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

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15 Abstract

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

41 Keywords: aquatic biodiversity; pond size; road runoff; road salt; sedimentation ponds;
42 water quality

43 1. Introduction

44 It is widely accepted that roads have major environmental impacts on aquatic
45 ecosystems. For example, habitat quality can be altered through sediment loading
46 (Angermeier et al., 2004) and pollutants released from transportation (Le Viol et al.,
47 2009, Scher and Thiéry, 2005). Runoff from roads contains a plethora of pollutants and
48 is considered a major source of diffuse pollution (Bohemen and Janssen Van De Laak,
49 2003), causing negative impacts on the receiving water bodies (Meland et al., 2010a,
50 Jensen et al., 2014). Therefore, the national road administrations as well as the
51 environmental authorities consider pollution reduction to be important. In most
52 countries, blue-green solutions such as engineered sedimentation ponds and wetlands
53 are the preferred mitigation measure for protecting receiving waters both from peak
54 runoff volumes and elevated pollution loadings and concentrations (Meland, 2016). In
55 addition to pollution, roads and the construction of them may disturb or even destroy
56 aquatic habitats physically. Disruption of connectivity by roads may also negatively
57 affect the movement of animals (Forman et al., 2003). In comparison to terrestrial
58 habitats, freshwater habitats suffer greater biodiversity decline due to various
59 stressors dominated by anthropogenic variables, such as habitat loss and degradation,
60 and pollution (Hassall, 2014, Burroni et al., 2011).

61 Ponds are defined as engineered and natural water bodies between 1 m² and 2 ha in
62 area, that may be permanent or temporary (Biggs et al., 2005). A highway
63 sedimentation pond, which reduces the peak flow during major storm events and
64 prevents water from either chronic or acute contamination reaching streams and lakes
65 (Scher et al., 2004), functions as a part of an urban drainage system. Sedimentation
66 ponds have also recently gained interest in an ecological context due to their potential
67 capacity to conserve and promote aquatic biodiversity e.g. Le Viol et al. (2009) and
68 Chester and Robson (2013). Only in Europe, thousands of ponds and other blue-green
69 solutions are built along major roads (Meland, 2016). The high number may in fact
70 underline their importance and relevance in an ecological context. Compared with
71 other freshwater habitats, natural ponds can support significantly more species,
72 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

103 certain factors on biodiversity in ponds, few of them have combined water quality and
104 physical factors into a single comprehensive analysis. Moreover, those few studies that
105 have combined various factors, included only a very limited number of chemicals such
106 as nutrients. Without this information, biodiversity conservation is likely to be
107 impeded or even impossible. It has been questioned whether ponds that are designed
108 for treating stormwater runoff are also able to enhance or maintain regional
109 biodiversity (Hassall and Anderson, 2015). Therefore, it is necessary to examine the
110 relationship between different factors and aquatic biodiversity.

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

128 2. Material and methods

129 2.1 Study area

130 Twelve highway sedimentation ponds situated along the major four-lane highway E6
131 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.

136 2.2 Field work and laboratory analyses

137 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 (NO₃⁻) and sulphate(SO₄²⁻); 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.

154 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.

161 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 $\frac{1}{2}$ 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 \times 4 months, $n = 48$),
243 while each physical variable was represented by one value constant during the
244 sampling campaign (12 ponds \times 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.

250 3. Results

251 3.1 General trends in water quality variables

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

265 The ecological status of surface water is categorized into classes from 1 to 5, with 1
266 being background level and 5 being very poor quality (Pettersen, 2016). For As, most
267 ponds belonged to class 2, and some belonged to class 3; only the pond Taraldrud
268 north (in August) belonged to class 4. For Cr, most ponds belonged to class 2, while the
269 ponds Såstad (in August and October), Fiulstad (in October), Idrettsveien (in October)
270 and Enebekk (in October) belonged to class 4. For Cd, most ponds had very low
271 concentrations and belonged to class 1, and only the pond Såstad (in October)
272 belonged to class 5. For Pb, 30 samples belonged to class 2, and 17 samples belonged
273 to class 3; only the pond Karlshusbunn (in October) belonged to class 5. For Ni, most
274 ponds belonged to class 2, and some belonged to class 3; only the ponds Taraldrud
275 north (in April and August) belonged to class 1. For Zn, 11 samples belonged to class 2,
276 and 25 samples belonged to class 4; the ponds that belonged to class 5 were Nøstvedt
277 (in April, and October), Vassum (in June, August and October), Enebekk (in April, June,
278 August and October), Såstad (in October), Idrettsveien (in October) and Karlshusbunn
279 (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 SO₄²⁻), 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 SO₄²⁻), 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 SO₄²⁻) 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.

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.

374 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

398 when it was applied to ponds. The species-area relationship was apparent for Odonata,
399 but not relevant for Coleoptera and Sphaeriidae (Oertli et al., 2002). Biggs et al. (2005)
400 also found that the trend that larger ponds support more species was stronger for
401 macrophytes, but weaker for invertebrates. A possible explanation to such
402 phenomenon might be the small island effect, in which species-area relationships are
403 not valid for small pond sizes (Hassall et al., 2011, Lomolino, 2000). The small island
404 effect suggests that in small pond patches, extrinsic, stochastic processes have a larger
405 effect compared to intrinsic, ecological processes in structuring communities (Hassall
406 and Anderson, 2015). In urban environments, such effect could be aggravated by
407 numerous interacting stressors that act on top of natural processes (Hassall and
408 Anderson, 2015).

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

420 Most taxa were negatively correlated with the distance from the study pond to the
421 nearest neighbouring pond. This is potentially attributed to the higher connectivity
422 that facilitates the mobility of invertebrates between ponds (Gledhill et al., 2008). The
423 importance of nearby ponds is in agreement with previous studies, which indicate that
424 pond density and connectivity appeared to be the major contributing variables to
425 biodiversity (Noble and Hassall, 2015, Staddon et al., 2010). This highlights the
426 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 Houlihan 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.

487 5. Conclusions

488 We studied the impacts of multiple environmental factors, including water quality and
489 physical variables, on the biological community composition in sedimentation ponds.

490 In the present analysis, the key variables controlling the aquatic biodiversity were the
491 pond size, distance to the closest pond from study pond, AADT and a combination of
492 various contaminants such as metals, phosphorus, road salt, dissolved oxygen and
493 hydrocarbons. The pond size plays a crucial role in affecting biological community
494 composition, as more species tend to live in the larger ponds. Our study indicates that
495 sedimentation ponds have the potential to contribute to biodiversity conservation. In
496 order to promote and conserve aquatic biodiversity in road sedimentation ponds,
497 larger ponds would be preferable due to the “species-area effect” and the dilution of
498 harmful pollutants. In addition, the shorter distance between ponds allows organisms
499 to spread more easily due to the higher connectivity, which maintains biodiversity.

500 More studies are still needed to investigate the influence of additional environmental
501 factors using different approaches and methods, such as process-based modelling.

502 Furthermore, measurements of the pollutants in the pond sediments, which may act
503 as a more accurate proxy for the overall pollution level compared to water samples,
504 should be included in such studies. These studies could then provide recommendations
505 for optimising aquatic biodiversity in the road sedimentation ponds.

506 Authors' Contributions

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

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516 Ole Wiggo Røstad verified the identification of Dytiscidae. Trond Bremnes verified
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

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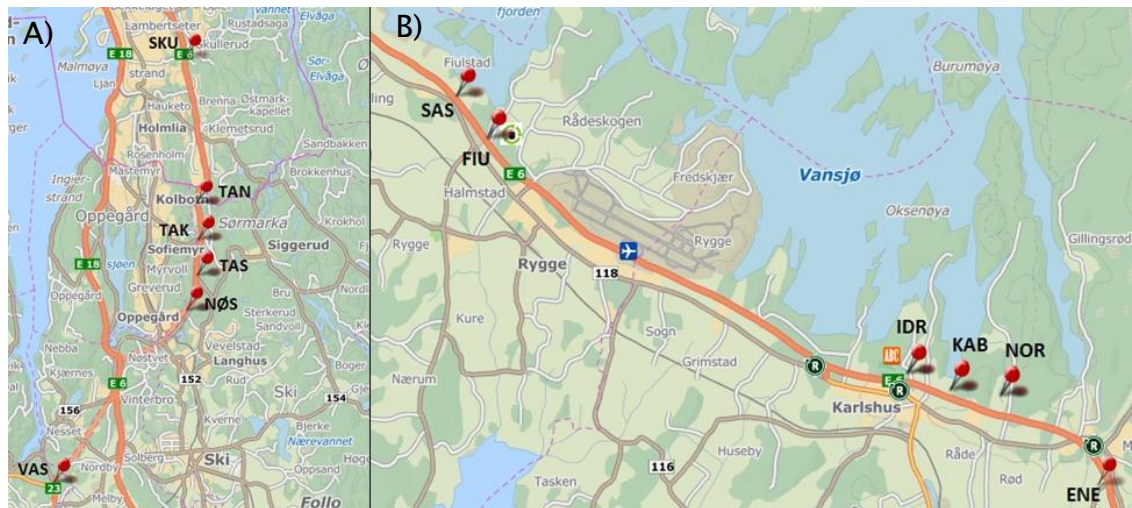


Figure 1. Location of the studied sedimentation ponds (red dots) in Oslo and Akershus county (A) and in Østfold county (B). In A), the ponds are SKU - Skullerud, TAN - Taraldrud north, TAK - Taraldrud crossing, TAS - Taraldrud south, NØS - Nøstvedt, and VAS - Vassum. In B), the ponds are SÅS - Såstad, FIU - Fiulstad, IDR - Idrettsveien, KAB - Karlshusbunn, NOR - Nordby, ENE - Enebekk. The distance between the two farthest ponds (Skullerud and Enebekk) is 71 km.

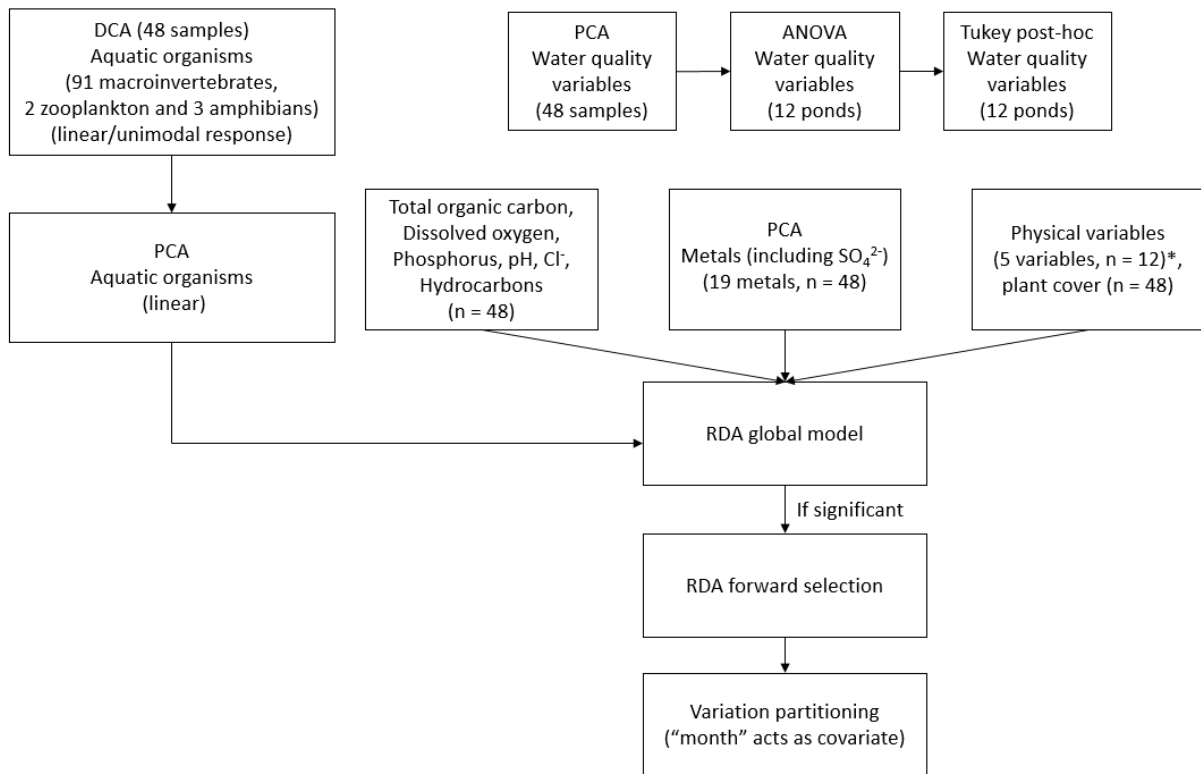


Figure 2. Schematic overview of the methods that were used for the data analysis: DCA - Detrended Correspondence Analysis, PCA - Principal Component Analysis, RDA - Redundancy Analysis, and ANOVA – One-way Analysis of Variance. * In order to run the model, the physical variables were upscaled to n=48 to match the water quality variables.

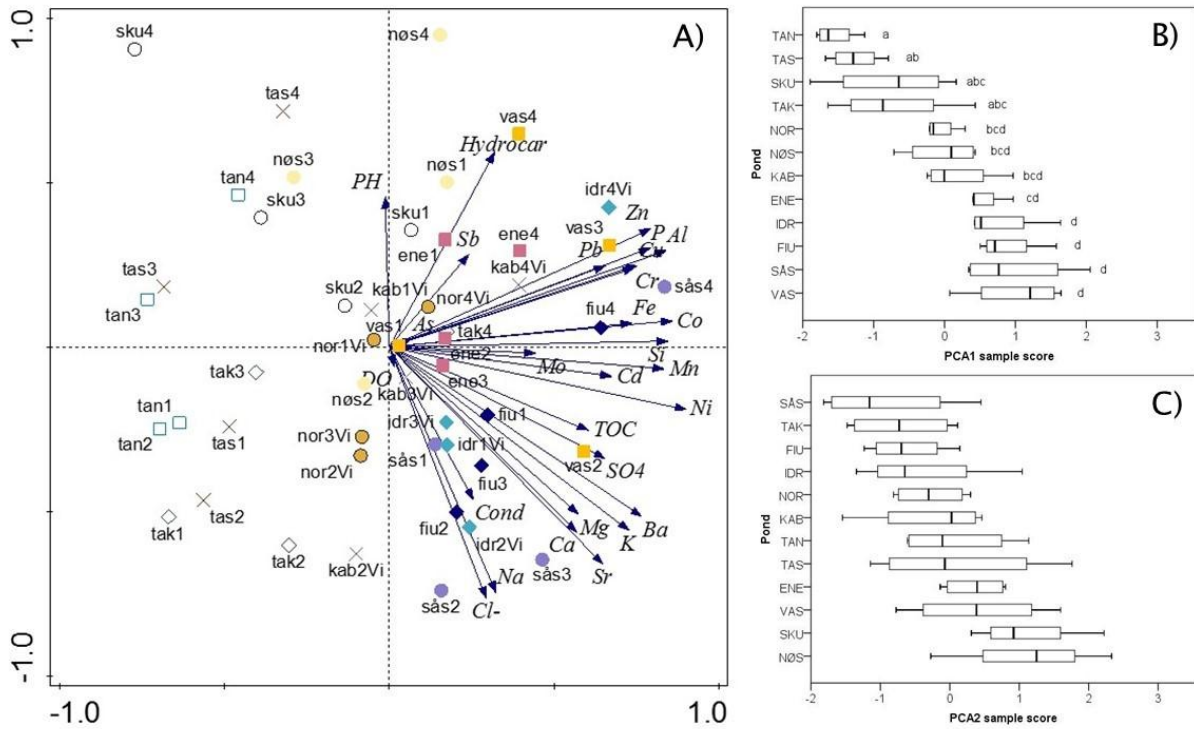


Figure 3. A) Principal components analysis (PCA) for water quality variables. The same symbol with the same colour indicates that samples were collected from the same pond; the first three letters indicate the name of the pond; “1”, “2”, “3” and “4” indicate that the samples were collected in April, 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.

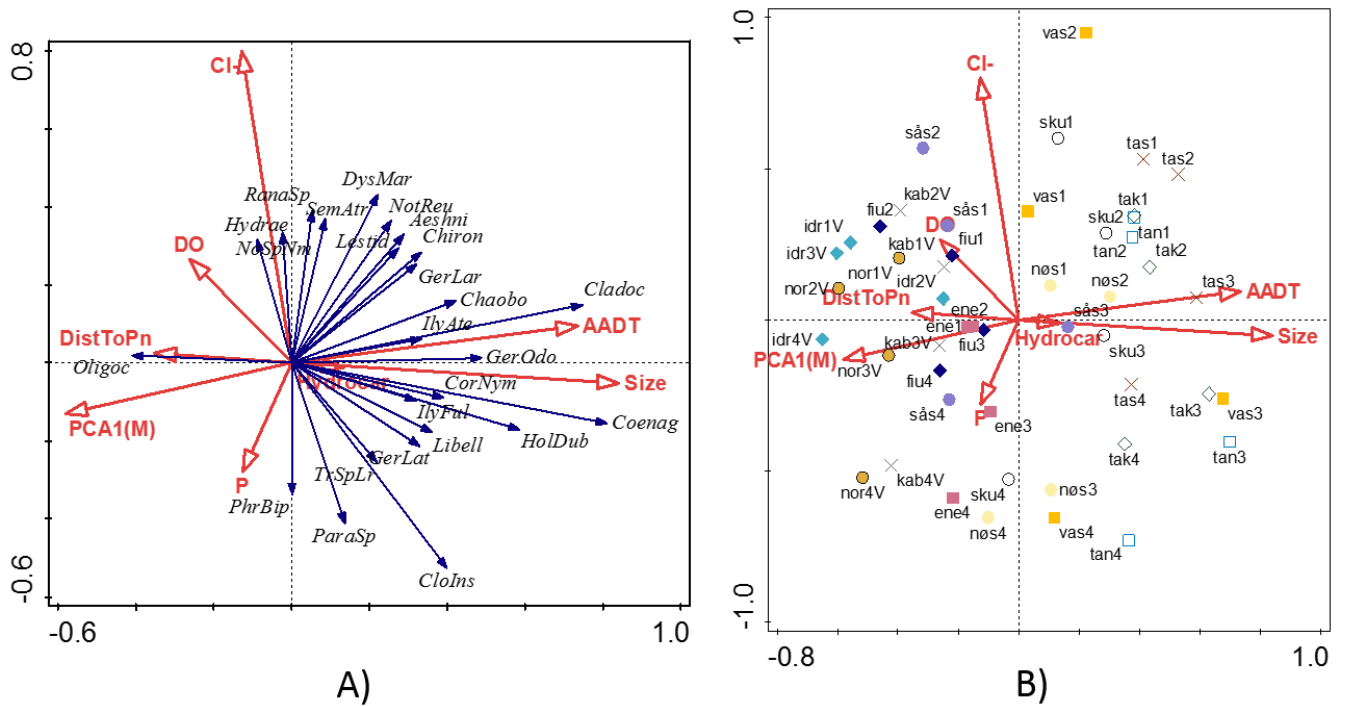


Figure 4. Redundancy analysis (RDA) with forward selection of taxa in relation to the water quality and physical variables. The effect of covariate “month” was removed. PCA1 (M) represents concentrations of metals (including SO_4^{2-}), DO - dissolved oxygen, and P – phosphorus; “DistToPn” represents 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.

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