



ALMA MATER STUDIORUM  
UNIVERSITÀ DI BOLOGNA

ARCHIVIO ISTITUZIONALE  
DELLA RICERCA

## Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

Assessing the impact of artificial summer drainage on the benthic macroinvertebrates in a freshwater wetland in northeast Italy

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

*Published Version:*

Beltrami R., Greggio N., Dinelli E., Pasteris A. (2022). Assessing the impact of artificial summer drainage on the benthic macroinvertebrates in a freshwater wetland in northeast Italy. *HYDROBIOLOGIA*, 849(3), 571-587 [10.1007/s10750-021-04708-5].

*Availability:*

This version is available at: <https://hdl.handle.net/11585/863122> since: 2024-05-21

*Published:*

DOI: <http://doi.org/10.1007/s10750-021-04708-5>

*Terms of use:*

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).  
When citing, please refer to the published version.

(Article begins on next page)

[Click here to view linked References](#)

1 **Assessing the impact of artificial summer drainage on the**  
2 **benthic macroinvertebrates in a freshwater wetland in**  
3 **northeast Italy**

4

5

6 Riccardo Beltrami, Nicolas Greggio, Enrico Dinelli, Andrea Pasteris\*

7

8 *Department of Biological, Geological and Environmental Sciences, University of*  
9 *Bologna, via Sant'Alberto 163, 48123 Ravenna, Italy*

10 *Interdepartmental Research Centre for Environmental Sciences, University of Bologna,*  
11 *via Sant'Alberto 163, 48123 Ravenna, Italy*

12

13 \* Corresponding author.

14 *E-mail address:* andrea.pasteris@unibo.it.

15

16 **ORCID ID**

17 Riccardo Beltrami: not available

18 Nicolas Greggio: 0000-0002-7323-134X

19 Enrico Dinelli: 0000-0002-3870-544X

20 Andrea Pasteris: 0000-0001-8958-5698

21

22

23

24

25 **Acknowledgments**

26 The authors are grateful to Dr Giorgio Lazzari for the valuable information and suggestions.

27 This study was founded by the Ricerca Fondamentale Orientata program of the Italian Ministry of

28 University and Research. The Ministry did not have any role in study design, in the collection, analysis

29 and interpretation of data, in the writing of the report and in the decision to submit the article for

30 publication.

31

32

33 **Abstract**

34 Valle Mandriole is one of the two last remaining freshwater wetlands in the coastal area of Ravenna (NE  
35 Italy). In 2011 a management technique that involves the complete drainage of the southern portion of Valle  
36 Mandriole during summer has been undertaken. In the present study, the effects of this artificial drying on  
37 the benthic macroinvertebrate fauna were assessed using a beyond before-after-control-impact (beyond  
38 BACI) sampling design. Macroinvertebrates dwelling on macrophytes and in bare sediments were sampled  
39 in the impact location and in four control locations, two times before and two times after the drying period.  
40 Simultaneously, water samples were collected to monitor chemical properties potentially affecting the  
41 studied organisms. Biological and chemical data were analysed by multivariate statistical methods.  
42 The statistical analysis did not detect any significant effect of the management action on the benthic  
43 macroinvertebrates or on the water chemical and physical properties. This contrasts with some previous  
44 results, suggesting that the effects of a management strategy based on draining completely dry and then  
45 reflooding a wetland area are site specific. However, it is necessary to consider that the present study does  
46 have some limitations, in particular the differences between impact and control locations and the timing of  
47 the sampling. The highest biodiversity was observed in one small and isolated control location; this  
48 highlights how maintaining, protecting, restoring and even creating small ponds may play an important role  
49 in biodiversity conservation.

50

51 **Keywords:** impact assessment; beyond BACI; benthic macroinvertebrates; water quality; water level  
52 management; managed wetlands

53

54 **Declarations**

55

56 ***Funding***

57 This study was founded by the Ricerca Fondamentale Orientata program of the Italian Ministry of  
58 University and Research.

59

60 **Conflicts of interest/Competing interests**

61 The Authors have no conflict of interest or competing interest to declare.

62

63 **Ethics approval**

64 Not applicable

65

66 **Consent to participate**

67 All the participants voluntarily agreed to participate in study on which the present manuscript is based.

68

69 **Consent for publication**

70 All the Authors gave their consent to publish the present manuscript in *Hydrobiologia*.

71

72 **Availability of data and material**

73 Research data are provided as electronic supplementary material to the present manuscript.

74

75 **Code availability**

76 Not applicable.

77

78

79

## 80 **Introduction**

81 Wetlands are extremely diverse in terms of habitats and biological communities (Moss, 2000;  
82 Dudgeon et al., 2006) and are among the most productive environments all over the world (Ramsar  
83 Convention Secretariat, 2013). The value of wetlands, related to the number of ecosystem services they  
84 provide, has been repeatedly highlighted during the last forty years (Tiner, 1984; Postel & Carpenter, 1997;  
85 Mitsch & Gosselink, 2000; Barbier, 2011; Maltby & Acreman, 2011; Sievers et al., 2018).

86 Despite their importance, over the centuries the extent of wetlands on our planet has been considerably  
87 reduced as a result of human activities, *in primis*, land reclamation (Denny, 1994; Gordon et al., 2010,  
88 Antonellini et al., 2015).

89 In natural alluvial landscapes, the extension of wetlands is influenced by many factors, such as the  
90 hydrological cycle, the landscape morphology and the riverine regime, which can lead wetlands to evolve  
91 into terrestrial environments (Ward, 1997). Under these conditions, the natural burying processes can be  
92 counterbalanced by flooding of new areas. However, in inhabited regions river basin management prevents  
93 these dynamics. Thus, maintaining the water level and bathymetry of wetlands present in these areas often  
94 requires an active human intervention.

95 In the coastal areas of the Po Valley (northeast Italy), the alluvial wetlands generated by the Po River  
96 have been reduced considerably to make room for crops (Cencini, 1998; Buscaroli et al., 2011; Antonellini  
97 et al., 2015). After the reclamation process, excluding minor and hunting ponds, in the coastal area  
98 surrounding the city of Ravenna only two freshwater coastal wetlands have survived to nowadays: Valle  
99 Mandriole and Punte Alberete. Recognition of their important role as unique habitats that survived the  
100 reclamation process has triggered actions aimed at preventing progressive burying and onset of anoxic  
101 conditions, such as hydraulic intervention and vegetation clearing (Buscaroli et al., 2011).

102 A particularly drastic measure has been taken in Valle Mandriole, due to the recurrence of anoxia and  
103 deterioration of vegetation. Starting from year 2011, the southern portion of Valle Mandriole has been  
104 completely drained every July to allow for mineralization of sediment organic matter and to facilitate  
105 vegetation clearing, then flooded again at the end of September to maintain wintering habitat for waterbirds.

106 Information on the benthic macroinvertebrates is highly relevant to the whole wetland ecosystem,  
107 since they participate in the transformation of organic matter and they contribute to the cycle of energy and

108 nutrients of the ecosystem. In particular they are a primary food source for organisms at the top of the food  
109 chain, i.e., fish, birds, mammals and reptiles (Covich et al., 1999, Cooper et al., 2009). Benthic  
110 macroinvertebrates are often used in biomonitoring to obtain information to implement management plans  
111 and improvement of environmental quality (Rosenberg & Resh, 1993). In particular, changes in the  
112 hydrological regime can significantly influence the composition of the benthic macroinvertebrate  
113 assemblages (Kaster & Jacobi, 1978; Furey et al., 2006; White et al., 2008; McEwen & Butler, 2010).

114 The aim of the present study was to assess if the dry period after complete drainage of the wetland  
115 impacted on the benthic macroinvertebrate assemblages, by comparison of the drained location with other  
116 reference locations in the same area, using a beyond before-after-control-impact (beyond BACI) sampling  
117 design (Underwood, 1992, 1994). Along with the benthic macroinvertebrate assemblages, the main water  
118 chemical and physical parameters have been evaluated pre and post the draining event.

119 Although it could be trivial to state that the benthos is affected in the period when the basin is  
120 completely dry, the point was to assess how the imposed dry period affected the benthic assemblages when  
121 the basin returned to be flooded. The drainage is supposed to improve the oxygenation conditions, of the  
122 wetland, historically affected by anoxia. This improvement is hypothesized to increase the diversity of the  
123 benthic assemblages and the abundance of taxa that are more sensitive to the oxygenation conditions. On  
124 the other hand, this intervention annihilates the benthos for a few months, and this could result in lower  
125 abundance and diversity for several months after the basin returns to be flooded (Lindegarth & Chapman,  
126 2001; Bedford & Powell, 2005).

127 Although this study focuses on a specific wetland of limited extension, its scope is beyond the local  
128 interest, as it can contribute to understand the implications of a management practice of simple applicability  
129 and wide exportability.

130

## 131 **Methods**

### 132 *Study area*

133 The freshwater wetlands complex of Valle Mandriole (VM) and Punte Alberete (PA) is located in the  
134 Po Plain, northeast Italy, 4 km from the coast of the north Adriatic Sea, 3.5 km south of the brackish  
135 Comacchio lagoons, 10 km north of the city of Ravenna, covering an area of 420 hectares (Fig. 1). The two  
136 wetlands are separated by the River Lamone and are the remnants of a larger complex (about 8000 hectares),

137 mostly reclaimed during the 1960s. The extension of the whole complex, as it appears today, was finally  
138 established in 1972 (Lazzari, 1994; Buscaroli et al., 2011).

139

140  
141

### Fig. 1

142 The entire study area lies in an anthropized landscape characterized by intensive farming and by the  
143 presence of a highway running along the eastern edge of both PA and VM. Moreover, the international port  
144 and the industrial area of Ravenna are located just a few kilometres to the South (Fig.1).

145 The area is part of the Po Delta Regional Park, established in 1988. In 1977, both wetlands were designated  
146 Ramsar sites, according to Ramsar Convention Secretariat (2013). In 1994 they were included in the Natura  
147 2000 network as *site of community importance* (Council Directive 92/43/EEC) and as *special protected*  
148 *areas* (European Parliament and Council Directive 2009/147/EC).

149 Nevertheless, strong pressures on these habitats driven by alien organisms such as *Procambarus*  
150 *clarkii* (red swamp crayfish or Louisiana crawfish) and *Myocastor coypus* (coypu), saltwater intrusion and  
151 water turbidity, have compromised over time their ecological status (Antonellini et al., 2010; Studio Silva,  
152 2012; Mollema et al., 2013).

153 Valle Mandriole, extending over 240 ha, lies in the north of the complex and is mostly made up of  
154 marshes with populations of *Phragmites australis*. One single sluice, located northeast, takes water in and  
155 out of the River Reno through an artificial channel.

156 Until 2011, to offset the significant lowering of the water level in the summer, VM was loaded to  
157 +0.80 m above mean sea level (AMSL) in September/October. This was implemented because Valle  
158 Mandriole was used as a reservoir for the water supply of Ravenna. However, this practice has led to a  
159 deterioration of emergent vegetation. Thus, starting from 2011 a management plan aimed at restoring the  
160 functionality of the biotope has been implemented. A complete drainage of the southern half of the wetland  
161 has been carried out every year during summer to foster mineralization of sediment organic matter. This  
162 intervention was accompanied in 2013 by the dredging of submerged channels to increase water circulation  
163 and guarantee refuge habitats for fishes.

164 Punte Alberete, extending over 186 ha, occupies the southern portion of the complex and consists  
165 mostly in alluvial forests alternating with low-lying perennially submerged marshes that are connected by  
166 ditches. The water level is controlled by two sluices. The first one, located in the southeast, is used to intake

167 water from River Lamone through an artificial channel. The second one, in the northeast, is used to drain  
168 water to Adriatic Sea, through the Pialassa Baiona lagoon.

169 The water level of Punte Alberete is usually not actively managed. However, occasionally it is set by  
170 acting on the sluices to allow for vegetation clearing. The average water level calculated on three years  
171 (2010-2012) observed in the northeast sluice was +0.64 m AMSL in March and -0.18 m AMSL in August.  
172 A small interdunal wetland (0.1 ha), here referred to as SV, was also included in this study (see below for  
173 the sampling design). The pond is located within the San Vitale Pinewood, established on the sand dune  
174 system that extends immediately east of PA and VM (Fig.1). This habitat differs from the other sampling  
175 locations due to the vegetation, that includes *Thypha latifolia*, and to the higher water salinity.

176

### 177 *Field and laboratory methods*

178 A beyond before-after-control-impact (beyond BACI) sampling design was adopted here to assess the  
179 effect on the benthic macroinvertebrate assemblages. In particular, we studied the effect of the drainage of  
180 the southern part of VM, that occurred during summer 2013. In the beyond BACI design a single putatively  
181 impacted location is compared with multiple control locations. All the locations are sampled at multiple  
182 times before and after the impacting event has occurred.

183 Five sampling locations (one impact, four controls) were identified in the study area. VM was divided  
184 into two portions (Fig. 1). The southern portion (VMS), extending approximately 120 ha, was identified as  
185 the impact location, since it was completely drained and kept dry from July to September. The control  
186 locations were the northern portion of Valle Mandriole (VMN), two marshes within Punte Alberete, one in  
187 the northern part (PAN), one in the southern part (PAS), and the pond within the San Vitale Pinewood (SV).  
188 Control locations maintained their own water level throughout the year, although minimal during summer.  
189 Within each location, two sites were identified, labelled as 1 and 2 (e.g., VMS1 and VMS2). All sites were  
190 located at the border between bare sediments and thickets of *Phragmites australis*, or *Typha latifolia* in the  
191 case of the SV pond where *P. australis* was not present. None of the sampled sites, neither in the impact  
192 location nor in the control locations, was subject to vegetation clearing during 2013. All ten sites were  
193 sampled twice before summer drainage (May and June), and twice after (October and November).

194 At each site, the macroinvertebrates were sampled separately from bare sediments and from emergent  
195 vegetation. For bare sediments, one sample for each site was taken using a PONAR grab with opening



196 155x175 mm (sampling surface: 0.027 m<sup>2</sup>). On vegetation, a squared-frame kick net with opening 210x210  
197 mm and a 0.5 mm mesh was used by scraping the immersed portion of the stems from the base upward for  
198 two minutes. Both types of samples were sieved on site with a 0.5 mm mesh conical net and then  
199 immediately preserved with 10% formaldehyde. In the laboratory, all sampled specimens were identified  
200 to family level and counted using a stereo microscope.

201 At each site, temperature (°C), electrical conductivity (µS/cm), pH, redox potential (mV) and  
202 dissolved oxygen concentration (mg/l) of the water were measured in the field, both on the surface and at  
203 the bottom, just above the sediment, using OX22 Aqualytic probe for dissolved oxygen and PCD 650  
204 (EUTHECH Instruments) for the other parameters. Water alkalinity (meq/l) was also measured in the field  
205 by titration using alkalinity titration test (Merck Mcolorstest™). Depth was determined using a graduated  
206 pole. In addition, a 2 l sample of surface water was taken and brought to the lab where dissolved sulphate  
207 (SO<sub>4</sub><sup>2-</sup>-S, mg/l), ammonium (NH<sub>4</sub><sup>+</sup>-N, mg/l), nitrite (NO<sub>2</sub><sup>+</sup>-N, mg/l), nitrate (NO<sub>3</sub><sup>+</sup>-N, mg/l) and phosphate  
208 (PO<sub>4</sub><sup>3-</sup>-P, mg/l) were measured using a Hach DR/2010 spectrophotometer, and total and volatile suspended  
209 solids (g/l) were determined gravimetrically using GF/F Whatman® glass microfiber filters (Eaton &  
210 Franson, 2005).

211

## 212 *Data analysis*

213 Three different multivariate data sets were developed and then analysed separately: i) abundance of  
214 taxa collected from vegetation, expressed as number of individuals per minute of sampling; ii) abundance  
215 of taxa sampled from bare sediments, expressed as number of individuals per square meter; iii) physical  
216 and chemical properties of the water. In addition, number of taxa, Shannon index of diversity and Pielou's  
217 index of evenness were calculated for invertebrates from both vegetation and bare sediments.

218 To assess the impact of the drainage of VMN and the effects of the other factors, each data set was  
219 analysed by PERMANOVA (Anderson, 2001; Anderson et al., 2008). PERMANOVA is a non-parametric  
220 analysis analogue to the analysis of variance (ANOVA) that allows for multivariate (and univariate) data  
221 to be analysed and tested based on any resemblance measure. Analysis of complex designs, involving  
222 several orthogonal and nested factors is possible. All tests of hypothesis, including those regarding  
223 interactions between orthogonal factors, are performed using permutation techniques; 9999 permutations  
224 were used for all the analyses presented here.

225 The Bray-Curtis distance was used as the resemblance measure for taxa abundances, after square root  
226 transformation of the raw data. The Euclidean distance was used as the resemblance measure for chemical  
227 and physical properties, after standardization of the raw data. The Euclidean distance was used also for the  
228 diversity indices.

229 PERMANOVA was applied in accordance with the principles of Beyond BACI, following the  
230 indications of Anderson et al. (2008) for the analysis of asymmetrical designs.

231 The factors included in the analysis were:

- 232 – control vs. impact (I, fixed, two levels: control and impact);
- 233 – location (L, random, nested in the factor control vs. impact);
- 234 – site (S, random, nested in the factor location, two sites for each location);
- 235 – period (P, fixed, two levels: before and after the drainage of VMS);
- 236 – time (T, random, nested in the factor period, two times before and two times after the drainage  
237 of VMS).

238 The design is asymmetrical, since only one impact location was available (VMS), as opposed to four  
239 control locations (VMN, PAN, PAS and SV). For each combination of site and time, only one sample was  
240 collected for the benthos on vegetation, one for the benthos in bare sediments and one for the water.  
241 Replicated sites were sampled within each location, but there were no replicates within each site; as a  
242 consequence, the lowest-level interaction, i.e., site×time, had to be used as the residual source of variation  
243 and could not be tested.

244 According to the beyond BACI rationale, an effect of the drainage of VMS would cause the temporal pattern  
245 of benthic abundances or of chemical properties in the impacted location to differ from the temporal patterns  
246 in the control locations. Thus, an impact would be detected if the I×P interaction or the I×T interaction were  
247 significant.

248 To complement PERMANOVA, two additional multivariate methods, based on resemblance  
249 measures, were applied. Non-metric multidimensional scaling (MDS) was used to graphically represent the  
250 relationships among samples. Similarity percentage (SIMPER) was used to quantify the contribution of  
251 each taxon to the similarity within and to the dissimilarity between groups of samples (Clarke, and  
252 Warwick, 2001). Ordination of samples based on water chemistry variables was performed by discriminant  
253 function analysis, using the location as the grouping criterion (Legendre & Legendre, 2012).

254 The software StatSoft Statistica was used to carry out the discriminant function analysis. All the other  
 255 calculations were performed using the software PRIMER 6 with the PERMANOVA+ add-on.

256

## 257 **Results**

### 258 *Macroinvertebrates on vegetation*

259 It was not possible to demonstrate any impact of the drainage of VMS on the abundances of benthic  
 260 macroinvertebrate taxa sampled from vegetation, since the PERMANOVA analysis did not detect a  
 261 significant I×P or I×T interaction (Table 1).

262

263 **Table 1** Results of the PERMANOVA test for the benthic macroinvertebrates sampled from vegetation.

264 \*: significant ( $P < 0.05$ ); \*\*: highly significant ( $P < 0.01$ )

Source of variation	Degrees of freedom	P value			
		Abundances	N of taxa	H'	J'
I: impact vs. control	1	0.771	0.934	0.980	0.522
P: period, before vs. after	1	0.522	0.262	0.948	0.988
L: location (nested in I)	3	0.005**	0.003**	0.006**	0.001**
T: time (nested in P)	2	0.025*	0.880	0.034*	0.013**
I×P	1	0.636	0.516	0.272	0.254
S: site (nested in L)	5	<0.001**	0.192	0.236	0.834
I×T	2	0.181	0.140	0.411	0.567
L×P	3	0.433	0.008**	0.206	0.334
L×T	6	<0.001**	0.445	0.781	0.776
S×P	5	0.042*	0.960	0.584	0.580
Residual	10	–	–	–	–
Total	39	–	–	–	–

265

266 The L×T interaction was significant, denoting that the temporal patterns of benthic abundances  
 267 differed among locations; however, these differences could not be ascribed to an effect of the drainage of  
 268 VMS, since they were present also among control locations.

269 The factor location was significant also as a main effect, indicating that the abundances of benthic  
 270 organisms differed among locations not only for their temporal pattern of variation but also for their mean  
 271 values over the considered time span.

272 In fact, The MDS plot (Fig. 2) shows that samples from different locations are clearly separated on  
273 the ordination plane; more precisely the samples from the pond in San Vitale Pinewood (SV) are grouped  
274 on the higher part of the diagram, the samples from both locations of Punte Alberete (PA) are grouped on  
275 the lower right and the samples from both locations in Valle Mandriole (VM) are grouped on the lower left  
276 side of the plane.

277

278  
279

## Fig. 2

280 According to the SIMPER analysis, the taxa that most contributed to the distance between the samples  
281 from SV and those from PA are Physidae, Baetidae, Chironomidae, which were more abundant in SV  
282 samples, and Naididae, more abundant in PA samples (Tables S1, S2 in the Supplementary Data). The same  
283 taxa gave the highest contribution to the distance between SV and VM, all being more abundant in SV  
284 samples (Tables S1, S3 in the Supplementary Data). The pinewood pond was characterized by the highest  
285 abundance of all taxa, excluding Naididae and Cambaridae, and by the presence of several families of  
286 Diptera, Coleoptera, Hetroptera and Odonata, absent in the other areas. Naididae is the taxon that by far  
287 gave the highest contribution to the distance between PA and VM, due to its high abundances in PA (Tables  
288 S1, S4 in the Supplementary Data). In general, VM was characterized by the highest abundances of  
289 Cambaridae and low abundances of other taxa. However, Baetidae were more abundant than in PA.

290 The horizontal sorting of the points in the MDS plot is mostly determined by the abundance of  
291 Naididae, which increases from left to right (Fig. S1a in the Supplementary Data). The vertical sorting is  
292 determined by the abundances of several taxa (notably Chironomidae, Baetidae, Physidae) that increase  
293 from bottom to top (Fig. S1b–d).

294 As for the temporal variation, in SV, Chironomidae, Baetidae and Physidae decreased in abundance  
295 over time, while Naididae increased in abundance. Coenagrionidae were sampled only in June (second time  
296 of the before period). In both locations of PA the abundance of Naididae increased from May to June and  
297 then decreased to the lowest values in the after period. Corixidae were relatively abundant in May and June  
298 and almost absent in October and November. One difference between PAS and PAN is the relatively high  
299 abundance of Gammaridae in the former in May and June. It is difficult to identify clear temporal patterns  
300 for VM due to the general low abundance of macrobenthic organisms. However, Cambaridae were

301 generally more abundant in the after period, particularly in October. The highest abundance of Baetidae  
 302 was recorded in June for VMS and in October for VMN.

303 The I×P and I×T interactions were not significant also for the diversity indices, while the main factor  
 304 location was highly significant for all of them (Table 1). SV had the highest richness (14-20 taxa), VM  
 305 constantly had a low richness (4-6 taxa) and PA had a decreasing richness over time from 9-11 taxa to 1-4  
 306 taxa (Fig. S2a in the Supplementary Data). The highest values of Shannon's index of diversity H' were  
 307 recorded in SV, the lowest in PA, were they decreased over time alongside with the reduction of the number  
 308 of taxa (Fig. S2b). Despite the low number of taxa, H' values in VM were higher than in PA, since all taxa  
 309 had low abundance, and none were dominant. In fact, the highest values of Pielou's index of evenness J'  
 310 were recorded in VM, the lowest in PA, where Naididae were highly dominant (Fig. S2c).

311

### 312 *Macroinvertebrates in bare sediments*

313 It was not possible to demonstrate any impact of the drainage of VMS on the abundances of benthic  
 314 macroinvertebrate taxa sampled from bare sediments, since the PERMANOVA analysis did not detect a  
 315 significant I×P or I×T interaction (Table 2).

316

317 **Table 2** Results of the PERMANOVA test for the benthic macroinvertebrates sampled from bare  
 318 sediments. \*: significant (P < 0.05); \*\*: highly significant (P < 0.01)

Source of variation	Degrees of freedom	P value			
		Abundances	N of taxa	H'	J'
I: impact vs. control	1	0.962	0.848	0.979	0.994
P: period, before vs. after	1	0.021*	0.993	0.736	0.384
L: location (nested in I)	3	0.028*	0.009**	0.003**	0.013*
T: time (nested in P)	2	0.893	0.735	0.488	0.393
I×P	1	0.513	0.949	0.984	0.980
S: site (nested in L)	5	0.041*	0.409	0.330	0.310
I×T	2	0.878	0.599	0.158	0.091
L×P	3	0.507	0.022*	<0.001**	0.079
L×T	6	0.016*	0.410	0.208	0.223
S×P	5	0.156	0.108	0.191	0.399
Residual	10	–	–	–	–
Total	39	–	–	–	–

319

320 Similarly to the vegetation samples, both the L×T interaction and the location main factor were  
321 significant, even if at higher P values, indicating that the mean values and the temporal patterns of the  
322 abundances of benthic taxa were different among locations, but that these differences could not be ascribed  
323 to an impact of the drainage.

324 The MDS plot (Fig. 3) shows that the points that represent the samples from VM and PA are clustered  
325 in the lower part of the diagram and largely overlap, indicating that the benthic assemblages in the two  
326 areas were rather similar in bare sediments, while on vegetation they were quite distinct. The samples from  
327 SV are scattered in the upper part of the plot, fairly spaced from each other and mostly clearly separated  
328 from those from VM and PA, indicating that the benthic assemblages of bare sediments of SV were not  
329 only different from those of the other areas but were also more heterogeneous.

330

331  
332

### Fig. 3

333 According to the SIMPER analysis, the samples from SV were characterized by the lowest mean  
334 abundance of Naididae and by the significant abundance of other taxa, in particular Chironomidae,  
335 Cerataopognidae and Chaoboridae (Tables S5-S7 in the Supplementary Data). On the other hand, the  
336 macrobenthos of bare sediments in VM and PA consisted almost exclusively of Naididae and the main  
337 difference between the two locations was that these organisms were on average more abundant in PA  
338 (Tables S5, S8 in the Supplementary Data).

339 The sorting of the points in the MDS plot is mostly determined by the abundance of Naididae, which  
340 increases moving to the lower right corner of the plot (Fig. S3a in the Supplementary Data) and by the  
341 abundance of Chironomidae, which increases moving in the opposite direction (Fig. S3b).

342 The abundance of Naididae decreased over time in all the locations, although to a different extent in  
343 each of them.

344 Again, the I×P and I×T interactions were not significant for the diversity indices, while the location  
345 factor was significant as main effect for all the indices and the L×P interaction was significant for H' (Table  
346 2). The highest values were observed in SV, where they further increased in the after period (Fig. S4 in the  
347 Supplementary Data).

348 In summary, the temporal pattern of variation of the benthic assemblages at the impact location was  
349 within the range defined by the other wetlands in the area, both on vegetation and in bare sediments. The  
350 structure of the benthic assemblages and their temporal patterns were related to the wetland where each  
351 location was situated (SV, PA or VM) and not to the classification of the location as impact or control.  
352 Consequently, it was not possible to detect any statistically significant impact of the drainage of VMS on  
353 the macrobenthic fauna.

354

### 355 *Water chemistry*

356 Fig 4. shows the range of variation of chemical and physical properties at the five locations and at  
357 three sites on the main rivers and channels in the surrounding area. Dissolved oxygen below 1 mg/l was  
358 measured only once (site PAS1, October); PAN was the location with lower values on average. The  
359 dissolved oxygen recorded at VMS and VMN was always relatively high, with no indications of anoxia;  
360 however, the months from July to September in which anoxic events are more frequent were excluded from  
361 the sampling.

362

363

### 363 **Fig. 4**

364

365 It was not possible to demonstrate any impact of the drainage of VMS on the water chemistry  
366 variables, since the PERMANOVA analysis did not detect a significant I×P or I×T interaction (Table 3).

367

368 **Table 3** Results of the PERMANOVA test for the chemical properties of the water. \*: significant (P <  
 369 0.05); \*\*: highly significant (P < 0.01)

Source of variation	Degrees of freedom	P value
I: impact vs. control	1	0.957
P: period, before vs. after	1	0.369
L: location (nested in I)	3	0.007**
T: time (nested in P)	2	0.057
I×P	1	0.643
S: site (nested in L)	5	0.010*
I×T	2	0.690
L×P	3	0.079
L×T	6	<0.001**
S×P	5	0.289
Residual	10	–
Total	39	–

370

371 The L×T interaction and the location main factor were significant, as for the macrobenthos on  
 372 vegetation and in bare sediments, indicating that the mean values and the temporal patterns of the  
 373 considered chemical variables were different among locations, but that these differences could not be  
 374 ascribed to an impact of the draining.

375 Fig. 5 shows the ordination plot based on the discriminant function analysis, using the location as the  
 376 grouping criterion. The analysis identified electrical conductivity, sulphates, volatile suspended solids and  
 377 phosphates as the variables giving a significant contribution to the separation between locations. The first  
 378 discriminant axis has a strong negative correlation with electrical conductivity and clearly separates SV  
 379 (average conductivity around 4 mS/cm) from the other locations, characterized by conductivity below 1  
 380 mS/cm. The second discriminant axis is positively correlated with volatile suspended solids, on average  
 381 higher in the PA locations, and is negatively correlated with sulphates, higher in the VM locations. The  
 382 samples from the two locations within Valle Mandriole, the impact location (VMS) and the control location  
 383 (VMN), largely overlap. The two locations from PA, that are actually two separate ponds, are close to each  
 384 other on the ordination plane, but still clearly distinct.

385  
 386

387  
 388

**Fig. 5**



389 In summary, similarly to what observed for the benthic assemblages, the values of the water chemistry  
390 and their temporal patterns were related to the area where the location was situated and not to the  
391 classification of the location as impact or control.

392

## 393 **Discussion**

394 The preservation and protection of wetlands is a goal that has been repeatedly emphasized in recent  
395 years (e.g., Mitsch & Gosselink, 2000; Sievers et al., 2018). In many cases this goal is pursued with specific  
396 management actions, of which the summer drainage of the southern part of Valle Mandriole is an example.  
397 Assessing the consequences of these actions is fundamental to identify the most effective practices.

398 The present study did not detect any adverse effect of the drainage on the benthic macroinvertebrates,  
399 suggesting that this practice is acceptable, at least as regards the investigated assemblages. On the other  
400 hand, the results also suggested that the drainage did not produce any benefit on the macrobenthic fauna, at  
401 least in the short term.

402 To adequately evaluate this result, it is necessary to take into consideration the characteristics of the  
403 studied wetlands and of their benthic assemblages. In addition, it is important to be aware of the limitations  
404 of the present study and, in general, of the problems involved in assessing the environmental impact of a  
405 specific event.

406

### 407 *The studied wetlands and their benthic macroinvertebrate assemblages*

408 In the present study benthic macroinvertebrates were identified at family level. We acknowledge that  
409 a more detailed taxonomical resolution would have been preferable and that, working at family level,  
410 relevant information is lost, in particular regarding the assessment of biodiversity. This was considered an  
411 acceptable compromise based on Bowman & Bailey (1997) and Mueller et al. (2013). These studies  
412 analysed several independent benthic macroinvertebrate datasets collected in freshwater ecosystems, both  
413 lotic and lentic. Both studies acknowledge the importance of identification to species or genus level in some  
414 circumstances. However, both studies concluded that multivariate characterizations of community  
415 composition do not seem to be sensitive to taxonomic resolution, at least to family level, if quantitative data  
416 (abundances) are considered. In fact, in many ecological studies on the benthic macroinvertebrates of

417 shallow lentic freshwater habitats organisms are identified at the family level, including those cited in the  
418 next section.

419 Even if based only on identification at family level, the present study evidenced that the  
420 macroinvertebrate assemblages dwelling on vegetation and in bare sediments of Valle Mandriole (VM) and  
421 Punte Alberete (PA) had low abundance and diversity in comparison to the pond in the San Vitale Pinewood  
422 (SV). In particular, the macrobenthic fauna of bare sediments was almost entirely composed by Naididae.

423 A comparison with the results of Zanni (1998) shows that the taxa richness radically decreased over  
424 the last decades. In particular, several families of Coleoptera (Dytiscidae, Haliplidae, Dryopidae), Odonata  
425 (Lestidae, Libellulidae and Aeshnidae), and Heteroptera (Pleidae, Mesovelidae) once present in both VM  
426 and PA were not sampled during the present study.

427 The low water quality in the two wetlands and in the rivers that supply them water could have played  
428 a role in the decreased diversity of the benthic fauna. In this regard, both the Lamone River, which supplies  
429 Punte Alberete, and the Reno River, which supplies Valle Mandriole, failed to achieve good chemical  
430 status, under the European Water Framework directive in the period 2010–2012 (ARPAE, 2015).

431 In addition, the annual mean concentrations of ammoniacal nitrogen in the Reno River in the period  
432 2010–2013 were in the range 0.43–0.64 mg/l, placing the river at the fifth level (worst quality class) for  
433 this indicator, under the Italian regulations (Fig. 4). Even higher concentrations of ammoniacal nitrogen  
434 were recorded in the Destra Reno channel. This artificial channel does not supply water to VM or PA;  
435 however, since it drains the farmland area surrounding VM and PA, its water properties give some  
436 indications on the surface runoff entering the two wetlands. Indeed, high concentrations of ammoniacal  
437 nitrogen (up to 1.1 mg/l) were measured in several water samples collected from PA.

438 A feature of PA is the recurrence of suspended mucilage, often observed in the water also during the  
439 present study, consistently with the high values of volatile suspended solids, generating strong water  
440 turbidity (Fig. 4).

441 A second possible reason for the impoverishment of the benthic assemblages is the presence the  
442 invasive alien crayfish *Procambarus clarkii*. This species was not recorded by Zanni (1998); on the  
443 contrary, it was frequently sampled during the present study. In Europe, the first introductions of *P. clarkii*  
444 from North America occurred in Spain in 1973 for aquaculture. The species was introduced in Italy in 1989,  
445 for the same purpose. After escaping into freshwater bodies, it has since steadily spread across Europe due

446 to its ecological plasticity, high fecundity, rapid life cycle and dispersal capacities. *P.clarkii* has been  
447 documented to heavily affect abundance and diversity of benthic invertebrate assemblages both by direct  
448 predation and by modifying habitat composition, through consumption of living macrophytes and litter  
449 (Souty-Grosset et al., 2016).

450 Thirty-one families were identified in the macrobenthic fauna of the pond in the San Vitale Pinewood (SV),  
451 including several recorded by Zanni (1998) in PA and VM and not sampled in these wetlands during the  
452 present study (Culicidae, Tipulidae, Aeshnidae, Lestidae, Libellulidae, Mesovelidae, Dytiscidae,  
453 Haliplidae). Among the features of SV that might concur in determining the higher taxonomic diversity is  
454 the origin of its water, which comes mainly from the local underneath aquifer. The San Vitale Pinewood is  
455 established on a paleodune system constituted by 20 m thick coarse sand deposits (Amorosi et al., 1999 and  
456 Greggio et al., 2018) where the coastal aquifer is phreatic and rainwater infiltrate and freshen the underneath  
457 saline groundwater (Antonellini et al., 2008). If the budget between precipitation and evapotranspiration is  
458 positive, freshwater lenses settle down on top of saline groundwater; where the shallow water table meets  
459 low topographic surface, interdunal wetlands appear (Cozzolino et al., 2017). So, SV is not affected by the  
460 sources of pollution occurring to PA and VM where water is supplied by the final stretch of Lamone and  
461 Reno rivers running through a heavily anthropized lowland area. Accordingly, most of the chemical  
462 properties of the water of SV such as nutrients and suspended solids, are indicative of a good state (Fig. 4).  
463 On the other hand, the electrical conductivity of the water is rather high (about 4 mS/cm) compared to VM  
464 and PA (less than 1 mS/cm), even if it can be considered relatively fresh for Ravenna coastal aquifer  
465 (Mollema et al., 2013; Greggio et al., 2020). However, this does not seem to hinder the development of a  
466 well differentiated, typically freshwater, biological community. Based on the comparison with SV, it can  
467 be inferred that the richness of the benthic fauna in VM and PA was clearly below the potential of the area.

468

#### 469 *The effect of drainage on benthic macroinvertebrates*

470 The impoverished fauna of VM could be a reason why no effects of the drainage were detected in the  
471 present study: there was little potential for change due to the presence of few taxa and low abundances. The  
472 benthic assemblages of both VM and PA testify the low habitat quality of the whole wetland system. The  
473 draining of VMS was apparently inadequate to increase abundance and diversity of the benthic  
474 macroinvertebrate assemblages, possibly because the starting point was so disadvantaged. In addition, even

475 if the environmental conditions had improved in VMS, there would have been little possibility to develop  
476 a richer fauna by colonization from nearby areas. On the other hand, it could be argued that it was  
477 impossible to further worsen an already compromised situation.

478         However, the absence of lasting effects on the benthic assemblages in cases of drying events has been  
479 documented also in previous studies. Vander Vorste et al. (2016) applied a multisite before-after-control-  
480 impact (BACI) design to quantify the effects of drying events of different durations on benthic invertebrates  
481 in gravel-bed, braided rivers in southeast France and concluded that taxonomic composition, functional  
482 diversity, trait richness, and trait composition showed no effects of drying events.

483         On the other hand, effects of dry periods on the macrobenthic fauna, lasting long after the water level  
484 had recovered, were documented, both in cases of management actions and in cases of drought. Lindegarth  
485 & Chapman (2001) tested the effect on benthic invertebrates of controlled drainage and subsequent refilling  
486 in a small managed marsh in Australia. The goal of the water level manipulation was to improve the habitat  
487 for water birds and an increase in abundances of benthic invertebrates was expected. Contrary to the  
488 expectations, a decrease in abundance and taxa richness of benthic invertebrates, chironomids in particular,  
489 was evidenced.

490         Bedford & Powell (2005) monitored the invertebrate fauna associated with the litter of *Phragmites*  
491 *australis* in a managed reedbed in northwest England by bi-monthly sampling over 6 years. Management  
492 involved an annual cycle of summer draw-down and winter reflooding. Based on comparisons with data  
493 from previous studies on permanently flooded reedbeds, these Authors concluded that the manipulation of  
494 water levels caused low invertebrate abundances and prevented the development of diverse communities.

495         Bertoncin et al. (2019) assessed the effect of a prolonged drought on the benthic invertebrates of a  
496 small (60×15 m) pond located within an island of the upper Paraná River, Brazil. The benthic assemblages  
497 became more homogeneous after the drought, in the sense that the differences between the three sites within  
498 the pond decreased. However, both diversity and abundance increased within each site, in contrast to the  
499 results of Lindegarth & Chapman (2001).

500         Unambiguously measuring the impact of a specific event in a natural ecosystem is challenging (see  
501 next section) and the above cited studies, including ours, have limitations. However, taken together, they  
502 indicate that the effects of a management strategy based on completely drying and then reflooding a wetland  
503 area are site specific. As for benthic invertebrates both an increase and a decrease in abundance and diversity

504 is possible, as well as it is possible that no substantial changes occur. In our view, this depends on the  
505 balance between the actual improvement of the oxygenation conditions and the disturbance caused by the  
506 temporary cancellation of the aquatic habitat. The relative importance of these two drivers is influenced by  
507 climate, morphology, hydrology and water quality, along with the ecological community present at the site  
508 and the availability of refuges or nearby habitats from which recolonization can occur.

509

### 510 *Impact assessment*

511 Evaluating the outcomes of management actions is an essential but often neglected aspect of  
512 environmental management (Walsh et al., 2012). The effect of a specific action can only be evaluated within  
513 an experimental framework that should be planned before the action takes place (Lindgarth & Chapman,  
514 2001). Even when this condition is met, achieving a quantitative and unambiguous assessment is  
515 challenging.

516 The major problem in assessing the impact of a specific event in a natural ecosystem is that there is  
517 usually only one potentially affected location (Stewart-Oaten et al., 1986; Underwood, 1992). This lack of  
518 replication makes it difficult to separate the anthropogenic effects from natural variability in space and time.

519 In an attempt to overcome this problem, the before-after-control-impact (BACI) sampling design was  
520 proposed. The BACI approach dictates that a single impact location, putatively affected by the event of  
521 interest, is compared with a single control location, surely unaffected by the same event. Both the impact  
522 and the control locations are sampled at multiple times both before and after the event has occurred  
523 (Bernstein & Zalinsky, 1983; Stewart-Oaten et al., 1986).

524 The shortcomings of this approach were highlighted by Underwood (1992, 1994), that proposed the  
525 beyond before-after-control-impact (beyond BACI) sampling design, where the single impact location is  
526 compared with multiple control locations, the same design we adopted in the present study. While the  
527 approach was originally devised to deal with human interventions expected to cause adverse effects on the  
528 ecosystems, it is equally suited to assess the effectiveness of actions aimed at some improvement.

529 Some authors (notably Stewart-Oaten & Bence, 2001; Stewart-Oaten, 2008; Paul, 2011), strongly  
530 criticized the Beyond BACI and other “design based” approaches, which are founded on sampling design  
531 and ANOVA or related statistical methods. In open contrast with Underwood (1992, 1994), they maintain  
532 that multiple control locations are not indispensable to properly measure the ecological effects of an event.

533 These authors advocate “model based” approaches, i.e., the use of predictive models, parametrized using  
534 data collected from the putatively impacted location before the event of interest occurs. The ecological  
535 impact should then be measured as the difference between the predictions of the model and the actual  
536 observations made after the event has occurred.

537 One of the fundamental tenets stated by Underwood (1992, 1996) is that impact assessment studies  
538 should be treated as experiments. This is the most criticized aspect of the approach. As Stewart-Oaten &  
539 Bence (2001) and Stewart-Oaten (2008) pointed out, statistical inferences in experiment are based on the  
540 assumption that the experimental units are randomly chosen from the same large population or, at least,  
541 randomly assigned to the treatments. Conversely, in an impact assessment study, the impact location is not  
542 chosen at random, either from a population or from the sites used in the study.

543 We acknowledge that the theoretical framework of the beyond BACI approach may have weaknesses,  
544 if considered from a formal statistical standpoint. However, we adopted this approach in the present study,  
545 because it is our opinion that its fundamental rationale is sound: an ecological effect is detected if the  
546 temporal pattern of variation observed at the impact location is outside the range defined by a set of control  
547 locations, which are unaffected by the event of interest but otherwise comparable with the impact location.  
548 The use of more than one control location takes into account that different unaffected locations may exhibit  
549 different temporal patterns. If only one control location is sampled, differences that could exist even if the  
550 impact location were actually unaffected could be interpreted as an ecological effect of the event of interest.

551 On the other hand, a model based impact assessment requires a validated model that can predict how  
552 the value of the response variables would change over time at the impact location if this were unaffected  
553 by the planned intervention. Complete confidence in the model is required since any deviation from its  
554 predictions would be interpreted as an actual impact. A long data series, collected before the planned  
555 disturbance, is essential to develop and validate the model. We did not follow this approach as it appeared  
556 unrealistic to develop a sufficiently reliable model for the abundances of benthic invertebrates and the  
557 values of the chemical properties of Valle Mandriole.

558 While we think that, under the circumstances, there were no better alternatives to evaluate the effect  
559 of the summer drainage of VM on the benthic invertebrate fauna, the specific application of the Beyond  
560 BACI approach carried out in the present study does have some important limitations.

561 The real situation hardly fitted to the reference model of a population of locations from which the  
562 impact locations and the control locations were independently and randomly selected. While all the sampled  
563 locations are freshwater wetlands, the benthic assemblages and the chemical properties of Valle Mandriole  
564 (impact location and one control location), of Punte Alberete, (two control locations) and of the pine wood  
565 pond were quite distinct.

566 According to Underwood (1994) in the application of the beyond BACI approach, there is no need to  
567 attempt to choose places with identical characteristics or abundances of the investigated populations. The  
568 set of locations chosen to serve as controls must simply represent the range of habitats like the one that  
569 might be affected (the Impact location). Obviously, the control locations must be a representative sample  
570 of places of the same general habitat as the impact location.

571 However, what can be considered the same general habitat, is rather subjective. Our strategy was to  
572 sample the highest possible number of locations and to represent the whole range of freshwater wetlands in  
573 the area. Since the control locations define the norm against which the temporal pattern observed in the  
574 impact location is evaluated, our idea was that the norm should have some degree of generality. In our  
575 opinion this is consistent with the rationale of the Beyond BACI approach.

576 The opposite strategy would have been to favour the homogeneity between the impact and the control  
577 locations. It could be argued that the differences between SV and the other locations are too large to  
578 represent the same general habitat. Admittedly, the benthic assemblages of SV are clearly distinct from  
579 those of VM and PA. However, regarding the temporal patterns (how the structure of the benthic  
580 assemblages changes over time) the difference between SV and the other locations do not seem to be larger  
581 than the differences among the other locations, at least for the fauna sampled from vegetation (Fig 2, Fig.  
582 S2 in the Supplementary Data). In any case, omitting the SV from the statistical analyses does not cause  
583 major changes in the results and the interaction terms relevant to the detection of the impact are still not  
584 significant (data not shown).

585 In fact, while PA and VM both had an impoverished benthic fauna, their benthic assemblages and the  
586 properties of their water were still clearly distinct. Adopting homogeneity with the impact location as the  
587 sole criterion to select the control locations, would imply that VMN is the only legitimate control location  
588 for VMS, and would make it impossible to apply a beyond BACI approach.

589 Arguably, in the present study, the range of temporal patterns observed at the control locations, which  
590 defined the norm, was so wide that possibly prevented the detection of any deviation of the impact location  
591 from the norm itself, except for extreme deviations.

592 Indeed, the most important shortcoming of the present application of the Beyond BACI approach is  
593 probably that all the samples were collected during one single year (2013) and “before” and “after” are  
594 referred to a single drainage of VMN. Since the drainage was performed on summer every year, starting  
595 from 2011, sampling over a longer time span, including a period before the first occurrence of the summer  
596 drainage would have been more relevant to the problem considered. Unfortunately, the local authority that  
597 planned and funded the intervention, apparently did not deem important to plan a study to properly assess  
598 its effectiveness.

599

## 600 **Conclusions**

601 The present study, even taking account of its limitations, did not detect any statistically significant  
602 effect on the benthic macroinvertebrates, suggesting that this practice is acceptable, at least in regard to the  
603 investigated assemblages. On the other hand, the results also suggest that the drainage does not produce  
604 any benefit on the macrozoobenthic fauna, at least in the short term. The effects of a management strategy  
605 based on draining completely dry and then reflooding a wetland area appear to be site specific.

606 Our study, by comparison to previous data, evidenced a severe impoverishment of the benthic fauna  
607 of Valle Mandriole and Punte Alberete, as can be inferred by comparison to the San Vitale pinewood pond  
608 which, despite being of lesser extension, sustains a much more diverse fauna. This also highlights how  
609 small water bodies can preserve the diversity of aquatic species of an area, even when larger water bodies  
610 are degraded, and that they are potentially important pools of species that could recolonize larger habitats,  
611 once the environmental quality of the latter is restored. Maintaining, protecting, restoring and even creating  
612 small ponds may play an important role in nature conservation.

613

## 614 **References**

615 Amorosi, A., M. Colalongo, G. Pasini, & D. Preti, 1999. Sedimentary response to Late Quaternary sea-  
616 level changes in the Romagna coastal plain (northern Italy). *Sedimentology* 46: 99-121.  
617 <https://doi.org/10.1046/j.1365-3091.1999.00205.x>.



618 Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. *Austral*  
619 *Ecology* 26: 32–46. <https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x>.

620 Anderson, M.J., R.N. Gorley, K.R. Clarke, , 2008. PERMANOVA+ for PRIMER: Guide to software and  
621 statistical methods. PRIMER-E, Plymouth, UK.

622 Antonellini, M., P.N. Mollema, B.M.S. Giambastiani, K. Bishop, L. Caruso, A. Minchio, L. Pellegrini, M.  
623 Sabia, E. Ulazzi, & G. Gabbianelli, 2008. Salt water intrusion in the coastal aquifer of the southern  
624 Po Plain, Italy. *Hydrogeology journal* 16: 1541–1556. <https://doi.org/10.1007/s10040-008-0319-9>.

625 Antonellini, M., & Mollema, P. N. 2010. Impact of groundwater salinity on vegetation species richness in  
626 the coastal pine forests and wetlands of Ravenna, Italy. *Ecological Engineering*, 36(9), 1201-1211.  
627 <https://doi.org/10.1016/j.ecoleng.2009.12.007>.

628 Antonellini, M., D.M. Allen, P.N. Mollema, D. Capo & N. Greggio, 2015. Groundwater freshening  
629 following coastal progradation and land reclamation of the Po Plain, Italy. *Hydrogeology Journal*  
630 23: 1009–1026. <https://doi.org/10.1007/s10040-015-1263-0>.

631 ARPAE, 2015. Valutazione dello stato delle acque superficiali fluviali 2010-2013 [Assessment of the  
632 status of riverine surface waters]. Regional Agency for Prevention, Environment and Energy of  
633 Emilia-Romagna, Bologna, Italy. Retrieved from:  
634 [https://www.arpae.it/cms3/documenti/\\_cerca\\_doc/acqua/report\\_acque\\_dolci\\_2010-](https://www.arpae.it/cms3/documenti/_cerca_doc/acqua/report_acque_dolci_2010-)  
635 [13/report\\_fiumi\\_2010\\_2013.pdf](https://www.arpae.it/cms3/documenti/_cerca_doc/acqua/report_acque_dolci_2010-13/report_fiumi_2010_2013.pdf).

636 Barbier, E. B., 2011. Wetlands as natural assets. *Hydrological Sciences Journal* 56: 1360–1373.  
637 <https://doi.org/10.1080/02626667.2011.629787>.

638 Bedford, A.P. & I. Powell, 2005. Long-term changes in the invertebrates associated with the litter of  
639 *Phragmites australis* in a managed reedbed. *Hydrobiologia* 549: 267–285.  
640 <https://doi.org/10.1007/s10750-005-5439-9>.

641 Bernstein, B.B. & J. Zalinsky, 1983. An optimum sampling design and power tests for environmental  
642 biologists. *Journal of Environmental Management* 16: 35–43.

643 Bertoncin, A.P. dos S., G.D. Pinha, M.T. Baumgartner & R.P. Mormul, 2019. Extreme drought events  
644 can promote homogenization of benthic macroinvertebrate assemblages in a floodplain pond in  
645 Brazil. *Hydrobiologia* 826: 379–393. <https://doi.org/10.1007/s10750-018-3756-z>.

646 Bowman, M. F., & R. C. Bailey, 1997. Does taxonomic resolution affect the multivariate description of  
647 the structure of freshwater benthic macroinvertebrate communities? *Canadian Journal of Fisheries*  
648 *and Aquatic Sciences* 54: 1802–1807.

649 Buscaroli, A., E. Dinelli & D. Zannoni, 2011. Geohydrological and environmental evolution of the area  
650 included among the lower course of the Lamone river and the Adriatic coast. *EQA—Environmental*  
651 *quality/Qualité de l’Environnement/Qualità ambientale* 5: 11–22.

652 Cencini, C., 1998. Physical processes and human activities in the evolution of the Po Delta, Italy. *Journal*  
653 *of Coastal Research* 14: 774–793.

654 Clarke, K.R. & R.M. Warwick, 2001. Change in marine communities: an approach to statistical analysis  
655 and interpretation, 2<sup>nd</sup> edition. PRIMER-E. Plymouth.

656 Cooper, M.J., D.G. Uzarski & T.M. Burton, 2009. Benthic Invertebrate Fauna, Wetland Ecosystems, in:  
657 *Encyclopedia of Inland Waters*. Elsevier, Amsterdam, pp. 232–241. [https://doi.org/10.1016/B978-](https://doi.org/10.1016/B978-012370626-3.00165-4)  
658 [012370626-3.00165-4](https://doi.org/10.1016/B978-012370626-3.00165-4).

659 Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora [1992]  
660 OJ L 206/7.

661 Covich A.P., M.A. Palmer & T.A. Crowl, 1999. The role of benthic invertebrate species in freshwater  
662 ecosystems: Zoobenthic species influence energy flows and nutrient cycling. *BioScience* 49: 119–  
663 127. <https://doi.org/10.2307/1313537>.

664 Cozzolino, D., N. Greggio, M. Antonellini & B. M. S. Giambastiani, 2017. Natural and anthropogenic  
665 factors affecting freshwater lenses in coastal dunes of the Adriatic coast. *Journal of Hydrology*  
666 551: 804–818. <https://doi.org/10.1016/j.jhydrol.2017.04.039>

667 Denny, P., 1994. Biodiversity and wetlands. *Wetlands Ecology and Management* 3: 55–61.

668 Dudgeon, D., A.H. Arthington, M.O. Gessner, Z. Kawabata, D.J. Knowler, C. Lévêque, R.J. Naiman,  
669 H.E. Prieur-Richard, D. Soto, M.L.J. Stiassny & C.A. Sullivan, 2006. Freshwater biodiversity:  
670 importance, threats, status and conservation challenges. *Biological Reviews* 81: 163–182.  
671 <https://doi.org/10.1017/S1464793105006950>.

672 Eaton ,A.D. & M.A.H. Franson. 2005 (eds). *Standard methods for the examination of water &*  
673 *wastewater*, 21th edition. American Public Health Association, American Water Works  
674 Association, Water Environment Federation, Washington.

675 European Parliament and Council Directive 2009/147/EC on the conservation of wild birds [2010] OJ L  
676 20/7.

677 Furey, P.C., R.N. Nordin & A. Mazumder, 2006. Littoral benthic macroinvertebrates under contrasting  
678 drawdown in a reservoir and a natural lake. *Journal of the North American Benthological Society*  
679 25: 19–31. [https://doi.org/10.1899/0887-3593\(2006\)25\[19:LBMUCD\]2.0.CO;2](https://doi.org/10.1899/0887-3593(2006)25[19:LBMUCD]2.0.CO;2).

680 Gordon, L.J., C.M. Finlayson & M. Falkenmark, 2010. Managing water in agriculture for food production  
681 and other ecosystem services. *Agricultural Water Management* 97: 512–519.  
682 <https://doi.org/10.1016/j.agwat.2009.03.017>.

683 Greggio, N., B.M.S. Giambastiani, B. Campo, E. Dinelli, & A. Amorosi, 2018. Sediment composition,  
684 provenance, and Holocene paleoenvironmental evolution of the Southern Po River coastal plain  
685 (Italy). *Geological Journal* 53: 914-928. <https://doi.org/10.1002/gj.2934>

686 Greggio, N., B.M.S. Giambastiani, P.N. Mollema, M. Laghi, D. Capo, G. Gabbianelli, ... & E. Dinelli,  
687 2020. Assessment of the Main Geochemical Processes Affecting Surface Water and Groundwater  
688 in a Low-Lying Coastal Area: Implications for Water Management. *Water* 12: 1720-1739.  
689 <https://doi.org/10.3390/w12061720>.

690 Kaster, J.L. & G.Z. Jacobi, 1978. Benthic macroinvertebrates of a fluctuating reservoir. *Freshwater*  
691 *Biology* 8: 283–290. <https://doi.org/10.1111/j.1365-2427.1978.tb01449.x>.

692 Lazzari, G., 1994. Punte Alberete, la storia [Punte Alberete: history]. In: WWF sezione di Ravenna (Ed.),  
693 *La foresta allagata [The flooded forest]*. COOP Libreria e di Informazione, Ravenna, Italy, pp. 9–  
694 22.

695 Legendre, P. & L. Legendre, 2012. *Numerical Ecology*, 3rd edition. Elsevier, Amsterdam.

696 Lindegarth, M. & M.G. Chapman, 2001. Testing hypotheses about management to enhance habitat for  
697 feeding birds in a freshwater wetland. *Journal of Environmental Management* 62: 375–388.  
698 <https://doi.org/10.1006/jema.2001.0441>.

699 Maltby, E. & M.C. Acreman, 2011. Ecosystem services of wetlands: pathfinder for a new paradigm.  
700 *Hydrological Sciences Journal* 56: 1341–1359. <https://doi.org/10.1080/02626667.2011.631014>.

701 McEwen, D.C. & M.G. Butler, 2010. The effects of water-level manipulation on the benthic invertebrates  
702 of a managed reservoir. *Freshwater Biology* 55: 1086–1101. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2427.2009.02382.x)  
703 [2427.2009.02382.x](https://doi.org/10.1111/j.1365-2427.2009.02382.x).

704 Mitsch, W.J. & J.G. Gosselink, 2000. The value of wetlands: importance of scale and landscape setting.  
705 Ecological Economics 35: 25–33. [https://doi.org/10.1016/S0921-8009\(00\)00165-8](https://doi.org/10.1016/S0921-8009(00)00165-8).

706 Mollema, P.N., M. Antonellini, E. Dinelli, G. Gabbianelli, N. Greggio & P.J. Stuyfzand, 2013.  
707 Hydrochemical and physical processes influencing salinization and freshening in Mediterranean  
708 low-lying coastal environments. Applied Geochemistry 34: 207–221.  
709 <https://doi.org/10.1016/j.apgeochem.2013.03.017>.

710 Moss, B., 2000. Biodiversity in fresh waters - An issue of species preservation or system functioning?  
711 Environmental Conservation 27: 1–4.

712 Mueller, M., J. Pander, & J. Geist, 2013. Taxonomic sufficiency in freshwater ecosystems: Effects of  
713 taxonomic resolution, functional traits, and data transformation. Freshwater Science 32: 762–778.  
714 <https://doi.org/10.1899/12-212.1>.

715 Paul, W.L., 2011. A causal modelling approach to spatial and temporal confounding in environmental  
716 impact studies. Environmetrics 22: 626–638. <https://doi.org/10.1002/env.1111>.

717 Postel, S. & S. Carpenter, 1997. Freshwater Ecosystem Services. In: Daily, G.C. (Ed), Nature’s services:  
718 societal dependence on natural ecosystems. Island Press, Washington, D.C., USA, pp. 195–214.

719 Ramsar Convention Secretariat., 2013. The Ramsar Convention Manual: a guide to the Convention on  
720 Wetlands (Ramsar, Iran, 1971) (6th edition). Gland, Switzerland: Ramsar Convention Secretariat.

721 Rosenberg, D.M. & V.H. Resh, 1993. Introduction to freshwater biomonitoring and benthic  
722 macroinvertebrates. In: Rosenberg, D.M., Resh, V.H. (Eds.), Freshwater biomonitoring  
723 Sievers, M., R. Hale, K.M. Parris & S.E. Swearer, 2018. Impacts of human-induced environmental  
724 change in wetlands on aquatic animals. Biological Reviews 93: 529–554.  
725 <https://doi.org/10.1111/brv.12358>.

726 Souty-Grosset, C., P.M. Anastácio, L. Aquiloni, F. Banha, J. Choquer, C. Chucholl & E. Tricarico, 2016.  
727 The red swamp crayfish *Procambarus clarkii* in Europe: Impacts on aquatic ecosystems and  
728 human well-being. Limnologica 58: 78–93. <https://doi.org/10.1016/j.limno.2016.03.003>.

729 Stewart-Oaten, A., 2008. Chance and randomness in design versus model-based approaches to impact  
730 assessment: comments on Bulleri et al. (2007). Environmental Conservation 35: 8–10.  
731 <https://doi.org/10.1017/S0376892908004566>.

732 Stewart-Oaten, A. & J.R. Bence, 2001. Temporal and spatial variation in environmental impact  
733 assessment. *Ecological Monographs* 71: 305–339. [https://doi.org/10.1890/0012-](https://doi.org/10.1890/0012-9615(2001)071[0305:TASVIE]2.0.CO;2)  
734 [9615\(2001\)071\[0305:TASVIE\]2.0.CO;2](https://doi.org/10.1890/0012-9615(2001)071[0305:TASVIE]2.0.CO;2).

735 Stewart-Oaten, A., W.W. Murdoch & K.R. Parker, 1986. Environmental impact assessment:  
736 “pseudoreplication” in time? *Ecology* 67: 929–940. <https://doi.org/10.2307/1939815>.

737 Studio Silva, 2012. Piano di gestione del SIC-ZPS IT4070001 "Punte Alberete, Valle Mandriole", Quadro  
738 Conoscitivo. [Management plan for the SCI-SPA IT4070001 "Punte Alberete, Valle Mandriole".  
739 Baseline information]. Comacchio, Italy: Parco del Delta del Po.

740 Tiner, R.W. Jr., 1984. Wetlands of the United States: current status and recent trends. US Department of  
741 the Interior. US Fish and Wildlife Service, Washington DC, USA.

742 Underwood, A.J., 1992. Beyond BACI: the detection of environmental impacts on populations in the real,  
743 but variable, world. *Journal of Experimental Marine Biology and Ecology* 161: 145–178.  
744 [https://doi.org/10.1016/0022-0981\(92\)90094-Q](https://doi.org/10.1016/0022-0981(92)90094-Q)

745 Underwood, A.J., 1994. On beyond BACI: Sampling designs that might reliably detect environmental  
746 disturbances. *Ecological Applications* 4: 3–15. <https://doi.org/10.2307/1942110>.

747 Vander Vorste, R., R. Corti, A. Sagouis & T. Datry, 2016. Invertebrate communities in gravel-bed,  
748 braided rivers are highly resilient to flow intermittence. *Freshwater Science* 35: 164 – 177.  
749 <https://doi.org/10.1086/683274>.

750 Ward, J.V., 1997. Riverine landscapes: Biodiversity patterns, disturbance regimes, and aquatic  
751 conservation. *Biological Conservation* 83: 269–278. [https://doi.org/10.1016/S0006-](https://doi.org/10.1016/S0006-3207(97)00083-9)  
752 [3207\(97\)00083-9](https://doi.org/10.1016/S0006-3207(97)00083-9).

753 Walsh, J.C., K.A. Wilson, J. Benshemesh & H.P. Possingham, 2012. Unexpected outcomes of invasive  
754 predator control: The importance of evaluating conservation management actions. *Animal*  
755 *Conservation* 15: 319–328. <https://doi.org/10.1111/j.1469-1795.2012.00537.x>

756 White, M.S., M.A. Xenopoulos, K. Hogsden, R.A. Metcalfe & P.J. Dillon, 2008. Natural lake level  
757 fluctuation and associated concordance with water quality and aquatic communities within small  
758 lakes of the Laurentian Great Lakes region. *Hydrobiologia* 613: 21–31.  
759 <https://doi.org/10.1007/s10750-008-9469-y>.

760 Zanni, F., 1998. Studio delle interazioni fra caratteristiche chimiche delle acque, macrozoobenthos e  
761 avifauna nelle zone umide: Punte Alberete e Valle Mandriole [Study of the interactions between  
762 water chemistry, macrozoobenthos and avifauna in wetlands: Punte Alberete and Valle  
763 Mandriole]. (Unpublished master's thesis). University of Bologna, Ravenna.  
764

765 **Captions for figures**

766

767 **Fig.1** Study area and sampling sites

768

769 **Fig. 2** Non-metric multidimensional scaling (MDS) plot of 10 sites sampled four times, two times before  
770 (May, June) and two after (October, November) the drainage of the southern part of Valle Mandriole  
771 (VMS). The MDS is based on square root transformed abundances of families of benthic  
772 macroinvertebrates sampled from vegetation and Bray-Curtis distance

773

774 **Fig. 3** Non-metric multidimensional scaling (MDS) plot of 10 sites sampled in four times, two times before  
775 (May, June) and two after (October, November) the drainage of the southern part of Valle Mandriole  
776 (VMS). The MDS is based on square root transformed abundances of families of benthic  
777 macroinvertebrates sampled from bare sediments and Bray-Curtis distance

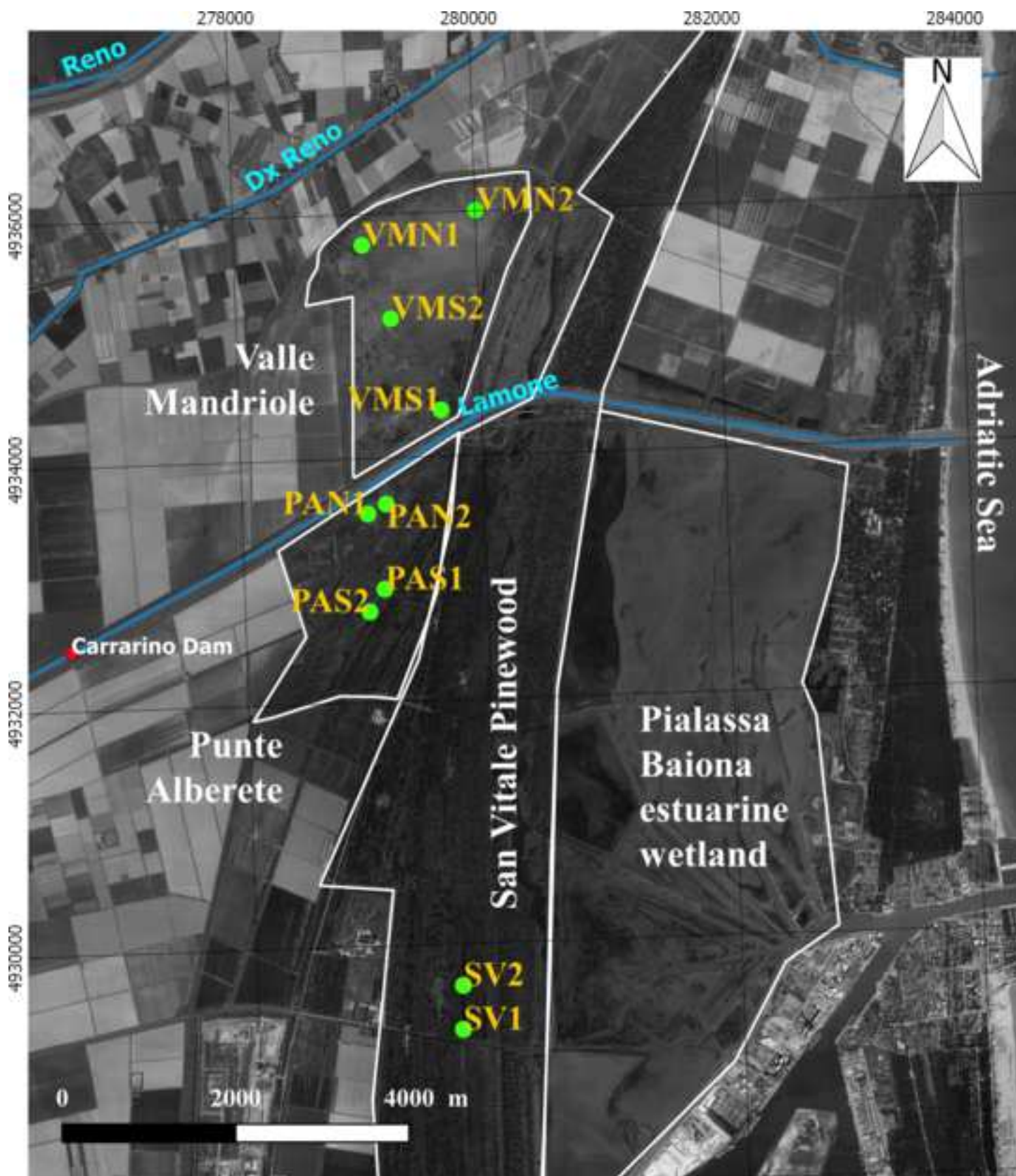
778

779 **Fig. 4** Range of variation of water column properties in the five wetland locations and three related  
780 riverine locations in year 2013. The wetland data are from the present study, the riverine data were  
781 collected by the regional environmental agency (available at:  
782 [https://www.arpae.it/dettaglio\\_documento.asp?id=6312&idlivello=2020](https://www.arpae.it/dettaglio_documento.asp?id=6312&idlivello=2020)). VMS: Valle Mandriole South  
783 (impact location); VMN: VMN: Valle Mandriole North; PAN, PAS: Punte Alberete north and south; SV:  
784 San Vitale. Riverine locations: Lamone, Destra Reno, Reno. Boxes: 2<sup>nd</sup> and 3<sup>rd</sup> quartile; the whiskers  
785 represent the whole range without outliers. Outliers are defined as value outside 1.5 times the interquartile  
786 range above the 3<sup>rd</sup> quartile and below the 2<sup>nd</sup> quartile

787

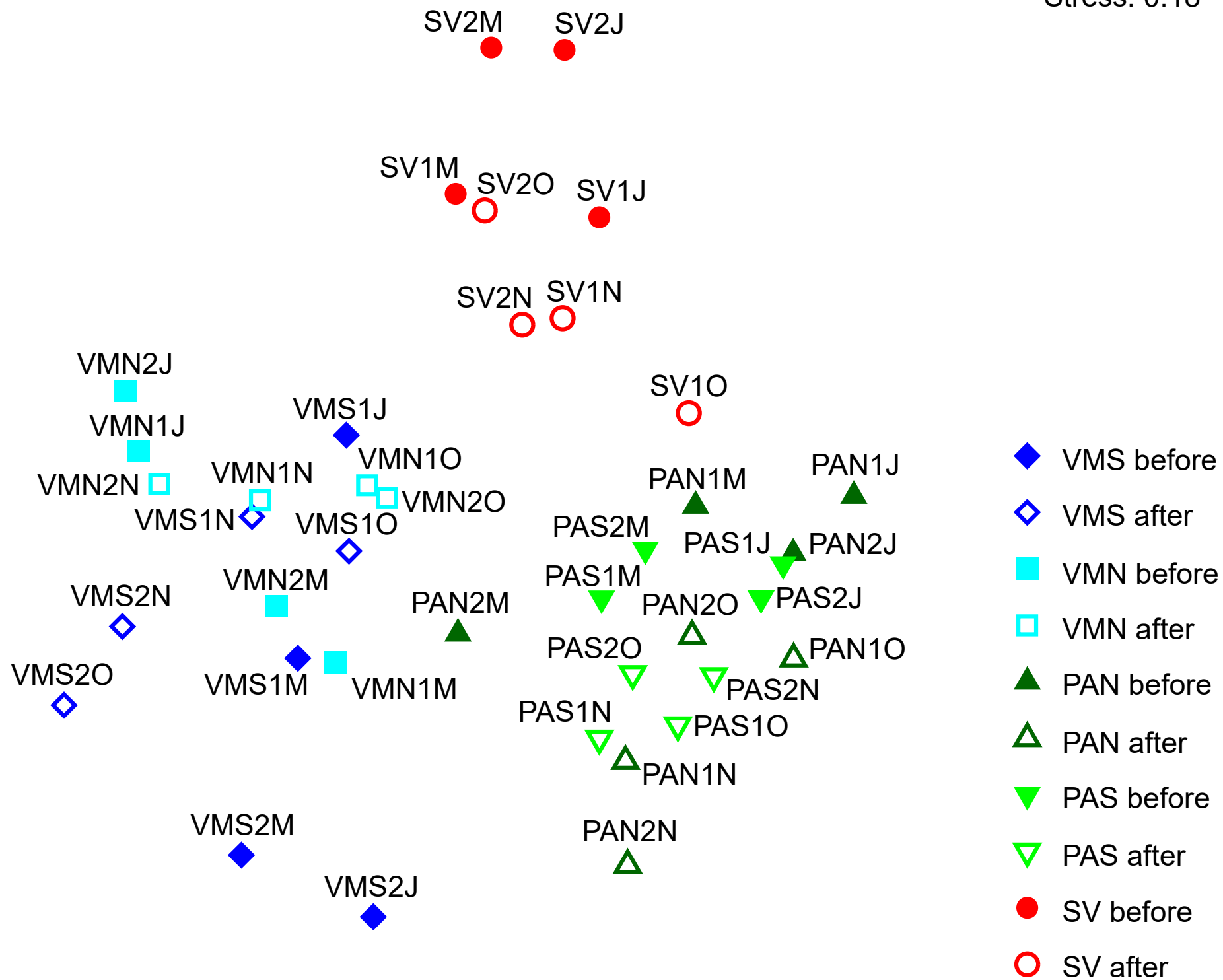
788 **Fig. 5** Discriminant function analysis ordination plot using the location as the grouping criterion and the  
789 water chemistry variables as predictors

Fig. 1 .tif

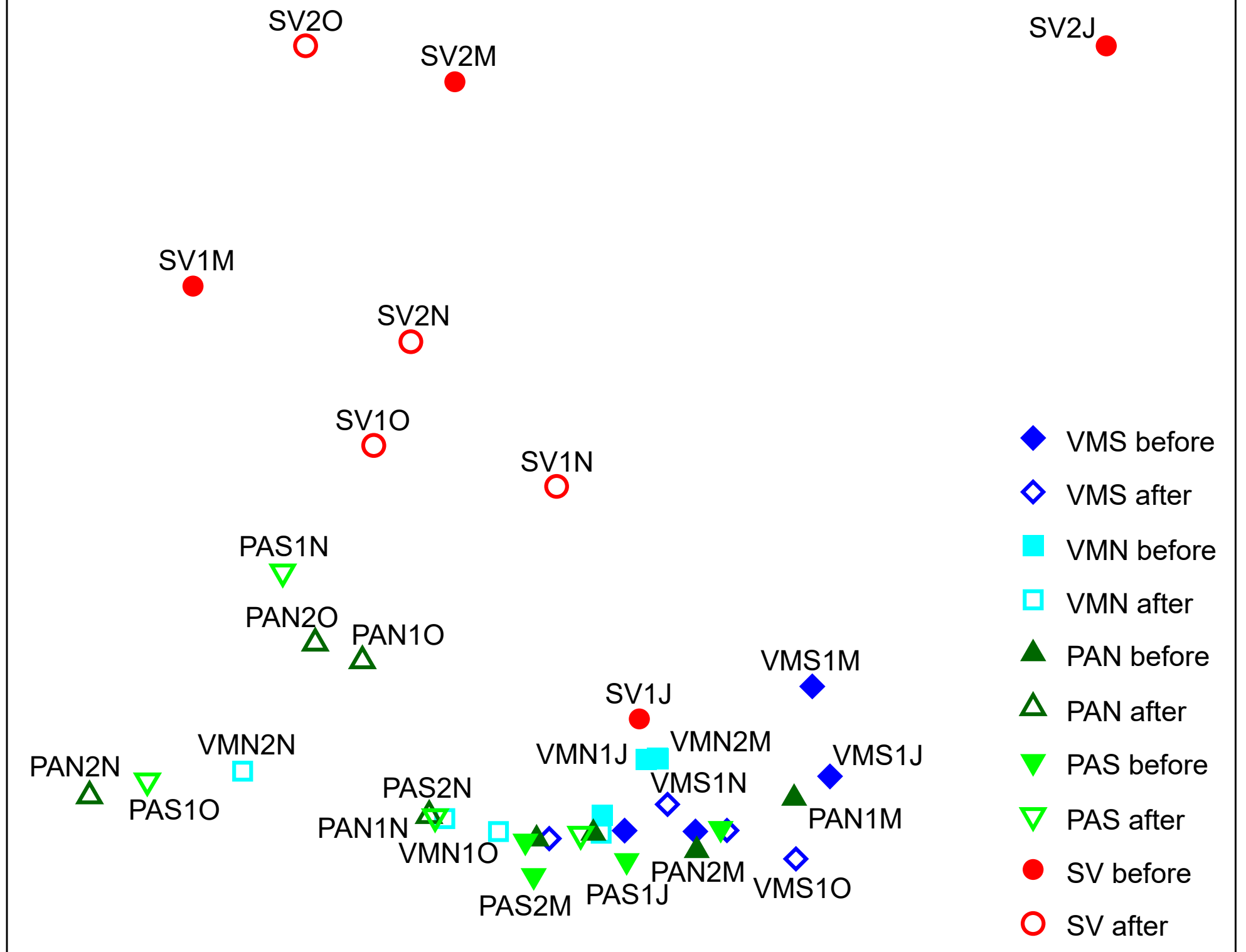


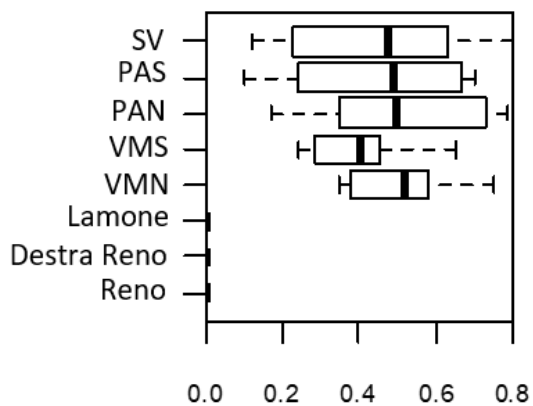
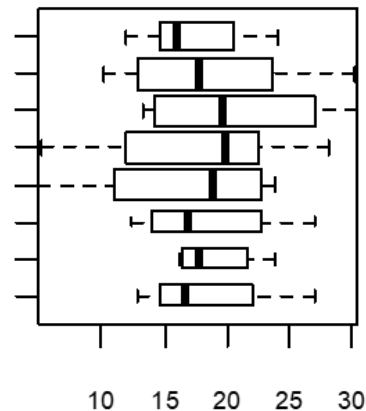
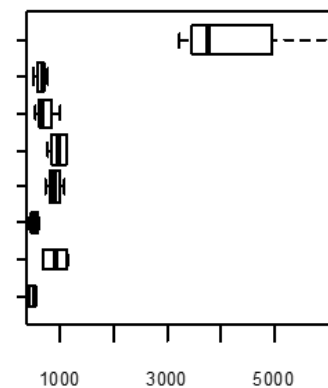
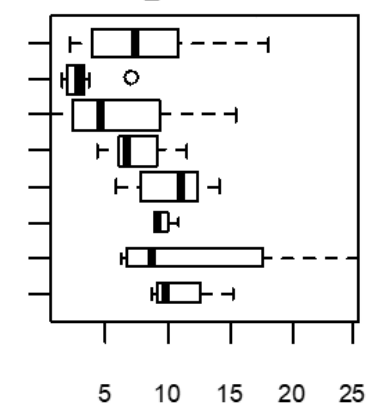
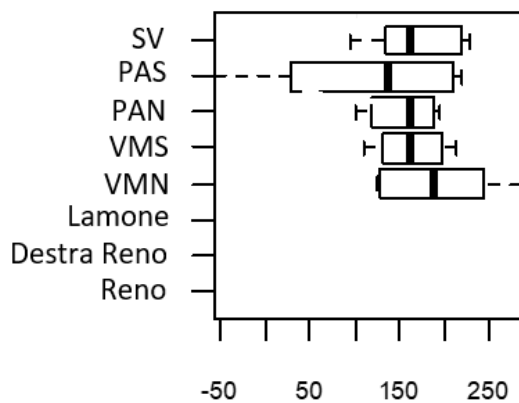
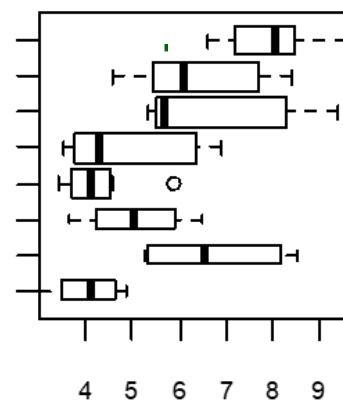
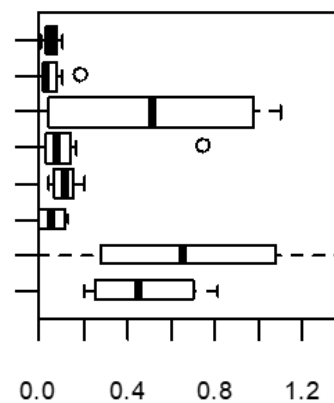
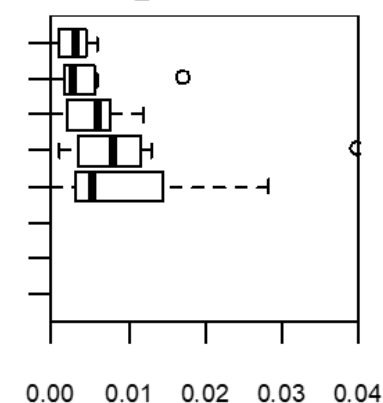
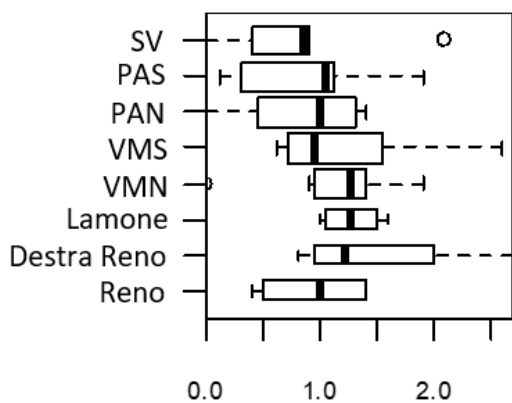
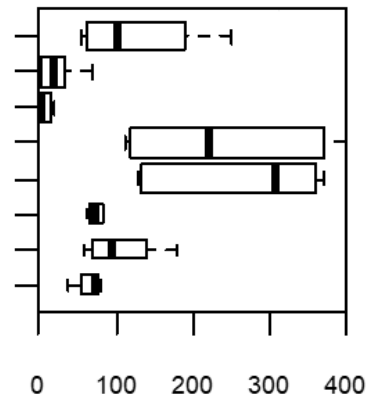
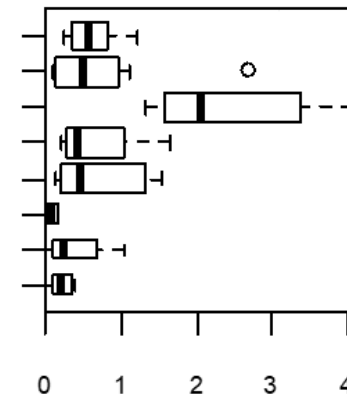


Stress: 0.18



Stress: 0.06



**Water depth [m]****T [°C]****Elect. cond. [μS/cm]****O<sub>2</sub> [mg/l]****Eh [mV]****Alkalinity [meq/l]****NH<sub>4</sub><sup>+</sup>-N [mg/l]****NO<sub>2</sub><sup>-</sup>-N [mg/l]****NO<sub>3</sub><sup>-</sup>-N [mg/l]****SO<sub>4</sub><sup>2-</sup>-S [mg/l]****PO<sub>4</sub><sup>3-</sup>-P [mg/l]****Tot. suspended solids [g/l]**