# 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 Thièry, 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 $1 \text{ m}^2$ 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

73　multiple ecosystem services provided by ponds make them excellent candidates to be

74　incorporated into road construction.

75　Some studies found that pond density is a major variable that determines aquatic

76　macroinvertebrate richness (Gledhill et al., 2008, Staddon et al., 2010, Hassall, 2014).

77　Gledhill et al. (2008) indicated that species richness for macroinvertebrates was higher

78　in an area with more ponds, potentially due to higher connectivity between ponds.

79　Hassall (2014) suggested maximizing the habitat connectivity between ponds to

80　enhance and protect biodiversity. Plant cover is another factor that influences the

81　distribution of aquatic invertebrates by, for instance, affecting predation and food

82　availability (De Szalay and Resh, 2000). The richness and density of aquatic

83　macroinvertebrates in ponds with vegetated areas are significantly greater than in

84　ponds lacking vegetation (Hsu et al., 2011). Pond size is also likely to affect aquatic

85　biodiversity; larger ponds tend to contain more species. However, Oertli et al. (2002)

86　demonstrated that this biogeographic principle has limitations when it is applied to

87　ponds; they found that it was only relevant for a few taxa, such as Odonata. Regarding

88　pond age, Williams et al. (2008) found that compared with older ponds, 6-12-year-old

89　ponds were able to support significantly more species and more uncommon species,

90　while Gee et al. (1997) demonstrated that the number of taxa of macroinvertebrates

91　was not significantly related to pond age. In addition, owing to the pollutant retention

92　function, ponds normally contain high levels of pollution (Karlsson et al., 2010,

93　Vollertsen et al., 2007), and may become sink-habitats and ecological traps. Chemical

94　pollutants have lethal and sublethal effects on aquatic organisms via physiological and

95　behavioural processes (Foltz and Dodson, 2009). Even if the concentration of a

96　pollutant is low, chemical accumulation in roadside ponds can be an issue, as in the

97　case of metals (Chester and Robson, 2013). Accumulation of metals and organic

98　pollutants in the sediments may have long-term adverse effects on aquatic organisms

99　(Grung et al., 2016), and it has been shown that metals and PAHs are easily

100　accumulated in aquatic organisms (Meland et al., 2013, Grung et al., 2016).

101　There is still a lack of comprehensive understanding of factors that influence aquatic

102　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, García 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

132 the City of Oslo, while five and six sedimentation ponds are located in the counties of

133 Akershus and Østfold, respectively. The ponds were visited four times during the

134 study: in April, June, August and October 2012. Water and biological samples were

135 obtained on each visit.

## 2.2 Field work and laboratory analyses

### 2.2.1 Water quality variables

138 Twenty-eight water quality variables were analysed in this study. Water samples were

139 collected close to the inlet of the ponds in April, June, August and October 2012.

140 Sampling was performed using separate bottles for different parameters: one 125 mL

141 acid washed polyethylene (PE)-bottle for metals (aluminium (Al), antimony (Sb),

142 arsenic (As), barium (Ba), cadmium (Cd), calcium (Ca), chromium (Cr), cobalt (Co),

143 copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg),

144 molybdenum (Mo), nickel (Ni), potassium (K), silicon (Si), silver (Ag), sodium (Na),

145 strontium (Sr) and zinc (Zn)); one 1 L glass bottle for oil (hydrocarbons); one 1 L glass

146 bottle for polycyclic aromatic hydrocarbons (US EPA 16 PAHs); one 125 mL PE-bottle

147 for the anions, chloride ($Cl^-$), nitrate ($NO_3^-$) and sulphate($SO_4^{2-}$); one 125 mL PE-bottle

148 for total organic carbon (TOC). The chemical analyses were performed by ALS

149 Laboratory Group, Skøyen, Oslo.

150 Dissolved oxygen (DO), conductivity, pH and temperature were measured near the

151 inlet of each pond. In the first two surveys, handheld probes Extech Exstick 11 DO600

152 and Extech Exstick EC500 were used, while during the last two surveys, a multi-

153 parameter probe YSI 6600 V2-4 was used.

### 2.2.2 Physical variables

155 The data on several physical variables considered to be relevant for the composition of

156 the macroinvertebrate community were collected either from digital maps (Norwegian

157 Mapping Authorities) or directly from the Norwegian Public Roads Administration

158 (NPRA) (Table 1). Plant cover within and around the ponds was estimated in the field

159 as "little", "medium" and "extensive" and represented in the model with percentages

160 33%, 66% and 100%, respectively.

### 2.2.3 Aquatic organisms

162   Aquatic organisms, including 91 macroinvertebrates, 2 zooplankton (Cladocera and

163   Copepoda) and 3 amphibians, were sampled using a kick net with an opening of 30×30

164   cm and a mesh size of 0.45 mm. The kick samples were taken at the bottom of the

165   pond, if the substrate was stony; and at approximately 50 cm above the bottom, if the

166   substrate was muddy or containing a lot of organic material. In all cases, five sweeps

167   were made. Sampling was done once in the inlet basin and twice on either side of the

168   main pond.

169   In addition to kick sampling, traps made of 1.5 L transparent plastic bottles were used.

170   The bottles were cut in two where the bottleneck starts to form the spout; the

171   bottleneck was turned around placing the spout inside the bottle and attached using

172   transparent tape. Two traps were placed into the main pond at the places where the

173   kick samples were taken and left for 1 – 4 days, depending on time of the year. After

174   sampling, the organisms, except larger specimens such as amphibians, were preserved

175   in 70% ethanol.

176   Biological samples were sorted in the laboratory and identified to order, family, or

177   species level: Odonata were identified to family level, while Trichoptera,

178   Ephemeroptera, Coleoptera, Plecoptera and Heteroptera were, where possible,

179   identified to species level. Literature that was used for identification included Elliott et

180   al. (1988), Hynes (1993), and Nilsson (1996, 1997).

181   ### 2.3 Statistical analyses

182   Both univariate and multivariate statistical methods were applied to analyse the

183   collected data. The IBM SPSS Statistics Version 22 was used for univariate statistical

184   analysis, while the CANOCO5 software (Microcomputer Power) was used for

185   multivariate statistical analysis. The different statistical methods used in the present

186   study are summarised in a schematic overview (Figure 2).

187   ### 2.3.1 Water quality

188   The general trends in water quality were analysed using principal component analysis

189   (PCA). The data were log(x+1) transformed prior to the PCA in order to reduce the

190 skewness and improve the normality of the data. The concentrations below the limit of

191 quantification (LOQ) were substituted with ½ LOQ. If the concentrations for a variable

192 were below LOQ in more than 15% of the total number of samples, the variable was

193 excluded from the analysis. This was the case for PAH compounds, $NO_3^-$ and Hg.

194 To disclose any differences in water quality between the different sedimentation

195 ponds, one-way analysis of variance (ANOVA) followed by Tukey post-hoc tests were

196 conducted on the sample scores extracted from axes 1 and 2 of the PCA analysis for

197 the water quality data. The sample scores were checked for normality and

198 homogeneity prior to the analysis. Results with $p < 0.05$ were considered statistically

199 significant.

200 Datasets with water quality variables often display high co-linearity. The risk of

201 overfitting the RDA model is high when too many explanatory variables are included.

202 For example, it is likely that some of the explanatory variables become statistically

203 significant just by chance. Therefore, the number of water quality variables was

204 reduced by using sample scores extracted from axis 1 of the PCA analysis for metals

205 (Figure S3) as a proxy for metal concentrations. In this way, the number of variables

206 was reduced from 28 to 7, as well as reducing the risk of overfitting the RDA model.

207 ### 2.3.2 Aquatic organisms – community analyses

208 The evaluation of the biological community composition was conducted by using

209 ordination analyses (multivariate statistics) in several steps (Figure 2). Prior to the

210 analyses, the data were log(x+1) transformed. A Detrended Correspondence Analysis

211 (DCA) was applied to disclose whether the data followed a linear or a unimodal

212 response. The response is defined according to the species turnover in the data,

213 measured as standard deviation (SD) units and termed gradient length in the DCA

214 (Šmilauer and Lepš, 2014). If the length is less than 3 SD, the linear method is

215 recommended; if the length is more than 4 SD, the unimodal method is recommended.

216 The output of the DCA in this study showed that the data had a gradient of 3.8 SD; and

217 therefore no clear decision whether the data followed a linear or unimodal response

218 could be made. Both linear and unimodal methods were applied to test the biological

219 data. The results showed that the linear methods explained more variation than the

220  unimodal methods. Hence, PCA (unconstrained) and Redundancy Analysis (RDA,

221  constrained) were used in the final analyses. PCA was undertaken to reveal the

222  maximum variation in the biological community, while RDA was used to evaluate the

223  relationship between the biological community composition and the environmental

224  data (i.e. water quality and physical variables).

225  An RDA with a global permutation test (RDA global) was conducted on the entire

226  environmental dataset to disclose the overall impact of the variables on the

227  community composition. In addition, the output of the significance test ($p < 0.05$) was

228  used as a criterion for conducting a second RDA with forward selection (Šmilauer and

229  Lepš, 2014). The RDA with forward selection was conducted in order to disclose a

230  subset of the most important and significant environmental variables. The conditional

231  (partial) effect of each variable was tested, and the effect size and significance of

232  variables depend on the already selected variable(s). Month was included as a

233  covariate in order to remove any seasonal effects on the community composition.

234  After the selection, the effects of the selected groups of explanatory variables (water

235  quality and physical groups), including their overlap, were quantified using variation

236  partitioning. Monte Carlo permutation tests (499 permutations, $p < 0.05$) were used

237  for determination of the statistical significance in the RDAs. The significance tests

238  performed during the forward selection were conducted without $p$-adjustment (i.e.

239  preventing Type I error). This is considered valid as the number of variables (e.g.

240  metals) was reduced prior to the RDA global test and in addition the RDA global test

241  was significant (Šmilauer and Lepš, 2014). Each water quality variable was represented

242  by four values measured during the sampling campaign (12 ponds × 4 months, n = 48),

243  while each physical variable was represented by one value constant during the

244  sampling campaign (12 ponds × 1, n =12). Thus, the dataset for the physical variables

245  was unequal in size to the datasets for the water quality and the biological community.

246  In order to run the RDA with the complete environmental dataset (i.e. water quality

247  and physical variables), the physical dataset was upscaled from n = 12 to n = 48.

248  Therefore, the tests of the physical variables must be interpreted with caution as the

249  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

280    4; the ponds that belonged to class 5 were Vassum (in April, June and October),

281    Fiulstad (in October) and Såstad (in October). As mentioned above, Hg was not

282    considered in the analysis, since the concentrations in most samples were below LOQ.

283    Chloride ($Cl^-$) concentrations, representing road salt pollution, were compared to the

284    criteria set by the United States Environmental Protection Agency (US EPA): a

285    maximum concentration of 860 mg/L and a continuous concentration of 230 mg/L

286    (United States Environmental Protection Agency, 2017). The $Cl^-$ concentrations were

287    above 230 mg/L in 22 samples, while in the pond Vassum in June the concentration

288    was 2090 mg/L. The DO concentrations in most of the ponds were above 10 mg/L, and

289    none were below the threshold set by the US EPA of 2.3 mg/L (EPA, 2000). Therefore,

290    the DO levels were generally good.

291    Axes 1 and 2 in the PCA captured 44% and 18% of the total variation in the water

292    quality data, respectively (Figure 3). Many of the water quality variables were

293    positively correlated to each other, and as displayed in the ordination plot some

294    clusters were evident. For example, the cluster of Fe, Co, Si, Mn, Mo, Cd and Ni was

295    highly correlated with axis 1, while the cluster of Zn, Pb, P, Al and Cr and the cluster of

296    TOC, $SO_4^{2-}$, Ba, K, Mg, Ca, Sr were located on either side of the first cluster. The cluster

297    of pH, hydrocarbons and Sb, and the cluster of $Cl^-$, Na, conductivity and DO were

298    negatively correlated with each other along axis 2. The PCA revealed that there were

299    differences in water quality between different sedimentation ponds.

300    To better illustrate the differences in water quality between ponds, the sample scores

301    from PCA axes 1 and 2 were displayed in box-plots (Figure 3) and tested for statistical

302    differences using the ANOVA followed by Tukey post-hoc tests. Based on the sample

303    scores extracted from axis 1, some of the ponds were significantly different from each

304    other. The ponds Vassum, Såstad, Fiulstad, Idrettsveien and Enebekk appeared to have

305    higher concentrations for the variables related to axis 1, while Taraldrud north and

306    south, Skullerud and Taraldrud crossing appeared to have lower concentrations for the

307    variables related to axis 1. Based on the sample scores extracted from axis 2, none of

308    the ponds were significantly different. The ponds Såstad, Taraldrud crossing, Fiulstad,

309     Idrettsveien and Karlshusbunn appeared to have high concentrations for the variables

310     related to axis 2, mainly because of the road salt (indicated by $Cl^-$, Na and

311     conductivity).

312     According to ANOVA followed by Tukey post-hoc tests, there were no statistically

313     significant differences in water quality between sampling periods when using PCA

314     scores extracted from axis 1, but there were statistically significant differences for PCA

315     scores extracted from axis 2 (Figure S1-S2). The PCA scores extracted from axis 2

316     indicated that the lowest and highest levels of road salt ($Cl^-$, Na and conductivity) were

317     observed in October and June, respectively (3.6 – 2090 mg/L). The opposite pattern

318     was observed for pH (4.3 – 9.7).

319     ## 3.2 Biological community composition in relation to water quality and physical

320     variables

321     Of the 96 taxa found in the studied sedimentation ponds (Tables S6.1 – 6.8), 7 taxa

322     occurred in all investigated ponds (Hydracarina, Hirudinea, *Notonecta reuteri*

323     (Hemiptera), Chironomidae, Chaoboridae, *Caenis horaria* (Ephemeroptera)*,

324     Coenagrionidae), while 32 taxa were present in two or more of the sedimentation

325     ponds.

326     The result of the unconstrained PCA for biological data showed that 40% of the

327     variation in the biological community could be explained by axes 1 and 2; most taxa

328     were gathered along axis 1 and the rest along axis 2 (Figure S4). For clarity, only 25

329     taxa that were well characterised by the first four ordination axes are displayed; the

330     same was done for RDA.

331     The results of PCA for water quality variables (Figure 3A) showed that all metals were

332     correlated with each other as well as with $SO_4^{2-}$; thus, $SO_4^{2-}$ was analysed together with

333     metals. PCA was repeated for metals (including $SO_4^{2-}$) to extract the PCA scores. Axis 1

334     from PCA for metals (including $SO_4^{2-}$) explained 58% of the variance (Figures S3); thus,

335     the PCA scores extracted from axis 1 were used (denoted PCA1 (M)). Moreover, $Cl^-$

336     content was highly correlated with conductivity and Na; thus, the concentration of $Cl^-$

337     was used to represent road salt. Therefore, seven variables (TOC, DO, P, hydrocarbons,

338  Cl⁻, pH and PCA1 (M)) were used in the RDA to analyse the effects of water quality

339  variables on the biological community composition.

340  The RDA analysis showed that the overall RDA global model (Figure S5) was significant

341  ($p$ = 0.002). The RDA with forward selection showed that out of the 14 variables

342  (metals (including $SO_4^{2-}$), P, TOC, DO, pH, hydrocarbons, Cl⁻, size, age, AADT, number of

343  ponds/water bodies within 1 km, distance to the closest pond from study pond, as well

344  as plant cover within and around the ponds), 8 variables were statistically significant:

345  metals (including $SO_4^{2-}$), Cl⁻, P, DO, hydrocarbons, AADT, distance and pond size (Figure

346  4); the simple effects of each variable are presented in Table S7 (i.e. the explained

347  variation as if the variable is used alone in the RDA). Axes 1 and 2 explained 19% and

348  7% of the variation in the biological community composition. The RDA plot indicated

349  that the variable pond size had the greatest impact on the biological community

350  composition. Metals (including $SO_4^{2-}$) and AADT also contributed considerably to

351  explaining the variation in the biological community composition. Most taxa were

352  positively correlated to the pond area, with some exceptions, e.g. Hydraenidae and

353  Oligochaeta. Most taxa were positively correlated with AADT. Most taxa were

354  negatively correlated with metals, with some exceptions, e.g. *Phryganea bipunctata*

355  (Trichoptera) and Oligochaeta. Moreover, most taxa were negatively correlated with

356  the distance to the nearest neighbouring pond. Among the 25 dominant taxa, some

357  taxa were positively correlated with Cl⁻, e.g. *Rana* sp, *Notonecta* sp. Nymphs

358  (Heteroptera) and *Semblis atrata* (Trichoptera), while others were negatively

359  correlated with Cl⁻, e.g. *Cloeon inscriptum* and *Paraleptoplebia* sp. (Ephemeroptera).

360  Some taxa were positively correlated with P and DO, while other taxa were negatively

361  correlated. Compared with the other selected variables, hydrocarbons had the least

362  contribution to the biological community composition; most organisms were positively

363  correlated with hydrocarbons.

364  The result of the RDA after removing the seasonal effect (i.e. month used as a

365  covariate) showed that the variation in taxa explained by the eight selected variables

366  decreased from 42% to 39%. This indicates that seasons had a minor influence on the

367  variation in the biological community composition in the present study.

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368    The variation partitioning (Table 2) showed that the group of water quality variables

369    (metals (including $SO_4^{2-}$), $Cl^-$, DO, P, and hydrocarbons) explained 48%, while the group

370    of physical variables (size of ponds, AADT, and distance to the nearest neighbouring

371    pond) explained 41% of the total variation in the biological community composition.

372    The shared effects of these two groups of variables accounted for 11% of the total

373    variation.


## 4. Discussion

375    Due to the lack of studies that combine water quality and physical variables into a

376    single analysis of species community over several ponds, there is a lack of

377    understanding of the relative impacts of such variables on species richness (Hassall et

378    al., 2011). In our study, the effects of water quality and physical variables on 96 taxa,

379    including 91 macroinvertebrates, 2 zooplankton and 3 amphibians, were analysed.

380    Among the identified taxa, 4 macroinvertebrate species (*Brychius elevates*, *Hygrotus*

381    *confluens, Ilybius guttiger, Ilybius quadriguttatus)* and one amphibian species (*Triturus*

382    *vulgaris)* belong to the "near threatened" category, while *Plateumaris braccata*

383    belongs to the "vulnerable" category in the Norwegian Red List (Artsdatabanken,

384    2011). The water quality variables included 19 metals, hydrocarbons, P, $Cl^-$, $SO_4^{2-}$, TOC,

385    DO, pH and conductivity. It should be stressed that the sampling strategy in the

386    present study did not aim to collect water samples after runoff episodes. Therefore,

387    the measured concentrations can be considered representative of the general water

388    quality levels in the studied ponds and not an indication of extreme concentrations

389    that may occur after runoff episodes. Nevertheless, some of the metals were present

390    at relatively high concentrations.

391    Pond size is the most important physical variable that was statistically selected by RDA

392    forward selection method. The results showed that large ponds can support more

393    species than small ones; this is in accordance with the conventional species-area

394    relationships. Gotelli and Graves (1996) mentioned that small ponds have low species

395    richness due to their higher vulnerability to disturbance, such as degradation resulted

396    from pollutant loads. Nevertheless, the results of studies involving the pond size are

397    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.

427     Hassall (2014) referred to such kind of networks as "pondscapes", constituting a

428     network of distributed discrete habitat patches.

429     Most taxa were negatively correlated with the metals concentrations in water. Metals

430     in road runoff arise from various sources, including automobile sources (e.g. fuel

431     components, brakes and tyres), traffic barriers, road signs and road lightning

432     infrastructures (Meland, 2010). It has been demonstrated that increases in heavy

433     metal concentrations lead to decrease in biodiversity (Phillips et al., 2015). Although

434     several metals can act as essential nutrients for living organisms (e.g. Ca, Na, K, Mg, Fe,

435     Zn, Cr and Se), these metals are harmful to living organisms when they reach excessive

436     levels or enter certain oxidation states (Weiner, 2008). Compared to the WFD EQS for

437     the priority pollutants and the River Basin Specific Pollutants for Norway (Pettersen,

438     2016), the concentrations of Zn and Cu in most ponds in our study were high, while the

439     concentrations of As, Cd, Cr, Ni and Pb were generally relatively low, but could

440     occasionally reach high levels. It is important to stress that we did not collect water

441     samples directly after runoff episodes when the concentrations are believed to be the

442     highest.

443     The aquatic organisms were greatly affected by $Cl^-$. The reason why $Cl^-$ was quite high

444     in some ponds (the maximum concentration recorded in our study reached 2090 mg/L)

445     is because sodium chloride (NaCl) is widely used on roads in Norway as a de-icing

446     agent. Thus, road runoff and snowmelt-induced runoff normally contain high

447     concentrations of $Cl^-$ in these areas during winter and spring, thereby considerably

448     affecting the water quality of receiving ponds. Different from rainstorms, snowmelt

449     runoff can persist for several days to weeks. Furthermore, in the areas with frozen soil,

450     both pervious and impervious surfaces contribute with snowmelt runoff (Semadeni-

451     Davies, 2006). Marsalek et al. (1999) has demonstrated that in winter, road runoff

452     exhibited the highest frequency of severe toxic effects. The elevated concentration of

453     $Cl^-$ can cause toxicity due to the osmotic stress related to overall ionic strength (Elphick

454     et al., 2011, Blasius and Merritt, 2002). In addition, high $Cl^-$ concentrations may kill

455     roadside vegetation resulting in increased erosion and sediment load that have a

456     negative impact on the abundance of invertebrates (Blasius and Merritt, 2002). Other

457 severe consequences resulting from Cl⁻ deposition and retention are the prevention of

458 water circulation leading to anoxic conditions in bottom waters, and release of trapped

459 metals from sediment causing lethal toxicity to pond organisms (Van Meter et al.,

460 2011). Therefore, due to the characteristics of snowmelt runoff and excessive amount

461 of Cl⁻, future studies need to further investigate the influence of road salt on biological

462 community composition during winter and spring, especially in cold regions.

463 Although of apparently lower importance, P, DO, and hydrocarbons were included as

464 statistically significant variables in the forward selection procedure. P is the primary

465 growth limiting nutrient in most freshwater systems (Yang et al., 2009). Some taxa

466 were positively related to the P concentration, but some were negatively correlated.

467 Houlahan et al. (2006) also found that total species richness in wetlands was negatively

468 correlated with water nutrient levels. If nutrient loading rates exceed the critical level,

469 species composition can be altered over a short time (Verhoeven et al., 2006). For

470 example, P concentration in the runoff could result in eutrophication in receiving

471 water bodies. Eutrophication is considered to be one of the main impacts on small

472 standing water bodies (Menetrey et al., 2005) causing episodes of noxious blooms,

473 reduction in aquatic macrophyte communities and the depletion of DO in bottom

474 waters (Conley, 1999). As one of the crucial limnological variables, DO affects the

475 distribution of many species and maintains aquatic life (de Moura Guimaraes Souto et

476 al., 2011, Connolly et al., 2004). In addition, DO plays the crucial role in speciation of

477 metals, influencing their biomobility and toxicity (Rabajczyk, 2010). Since the DO

478 concentrations in the studied ponds were above the threshold value for the aquatic

479 organisms to live, DO levels do not appear to be a major limiting factor in the present

480 study. Nevertheless, the RDA plot indicated that different taxa may have different

481 oxygen requirements and tolerance to hypoxia; this can be attributed to a diverse

482 array of structural and behavioural respiratory adaptations among various aquatic

483 organisms (Connolly et al., 2004). Lastly, there was an indication of a small positive

484 correlation between the hydrocarbons and the abundance of macroinvertebrates

485 which may be somewhat obscure. Further research is needed to evaluate the effects of

486 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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517    Trichoptera and checked random samples of Heteroptera and Odonata.

## 518    Colour for figures

519    Colour should be used for all figures in print.

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686

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

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

[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).

| Fraction | % of Explained | % of All | $p$ – value |
|---|---|---|---|
| Metals (including $SO_4^{2-}$), $Cl^-$, DO, P and hydrocarbons | 48% | 13% | 0.002 |
| Pond size, AADT, and distance to the nearest neighbouring pond | 41% | 11% | 0.002 |
| Shared parts of two groups | 11% | 3% | 0.002 |
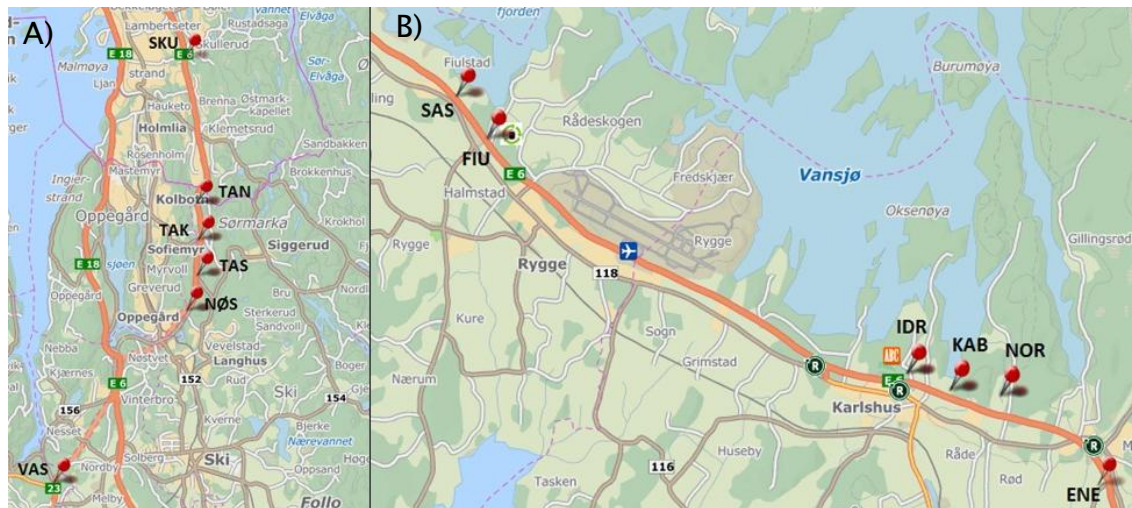
Figure 1. Location of the studied sedimentation ponds (red dots) in Oslo and Akershus county (A) and in Østfold county (B). In A), the ponds are SKU - Skullerud, TAN - Taraldrud north, TAK - Taraldrud crossing, TAS - Taraldrud south, NØS - Nøstvedt, and VAS - Vassum. In B), the ponds are SÅS - Såstad, FIU - Fiulstad, IDR - Idrettsveien, KAB - Karlshusbunn, NOR - Nordby, ENE - Enebekk. The distance between the two farthest ponds (Skullerud and Enebekk) is 71 km.
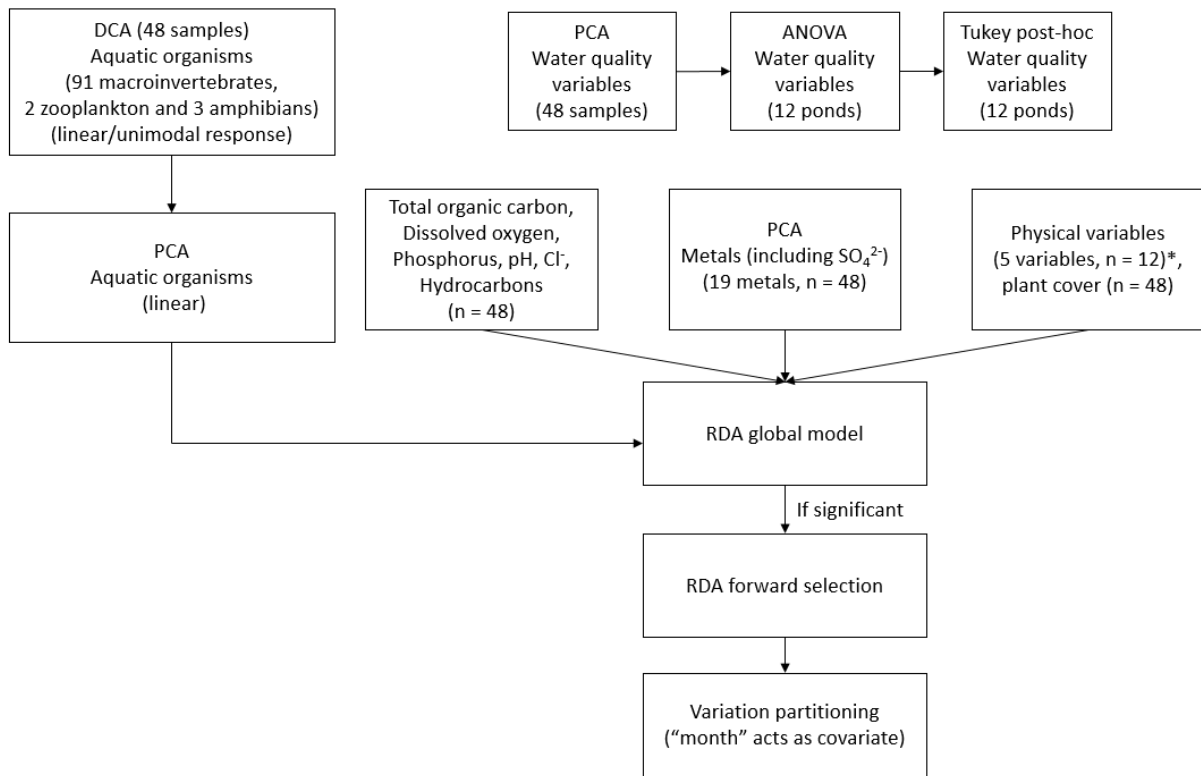
Figure 2. Schematic overview of the methods that were used for the data analysis: DCA - Detrended Correspondence Analysis, PCA - Principal Component Analysis, RDA - Redundancy Analysis, and ANOVA – One-way Analysis of Variance. * In order to run the model, the physical variables were upscaled to n=48 to match the water quality variables.
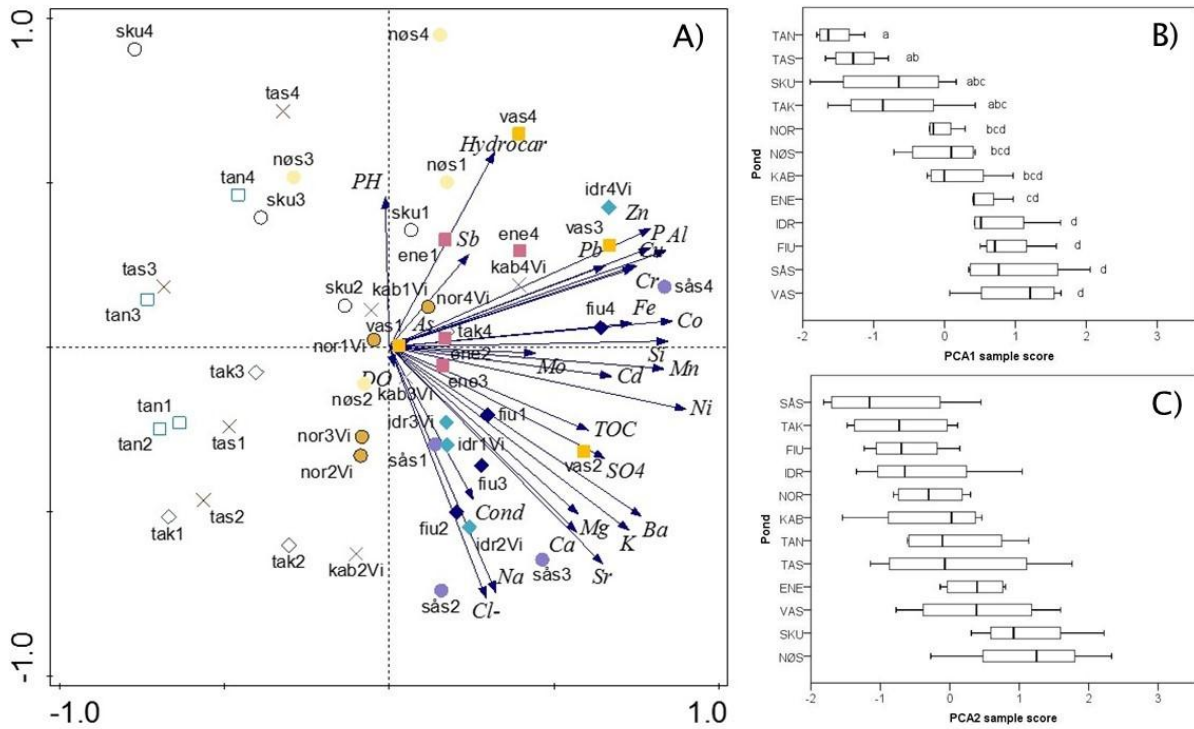
Figure 3. A) Principal components analysis (PCA) for water quality variables. The same symbol with the same colour indicates that samples were collected from the same pond; the first three letters indicate the name of the pond; "1", "2", "3" and "4" indicate that the samples were collected in April, 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.
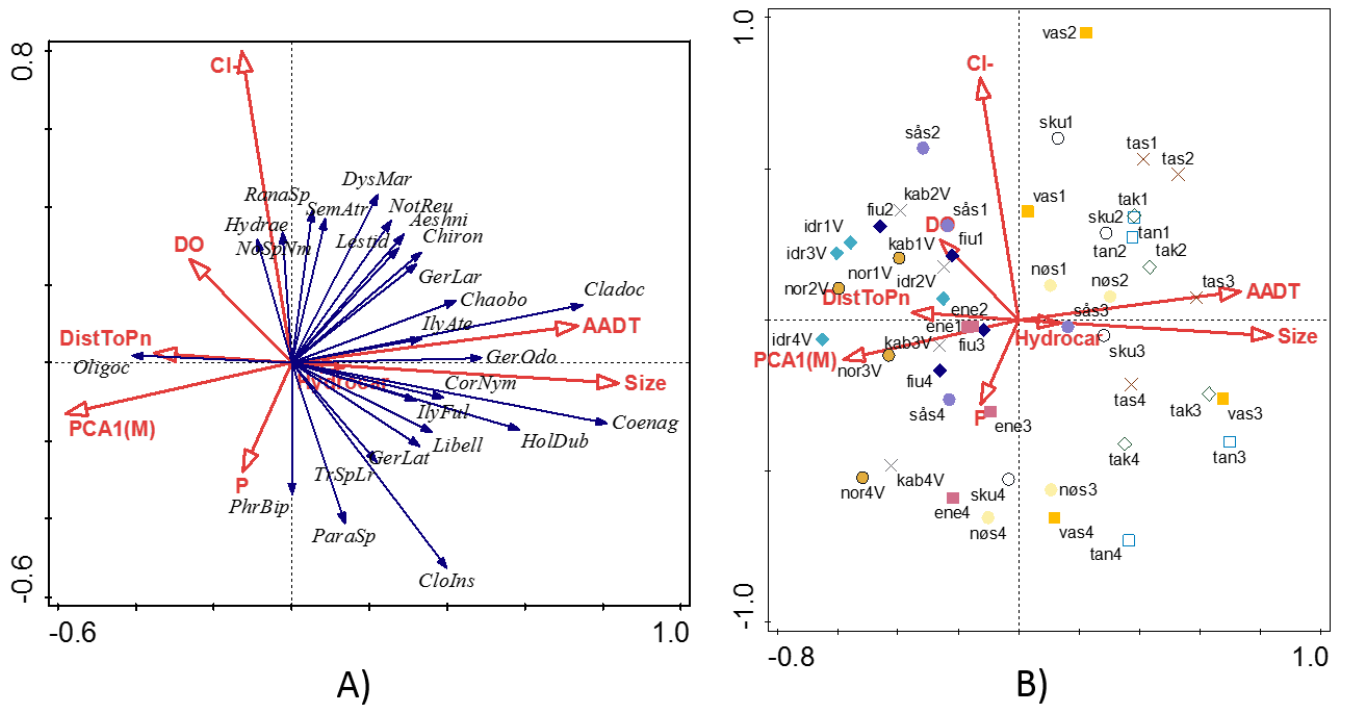
Figure 4. Redundancy analysis (RDA) with forward selection of taxa in relation to the water quality and physical variables. The effect of covariate "month" was removed. PCA1 (M) represents concentrations of metals (including $SO_4^{2-}$), DO - dissolved oxygen, and P – phosphorus; "DistToPn" represents 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.

**Supplementary material for on-line publication only**