of aquatic organisms were age and size of ponds, average annual daily traffic (AADT), the number of ponds/water bodies within 1 km radius, the distance to the closest pond from each study pond as well as plant cover within and around the ponds. Macroinvertebrates were selected as the main study organisms because many of them are sensitive to pollution and have rapid response to a variety of changing environmental conditions (Vermonden et al., 2009, Garcia et al., 2014). Moreover, loss of biodiversity in macroinvertebrate communities could easily be attributed to anthropogenic pressure (Giorgio et al., 2016).

2. Material and methods

2.1 Study area

Twelve highway sedimentation ponds situated along the major four-lane highway E6 were included in the present study (Figure 1). One sedimentation pond is located in
the City of Oslo, while five and six sedimentation ponds are located in the counties of Akershus and Østfold, respectively. The ponds were visited four times during the study: in April, June, August and October 2012. Water and biological samples were obtained on each visit.

2.2 Field work and laboratory analyses

2.2.1 Water quality variables

Twenty-eight water quality variables were analysed in this study. Water samples were collected close to the inlet of the ponds in April, June, August and October 2012. Sampling was performed using separate bottles for different parameters: one 125 mL acid washed polyethylene (PE)-bottle for metals (aluminium (Al), antimony (Sb), arsenic (As), barium (Ba), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), molybdenum (Mo), nickel (Ni), potassium (K), silicon (Si), silver (Ag), sodium (Na), strontium (Sr) and zinc (Zn)); one 1 L glass bottle for oil (hydrocarbons); one 1 L glass bottle for polycyclic aromatic hydrocarbons (US EPA 16 PAHs); one 125 mL PE-bottle for the anions, chloride (Cl\(^-\)), nitrate (NO\(_3^-\)) and sulphate(SO\(_4^{2-}\)); one 125 mL PE-bottle for total organic carbon (TOC). The chemical analyses were performed by ALS Laboratory Group, Skøyen, Oslo.

Dissolved oxygen (DO), conductivity, pH and temperature were measured near the inlet of each pond. In the first two surveys, handheld probes Extech Exstick 11 DO600 and Extech Exstick EC500 were used, while during the last two surveys, a multi-parameter probe YSI 6600 V2-4 was used.

2.2.2 Physical variables

The data on several physical variables considered to be relevant for the composition of the macroinvertebrate community were collected either from digital maps (Norwegian Mapping Authorities) or directly from the Norwegian Public Roads Administration (NPRA) (Table 1). Plant cover within and around the ponds was estimated in the field as “little”, “medium” and “extensive” and represented in the model with percentages 33%, 66% and 100%, respectively.
2.2.3 Aquatic organisms

Aquatic organisms, including 91 macroinvertebrates, 2 zooplankton (Cladocera and Copepoda) and 3 amphibians, were sampled using a kick net with an opening of 30×30 cm and a mesh size of 0.45 mm. The kick samples were taken at the bottom of the pond, if the substrate was stony; and at approximately 50 cm above the bottom, if the substrate was muddy or containing a lot of organic material. In all cases, five sweeps were made. Sampling was done once in the inlet basin and twice on either side of the main pond.

In addition to kick sampling, traps made of 1.5 L transparent plastic bottles were used. The bottles were cut in two where the bottleneck starts to form the spout; the bottleneck was turned around placing the spout inside the bottle and attached using transparent tape. Two traps were placed into the main pond at the places where the kick samples were taken and left for 1 – 4 days, depending on time of the year. After sampling, the organisms, except larger specimens such as amphibians, were preserved in 70% ethanol.

Biological samples were sorted in the laboratory and identified to order, family, or species level: Odonata were identified to family level, while Trichoptera, Ephemeroptera, Coleoptera, Plecoptera and Heteroptera were, where possible, identified to species level. Literature that was used for identification included Elliott et al. (1988), Hynes (1993), and Nilsson (1996, 1997).

2.3 Statistical analyses

Both univariate and multivariate statistical methods were applied to analyse the collected data. The IBM SPSS Statistics Version 22 was used for univariate statistical analysis, while the CANOCO5 software (Microcomputer Power) was used for multivariate statistical analysis. The different statistical methods used in the present study are summarised in a schematic overview (Figure 2).

2.3.1 Water quality

The general trends in water quality were analysed using principal component analysis (PCA). The data were log(x+1) transformed prior to the PCA in order to reduce the
skewness and improve the normality of the data. The concentrations below the limit of quantification (LOQ) were substituted with ½ LOQ. If the concentrations for a variable were below LOQ in more than 15% of the total number of samples, the variable was excluded from the analysis. This was the case for PAH compounds, NO$_3^-$ and Hg.

To disclose any differences in water quality between the different sedimentation ponds, one-way analysis of variance (ANOVA) followed by Tukey post-hoc tests were conducted on the sample scores extracted from axes 1 and 2 of the PCA analysis for the water quality data. The sample scores were checked for normality and homogeneity prior to the analysis. Results with $p < 0.05$ were considered statistically significant.

Datasets with water quality variables often display high co-linearity. The risk of overfitting the RDA model is high when too many explanatory variables are included. For example, it is likely that some of the explanatory variables become statistically significant just by chance. Therefore, the number of water quality variables was reduced by using sample scores extracted from axis 1 of the PCA analysis for metals (Figure S3) as a proxy for metal concentrations. In this way, the number of variables was reduced from 28 to 7, as well as reducing the risk of overfitting the RDA model.

**2.3.2 Aquatic organisms – community analyses**

The evaluation of the biological community composition was conducted by using ordination analyses (multivariate statistics) in several steps (Figure 2). Prior to the analyses, the data were log(x+1) transformed. A Detrended Correspondence Analysis (DCA) was applied to disclose whether the data followed a linear or a unimodal response. The response is defined according to the species turnover in the data, measured as standard deviation (SD) units and termed gradient length in the DCA (Šmilauer and Lepš, 2014). If the length is less than 3 SD, the linear method is recommended; if the length is more than 4 SD, the unimodal method is recommended. The output of the DCA in this study showed that the data had a gradient of 3.8 SD; and therefore no clear decision whether the data followed a linear or unimodal response could be made. Both linear and unimodal methods were applied to test the biological data. The results showed that the linear methods explained more variation than the
unimodal methods. Hence, PCA (unconstrained) and Redundancy Analysis (RDA, constrained) were used in the final analyses. PCA was undertaken to reveal the maximum variation in the biological community, while RDA was used to evaluate the relationship between the biological community composition and the environmental data (i.e. water quality and physical variables).

An RDA with a global permutation test (RDA global) was conducted on the entire environmental dataset to disclose the overall impact of the variables on the community composition. In addition, the output of the significance test ($p < 0.05$) was used as a criterion for conducting a second RDA with forward selection (Šmilauer and Lepš, 2014). The RDA with forward selection was conducted in order to disclose a subset of the most important and significant environmental variables. The conditional (partial) effect of each variable was tested, and the effect size and significance of variables depend on the already selected variable(s). Month was included as a covariate in order to remove any seasonal effects on the community composition.

After the selection, the effects of the selected groups of explanatory variables (water quality and physical groups), including their overlap, were quantified using variation partitioning. Monte Carlo permutation tests (499 permutations, $p < 0.05$) were used for determination of the statistical significance in the RDAs. The significance tests performed during the forward selection were conducted without $p$-adjustment (i.e. preventing Type I error). This is considered valid as the number of variables (e.g. metals) was reduced prior to the RDA global test and in addition the RDA global test was significant (Šmilauer and Lepš, 2014). Each water quality variable was represented by four values measured during the sampling campaign (12 ponds $\times$ 4 months, $n = 48$), while each physical variable was represented by one value constant during the sampling campaign (12 ponds $\times$ 1, $n = 12$). Thus, the dataset for the physical variables was unequal in size to the datasets for the water quality and the biological community.

In order to run the RDA with the complete environmental dataset (i.e. water quality and physical variables), the physical dataset was upscaled from $n = 12$ to $n = 48$. Therefore, the tests of the physical variables must be interpreted with caution as the number of the degrees of freedom is incorrect for those variables.
3. Results

3.1 General trends in water quality variables

The concentrations for the water quality variables are presented in Table S1-S3. The priority metals that are able to induce toxicity at low levels of exposure are As, Cd, Cr, Pb, Ni and Hg (Tchounwou et al., 2012, Beasley G, 2002). In addition, Zn and Cu are also typical pollutants from road runoff. Although Zn and Cu are considered as essential elements for biological functioning, an excess can lead to tissue damage (Tchounwou et al., 2012). The concentrations of selected priority pollutants were compared to the Environmental Quality Standards (EQS) (Tables S4 and S5) according to the EU Water Framework Directive (EU WFD) and the Norwegian River Basin Specific Pollutants (Council Regulation (EC), 2008, Pettersen, 2016). Although the EQS for metals are based on the dissolved fraction and in our study the total concentrations were measured, the comparison indicates which metals may appear at toxic concentrations and have an impact on the aquatic organisms in the sedimentation ponds.

The ecological status of surface water is categorized into classes from 1 to 5, with 1 being background level and 5 being very poor quality (Pettersen, 2016). For As, most ponds belonged to class 2, and some belonged to class 3; only the pond Taraldrud north (in August) belonged to class 4. For Cr, most ponds belonged to class 2, while the ponds Såstad (in August and October), Fiulstad (in October), Idrettsveien (in October) and Enebekk (in October) belonged to class 4. For Cd, most ponds had very low concentrations and belonged to class 1, and only the pond Såstad (in October) belonged to class 5. For Pb, 30 samples belonged to class 2, and 17 samples belonged to class 3; only the pond Karlshusbunn (in October) belonged to class 5. For Ni, most ponds belonged to class 2, and some belonged to class 3; only the ponds Taraldrud north (in April and August) belonged to class 1. For Zn, 11 samples belonged to class 2, and 25 samples belonged to class 4; the ponds that belonged to class 5 were Nøstvedt (in April, and October), Vassum (in June, August and October), Enebekk (in April, June, August and October), Såstad (in October), Idrettsveien (in October) and Karlshusbunn (in October). For Cu, 26 samples belonged to class 2, and 17 samples belonged to class...
the ponds that belonged to class 5 were Vassum (in April, June and October), Fiulstad (in October) and Såstad (in October). As mentioned above, Hg was not considered in the analysis, since the concentrations in most samples were below LOQ.

Chloride (Cl\textsuperscript{−}) concentrations, representing road salt pollution, were compared to the criteria set by the United States Environmental Protection Agency (US EPA): a maximum concentration of 860 mg/L and a continuous concentration of 230 mg/L (United States Environmental Protection Agency, 2017). The Cl\textsuperscript{−} concentrations were above 230 mg/L in 22 samples, while in the pond Vassum in June the concentration was 2090 mg/L. The DO concentrations in most of the ponds were above 10 mg/L, and none were below the threshold set by the US EPA of 2.3 mg/L (EPA, 2000). Therefore, the DO levels were generally good.

Axes 1 and 2 in the PCA captured 44% and 18% of the total variation in the water quality data, respectively (Figure 3). Many of the water quality variables were positively correlated to each other, and as displayed in the ordination plot some clusters were evident. For example, the cluster of Fe, Co, Si, Mn, Mo, Cd and Ni was highly correlated with axis 1, while the cluster of Zn, Pb, P, Al and Cr and the cluster of TOC, SO\textsubscript{4}\textsuperscript{2−}, Ba, K, Mg, Ca, Sr were located on either side of the first cluster. The cluster of pH, hydrocarbons and Sb, and the cluster of Cl\textsuperscript{−}, Na, conductivity and DO were negatively correlated with each other along axis 2. The PCA revealed that there were differences in water quality between different sedimentation ponds.

To better illustrate the differences in water quality between ponds, the sample scores from PCA axes 1 and 2 were displayed in box-plots (Figure 3) and tested for statistical differences using the ANOVA followed by Tukey post-hoc tests. Based on the sample scores extracted from axis 1, some of the ponds were significantly different from each other. The ponds Vassum, Såstad, Fiulstad, Idrettsveien and Enebekk appeared to have higher concentrations for the variables related to axis 1, while Taraldrud north and south, Skuellerud and Taraldrud crossing appeared to have lower concentrations for the variables related to axis 1. Based on the sample scores extracted from axis 2, none of the ponds were significantly different. The ponds Såstad, Taraldrud crossing, Fiulstad,
Idrettsveien and Karlshusbunn appeared to have high concentrations for the variables related to axis 2, mainly because of the road salt (indicated by Cl\(^-\), Na and conductivity).

According to ANOVA followed by Tukey post-hoc tests, there were no statistically significant differences in water quality between sampling periods when using PCA scores extracted from axis 1, but there were statistically significant differences for PCA scores extracted from axis 2 (Figure S1-S2). The PCA scores extracted from axis 2 indicated that the lowest and highest levels of road salt (Cl\(^-\), Na and conductivity) were observed in October and June, respectively (3.6 – 2090 mg/L). The opposite pattern was observed for pH (4.3 – 9.7).

### 3.2 Biological community composition in relation to water quality and physical variables

Of the 96 taxa found in the studied sedimentation ponds (Tables S6.1 – 6.8), 7 taxa occurred in all investigated ponds (Hydracarina, Hirudinea, Notonecta reuteri (Hemiptera), Chironomidae, Chaoboridae, Caenis horaria (Ephemeroptera), Coenagrionidae), while 32 taxa were present in two or more of the sedimentation ponds.

The result of the unconstrained PCA for biological data showed that 40% of the variation in the biological community could be explained by axes 1 and 2; most taxa were gathered along axis 1 and the rest along axis 2 (Figure S4). For clarity, only 25 taxa that were well characterised by the first four ordination axes are displayed; the same was done for RDA.

The results of PCA for water quality variables (Figure 3A) showed that all metals were correlated with each other as well as with SO\(_4^{2-}\); thus, SO\(_4^{2-}\) was analysed together with metals. PCA was repeated for metals (including SO\(_4^{2-}\)) to extract the PCA scores. Axis 1 from PCA for metals (including SO\(_4^{2-}\)) explained 58% of the variance (Figures S3); thus, the PCA scores extracted from axis 1 were used (denoted PCA1 (M)). Moreover, Cl\(^-\) content was highly correlated with conductivity and Na; thus, the concentration of Cl\(^-\) was used to represent road salt. Therefore, seven variables (TOC, DO, P, hydrocarbons,
Cl⁻, pH and PCA1 (M) were used in the RDA to analyse the effects of water quality variables on the biological community composition.

The RDA analysis showed that the overall RDA global model (Figure S5) was significant ($p = 0.002$). The RDA with forward selection showed that out of the 14 variables (metals (including $\text{SO}_4^{2-}$), P, TOC, DO, pH, hydrocarbons, Cl⁻, size, age, AADT, number of ponds/water bodies within 1 km, distance to the closest pond from study pond, as well as plant cover within and around the ponds), 8 variables were statistically significant: metals (including $\text{SO}_4^{2-}$), Cl⁻, P, DO, hydrocarbons, AADT, distance and pond size (Figure 4); the simple effects of each variable are presented in Table S7 (i.e. the explained variation as if the variable is used alone in the RDA). Axes 1 and 2 explained 19% and 7% of the variation in the biological community composition. The RDA plot indicated that the variable pond size had the greatest impact on the biological community composition. Metals (including $\text{SO}_4^{2-}$) and AADT also contributed considerably to explaining the variation in the biological community composition. Most taxa were positively correlated to the pond area, with some exceptions, e.g. Hydraenidae and Oligochaeta. Most taxa were positively correlated with AADT. Most taxa were negatively correlated with metals, with some exceptions, e.g. *Phryganea bipunctata* (Trichoptera) and Oligochaeta. Moreover, most taxa were negatively correlated with the distance to the nearest neighbouring pond. Among the 25 dominant taxa, some taxa were positively correlated with Cl⁻, e.g. *Rana* sp, *Notonecta* sp. Nymphs (Heteroptera) and *Semblis atrata* (Trichoptera), while others were negatively correlated with Cl⁻, e.g. *Cloeon inscriptum* and *Paraleptoplebia* sp. (Ephemeroptera).

Some taxa were positively correlated with P and DO, while other taxa were negatively correlated. Compared with the other selected variables, hydrocarbons had the least contribution to the biological community composition; most organisms were positively correlated with hydrocarbons.

The result of the RDA after removing the seasonal effect (i.e. month used as a covariate) showed that the variation in taxa explained by the eight selected variables decreased from 42% to 39%. This indicates that seasons had a minor influence on the variation in the biological community composition in the present study.
The variation partitioning (Table 2) showed that the group of water quality variables (metals (including \(\text{SO}_4^{2-}\), \(\text{Cl}^-\), \(\text{DO}\), \(\text{P}\), and hydrocarbons) explained 48%, while the group of physical variables (size of ponds, AADT, and distance to the nearest neighbouring pond) explained 41% of the total variation in the biological community composition. The shared effects of these two groups of variables accounted for 11% of the total variation.

4. Discussion

Due to the lack of studies that combine water quality and physical variables into a single analysis of species community over several ponds, there is a lack of understanding of the relative impacts of such variables on species richness (Hassall et al., 2011). In our study, the effects of water quality and physical variables on 96 taxa, including 91 macroinvertebrates, 2 zooplankton and 3 amphibians, were analysed. Among the identified taxa, 4 macroinvertebrate species (\textit{Brychius elevates}, \textit{Hygrotus confluens}, \textit{Ilybius guttiger}, \textit{Ilybius quadriguttatus}) and one amphibian species (\textit{Triturus vulgaris}) belong to the “near threatened” category, while \textit{Plateumaris braccata} belongs to the “vulnerable” category in the Norwegian Red List (Artsdatabanken, 2011). The water quality variables included 19 metals, hydrocarbons, \(\text{P}\), \(\text{Cl}^-\), \(\text{SO}_4^{2-}\), TOC, \(\text{DO}\), pH and conductivity. It should be stressed that the sampling strategy in the present study did not aim to collect water samples after runoff episodes. Therefore, the measured concentrations can be considered representative of the general water quality levels in the studied ponds and not an indication of extreme concentrations that may occur after runoff episodes. Nevertheless, some of the metals were present at relatively high concentrations.

Pond size is the most important physical variable that was statistically selected by RDA forward selection method. The results showed that large ponds can support more species than small ones; this is in accordance with the conventional species-area relationships. Gotelli and Graves (1996) mentioned that small ponds have low species richness due to their higher vulnerability to disturbance, such as degradation resulted from pollutant loads. Nevertheless, the results of studies involving the pond size are conflicting. Oertli et al. (2002) found that the species-area relationship had limitations.
when it was applied to ponds. The species-area relationship was apparent for Odonata, but not relevant for Coleoptera and Sphaeriidae (Oertli et al., 2002). Biggs et al. (2005) also found that the trend that larger ponds support more species was stronger for macrophytes, but weaker for invertebrates. A possible explanation to such phenomenon might be the small island effect, in which species-area relationships are not valid for small pond sizes (Hassall et al., 2011, Lomolino, 2000). The small island effect suggests that in small pond patches, extrinsic, stochastic processes have a larger effect compared to intrinsic, ecological processes in structuring communities (Hassall and Anderson, 2015). In urban environments, such effect could be aggravated by numerous interacting stressors that act on top of natural processes (Hassall and Anderson, 2015).

Followed by pond size, AADT was selected by the RDA as the second most important physical variable. Most taxa appeared to be positively correlated with AADT; this is a bit unexpected as more traffic may be expected to cause higher concentrations of contaminants in road runoff and subsequently in the receiving ponds. However, in our study, the AADT was the highest in the areas with the largest ponds. Therefore, dilution may have been playing a crucial role in reducing the contaminant concentrations in these sedimentation ponds. Another possible explanation is that there is no obvious correlation between AADT and pollutants. For example, Kayhanian et al. (2003) found that although AADT has an influence on most road runoff constituents concentrations, there was no direct linear correlation between pollutant concentration in road runoff and AADT.

Most taxa were negatively correlated with the distance from the study pond to the nearest neighbouring pond. This is potentially attributed to the higher connectivity that facilitates the mobility of invertebrates between ponds (Gledhill et al., 2008). The importance of nearby ponds is in agreement with previous studies, which indicate that pond density and connectivity appeared to be the major contributing variables to biodiversity (Noble and Hassall, 2015, Staddon et al., 2010). This highlights the importance of pond and wetland density to maintain metapopulations of species.
Hassall (2014) referred to such kind of networks as “pondscapes”, constituting a network of distributed discrete habitat patches. Most taxa were negatively correlated with the metals concentrations in water. Metals in road runoff arise from various sources, including automobile sources (e.g. fuel components, brakes and tyres), traffic barriers, road signs and road lightning infrastructures (Meland, 2010). It has been demonstrated that increases in heavy metal concentrations lead to decrease in biodiversity (Phillips et al., 2015). Although several metals can act as essential nutrients for living organisms (e.g. Ca, Na, K, Mg, Fe, Zn, Cr and Se), these metals are harmful to living organisms when they reach excessive levels or enter certain oxidation states (Weiner, 2008). Compared to the WFD EQS for the priority pollutants and the River Basin Specific Pollutants for Norway (Pettersen, 2016), the concentrations of Zn and Cu in most ponds in our study were high, while the concentrations of As, Cd, Cr, Ni and Pb were generally relatively low, but could occasionally reach high levels. It is important to stress that we did not collect water samples directly after runoff episodes when the concentrations are believed to be the highest.

The aquatic organisms were greatly affected by Cl\(^{-}\). The reason why Cl\(^{-}\) was quite high in some ponds (the maximum concentration recorded in our study reached 2090 mg/L) is because sodium chloride (NaCl) is widely used on roads in Norway as a de-icing agent. Thus, road runoff and snowmelt-induced runoff normally contain high concentrations of Cl\(^{-}\) in these areas during winter and spring, thereby considerably affecting the water quality of receiving ponds. Different from rainstorms, snowmelt runoff can persist for several days to weeks. Furthermore, in the areas with frozen soil, both pervious and impervious surfaces contribute with snowmelt runoff (Semadeni-Davies, 2006). Marsalek et al. (1999) has demonstrated that in winter, road runoff exhibited the highest frequency of severe toxic effects. The elevated concentration of Cl\(^{-}\) can cause toxicity due to the osmotic stress related to overall ionic strength (Elphick et al., 2011, Blasius and Merritt, 2002). In addition, high Cl\(^{-}\) concentrations may kill roadside vegetation resulting in increased erosion and sediment load that have a negative impact on the abundance of invertebrates (Blasius and Merritt, 2002). Other
severe consequences resulting from Cl\(^-\) deposition and retention are the prevention of water circulation leading to anoxic conditions in bottom waters, and release of trapped metals from sediment causing lethal toxicity to pond organisms (Van Meter et al., 2011). Therefore, due to the characteristics of snowmelt runoff and excessive amount of Cl\(^-\), future studies need to further investigate the influence of road salt on biological community composition during winter and spring, especially in cold regions.

Although of apparently lower importance, P, DO, and hydrocarbons were included as statistically significant variables in the forward selection procedure. P is the primary growth limiting nutrient in most freshwater systems (Yang et al., 2009). Some taxa were positively related to the P concentration, but some were negatively correlated. Houlanah et al. (2006) also found that total species richness in wetlands was negatively correlated with water nutrient levels. If nutrient loading rates exceed the critical level, species composition can be altered over a short time (Verhoeven et al., 2006). For example, P concentration in the runoff could result in eutrophication in receiving water bodies. Eutrophication is considered to be one of the main impacts on small standing water bodies (Menetrey et al., 2005) causing episodes of noxious blooms, reduction in aquatic macrophyte communities and the depletion of DO in bottom waters (Conley, 1999). As one of the crucial limnological variables, DO affects the distribution of many species and maintains aquatic life (de Moura Guimaraes Souto et al., 2011, Connolly et al., 2004). In addition, DO plays the crucial role in speciation of metals, influencing their biomobility and toxicity (Rabajczyk, 2010). Since the DO concentrations in the studied ponds were above the threshold value for the aquatic organisms to live, DO levels do not appear to be a major limiting factor in the present study. Nevertheless, the RDA plot indicated that different taxa may have different oxygen requirements and tolerance to hypoxia; this can be attributed to a diverse array of structural and behavioural respiratory adaptations among various aquatic organisms (Connolly et al., 2004). Lastly, there was an indication of a small positive correlation between the hydrocarbons and the abundance of macrinhvertebrates which may be somewhat obscure. Further research is needed to evaluate the effects of hydrocarbons on biological community composition in sedimentation ponds.
5. Conclusions

We studied the impacts of multiple environmental factors, including water quality and physical variables, on the biological community composition in sedimentation ponds. In the present analysis, the key variables controlling the aquatic biodiversity were the pond size, distance to the closest pond from study pond, AADT and a combination of various contaminants such as metals, phosphorus, road salt, dissolved oxygen and hydrocarbons. The pond size plays a crucial role in affecting biological community composition, as more species tend to live in the larger ponds. Our study indicates that sedimentation ponds have the potential to contribute to biodiversity conservation. In order to promote and conserve aquatic biodiversity in road sedimentation ponds, larger ponds would be preferable due to the “species-area effect” and the dilution of harmful pollutants. In addition, the shorter distance between ponds allows organisms to spread more easily due to the higher connectivity, which maintains biodiversity. More studies are still needed to investigate the influence of additional environmental factors using different approaches and methods, such as process-based modelling. Furthermore, measurements of the pollutants in the pond sediments, which may act as a more accurate proxy for the overall pollution level compared to water samples, should be included in such studies. These studies could then provide recommendations for optimising aquatic biodiversity in the road sedimentation ponds.

Authors’ Contributions

Z. Sun, S. Meland, E. Sokolova, S. Rauch, S. Saltveit and J. Brittain gave substantial contributions to conception and design; H. Thygesen, S. Saltveit and J. Brittain collected the data; Z. Sun and S. Meland analysed the data; Z. Sun drafted the article; S. Meland, E. Sokolova, S. Rauch and J. Brittain revised the article critically for important intellectual content. All authors gave final approval for publication.

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Ole Wiggo Røstad verified the identification of Dytiscidae. Trond Bremnes verified Trichoptera and checked random samples of Heteroptera and Odonata.

Colour for figures

Colour should be used for all figures in print.

References


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Table 1. Physical variables for the twelve sedimentation ponds surveyed in the present study.

<table>
<thead>
<tr>
<th>Ponds</th>
<th>Constructed</th>
<th>Size (m$^2$)$^a$</th>
<th>No. of ponds$^b$</th>
<th>Distance (m)$^c$</th>
<th>AADT$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skullerud (SKU)</td>
<td>1998/1999</td>
<td>910</td>
<td>1</td>
<td>980</td>
<td>66500</td>
</tr>
<tr>
<td>Taraldrud north (TAN)</td>
<td>2004</td>
<td>780</td>
<td>3</td>
<td>450</td>
<td>42900</td>
</tr>
<tr>
<td>Taraldrud crossing (TAK)</td>
<td>2004</td>
<td>1400</td>
<td>6</td>
<td>120</td>
<td>42200</td>
</tr>
<tr>
<td>Taraldrud south (TAS)</td>
<td>2004</td>
<td>474</td>
<td>4</td>
<td>130</td>
<td>42200</td>
</tr>
<tr>
<td>Nøstvedt (NØS)</td>
<td>2009</td>
<td>340</td>
<td>3</td>
<td>15</td>
<td>35500</td>
</tr>
<tr>
<td>Vassum (VAS)</td>
<td>2000</td>
<td>363</td>
<td>5</td>
<td>30</td>
<td>41000</td>
</tr>
<tr>
<td>Fiulstad (FIU)</td>
<td>2004</td>
<td>150</td>
<td>3</td>
<td>330</td>
<td>33575</td>
</tr>
<tr>
<td>Såstad (SÅS)</td>
<td>2004</td>
<td>80</td>
<td>3</td>
<td>92</td>
<td>33575</td>
</tr>
<tr>
<td>Idrettsveien (IDR)</td>
<td>2004/2005</td>
<td>19</td>
<td>3</td>
<td>690</td>
<td>22735</td>
</tr>
<tr>
<td>Karlshusbunn (KAB)</td>
<td>2004/2005</td>
<td>87</td>
<td>3</td>
<td>240</td>
<td>22735</td>
</tr>
<tr>
<td>Nordby (NOR)</td>
<td>2004/2005</td>
<td>89</td>
<td>8</td>
<td>600</td>
<td>22735</td>
</tr>
<tr>
<td>Enebekk (ENE)</td>
<td>2004/2005</td>
<td>132</td>
<td>5</td>
<td>587</td>
<td>23837</td>
</tr>
</tbody>
</table>

$^a$Pond surface area  
$^b$Number of neighbouring ponds within a radius of 1 km  
$^c$Distance to the nearest neighbouring pond  
$^d$Annual Average Daily Traffic
Table 2. Variation partitioning analysis representing how much of the variation in the biological community composition could be ascribed to the water quality variables (metals (including $SO_4^{2-}$), chloride (Cl$^-$), phosphorus (P), dissolved oxygen (DO) and hydrocarbons) and the physical variables (pond size, average annual daily traffic (AADT), and distance to the nearest neighbouring pond).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>% of Explained</th>
<th>% of All</th>
<th>$p$ – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metals (including $SO_4^{2-}$, Cl$^-$, DO, P and hydrocarbons)</td>
<td>48%</td>
<td>13%</td>
<td>0.002</td>
</tr>
<tr>
<td>Pond size, AADT, and distance to the nearest neighbouring pond</td>
<td>41%</td>
<td>11%</td>
<td>0.002</td>
</tr>
<tr>
<td>Shared parts of two groups</td>
<td>11%</td>
<td>3%</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Figure 1. Location of the studied sedimentation ponds (red dots) in Oslo and Akershus county (A) and in Østfold county (B). In A), the ponds are SKU - Skollerud, 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 (Skollerud and Enebekk) is 71 km.
Figure 2. Schematic overview of the methods that were used for the data analysis: DCA - Detrended Correspondence Analysis, PCA - Principal Component Analysis, RDA - Redundancy Analysis, and ANOVA – One-way Analysis of Variance. * In order to run the model, the physical variables were upscaled to n=48 to match the water quality variables.
Figure 3. A) Principal components analysis (PCA) for water quality variables. 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, respectively; “V” indicates the basin receiving road runoff. B) Box-plot of PCA score extracted from axis 1 for twelve ponds. The small letters besides the boxes indicate which ponds were significantly different from each other. C) Box-plot for PCA score extracted from axis 2 for twelve ponds. None of the ponds were statistically different.
Figure 4. Redundancy analysis (RDA) with forward selection of taxa in relation to the water quality and 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, respectively; “V” indicates the basin receiving road runoff.