

1 **Structural and functional responses of macroinvertebrate communities in small wetlands of the Po**
2 **delta with different and variable salinity levels**

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14
15 **Abstract**

16 Coastal areas often host small water bodies described by high levels of biodiversity, which are threatened
17 by environmental changes such as saltwater intrusion. This work evaluates the salinization effects on
18 macroinvertebrate communities of 16 permanent small wetlands (ponds) located in a coastal
19 Mediterranean forest in Northern Italy, characterized by different salinity levels. From a preliminary
20 multivariate analysis (CCA), salinity was detected as the main driver affecting taxa distribution. Thus,
21 diversity in terms of taxa, biological and functional traits of macroinvertebrate communities were
22 analyzed considering three salinity classes (freshwater, oligo-mesohaline and polyhaline). The threshold
23 indicator taxa analysis (TITAN) was used for detecting changes in taxa abundance and trait distributions
24 within the salinity range and for assessing synchrony among their change points as evidence of
25 community thresholds. Taxonomic and functional diversity indices and single functional/biological traits
26 among the three salinity classes were also compared. The findings demonstrated that ponds'
27 macroinvertebrate communities are resilient to moderate increases of salinity, but salinization increase
28 to polyhaline levels causes loss of biodiversity and a drastic community simplification in terms of
29 functional evenness due to increased functional specialization. Since climate change and anthropogenic
30 activities are expected to exacerbate salinization, management measures are required for the conservation
31 of aquatic biodiversity in small coastal wetlands.

32
33 **Keywords:**

34 Macrozoobenthos, salinization, polyhaline, TITAN, taxonomic diversity indices, functional traits.

49 **Introduction**

50 Climate change is expected to generate significant ecosystems modifications worldwide, causing
51 changes in environmental conditions and ecosystem processes (Scholze et al., 2006). Transitional
52 environments as deltaic areas are markedly vulnerable to climatic and environmental changes because of
53 their sensitive hydrological balances and the increasing presence of human settlements and activities (Gu
54 et al., 2011; House et al., 2016; Gaglio et al., 2019). Deltas host different typologies of aquatic
55 environments, as river branches, wetlands, salt marshes and mudflats, that confer them a high
56 environmental diversity and provide several ecological functions, such as water retention and depuration,
57 habitat provision for species and recreation (Gaglio et al., 2017a). Since the changes of environmental
58 and climatic conditions are predicted to affect aquatic biotic and abiotic components underpinning deltas
59 ecological functions, the investigation of management consequences on river deltas are of paramount
60 importance to guarantee biodiversity conservation and human well-being.

61 The deltaic areas of the Mediterranean region are particularly vulnerable to climate change, since
62 an increase of temperature and a decrease of precipitation has been already identified (Cramer et al.,
63 2018). The combined effects of rising water demand and water scarcity will significantly affect future
64 water availability in Mediterranean basins (Saadi et al., 2015). Raising temperatures will increase
65 evapotranspiration rates, while the reduced amount of rainfall will enhance plant water stress, requiring
66 higher amounts of water withdrawals for crop irrigation. This trend will significantly influence the
67 wetlands biota by favoring species more tolerant to drought (Johansen et al., 2018; Oliver et al., 2015).
68 At the community level, the responses to such phenomena can be observed by investigating
69 macroinvertebrate assemblages. In Mediterranean coastal systems, climate change was observed to cause
70 losses of taxonomic and functional diversity in macroinvertebrate communities (Cardoso et al., 2008;
71 Pitacco et al., 2018).

72 The resilience of aquatic ecosystems to environmental changes relies on the capacity of aquatic
73 biota to reestablish living communities after perturbations (Downing and Leibold, 2010; Schaffner,
74 2010). Nonetheless, the newly established communities may present different levels of taxonomic and
75 functional diversity, as a consequence of adaptation to the new environmental conditions (Macleod et
76 al., 2008). This may lead to a general loss of both biodiversity and capacity to respond to additional
77 perturbations, which further threaten these ecosystems (Oliver et al., 2015). Moreover, aquatic
78 ecosystems are widely subjected to increasing human pressures harming their ecological status. The
79 intensification of human activities, such as agriculture, aquaculture, water withdrawals, in combination
80 with ongoing climatic changes can lead to detrimental effects in their ecological conditions and capacity
81 to support human well-being (Blann et al., 2009; Day et al., 2008; Gaglio et al., 2019; Xenopoulos et al.,
82 2005).

83 Salinization is one of the main stressors affecting deltaic areas that occur as an amplified result
84 of climatic changes and anthropogenic effects (Colombani et al., 2016). These transitional environments
85 are highly sensitive to changes of both terrestrial and marine components (Harley et al., 2006), as well
86 as climatic factors (Nielsen and Brock, 2009; Scavia et al., 2002). Different natural and human-related
87 factors concur to the increase of salinity levels of water bodies. Primary salinization is referred to natural
88 salt accumulation from rainwater and leached from terrestrial sources unaffected by human activities. On
89 the contrary, secondary salinization is caused by human-induced mechanisms, such as vegetation
90 clearance, intensive irrigation, river regulation and land reclamation (Gaglio et al., 2017a; Herbert et al.,
91 2015). Unlike primary salinization, secondary salinization occurs on a time frame of decades or less,
92 under the consequence of hydrological cycle alteration due to human effects (Herbert et al., 2015).

93 Salinity affects the presence of species both directly, for example through osmoregulation
94 physiology, and indirectly, by influencing biotic interactions (Liancourt et al., 2005; Pinder et al., 2005;
95 Withers, 1992). Freshwater invertebrates can withstand small salinity increases maintaining constant iso-
96 osmotic conditions between haemolymph and external solutions. With the increase of external solute

97 concentrations, many freshwater invertebrates suffer from dehydration, while salt-tolerant aquatic
98 invertebrates respond to the increased salinity by adopting osmoregulation strategies for maintaining
99 constant the osmotic concentration of body fluids (Evans, 2008).

100 Salinity also influences biotic interactions. For instance, the role of competition may vary with
101 the level of abiotic stress, such as salinity, as a result of different tolerance and competitive response of
102 species (La Peyre et al. 2001; Liancourt et al. 2005). Variation of salinity levels mediates trophic cascade
103 by influencing predators' abundance, thus altering their top-down control on preys (Cañedo-Argüelles et
104 al., 2016; Herbst, 2006; Herbst and Blinn, 1998). Moreover, salinity may interact with other
105 environmental factors to influence species composition (Larson and Belovsky, 2013).

106 The effects of increasing salinity levels on macroinvertebrate communities were described in
107 literature for coastal marine habitats (Zettler et al., 2014), estuarine (Little et al., 2017; Ritter et al., 2005)
108 and lagoons (Como et al., 2018), but very few studies exist for pond systems (Boix et al., 2008).

109 Ponds are small and isolated ecosystems ranging from 1 m² to few hectares, which temporarily
110 or permanently store water (De Meester et al., 2005). They are biodiversity hotspots both in terms of
111 species composition and biological traits, and provide ecosystem services to support human well-being
112 (Céréghino et al., 2014, 2012). Pond ecosystems host a large number of species and rare species,
113 exceeding those of other aquatic ecosystems such as streams and lakes (Williams et al., 2004).
114 Consequently, despite their limited dimensions, ponds are fundamental features for biodiversity
115 conservation at the landscape scale (Céréghino et al., 2014; Coccia et al., 2016). In fact, in addition to
116 the diversity of their own communities, ponds also play a role as stepping stones for aquatic mobile
117 species, thus mitigating the negative effects of habitat fragmentation and increasing aquatic habitats'
118 connectivity (Pereira et al., 2011; Rothermel, 2004). Moreover, ponds are ideal sentinels and early
119 warning systems of environmental changes due to their sensitivity to environmental changes, which is
120 associated to their small size and the high interaction with the groundwater (De Meester et al., 2005).
121 Particularly, pond macroinvertebrate communities can be sensitive indicators of how living communities
122 respond to environmental variations in coastal systems. However, the set of environmental variables
123 governing pond community composition and species traits is specific for each climatic/biogeographic
124 region (Céréghino et al., 2012; De Marco et al., 2014; Ruhí et al., 2013). Therefore, site-specific
125 investigations are needed to assess the response of macroinvertebrate communities of pond systems.

126 The aim of this study is to investigate macroinvertebrate communities' changes in a coastal
127 permanent system of ponds located in the Po river delta (Northern Italy) with different salinity levels, by
128 means of taxonomic and functional (i.e. biological traits) analyses. Macroinvertebrate communities were
129 studied as suitable indicators for detecting salinization effects on aquatic biota of small wetlands of
130 deltaic areas, such as the case of the "Bosco della Mesola" ponds in the Po river delta. Overall, the
131 assessment of macroinvertebrate community responses to salinity can shed light on how biodiversity and
132 ecological functions of aquatic ecosystems of deltaic areas are expected to change due to salinity
133 alterations.

135 **Material and Methods**

136 *Study area*

137 This study was carried out at "Bosco della Mesola" (44°50' 28'' N, 12°15' 12'' E), a National
138 Natural Reserve of 1058 ha located in the province of Ferrara (Northern Italy), with an altitude ranging
139 from -1 to +3 m a.s.l (Fig. 1).

140 The Nature Reserve "Bosco della Mesola" hosts a pond system formed by small water bodies
141 mainly located among the ancient dunes, where water stagnation is fed either by rainfall, by the coastal
142 aquifer that is hydrologically connected to the sea or, to a lesser extent, by incoming water from the canal
143 system. Their aquatic biota includes macroinvertebrate, amphibian and reptile communities. No fishes
144 were observed in ponds. The ponds' system is characterized by different salinity levels. Water bodies

145 near the shoreline are influenced by seawater rising from groundwater table and by occasional floods,
146 while the ponds at north receive freshwater from drainage canals.

147 The surrounding area was strongly altered by human interventions during the last century, e.g.
148 wetland reclamations, that affected the local hydrological balance (Gaglio et al., 2017a). Additionally,
149 the area is subjected to subsidence that causes the progressive intrusion of saltwater towards the inner
150 part of the Reserve, due to the progressive difficulties in recharging water table with freshwater, affecting
151 the communities of living organisms of local water bodies (Gerdol et al., 2018). Moreover, climatic
152 changes could speed up the salinization level of ponds' system, thus exacerbating such impacts.

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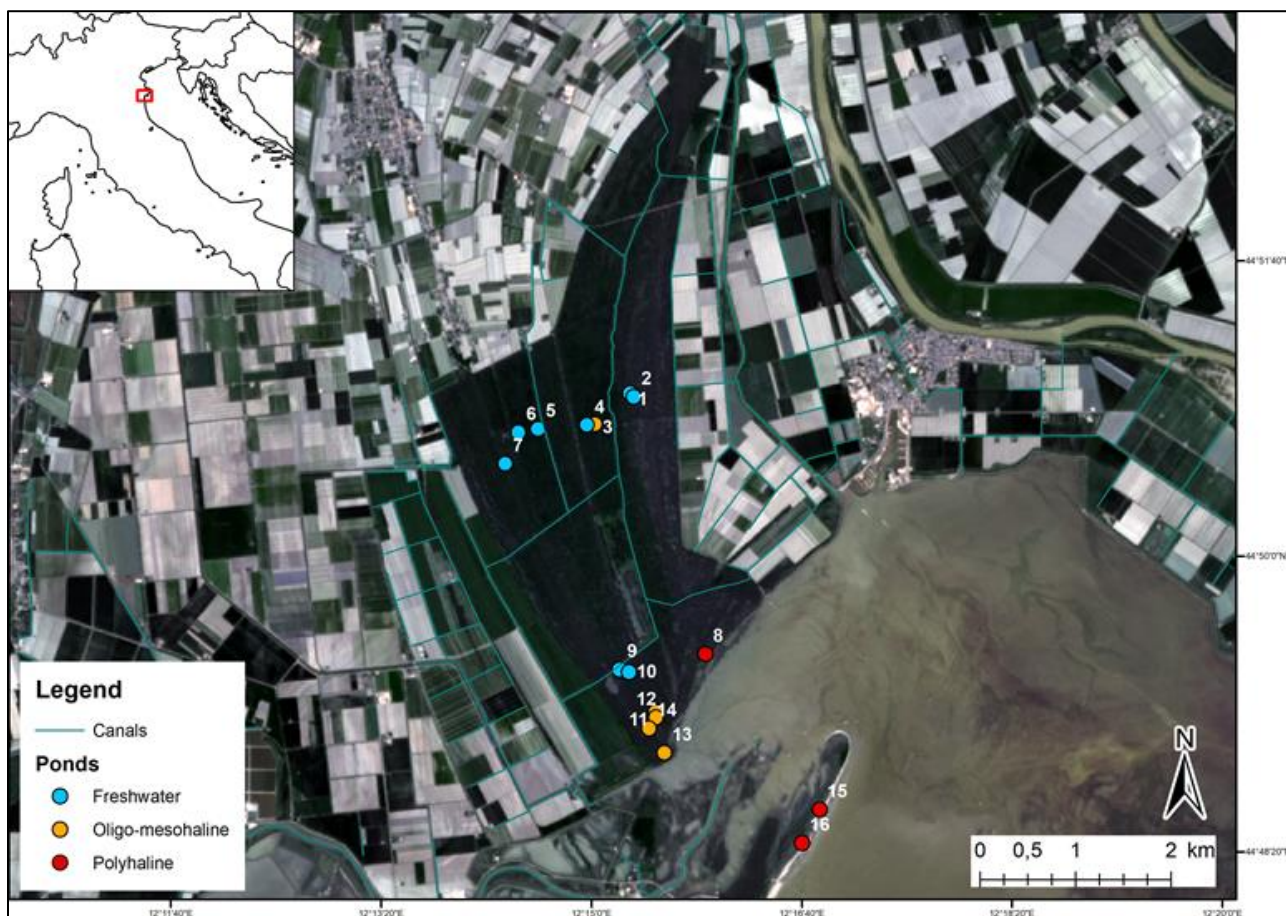


Fig.1 The study area of the 16 ponds where samplings were performed.

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157 *Sampling procedures*

158 The macroinvertebrate communities of 16 permanent coastal ponds were sampled during May
159 2017. Among these ponds, 14 are located inside the Nature Reserve of “Bosco della Mesola” in north-
160 eastern Italy. Moreover, two additional ponds were sampled outside the Reserve, located south in a
161 coastal outlet (Fig. 1). Temporary ponds were not considered in the analysis. The salinity levels ranged
162 between 0.2 and 29.3 psu. The ponds were classified into three salinity classes according to the
163 classification of Por (1972): freshwater (<0.5 psu) (8 ponds), oligo-mesohaline (0.5-18 psu) (5 ponds)
164 and polyhaline (>18 psu) (3 ponds). Benthic macrofauna was collected sweeping a distance of 2.5 m with
165 a 40 cm-wide D-frame hand net (mesh size = 500 μ m) (sampling area of 1 m²). Samplings were carried
166 out in 3 different sites within each pond, one in central and two in the outer parts, to capture intra-pond
167 variability. Animals were preserved in a 4% formalin solution and later identified in the laboratory up to
168 the genus level, and when it was not feasible, the family level was reached. Hence, the respective

169 biological/functional traits (feeding, mobility, adult life habitat, body size, life span, reproductive
170 frequency, habitat choice) were attributed to each taxon by means of bibliographic information using the
171 databases of Horton et al. (2017) and (MarLIN, 2006). When observed taxa were not covered by these
172 sources, Thorp and Covich (2010) was used as alternative literature. Table 1 provides the 39 taxa
173 observed in the sampling ponds while Table S1 in the Supplementary material provides the trait modalities
174 attributed to each taxon and Table S2 their mean abundance for each pond.

175 Eight environmental factors were measured/assessed during samplings (Table 2). Water
176 temperature, oxygen saturation and salinity were measured using a multi-parameter probe (YSI Model
177 85). Ponds' surface and maximum water depth were also measured in situ. The shape of the small ponds
178 was approximated as circle/ellipse. The area of circle is $A=\pi R^2$ while of ellipse is $A=\pi ab$ (where a and b
179 are the smaller and the larger radius). In order to avoid assumptions of shape, a laser meter was used to
180 take measurements of diameter from many positions of the pond shore. These measurements were used
181 to derive the mean diameter and consequently the mean radius (R_{mean}) of each pond. The area A was then
182 estimated as $A= \pi(R_{\text{mean}})^2$. For the larger ponds which were visible in Google Earth Pro, the area was
183 measured with the Ruler polygon tool of the respective software.

184 Euclidean nearest-neighbor distance (ENN) was computed using Fragstat 4.2 (McGarigal, 2014)
185 as a measure of pond isolation. ENN can be defined as the shortest straight-line distance between the
186 focal patch and its nearest neighbor of the same class. ENN was calculated for each pond, also
187 considering the presence of other water bodies, such as channels and coastal lagoons. Shapefiles of ponds
188 and channels were used to develop a reclassified raster file where water bodies were coded as "1" and
189 the remaining part of the landscape as "0". FRAGSTAT software used this raster file to calculate ENN,
190 considering the code "1" as foreground and "0" as background.

191 Both canopy coverage (i.e. the % of the pond surface shaded by surrounding trees) and aquatic
192 vegetation coverage (i.e. the % of the pond surface where aquatic plants were present) were measured by
193 analyzing photos taken on site (photos of water surface and photos of the sky at the shoreline were
194 elaborated in Arc-GIS).

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Table 1. Taxa observed in the 16 sampling ponds of Bosco della Mesola.

Phylum	Order	Family	Family abbr.	Genus
Anellida	Haplotaxidae	Naitidae	Nai	-
Anellida	Hirudinida	Hirudinidae	Hir	<i>Hirudo</i>
Anellida	Polychaeta	Nereidae	Ner	<i>Nereis</i>
Crustacea	Amphipoda	Corophiidae	Cor	<i>Corophium</i>
Crustacea	Amphipoda	Gammaridae	Gam	<i>Echinogammarus</i> <i>Gammarus</i>
Crustacea	Decapoda	Cambaridae	Cam	<i>Procambarus</i>
Crustacea	Decapoda	Portunidae	Por	<i>Carcinus</i>
Crustacea	Isopoda	Asellidae	Ase	<i>Asellus</i>
Crustacea	Isopoda	Sphaeromatidae	Sph	<i>Sphaeroma</i>
Insecta	Coleoptera	Dytiscidae	Dyt	-
Insecta	Coleoptera	Haliplidae	Hal	-
Insecta	Coleoptera	Hydrophilidae	Hyd	<i>Helochaers</i>
Insecta	Diptera	Ceratopogonidae	Cer	-
Insecta	Diptera	Chaoboridae	Chi	<i>Chaoborus</i> <i>Chironomus</i> <i>Cladopelma</i> <i>Cryptochironomus</i> <i>Parachironomus</i> <i>Polypedilum</i>
Insecta	Diptera	Orthocladiinae	Ort	<i>Orthocladus</i>
Insecta	Diptera	Tanyponidae	Tan	<i>Procladius</i> <i>Psectrotanypus</i> <i>Tanypus</i>
Insecta	Ephemeroptera	Baetidae	Bae	<i>Baetis</i> <i>Cloeon</i>
Insecta	Heteroptera	Corixidae	Crx	<i>Cymatia</i> <i>Micronecta</i>
Insecta	Heteroptera	Nepidae	Nep	<i>Nepa</i>
Insecta	Lepidoptera	Crambidae	Cra	<i>Cataclysta</i> <i>Paraponyx</i>
Insecta	Odonata	Lestidae	Les	<i>Chalcolestes</i>
Insecta	Odonata	Libellulidae	Lib	<i>Libellula</i>
Mollusca	Bivalvia	Corbulidae	Crb	<i>Corbula</i>
Mollusca	Gasteropoda	Bithyniidae	Bit	<i>Bithynia</i>
Mollusca	Gasteropoda	Lymnaeidae	Lym	<i>Lymnae</i>
Mollusca	Gasteropoda	Physidae	Phy	<i>Physa</i>
Mollusca	Gasteropoda	Planorbidae	Pla	<i>Planorbis</i>
Nematoda	-	-	Nem	-

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Table 2. Environmental parameters used in this study for analyzing taxa responses.

Parameter	Unit	Transformation	Abbrev.	Max	Min	Mean	SD
Temperature	°C	Log (x+1)	Temp	29.6	12.4	17.2	5.7
Oxygen saturation	%	arcsin (x/100) ^{0.5}	O2	100.0	8.5	56.3	30.5
Surface area	m ²	Log (x+1)	Area	6191.5	24.0	641.9	1514.0
Depth (maximum)	cm	Log (x+1)	Dep	63.0	10.0	34.3	16.6
Salinity	psu	Log (x+1)	Salt	29.3	0.2	6.3	9.8
Vegetation	%	arcsin (x/100) ^{0.5}	Veg	40.0	0.0	4.1	10.8
Canopy	%	arcsin (x/100) ^{0.5}	Can	50.0	0.0	14.4	18.6
ENN	m	Log (x+1)	ENN	356.5	28.3	103.9	88.0

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Methods of analysis

Detection and evaluation of salinity gradient effects on taxa abundance

225 All environmental parameters and taxa abundance were transformed to reduce normality
226 departures. Environmental parameters, which are ratios/percentages, were transformed using
227 arcsin(x/100)^{0.5} while the rest environmental parameters and taxa abundance (ind. m⁻²) using log(x+1)
228 (Aschonitis et al., 2016). Spearman correlations were performed among environmental variables of [Table](#)
229 [2](#).
230

231 Multiple gradient analysis was performed for assessing the effect of multiple descriptors
232 (environmental parameters) on multiple target variables (taxa) using Canonical Correspondence Analysis
233 (CCA) (Lepš and Šmilauer, 2003; ter Braak and Smilauer, 2002). CCA was performed using CANOCO
234 4.5, based on target variables correlations and their standardized scores (ter Braak and Smilauer, 2002).
235 The method was applied following the same steps as those described in a similar case study (Aschonitis
236 et al., 2016) and significant descriptors were identified using CANOCO's forward selection procedure
237 and Monte Carlo permutation test (499 permutations) (a default option in the CANOCO software).
238 Colinear variables with variance inflation factor VIF>8 (Zuur et al., 2007) or variables with statistical
239 significance $p>0.5$ were excluded from multiple gradient analysis. The multivariate analysis was
240 performed for macroinvertebrate communities at the family level, which is considered a sufficient level
241 for invertebrate community analysis (Gayraud et al., 2003). Moreover, when applied to multivariate
242 analyses, the results obtained considering higher taxa may more closely reflect effects related to gradients
243 or stresses than those based on finer level (e.g. genera or species), that are more affected by natural
244 "noise" (Warwick, 1988). This analysis detected that the only statistically significant descriptor variable
245 at $p<0.05$ level is salinity. For this reason, a method of single gradient analysis is more appropriate.

246 The threshold indicator taxa analysis TITAN (Baker and King 2010) is a single gradient analysis
247 method that is used in ecological studies for detecting changes in taxa abundance distributions along a
248 unique environmental gradient (i.e. salinity) and for assessing synchrony among taxa abundance change
249 points as evidence of community thresholds (Baker and King 2010). TITAN uses bootstrapping for
250 estimating purity and reliability criteria as well as uncertainty of change points related to individual taxa
251 abundances along the salinity gradient. Usually, a cut off value of 95% is used in both purity and
252 reliability criteria for identifying statistical robust responses of taxa abundance versus an environmental
253 gradient (i.e. salinity). The purity cut off value defines what is considered a pure response direction. A
254 purity value of 0.95 indicates that 95% of the results from bootstrap replicates agree with the observed
255 response direction. The reliability cut off value defines what is considered a reliable response magnitude.
256 A reliability value of 0.95 indicates that 95% of the results from bootstrap replicates have an IndVal p -
257 value less than or equal to 0.05, indicating a response magnitude at a given change point location that is

258 significantly different from what would expect from random permutation (Baker and King 2010) {for
259 more explanations about IndVal p -value, see Dufrêne and Legendre (1997) and Baker and King (2010)}.
260 In this study, the purity and reliability values were estimated and the response plots versus salinity
261 gradient were developed for all taxa without considering cut off values. The TITAN analysis was
262 performed with TITAN2 version 2.1 (Baker et al. 2015) in R language using 500 random permutations
263 of taxa abundances and creating 1000 new bootstrap datasets created by resampling the observed data
264 with replacement.

265

266 *Effects of salinity on biological traits and diversity measures*

267 The analysis considered both taxonomic and functional indices. The functional indices were
268 calculated using the “FD” package for R (Laliberté and Legendre, 2010), which takes into account
269 multidimensional (i.e. multiple traits) functional diversity. The differences in population indices among
270 the three salinity classes of ponds (i.e. Freshwater, Oligo-mesohaline, Polyhaline) were evaluated with
271 the non-parametric Kruskal-Wallis Test. When statistical significantly differences were found, a post-
272 hoc test was carried out (Bonferroni test). The analysis considered:

- 273 - Four taxonomic indices: species richness (S), Shannon's diversity index (H'), Pielou evenness index
274 (J') and Simpson index (D), and
- 275 - Four functional indices: Functional richness (FRic), Functional evenness (FEve), Functional
276 divergence (FDiv) and Rao quadratic entropy index (RaoQ).

277 Moreover, significant differences in single biological traits among the three respective salinity
278 classes of ponds were evaluated through the comparison of proportions with χ^2 test for P -value ≤ 0.01 .
279 The comparison of proportions takes into account the number of individuals characterized by each
280 specific trait modality. For each trait, the comparison was performed between the proportion of each trait
281 modality of the three salinity classes versus the overall proportion of the remaining modalities in the
282 respective salinity class (e.g. for the “adult life habitat” trait, the significance of difference of “aquatic”
283 type in the three salinity classes was evaluated comparing “aquatic” vs. “aeric”). The null hypothesis was
284 that the proportion of the two trait modalities (or of the proportion of the one trait type versus the
285 remaining ones) did not differ over the three salinity classes. Afterward, an analysis of means (ANOM)
286 plot with 99% confidence was applied to provide indications about the direction of the significant
287 differences based on the deviation from the grand mean of the ANOM plots (Fedrigotti et al., 2016;
288 Gaglio et al., 2017b). The ANOM analysis was also followed by TITAN analysis of biological traits
289 using salinity as a single gradient for assessing change points of trait groups abundance. TITAN was
290 performed following the same methodological steps as described in the case of taxa.

291

292 **Results**

293

294 *Effect of salinity gradient on taxa abundance*

295 As it was mentioned in the Methods section, CCA detected that the most important parameter from
296 [Table 2](#) but also the only parameter with statistical significance at $p < 0.05$ level describing the taxa
297 variance was salinity ([Table S3](#) in the Supplementary material). According to [Table S3](#), the CCA analysis
298 with all the variables of [Table 2](#) explained the 58.7% of taxa variance while only salinity explained 14.9%
299 of taxa variance.

300 The Spearman correlations among the environmental variables ([Table 3](#)) showed that salinity is
301 significantly positively correlated with temperature and pond area while it is significantly negatively
302 correlated with canopy coverage. Temperature is positively related to salinity due to higher evapo-
303 concentration (accumulation of salts due to higher water temperature that enhances evaporation). Pond
304 area is positively related to salinity, because larger ponds were closer to the coastline or to lower elevation
305 indicating higher salt water intrusion. Canopy coverage is negatively related to salinity because larger

306 canopy coverage reduces solar radiation interception by the water that leads to lower evapoconcentration
 307 (i.e. lower solar radiation leads to lower evaporation). Thus, salinity can also be used as a general
 308 surrogate descriptor of the aforementioned environmental parameters.
 309

310 Table 3. Spearman correlations among environmental variables of Table 2.

	Temp	O2	Area	Dep	Salt	Veg	Can	ENN
Temp	1							
O2	0.421	1						
Area	0.444	0.313	1					
Dep	-0.394	-0.345	-0.372	1				
Salt	0.519*	0.450	0.599*	-0.105	1			
Veg	0.004	0.303	-0.216	-0.111	-0.063	1		
Can	-0.462	-0.589*	-0.247	0.409	-0.556*	-0.282	1	
ENN	-0.092	0.142	0.389	-0.055	0.175	0.007	0.124	1

311 * Statistical significance for $0.01 < p < 0.05$ level.
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313 The results of TITAN analysis that concern the effects of salinity gradient on taxa abundance are
 314 provided in Table 4 and Fig. 2. Table 4 provides the indicator change point (CP) along the salinity
 315 gradient expressed in psu (median of 1000 bootstrap replicates), the purity and reliability % of CP, and
 316 the response (positive + or negative -) of each taxon versus the increase of salinity gradient. Fig. 2 shows
 317 the declining taxa on the left axis and the increasing taxa on the right axis. The observed change point is
 318 indicated by the circular symbol (its size corresponds to the median z-value of bootstrap replicates), while
 319 the horizontal line behind each circular symbol describes the 5-95% quantiles from the bootstrapped
 320 change-point distribution. Taking into account Table 4 and Fig. 2, the following observations were made:

- 321 • 18 out of 28 taxa showed purity >95%, while 9 out of 28 taxa showed both purity and reliability
 322 >95% (other taxa showing only reliability >95% were not observed). From the nine highly pure
 323 (>95%) and highly reliable (>95%) taxa, four showed a positive and three a negative response versus
 324 the salinity gradient.
- 325 • From the nine highly pure (>95%) and highly reliable (>95%) taxa, some of them (e.g. *Naitidae* -
 326 *Nai*) showed very low z-score (response magnitude) in the CP and large bootstrap interval. This
 327 indicates a very clear positive relationship (response) between this taxon and salinity but with high
 328 uncertainty in the CP value.
- 329 • The most sensitive to salinity taxa were found to be *Cambaridae* (Cam), *Sphaeromatidae* (Sph),
 330 *Dytiscidae* (Dyt), *Haliplidae* (Hal), *Orthocladiinae* (Ort), *Bithyniidae* (Bit) and *Planorbidae* (Pla),
 331 showing negative responses for indicator change points (CP) of salinity <0.5 psu.
- 332 • The taxa of highest tolerance to salinity were *Naitidae* (Nai), *Nereidae* (Ner), *Chaoboridae* (Chi),
 333 *Corbulidae* (Crb) and *Nematoda* (Nem) showing positive responses for indicator change points (CP)
 334 of salinity >10 psu.

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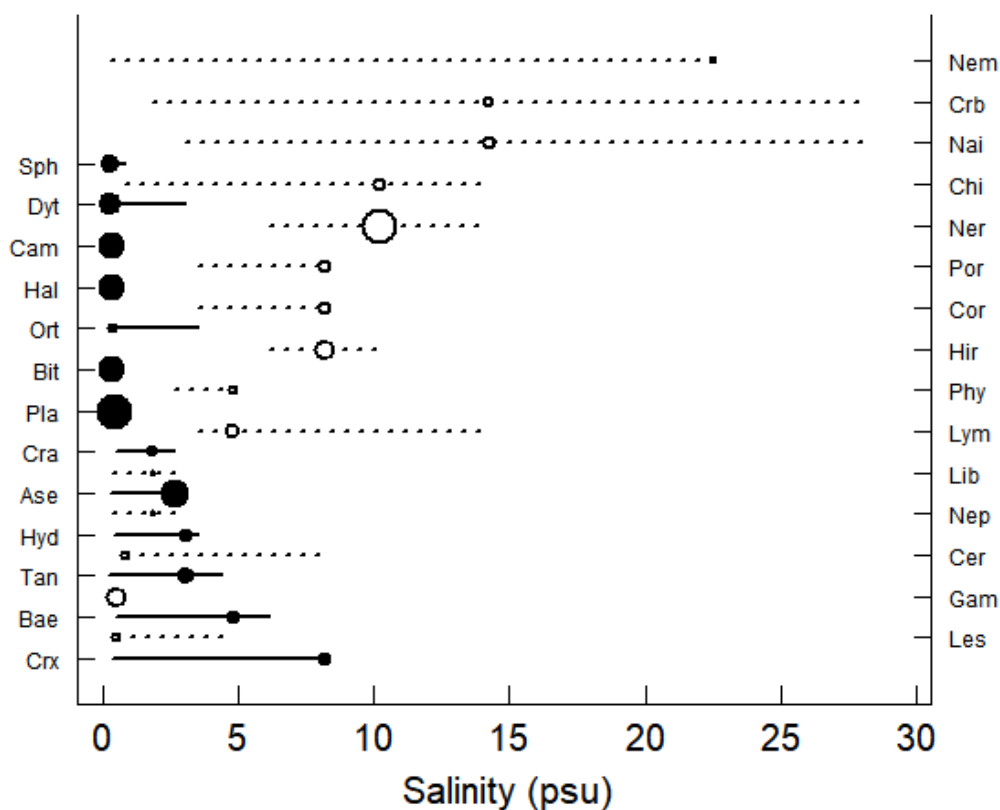
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Table 4. Indicator change point (CP) along the salinity gradient expressed in psu (median of bootstrap replicates), values of purity and reliability criteria, and response (positive + or negative -) of each taxon (see Table 1 for abbreviations) versus the increase of salinity gradient according to TITAN analysis.

Taxon	CP (psu)	Purity	Reliability	Response group	Taxon	CP (psu)	Purity	Reliability	Response group
Nai**	14.25	0.978	0.958	+	Ort	0.3	0.886	0.654	-
Hir**	8.15	0.998	0.985	+	Tan**	3.05	0.994	0.985	-
Ner**	10.2	1	1	+	Bae	4.8	0.82	0.871	-
Cor*	8.15	0.962	0.751	+	Crx*	8.15	0.96	0.939	-
Gam**	0.5	1	1	+	Nep	1.85	0.87	0.306	+
Cam*	0.3	0.967	0.76	-	Cra	1.85	0.665	0.668	-
Por*	8.15	0.962	0.719	+	Les	0.45	0.733	0.922	+
Ase**	2.6	1	1	-	Lib	1.85	0.87	0.317	+
Sph	0.25	0.862	0.807	-	Crb*	14.25	0.982	0.938	+
Dyt	0.25	0.921	0.857	-	Bit*	0.3	0.967	0.734	-
Hal*	0.3	0.967	0.734	-	Lym**	4.8	0.997	0.97	+
Hyd	3.05	0.771	0.841	-	Phy*	4.8	0.964	0.616	+
Cer*	0.8	0.982	0.936	+	Pla**	0.4	1	0.997	-
Chi**	10.2	1	1	+	Nem	22.5	0.754	0.694	+

348 *Statistically significant purity (>95%) or reliability (>95%)
349 **Statistically significant purity (>95%) and reliability (>95%)

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Fig.2 CP and response plots of each taxon versus the salinity gradient

355 according to TITAN analysis. Black circles are aligned and correspond to the negative-reponse taxa of
 356 the left vertical axis, whereas white circles are aligned and correspond to the positive-reponse taxa of
 357 the right vertical axis. The size of the circle is proportional to median z-score of bootstrap replicates.

358 Horizontal lines overlapping each circular symbol represent the interval of 5th and 95th percentiles
 359 among bootstrap replicates.
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362 ***Effect of salinity on diversity indices and biological traits***

363 The values of taxonomic and functional population indices of the three salinity classes of ponds
 364 (i.e. Freshwater, Oligo-mesohaline, Polyhaline) are given in Table 5. The only statistically significant
 365 differences among salinity classes were observed for the case of functional evenness (FEve) (p-
 366 value<0.05), when shifting from freshwater to oligo-mesohaline conditions. Even though not highlighted
 367 by statistical tests, a decreasing trend in the majority of the other indices and especially in species and
 368 functional richness values along the salinity gradient was observed.

369 Considering the ANOM analysis of differences in biological traits among the three respective
 370 salinity classes, all the considered traits showed significant variations (Table 6), highlighting taxa
 371 sensitivity to salinity in terms of functional and biological characteristics. From the 22 traits, 21 showed
 372 significant variations where 7 showed a positive response and 14 a negative response to salinity increase.
 373 The changes mainly occurred in the transition from oligo-mesohaline to polyhaline class (Table 6). The
 374 TITAN analysis of traits versus salinity is given in Table 7 and Fig.3, from which it was observed that
 375 12 out of 22 traits showed CPs with both purity and reliability >95%. TITAN analysis complements the
 376 ANOM analysis since it provides the possible range where a CP may appear.
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381 Table 5. Average values (\pm Standard error) of taxonomic (S, H', J' and D) and functional indices (FRic,
 382 FEve, FDiv and RaoQ) observed in each salinity class.

Pond salinity classes	Freshwater	Oligo-mesohaline	Polyhaline	p-value Kruskal-Wallis
Species Richness (S)	7.625 \pm 0.73a	5.8 \pm 2.853a	3.333 \pm 0.882a	0.074
Shannon's Diversity (H')	0.954 \pm 0.154a	0.82 \pm 0.201a	0.546 \pm 0.082a	0.235
Pielou Evenness (J')	0.481 \pm 0.07a	0.622 \pm 0.049a	0.561 \pm 0.205a	0.254
Simpson index (D)	0.466 \pm 0.07a	0.482 \pm 0.078a	0.376 \pm 0.129a	0.678
Functional Richness (FRich)	7.625 \pm 0.73a	5.6 \pm 2.65a	3.333 \pm 0.882a	0.074
Functional Evenness (FEve)	0.609 \pm 0.064a	0.345 \pm 0.092b	0.361 \pm 0.118b	0.029
Functional Divergence (FDiv)	0.219 \pm 0.034a	0.237 \pm 0.042a	0.116 \pm 0.045a	0.178
Rao index (RaoQ)	0.081 \pm 0.017a	0.088 \pm 0.026a	0.022 \pm 0.011a	0.112

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384 Table 6. Analysis of means (ANOM) for the different functional attributes in the three salinity classes.
 385 Statistical significances are highlighted in bold ($p < 0.05$). The three codes a, b and c denote the respective
 386 location of the proportion values (above, inside and below the upper and lower 95% confidence limits)
 387 of the three salinity classes for each trait type. The arrows show the positive (\uparrow), negative (\downarrow) or no trend
 388 (n.t.) of trait response to salinity increase.

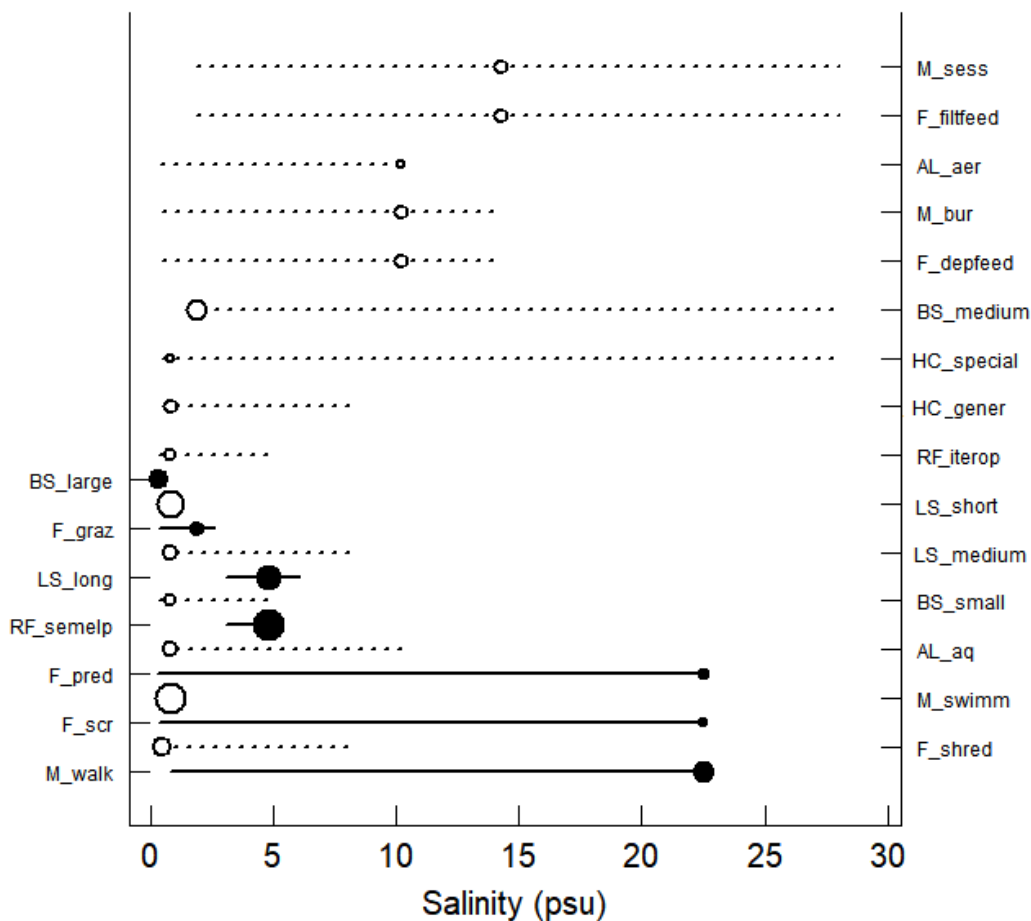
<i>Trait modality</i>	<i>Trend with salinity increase</i>	χ^2 (df=2)	<i>P value</i>	<i>Freshwater</i>	<i>Oligo-mesohaline</i>	<i>Polyhaline</i>
FEEDING						
<i>Predator</i>	\downarrow	1035.52	<0.001	a	a	c
<i>Grazer</i>	\downarrow	63.86	<0.001	a	b	b
<i>Shredder</i>	\downarrow	6158.39	<0.001	a	a	c
<i>Scraper</i>	\downarrow	814.6	<0.001	a	a	c
<i>Deposit feeder</i>	\uparrow	6038.5	<0.001	c	c	a
<i>Filter feeder</i>	\downarrow	8.3	0.0158	a	b	b
MOBILITY						
<i>sessile</i>	\downarrow	8.3	0.0158	a	b	b
<i>swimmer</i>	\downarrow	8245.1	<0.001	a	a	c
<i>burrower</i>	\uparrow	7195.53	<0.001	c	c	a
<i>walker</i>	\downarrow	3242.25	<0.001	a	a	c
ADULT LIFE HABITAT						
<i>aquatic</i>	\downarrow	830.57	<0.001	a	a	c
<i>aeric</i>	\uparrow	830.57	<0.001	c	c	a
BODY SIZE (g AFDW)						
<i>small (<0.01)</i>	<i>n.t.</i>	2.71	0.2575	b	b	b
<i>medium (0.01-0.05)</i>	\uparrow	1723.75	<0.001	c	c	a
<i>large (>0.05)</i>	\downarrow	134.61	<0.001	a	a	c
LIFE SPAN (years)						
<i>short (< 1)</i>	\downarrow	7709.88	<0.001	a	a	c
<i>medium (1-5)</i>	\uparrow	8024.5	<0.001	c	c	a
<i>long (>5)</i>	\downarrow	1518.22	<0.001	a	a	c
REPRODUCTIVE FREQUENCY						
<i>Iteroparous</i>	\uparrow	1905.59	<0.001	c	c	a
<i>Semelparous</i>	\downarrow	1905.59	<0.001	a	a	c
HABITAT CHOICE						
<i>generalist</i>	\uparrow	1679.05	<0.001	c	c	a
<i>specialist</i>	\downarrow	1679.05	<0.001	a	a	c

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396 Table 7. Indicator change point (CP) along the salinity gradient expressed in psu (median of bootstrap
 397 replicates), values of purity and reliability criteria, and response (positive + or negative -) of each trait
 398 class versus the increase of salinity gradient according to TITAN analysis (traits' abbreviations
 399 correspond to the traits of Table 6 in the same order).

Trait	CP (psu)	Purity	Reliability	Response group	Trait	CP (psu)	Purity	Reliability	Response group
F_pred	22.5	0.709	0.917	-	AL_aer*	8.15	1	0.912	+
F_graz*	1.85	0.985	0.912	-	BS_small**	0.8	1	1	+
F_shred*	0.4	0.875	0.998	+	BS_medium**	1.85	0.993	0.981	+
F_scr	22.5	0.529	0.919	-	BS_large*	0.35	0.961	0.758	-
F_depfeed**	8.15	1	0.994	+	LS_short**	0.8	1	1	+
F_filtfeed*	14.25	0.979	0.937	+	LS_medium**	1.85	1	0.999	+
M_sess*	14.25	0.979	0.937	+	LS_long**	4.8	1	1	-
M_swimm**	0.8	1	1	+	RF_iterop**	0.8	1	1	+
M_bur**	8.15	1	0.994	+	RF_semelp**	4.8	1	1	-
M_walk	22.5	0.876	0.804	-	HC_gener**	0.8	1	0.999	+
AL_aq**	0.8	1	1	+	HC_special	0.8	0.851	0.567	+

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Fig.3 CP and response plots of each trait versus the salinity gradient

404 according to TITAN analysis. Black circles are aligned and correspond to the negative-reponse traits of
405 the left vertical axis, whereas white circles are aligned and correspond to the positive-reponse traits of
406 the right vertical axis. The size of the circle is proportional to median z-score of bootstrap replicates.

407 Horizontal lines overlapping each circular symbol represent the interval of 5th and 95th percentiles
408 among bootstrap replicates.
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411 Discussion

412 Small wetlands are often under-investigated biodiversity hotspots that should be instead considered
413 with great attention under a conservation perspective (Viaroli et al., 2016). Particularly, the permanent
414 ponds system of Bosco della Mesola hosts a high number of taxa (39) if compared with ponds surrounded
415 by other land use types. Hill et al. (2016) investigated macroinvertebrate diversity in urban, arable and
416 floodplain ponds, recording 22, 30 and 32 taxa, respectively. The positive effect of surrounding forest is
417 further corroborated by the comparison of our data with those of Bazzanti (2015), who found a similarly
418 high number of taxa in temporary and permanent ponds located in a Mediterranean Tyrrhenian coastal
419 forest ecosystem. However, biodiversity of coastal ponds is seriously threatened by the pressing
420 environmental changes occurring in these areas. Our findings highlighted the role of salinity in shaping
421 macroinvertebrate community compositions in coastal permanent ponds and that these communities
422 could be significantly affected by salt water intrusion, which causes a relevant simplification of
423 taxonomic and functional diversity.

424 This finding is consistent with other studies describing community variations in different aquatic
425 environments (Castillo et al., 2018; Little et al., 2017; Piscart et al., 2005b; Zettler et al., 2014), including
426 temporary wetlands (Waterkeyn et al., 2008), and with Kefford et al. (2016) who found a limited
427 tolerance of aquatic insects to salinity increases in freshwater habitats.

428 Despite the fact that CCA detected salinity as the only statistically significant parameter of [Table](#)
429 [2](#) for describing taxa variation, the Spearman correlations among the environmental variables ([Table 3](#))
430 showed that salinity is significantly positively correlated with temperature and pond area while it is
431 significantly negatively correlated with canopy coverage. Thus, the observed salinity effect on
432 macroinvertebrate communities partly includes some effects of these parameters. Water depth and pond
433 isolation (described through ENN) were among the least important parameters affecting the communities
434 (according to preliminary CCA analysis) and the least associated parameters to salinity. The non-
435 significant effect of water depth was probably the result of the generally shallow profile of all analyzed
436 ponds. In the case of pond isolation, its non-significant effect can be attributed to the fact that the
437 recruitment of flying insects may be guaranteed by the surrounding forested landscape rather than by
438 other water bodies. Moreover, the potential role of isolation could be not evident at finer scale, as the one
439 applied in this study.

440 Taxonomic diversity indices were negatively influenced by salinity depicting an overall loss of
441 taxa diversity due to saltwater intrusion. Although freshwater ponds show higher values of taxonomic
442 diversity indices, oligo-mesohaline ones hosted higher taxa richness according to [Table 5](#). This partially
443 corroborates to the ‘intermediate disturbance hypothesis’ (Connell, 1978) of salinity gradient proposed
444 by Piscart, Lecerf, et al. (2005), according to which an intermediate level of salinity promotes a higher
445 level of biodiversity because of the co-occurrence of both halotolerant and freshwater species. Higher
446 values of taxa richness detected in oligo-mesohaline ponds are due to the occurrence of more insect and
447 crustacean taxa. However, this does not equal to higher values of taxonomic diversity indices because of
448 the decrease of evenness of taxa distribution along the salinity gradient. The increasing proportions of
449 crustacean taxa and crustaceans/insects ratio along salinity levels observed by Boix et al. (2008) were
450 confirmed by our findings only up to oligo-mesohaline levels, while opposite trends were observed at
451 polyhaline level.

452 As it was shown from [Table 5](#), salinization of permanent ponds above the polyhaline level leads to
453 a drastic loss of functional diversity. The results highlighted that functional traits' analysis is sensitive to
454 depict community responses mainly to high levels of salinity transitions. This also provides evidence that
455 functional variables can be used as indicators of drastic environmental perturbations and should always
456 be studied when assessing disturbance impacts on biota (Sandin and Solimini, 2009). However, it is
457 worth to be mentioned that the attribution of functional and biological trait modalities based on literature
458 survey introduces a slight bias due to the ecological plasticity of some taxa, i.e. the selection of different
459 functional strategies according to the environmental context.

460 Apparent incongruences between ANOM and TITAN tests were due to the different purposes of the
461 methods. In fact, ANOM tests the difference in terms of relative abundance of the specific trait
462 modalities, while TITAN considers their absolute abundances. Therefore, some trait attributes may
463 appear both negatively and positively related to salinity according the method of analysis. For example,
464 shredders (for feeding trait) and short life organisms (for lifespan trait) decrease significantly at
465 polyhaline level, while TITAN depicted a general positive trend with salinity. This can be explained by
466 the fact that such attributes benefit from slight increases of salinity (up to oligo-mesohaline levels) before
467 to decrease when reaching polyhaline levels.

468 The salinization of the ponds system toward polyhaline conditions caused the shift of
469 macroinvertebrate communities towards assemblages dominated by r-strategist taxa (*sensu* Pianka 1970),
470 which are generalist taxa with smaller body size and higher reproductive frequency. Polyhaline ponds
471 are dominated by burrowing deposit feeder taxa, mainly Chironomidae, Tubificinae and Nereidae. All
472 other taxa with different feeding and mobility attributes were rarely found in polyhaline conditions.
473 Piscart et al. (2006) found an increase in filter-feeding at intermediate salinity levels and an increase in
474 deposit-feeding thereafter, in accordance with an energy transfer from water column (i.e. suspended
475 organic material) to sediment (deposited organic material) along a salinity gradient. However, in our case
476 the low filter-feeding abundances observed in oligo-mesohaline ponds were insufficient to justify such
477 food web modification. On the other hand, other changes in the proportion of the feeding traits were
478 observed such as higher proportion of deposit feeder and lower proportions of predator/shredder/scrapper
479 in polihaline ponds.

480 The increase of ionic concentrations requires specific adaptations of macroinvertebrate
481 communities. The faster metabolic rates induced by the elevated maintenance costs for osmoregulation
482 are reflected by smaller body size (Woodward et al., 2005), supporting its use as an effective indicator
483 for assessing community variations in transitional environments (Basset et al., 2012). Some flying insect
484 taxa, as the case of Diptera, have aeric adult life stage in order to reduce the permanence in saltwater
485 environments.

486 Contrarily to the results presented by Venâncio et al. (2019), who found changes in community
487 structures and trophic relations even with small increments of salinity in laboratory experiment, the
488 macroinvertebrate communities of the permanent ponds of Bosco della Mesola were found to be resilient
489 to moderate salinization. This could be due to the functional redundancy phenomena (i.e. maintaining
490 functional traits over time when facing taxa extinctions) and, to a lesser extent, to the tolerance of some
491 taxa to moderate salinity levels. In fact, when analyzing single traits, the communities of oligo-
492 mesohaline ponds maintain all the functional traits occurring in freshwater ones, except for a lower grazer
493 abundance, which has found to be sensitive even at low salinity variations. Moreover, the observed
494 results for filter feeders and sessile mobility attributes are due to abundance variations of a single species
495 (*Corbula sp.*) that is the only observed organism with these functional traits, rather than to a general
496 pattern. Since the relation between salinity and abundance of grazer organisms is mediated by the
497 occurrence of microphytobenthos (De Jonge and Van Beuselom, 1992; Juneau et al., 2015), the relative
498 decrease of such functional attribute may highlight the sensitivity of microphytobenthic assemblages to

499 salinity variations (Waska and Kim, 2010). Therefore, changes in grazer relative abundances could be
500 regarded as a sentinel of salinization effects on microphytobenthic assemblages in the permanent ponds'
501 systems.

502 When considering functional multi-traits indices, only functional evenness was found statistically
503 sensitive to moderate salinization, as a consequence of the increasing dominance of few traits. This
504 confirms that functional diversity tends to be a better predictor of impacts of environmental changes on
505 ecosystem functioning, and therefore ultimately on ecosystem services, than taxonomic diversity (Sandin
506 and Solimini, 2009). The results also suggest that a single trait functional approach, as those performed
507 with the ANOM and TITAN, can be more informative than a multi-traits approach when linking
508 ecological functions with environmental gradients, as also stated by Butterfield and Suding (2013). In
509 fact, while multi-traits functional evenness expresses the overall evenness of traits abundance distribution
510 in filled niche space providing a comprehensive measure of functional modifications, ANOM and
511 TITAN, based on single traits, provide insights on which specific trait is sensitive to environmental
512 change and also allow to better identify the effects due to a single taxa extinction.

513 Since the salinity levels observed in the study area significantly increased during last two decades
514 (Gerdol et al., 2018), and this trend is expected to keep on in the next years, this investigation can provide
515 evidence to be exported on other deltaic contexts on how biodiversity of the pond systems is expected to
516 also respond to climate change. From an environmental conservation perspective, the ongoing
517 salinization of coastal water bodies observed in our and other study cases (Ketabchi et al., 2016) is a
518 serious threat to aquatic biodiversity. The described effects on macroinvertebrate communities in terms
519 of taxonomic and functional diversity are expected also to have consequences on higher trophic levels
520 and ecosystem stability, functioning and services (Landuyt et al., 2014; Pinto et al., 2014; Schratzberger
521 and Ingels, 2018). Future management measures should be designed for mitigating the impact of
522 salinization phenomena, which could be also the result of climate change, through the control of human
523 activities in coastal areas and through targeted environmental restoration works.

524 For instance, in the case of Bosco della Mesola ponds' systems new management measures have
525 recently been undertaken to decrease salinity levels in the near coastal lagoon (Gaglio et al., 2019). Such
526 interventions are expected to have beneficial outcomes in the coastal water bodies which are in contact
527 with groundwaters. However, this does not represent a definitive solution to the conservation of
528 biodiversity in a long period vision.

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532 **Supplementary Material**

533 The supplementary material contains the Tables S1, S2 and S3.

534

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