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Benthic community recovery from brine impact after the implementation of mitigation measures

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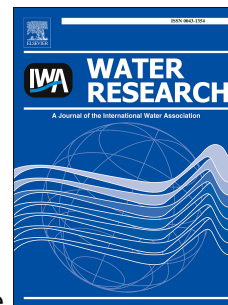
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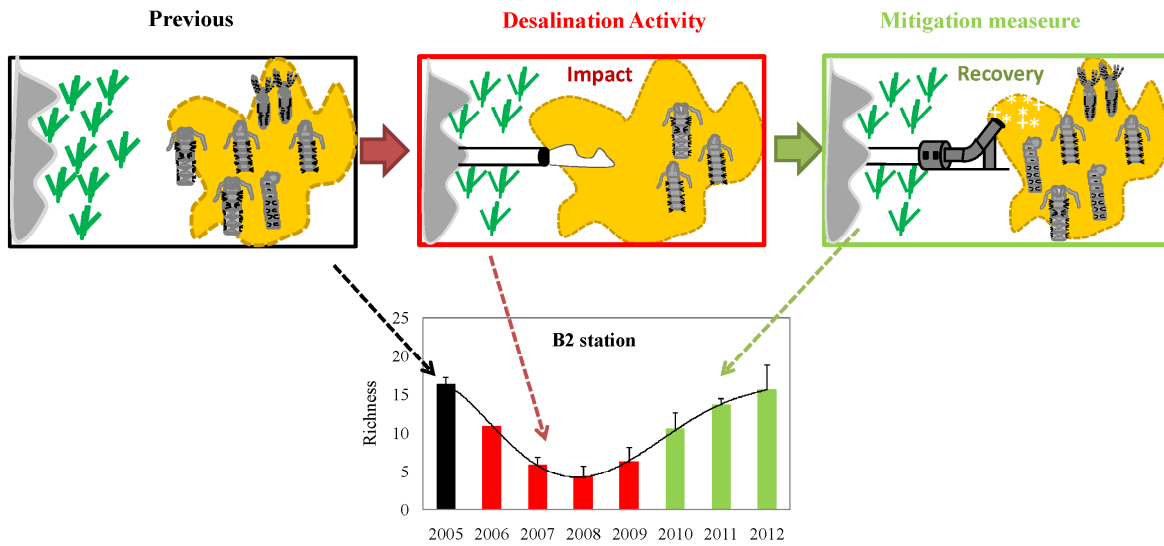
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Richness changes at B2 station related to salinity changes due to desalination activity and mitigation measures

1 **Benthic community recovery from brine impact after the**
2 **implementation of mitigation measures.**

3

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14

15

16 **Abstract**

17

18 In many regions, seawater desalination is a growing industry that has its impact on
19 benthic communities. This study analyses the effect on benthic communities of a
20 mitigation measure applied to a brine discharge, using polychaete assemblages as
21 indicator. An eight-year study was conducted at San Pedro del Pinatar (SE Spain)
22 establishing a grid of 12 sites at a depth range of 29-38 m during autumn. Brine
23 discharge started in 2006 and produced a significant decrease in abundance, richness
24 and diversity of polychaete families at the location closest to the discharge, where
25 salinity reached 49. In 2010, a diffuser was deployed at the end of the pipeline in order
26 to increase the mixing, to reduce the impact on benthic communities. After
27 implementation of this mitigation measure, the salinity measured close to discharge was
28 less than 38.5 and a significant recovery in polychaete richness and diversity was
29 detected, to levels similar to those before the discharge. A less evident recovery in
30 abundance was also observed, probably due to different recovery rates of polychaete
31 families. Some families like Paraonidae and Magelonidae were more tolerant to this
32 impact. Others like Syllidae and Capitellidae recovered quickly, although still affected
33 by the discharge, while some families such as Sabellidae and Cirratulidae appeared to
34 recover more slowly.

35

36 **Key words:** Polychaete assemblage, Brine discharge, Mitigation measures, Recovery,
37 Soft bottom, Mediterranean.

38

39 1. Introduction

40

41 Over the last few decades we have faced a global water crisis due to the increase in
42 domestic, industrial and agricultural water demand. The rapid growth in human
43 population and industrial-scale activities has contributed to this water scarcity (Medina,
44 2001). As a result many countries have pursued alternatives to conventional resources to
45 supply the additional water (Zhou et al., 2013). Desalination of seawater is
46 predominantly used for alleviating the problem of water scarcity in dry coastal regions.
47 It accounts for a worldwide production capacity of 24.5 million m³/day (IDA, 2006;
48 Lattemann and Höpner, 2008). In the Mediterranean Sea, the total production from
49 seawater is about 4.2 million m³/day (17% of the worldwide capacity) (Lattemann and
50 Höpner, 2008). Spain is considered a water-stressed country (European Environment
51 Agency, 2005); with 7% of the worldwide capacity it is the largest producer in the
52 region, with about 70% of its desalting plants located on the Mediterranean coast
53 (Lattemann and Höpner, 2008). Among the different technologies used, reverse osmosis
54 is the most common, mainly due to its low energy and space consumption and the
55 reduction in the cost of producing potable water (Einav et al., 2002). Nevertheless, such
56 facilities may give rise to several potentially adverse environmental impacts (Höpner
57 and Windelberg, 1996; Fernández-Torquemada et al., 2005; Sadhwani et al., 2005; Del-
58 Pilar-Ruso et al., 2009). The main impact of seawater reverse osmosis (SWRO)
59 desalination plants on marine communities is caused by the discharge of high salinity
60 effluent (Einav et al., 2002). This brine, characterised by high salt concentration and low
61 nutrients (Fernández-Torquemada et al., 2004), is usually returned to the sea. It can have
62 a detrimental effect on the marine environment, mainly on benthic communities that are

63 not adapted to these high salinities (Sanchez-Lizaso et al., 2008; Gacía and Ballesteros,
64 2001). The high-salt effluent remains on the bottom due to its greater density and
65 principally affects marine benthic communities adapted to a lower or more stable
66 salinity environment (Lattemann and Höpner, 2003). The magnitude of the impact
67 reached in an area close to the discharge will depend on the salinity, speed of dilution
68 and sensitivity of the ecosystem that receives it (Höpner and Windelberg, 1996;
69 Fernández-Torquemada et al., 2009). To protect the environment, most countries tend to
70 assess the environmental impacts produced by desalination activities (Sadhvani et al.,
71 2005), and attempt to minimise such impacts through mitigation measures. Brine
72 disposal impact may be reduced by the dilution of the effluent, either using diffusers or
73 by flushing with normal seawater added to the flow (Fernández-Torquemada et al.,
74 2009). At the San Pedro del Pinatar desalination plants, Environmental Impact
75 Assessments (EIAs) were carried out from the beginning of the activity and some
76 correction measures applied. Firstly, to avoid the impact on sensitive habitats (seagrass
77 meadows), since January 2006 the discharge has been carried via a pipeline to a deep
78 zone. However, due to the low levels of hydrodynamic activity there, salinity reached
79 higher values close to the discharge point and its effect on benthic communities was
80 detected, particularly on polychaete assemblages. Therefore, aimed at decreasing its
81 salinity, a diffuser was added at the end of the pipeline in May 2010 to facilitate the
82 mixture of the effluent with the ambient water (Loya-Fernández et al., 2012).

83

84 During the past ten years, studies have been conducted to assess the impacts of
85 desalination effluent on soft-bottom benthic communities (Raventos et al., 2006; Shute,
86 2009; Del-Pilar-Ruso et al., 2009; Riera et al., 2012). However, to date, few studies

87 assess benthic community recovery after the implementation of corrective measures.
88 One study conducted by Fernandez-Torquemada et al. (2013) showed the recovery of
89 echinoderm densities when the brine was diluted with seawater prior to discharge. In the
90 same area we also detected a recovery of polychaete assemblage after the dilution
91 (Unpublished data). The aim of the present study was to analyse the effectiveness of the
92 mitigation measure applied, using a polychaete assemblage as bioindicator of benthic
93 community status (Pocklington and Wells, 1992). Due to their ecological flexibility,
94 since this group present a wide geographical range and contains both tolerant and
95 sensitive species, they show an extraordinary ability to adapt to a whole range of
96 habitats and environmental variations (Fauchald and Jaumars, 1979). This taxonomic
97 group is therefore considered one of the best indicators of changes in environmental
98 disturbances (Pocklington and Wells, 1992). They can also be considered as an
99 alternative to studying the whole community (Olsgard et al., 2003; Martínez-García et
100 al., 2013; Del Pilar-Ruso et al., 2014).

101

102 **2. Material and Methods**

103

104

105 *2.1. Study area and desalination plants*

106

107 The present study was focused on the San Pedro desalination plants located in southeast
108 Spain ($37^{\circ}50'34.5''\text{N}$, $0^{\circ}46'15.6''\text{W}$) (Fig. 1), where two facilities are operating with a
109 maximum production capacity of 65,000 m³/day each. This output involves a high
110 volume of effluent water with a high salinity (around 70), from both plants via a 5 km

111 pipeline (1293 mm diameter) into a deep zone around 33 m in depth. The main chemical
112 discharged with the brine is the antiscalant PermaTreat® PC-1020T that is used at a
113 concentration of 1.1 ppm. The prevailing currents in the area run parallel to the shore-
114 line. Near the bottom, currents move mainly in a southerly direction but surface currents
115 move mainly northwards, with an average speed of 10.03 cm/s (Del-Pilar-Ruso et al.,
116 2009). A diffuser was added at the end of the pipeline in May 2010 to facilitate mixture
117 and dispersal (Supplementary material).

118

119 The study area was characterised by the heterogeneity of the sediments where two
120 anthropogenic impacts merge (a sewage outfall being the other effluent factor) (Fig. 1).

121 The sediment types remained consistent throughout the study period and a previous
122 investigation showed no synergic effects of both impacts together (Del-Pilar-Ruso et al.,
123 2009).

124

125 *2.2. Sampling method*

126

127 To monitor the brine plume (Fig. 2), the vertical distribution of salinity was measured
128 using a RBR XR-420 CTD sensor, range 0-70 and resolution ± 0.01 . The spatial data
129 was represented by means of the Surfer v9 program. The data was previously
130 interpolated using the kriging technique as a gridding method in each campaign
131 (Fernández-Torquemada et al., 2009). Two salinity surveys, one during the activity
132 (autumn 2008) and the other after the diffuser was installed (autumn 2010) were
133 represented to show the difference in plume extent before and after implementing the
134 mitigation measure.

135 To assess the polychaete assemblage, an eight-year study was conducted at 12 stations
136 with a depth range of 29-38 m during autumn 2005-2012, in the surroundings of a brine
137 discharge outfall (at station B2). One sampling period (T1 = 2005) took place prior to
138 the desalination activity (Previous), four during the activity (T2 = 2006; T3 = 2007; T4
139 = 2008 and T5 = 2009) (Activity) and the last three (T6 = 2010; T7 = 2011 and T8 =
140 2012) corresponding to the period after the diffuser piece installation (Mitigation
141 measure). Four replicates were taken using a Van Veen grab (0.04 m²), three for biotic
142 analyses and one for the analyses of environmental features. The biotic analysis samples
143 were sieved through a 0.5 mm mesh screen and fixed in 10% buffered formalin, then
144 preserved in 4% formalin for later sorting and identification of the polychaete
145 assemblage to the family level. The additional sample was used for granulometric
146 analyses following Buchanan's methodology (1984). Bottom salinity values were also
147 obtained by means of a CTD at each station and in each of the eight sampling periods.

148

149 2.3. Spatial analyses

150

151 Permutational multivariate analysis of variance (PERMANOVA) was used to analyse
152 changes in the structural parameters of the polychaete assemblage, such as total
153 abundance, family richness and diversity. The family richness was expressed as the
154 number of families found in each sample and the diversity index of each sample was
155 determined using the Shannon-Wiener index ($H'(\log_2)$) (Shannon and Weaver, 1963).

156

157 Changes in these parameters were evaluated between two fixed factors: 1) the level of
158 Exposure (EL) to the plume with two levels: Exposed (E) (B2, based on the measure

159 plume area) and Not Exposed (NE), and 2) Period of the activity, with three levels:
160 Previous to the activity (Pre), during the Activity (Act), and once the mitigation measure
161 was implemented (Mm). Pairwise comparisons were performed whenever significant
162 differences were detected among the interaction terms or the main factors (Anderson et
163 al., 2008). All PERMANOVAs were performed on untransformed data and similarities
164 among samples were calculated using the normalised Euclidean distance (Clarke and
165 Warwick, 1994). To obtain sufficient statistical power, p-values were generated after
166 4999 permutations of residuals (Anderson, 2001). These analyses were performed using
167 the PRIMER v.6 with the PERMANOVA+add-on (PRIMER-E Ltd., Plymouth, UK
168 (Anderson et al., 2008).

169

170 Additionally, graphical representation of multivariate patterns of the polychaete
171 assemblage was obtained by non-metric multidimensional scaling (nMDS), to help
172 detect any possible change in composition of the assemblage in relation to the activity.
173 Similarity percentage (SIMPER) procedure was used to determine the percentage
174 contribution of each polychaete family implicated in assemblage changes. Triangular
175 similarity matrices were calculated using the Bray-Curtis similarity coefficient on
176 untransformed abundance data for all taxa identified throughout the study period. All
177 multivariate analyses were performed using the PRIMER statistical package (Clarke and
178 Gorley, 2006).

179

180 A PERMANOVA was also applied to test differences in abundance of the polychaete
181 families involved in the changes detected at the station close to the brine discharge
182 thorough the three periods of the activity: (Previous, Activity and Mitigation measure).

183 Pairwise comparisons were also performed whenever significant differences were
184 detected.

185

186

187 **3. Results**

188

189 *3.1. Salinity campaigns related to the desalination activity and the corrective measures.*

190

191 The highest bottom salinity values were obtained around the discharge station (B2)
192 during autumn 2008 (Fig. 3), while the extension of the brine plume did not affect the
193 stations located 2 km away from the discharge, to both the North and South. Bottom
194 salinity values close to the pipeline outfall decreased significantly and the brine
195 dispersion was also reduced during autumn 2010, once the diffuser was installed (Fig.
196 3). Therefore, the initial impact of the desalination plant on the soft-bottom community
197 was confined to a very small area, since no impact was observed at stations at 250 m.

198

199

200 *3.2. Polychaete assemblage*

201

202 A total of 16069 individuals were counted at all the stations during all the surveys. They
203 were grouped into 47 families. The polychaete assemblage was mainly dominated by
204 the families Paraonidae (17.85%), Lumbrineridae (11.6%), Syllidae (9 %), Magelonidae
205 (7.6%), Cirratulidae (6.5%) and Nephtyidae (5%). A high percentage of rarity (57%)

206 was detected in the study area. Twenty seven families were represented by less than 1%
207 of the total abundance.

208

209 PERMANOVA of the total abundance data (including rare families) revealed significant
210 differences for the factor Exposure level ($p = 0.044$) and for the factor Period of the
211 activity ($p = 0.047$) (Table 1). A significant decrease in abundance took place in the
212 stations exposed to the discharge plume due to the desalination activity. Although the
213 recovery was not significant, a trend towards increase was observed (Fig. 4) (Table 2).
214 With respect to family richness and diversity, significant differences for the interaction
215 between two factors (EL X P) ($p = 0.0004$ and $p = 0.002$, respectively) were detected
216 (Table 1). The significant differences were mainly due to the decrease in these
217 parameters at station exposed to the plume during the desalination activity (Act), but no
218 differences were detected between the previous (Pre) and mitigation (Mm) periods. No
219 statistical differences were seen between the stations not exposed to the plume during
220 the three periods of the activity (Table 2, Fig. 5 and 6).

221

222 Temporal differences in these structural parameters (abundance, diversity and family
223 richness) showed inter-annual changes with similar distribution patterns for all the
224 stations. This pattern was interrupted at the station close to the discharge (Fig. 4, 5 and
225 6).

226

227 *3.3. Polychaete distribution pattern*

228

229 The nMDS plot also showed that the polychaete assemblage of the study area was
230 mainly segregated into two groups; muddy and heterogeneous bottoms (Fig. 7). The
231 SIMPER procedure indicated a 67.77 % dissimilarity between these groups. One group
232 was established by the stations A1, A2, A3, B1 and B4 in most of the time periods
233 (57.73 % similarity). This group, which corresponds with the area characterised as a
234 muddy bottom, was dominated by the families Paraonidae, Lumbrineridae,
235 Magelonidae and Cirratulidae (percentage of contribution higher than 5%). The other
236 group, characterised by the heterogeneity of the sediments, was established by stations
237 A4, B2, B3, C1, C2, C3 and C4 over most of the study time periods; except for station
238 B2, which segregated from the main group in 2007, 2008, 2009 and 2010. The families
239 Syllidae, Lumbrineridae, Paraonidae, Onuphidae, Eunicidae, Capitellidae, Cirratulidae
240 and Nephtyidae were responsible for its similarity (45.47%) (see Supplementary data).

241
242 The nMDS also showed changes in the structure of the polychaete assemblage at the
243 station closest to the brine effluent. Analysing the polychaete distribution pattern at this
244 station throughout the sampling period, a temporal segregation was detected with
245 respect to the pre-impact period (Fig. 7). This segregation appears to correspond to
246 changes detected in salinity, since the bottom salinity was around 37.41 ± 0.04 at all
247 stations except B2, where salinity exceeded 39 from 2006 to 2009 (reaching 49 in
248 2008). However these salinity values decreased once the diffuser was applied in 2010
249 (Fig. 3). Sabellidae, Syllidae, Cirratulidae, Capitellidae, Lumbrineridae, Paraonidae,
250 Flabelligeridae, Scalibregmatidae, Nephtyidae, Magelonidae and Dorvilleidae are the
251 families showing differences in B2 through time (Table 3). Examining Fig. 8 in more
252 detail, some families like Magelonidae ($p = 0.339$) and Paraonidae ($p = 0.141$) showed a

253 similar pattern to the rest of the stations (no differences between the periods of activity
254 were detected). Syllidae ($p = 0.002$) and Capitellidae ($p = 0.011$) decreased in
255 abundance during the activity, showing a recovery in abundance once the mitigation
256 measure was implemented. However, some families such as Sabellidae ($p = 0.001$) and
257 Cirratulidae ($p = 0.002$), which also decreased in abundance from the beginning of the
258 discharge, later show an upward trend in abundance, but significant differences were
259 still not detected between activity (Act) and mitigation (Mm) periods (Fig. 8).

260

261

262

263 **4. Discussion**

264

265 Brine discharge has been described as an activity that induces a pressure in a place
266 where the salinity values were once stable (Garcia and Ballesteros, 2001, Lattermann
267 and Höpner, 2003). This abrupt change in salinity is highest at the discharge outlet
268 (Del-Pilar-Ruso et al., 2009). However, the impoverishment of the community,
269 characterised by the dominance of a few opportunistic tolerant species, can extend to a
270 larger area if the activity remains without any mitigation measures.

271

272 As one of their main objectives, management interventions attempt to restore the
273 biodiversity of degraded ecosystems (Elliot et al., 2007). Possible mechanisms to reduce
274 potential environmental effects of brine would be to select a suitable discharge site
275 (Tsiourtis, 2008), to dilute the discharge (Baalousha, 2006) or to encourage more rapid
276 mixing (Altayaran and Madany, 1992). The diffuser added at the end of the pipeline

277 discharges the effluent at 60° to the horizontal, facilitating mixture of effluent, reducing
278 its salinity and dispersion area (Loya-Fernández et al., 2012). This shows that the
279 implementation of this corrective measure can lead to a recovery of the polychaete
280 assemblage, previously affected by the discharge of a high salinity effluent from a
281 SWRO desalination plant. The initial impact on the soft-bottom community seems to be
282 confined to a very small area since only the closest station was affected.

283

284 The recovery in polychaete assemblage descriptors, such as family richness and
285 diversity was notable, conforming a similar assemblage to that in the previous stage
286 (Fig. 4, 5, 6). The improvement in these descriptors was observed from 2010 onwards,
287 when the mitigation measure was applied. However, the recovery in abundance was not
288 so evident, due to inter-annual changes at all the stations except the closest to the
289 discharge (B2). Not only were the assemblage descriptors modified but also the
290 polychaete assemblage structure, showing changes in the composition of polychaete
291 families throughout the time (Fig. 7).

292

293 Benthic recovery processes vary considerably and are dependent on the type of stress,
294 and the temporal and spatial scales of disturbances (Johnson and Frid, 1995; Karakassis
295 et al., 1999; Gray et al., 2002). An understanding of the costs and trajectories of the
296 environmental recovery of degraded aquatic systems is increasingly necessary to allow
297 policy makers and regulators to formulate robust, cost efficient and feasible
298 management decisions (Pascual et al., 2012). Smith et al. (2006) discussed in their
299 survey how it took several years for the macrobenthos to recover from high pollution
300 levels after the cessation of stresses such as sewage sludge, pulp mill effluent and fish-

301 farming. Although in some cases substantial recovery has taken less than 5 years (Borja
302 et al., 2010; Kelley et al., 2014), full recovery of coastal marine and estuarine
303 ecosystems from over a century of degradation can take a minimum of 15-25 years to
304 attain the original biotic composition. Karakassis et al. (1999) showed that compared
305 with other studies the recovery from sediments left by fish farming is more rapid, as the
306 spatial scales of their impact are smaller and the constituents (fish-feed and faeces) are
307 more labile than most types of industrial or sewage waste. However, the recovery
308 succession in the case of fish-farm waste is not necessarily simple or monotonic, since a
309 complex of factors including different biogeochemical processes could be involved.

310

311 In this study, the benthic community improvement after the implementation of a
312 corrective measure, appears to be relatively rapid and may take place over months rather
313 than years. A recovery in polychaete assemblage parameters (abundance, family
314 richness and diversity) was observed 5 months after the implementation of this
315 mitigation measure. The reason may be because this effluent is characterized merely by
316 high salinity and low nutrient content. Once the main pressure is reduced by simply
317 improving its mixing with the surrounding water, the polychaete assemblage returns to
318 the pre-impacted state faster than after the cessation of other kinds of discharge that
319 potentially increase the long-term pollutants levels in the sediment. Fernández-
320 Torquemada et al. (2013) also detected a rapid increase in echinoderm densities in
321 seagrass meadows once the brine discharge was diluted.

322

323 In contrast to other types of pollution, the knowledge of the role of polychaetes as
324 indicators of brine impact is scarce. A detailed description of the behaviour of those

325 polychaete families involved in the structural changes of the community throughout the
326 periods of the activity suggests they have different levels of tolerance/sensitivity to it, as
327 well as different recovery rates. Families such as Syllidae and Capitellidae became less
328 abundant from 2006 onwards, showing recovery once the mitigation measure was
329 applied. However, other families such as Sabellidae and Cirratulidae, also decreasing in
330 abundance from the beginning of the activity, only seem to increase in the 2012 survey.
331 Contrastingly, families such as Paraonidae and Magelonidae seem not to be affected by
332 this activity. These families maintained similar distribution patterns at the discharge
333 point; even when the activity pressure was increased (Fig. 8).

334

335 Further experimental studies would be necessary to define the sensitivity levels of
336 different polychaete families at different salinity values and their recovery capacity.

337

338 **5. Conclusions**

339

340 In our study a direct link between the implementation of corrective measures and the
341 recovery of benthic community was detected. The deployment of a diffuser piece that
342 increases the mixing of the effluent with seawater reduces the impact of a desalination
343 plant and allows the improvement of the benthic community. Community recovery from
344 this impact was relatively rapid and takes place over months rather than years. To
345 reduce the impact of reverse osmosis desalination plants it is necessary to adopt the
346 measures that maximize the mixing of the effluent during the construction or, like in this
347 case, during the operation of the plant.

348

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350

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357

358

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Table 1.- Results of permutational multivariate analyses of variance (PERMANOVA) performed on the community structure measures parameters: total abundance, family richness and diversity of the polychaete assemblage between Exposure level to the plume, EL-(exposed (E) and not exposed (NE)) and Period of the activity, P- (Previous (Pre), Activity (Act) and Mitigation measure (Mm) (df: degrees of freedom; MS: mean squares, ns: no significant differences and significant results: * significant at $p < 0.05$; ** at $p < 0.01$; *** $p < 0.001$).

Factor	df	Abundance		Family richness		Diversity	
		MS	F	MS	F	MS	F
EL	1	5.79×10^6	24.906*	37.335	2.1243 ^{ns}	2.02×10^{-2}	6.01×10^{-2} ^{ns}
P	2	4.25×10^6	50.788*	156.68	8.915***	2.9019	8.6304**
EL x P	2	2.95×10^6	17.278 ^{ns}	151.95	8.6455***	3.2338	9.6174**
Res	282			17.575		0.33624	
Total	287						

Table 2. PERMANOVA pair-wise comparisons summary. P = factor Period: Pre: previous, Act: activity, Mm: mitigation measure; EL = factor Exposure level: E: Exposed, NE: not exposed. (ms: marginal significance ($p = 0.05$), ns: no significant differences and significant results: * significant at $p < 0.05$; ** at $p < 0.01$; *** $p < 0.001$). The significant results were highlighted to make it understandable.

Pair-wise	Factor	Level of factor	Abundance	
P	P	Previous_Activity	(Pre \neq Act)*	
		Previous_Mitigation	(Pre = Mm) ^{ns}	
		Activity_Mitigation	(Act = Mm) ^{ns}	
	Factor	Level of factor	Family richness	Diversity
	El (P)	Previous	(E = NE) ^{ns}	(E = NE) ^{ns}
		Activity	(E \neq NE) ***	(E \neq NE) ***
		Mitigation	(E = NE) ^{ns}	(E = NE) ^{ns}
EL x P	P (EL)	Exposed	(Pre \neq Act)**	(Pre \neq Act)*
			(Pre = Mm) ^{ns}	(Pre = Mm) ^{ns}
			(Act \neq Mm)***	(Act \neq Mm)**
		Not exposed	(Pre = Act) ^{ns}	(Pre = Act) ^{ns}
			(Pre \approx Mm) ^{ms}	(Pre = Mm) ^{ns}
			(Act = Mm) ^{ns}	(Act = Mm) ^{ns}

Table 3.- Summary of the results of SIMPER dissimilarities at the station B2 throughout the sampling periods: **Previous (Pre)** desalination activity T1 (2005); **during the activity (Act)** T2 (2006), T3 (2007), T4 (2008), T5 (2009) and once the **mitigation measure (Mm)** was implemented T6 (2010), T7 (2011) and T8 (2012). (Av. Abund: average abundance, Contrib. %: percentage of contribution; Cum. %: cumulative percentage, A.D = average dissimilarity).

	Av.Abund	Av.Abund	Contrib%	Cum.%
Time (A.D= 41.27%)	T1_Pre	T2_Act		
Sabellidae	275.00	0.00	21.15	21.15
Syllidae	241.67	41.67	15.38	36.54
Cirratulidae	233.33	75.00	12.18	48.72
Capitellidae	141.67	41.67	7.69	56.41
Lumbrineridae	275.00	183.33	7.05	63.46
Paraonidae	283.33	200.00	6.41	69.87
Flabelligeridae	75.00	0.00	5.77	75.64
Nephtyidae	183.33	116.67	5.13	80.77
Time (A.D= 76.70%)	T1_Pre	T3_Act		
Sabellidae	275.00	0.00	13.92	13.92
Syllidae	241.67	0.00	12.24	26.16
Cirratulidae	233.33	0.00	11.81	37.97
Lumbrineridae	275.00	58.33	10.97	48.95
Paraonidae	283.33	108.33	8.86	57.81
Nephtyidae	183.33	16.67	8.44	66.24
Capitellidae	141.67	25.00	5.91	72.15
Magelonidae	66.67	166.67	5.06	77.22
Time (A.D= 76.64%)	T1_Pre	T4_Act		
Sabellidae	275.00	12.53	13.88	13.88
Paraonidae	283.33	37.59	13.00	26.88
Syllidae	241.67	0.00	12.78	39.66
Cirratulidae	233.33	0.00	12.34	51.99
Lumbrineridae	275.00	75.19	10.57	62.56
Capitellidae	141.67	25.06	6.17	68.73
Nephtyidae	183.33	75.19	5.72	74.45
Time (A.D= 70.07%)	T1_Pre	T5_Act		
Sabellidae	275.00	0.00	15.29	15.29
Lumbrineridae	275.00	25.06	13.89	29.18
Cirratulidae	233.33	12.53	12.27	41.45
Syllidae	241.67	37.59	11.34	52.79
Paraonidae	283.33	125.31	8.78	61.58
Capitellidae	141.67	25.06	6.48	68.06
Nephtyidae	183.33	87.72	5.31	73.37
Time (A.D= 65.28%)	T1_Pre	T6_Mm		
Sabellidae	275.00	12.53	13.37	13.37
Paraonidae	283.33	25.06	13.16	26.54
Syllidae	241.67	0.00	12.31	38.85
Cirratulidae	233.33	12.53	11.25	50.10
Lumbrineridae	275.00	75.19	10.18	60.28
Scalibregmatidae	0.00	100.25	5.11	65.39
Time (A.D= 44.39%)	T1_Pre	T7_Mm		
Sabellidae	275.00	0.00	17.05	17.05
Cirratulidae	233.33	0.00	14.47	31.52
Paraonidae	283.33	87.72	12.13	43.65

Lumbrineridae	275.00	137.84	8.50	52.16
Magelonidae	66.67	175.44	6.74	58.90
Dorvilleidae	25.00	125.31	6.22	65.12
Time (A.D= 36.23%)	T1_Pre	T8_Mm		
Lumbrineridae	275.00	62.66	15.44	15.44
Sabellidae	275.00	75.19	14.53	29.97
Magelonidae	66.67	187.97	8.82	38.80
Cirratulidae	233.33	112.78	8.77	47.56
Paraonidae	283.33	175.44	7.85	55.41
Syllidae	241.67	137.84	7.55	62.96

Figure 1. Map of the study area showing the sampling stations around the brine discharge. The distances between 1 and 2 and between 2 and 3 were 250 m. The distance between 4 and 2 was 1 km. The distance between C3 and C4 was shorter due to sampling problems (UTM coordinate system. Grid zone 30S) (SO: sewage outfall; BD: brine discharge).

Figure 2. Spatial representation of the salinity distribution on the bottom, during the activity (A) and after the implementation of a mitigation measure (diffuser) (B) (UTM coordinate system. Grid zone 30S).

Figure 3. Salinity values at each station throughout the sampling period

Figure 4. Mean values of polychaete abundance (\pm SE) at all stations, throughout the three periods of the activity (Previous, Activity and Mitigation measure)

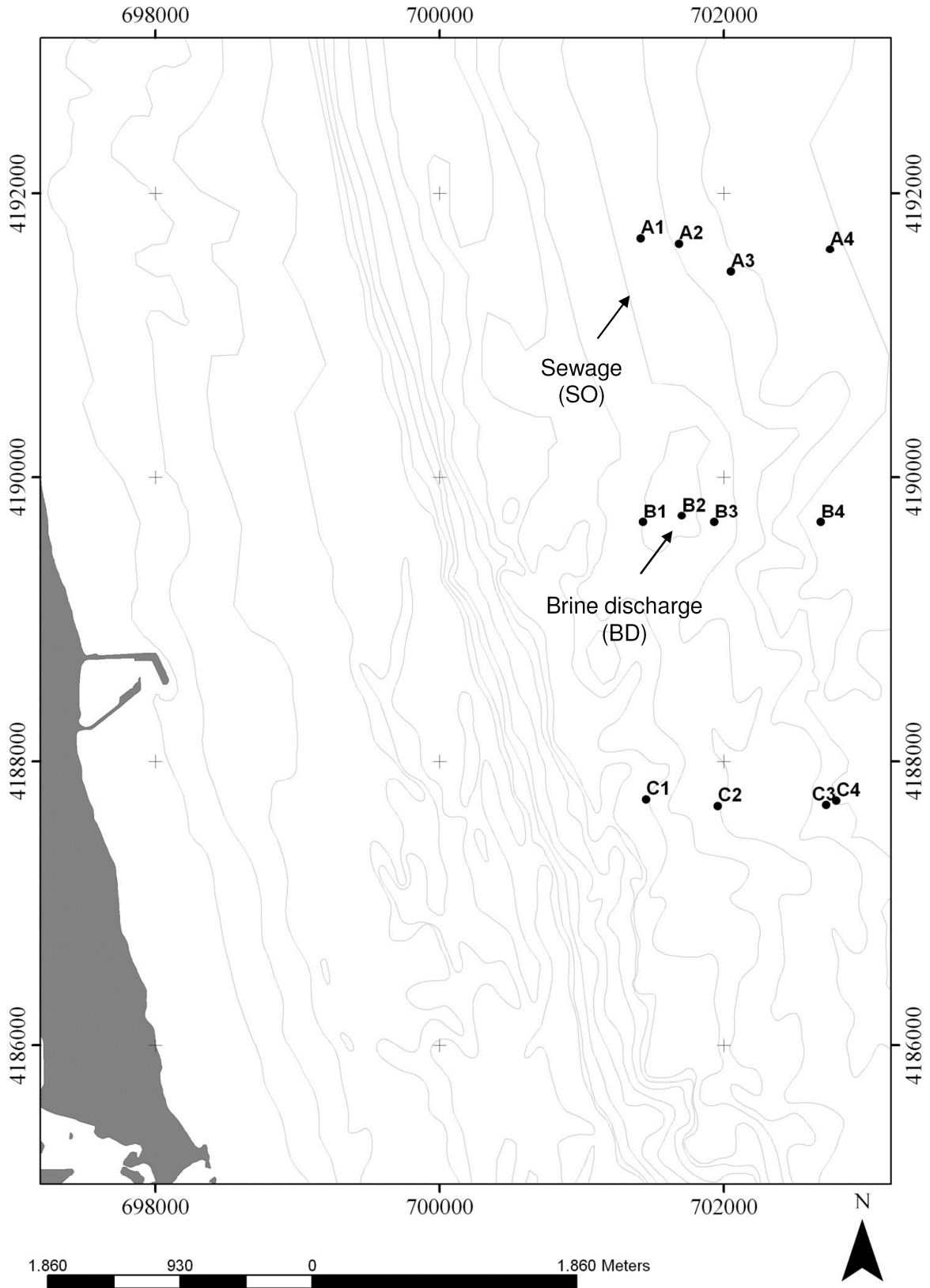
Figure 5. Mean values of polychaete family richness (\pm SE) at all stations, throughout the three periods of the activity (Previous, Activity and Mitigation measure)

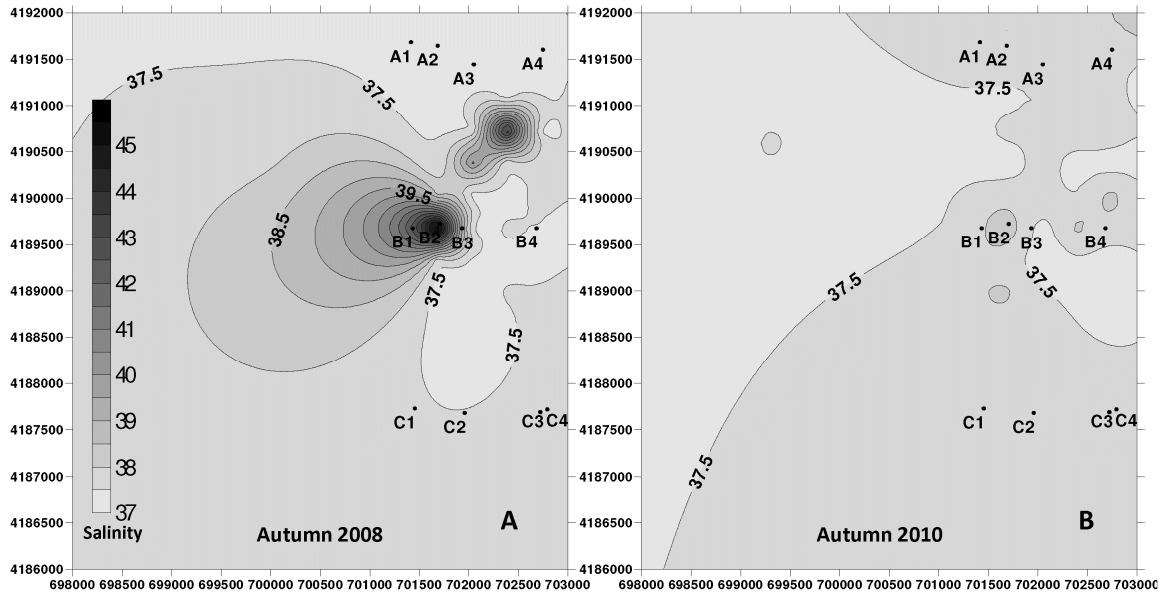
Figure 6. Mean values of polychaete diversity (\pm SE) at all stations, throughout the three periods of the activity (Previous, Activity and Mitigation measure)

Figure 7. MDS analyses based on the Bray-Curtis Similarity of non-transformed abundance data at each taxonomic scale considered throughout the sampling period. The two main groups have been highlighted. Temporal changes in the polychaete assemblage have been highlighted.

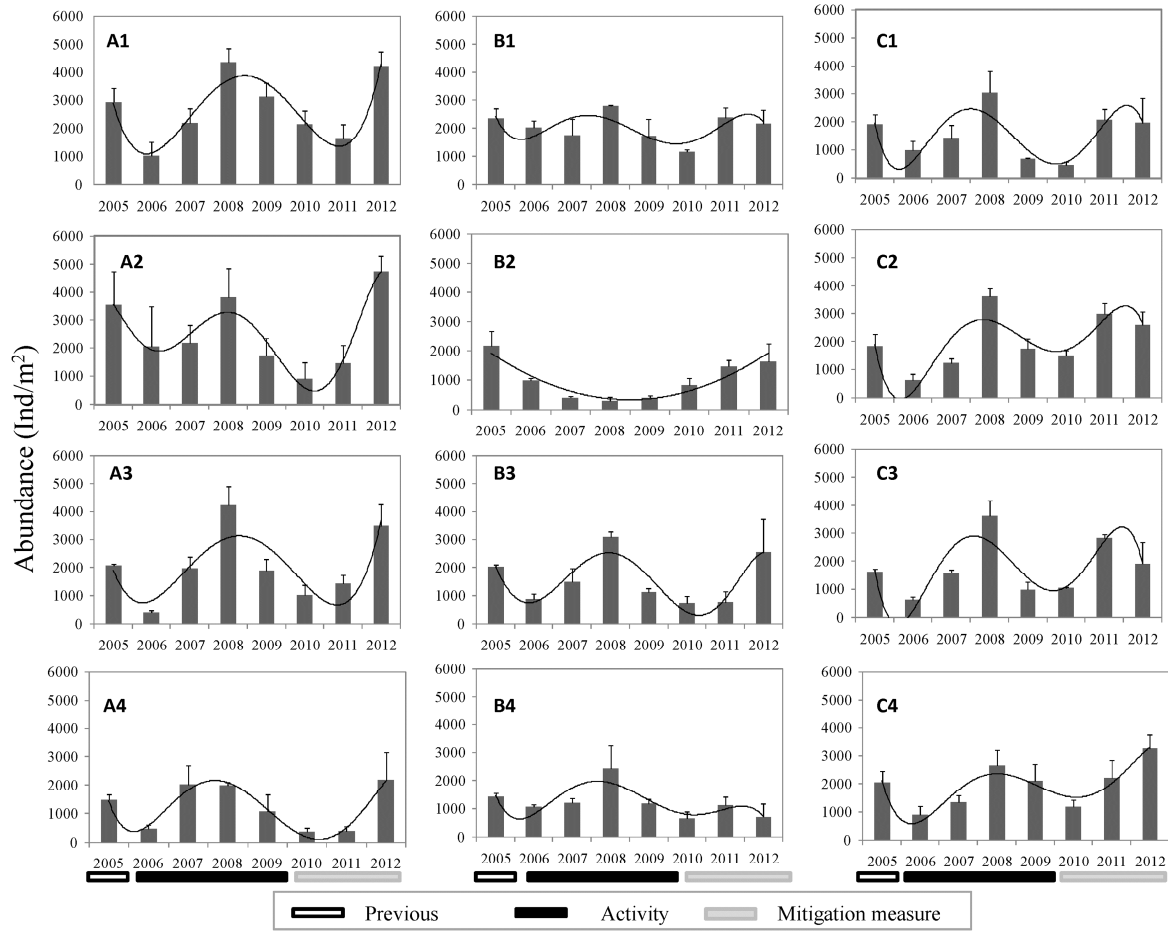
Figure 8. Changes in abundance of indicative polychaete families at the discharge point (B2) through the three periods of the activity: Previous, Activity and Mitigation measure Pairwise summary was included: a and b indicate which stations are statistically similar (those with the same letter) or different (those with different letters).

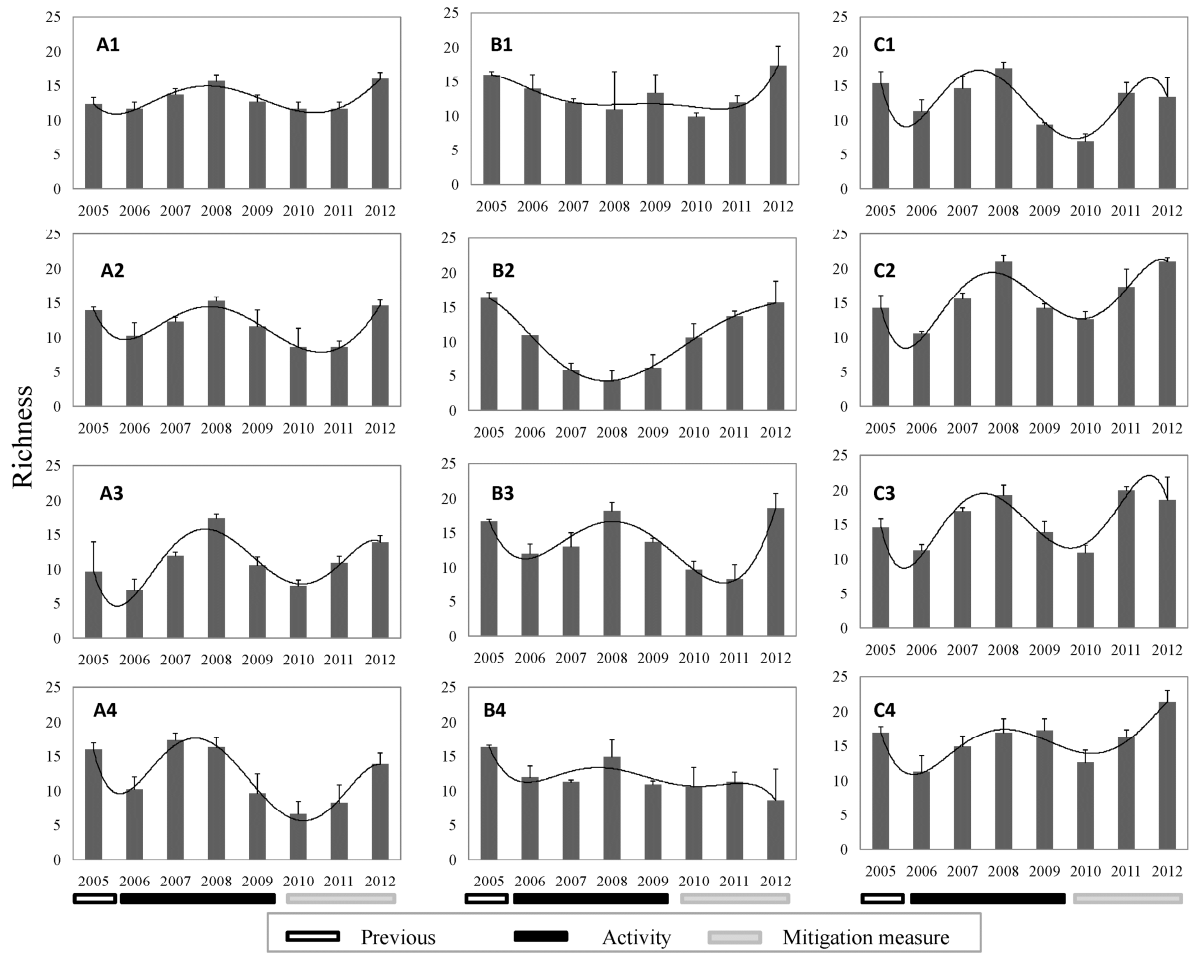
Supplementary material. Schematic of the diffuser piece added at the pipeline end.

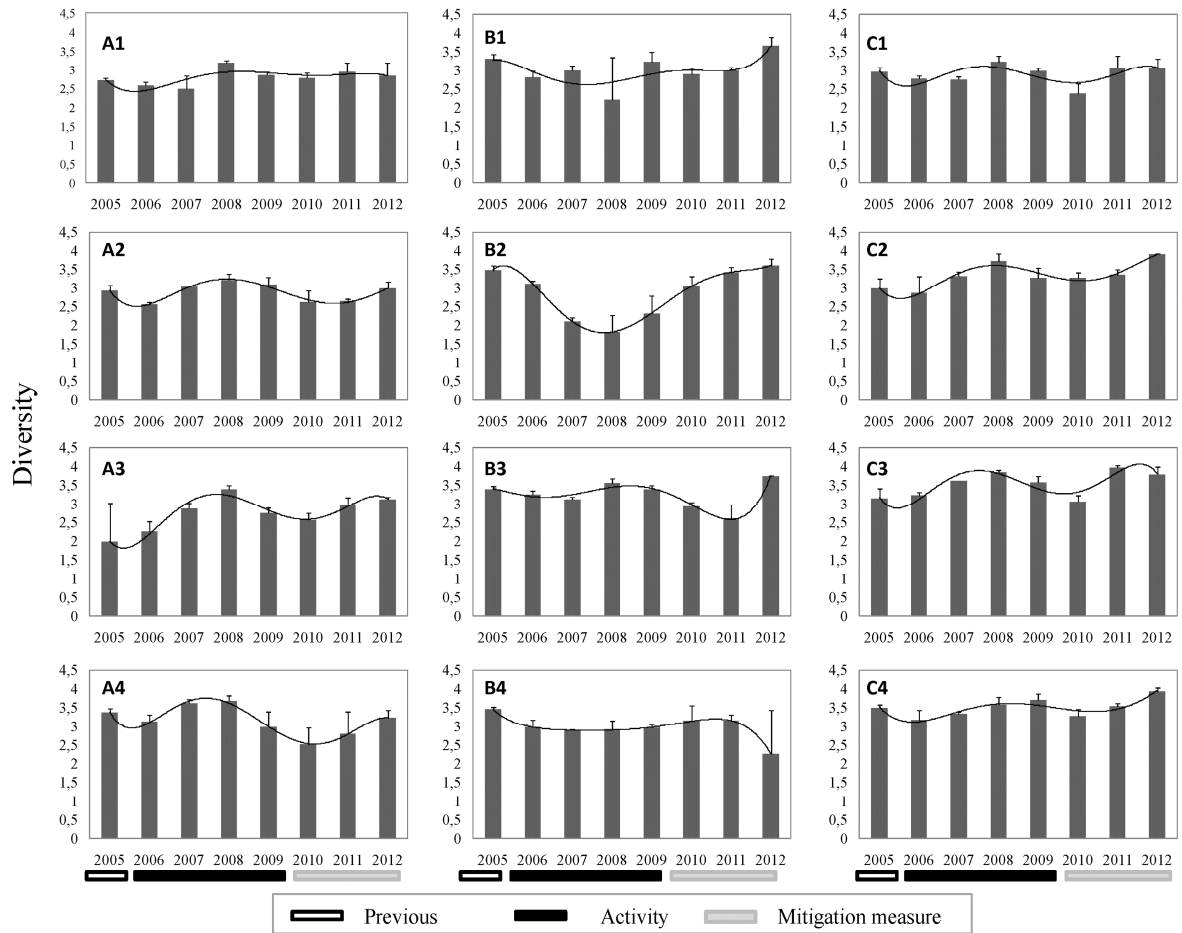


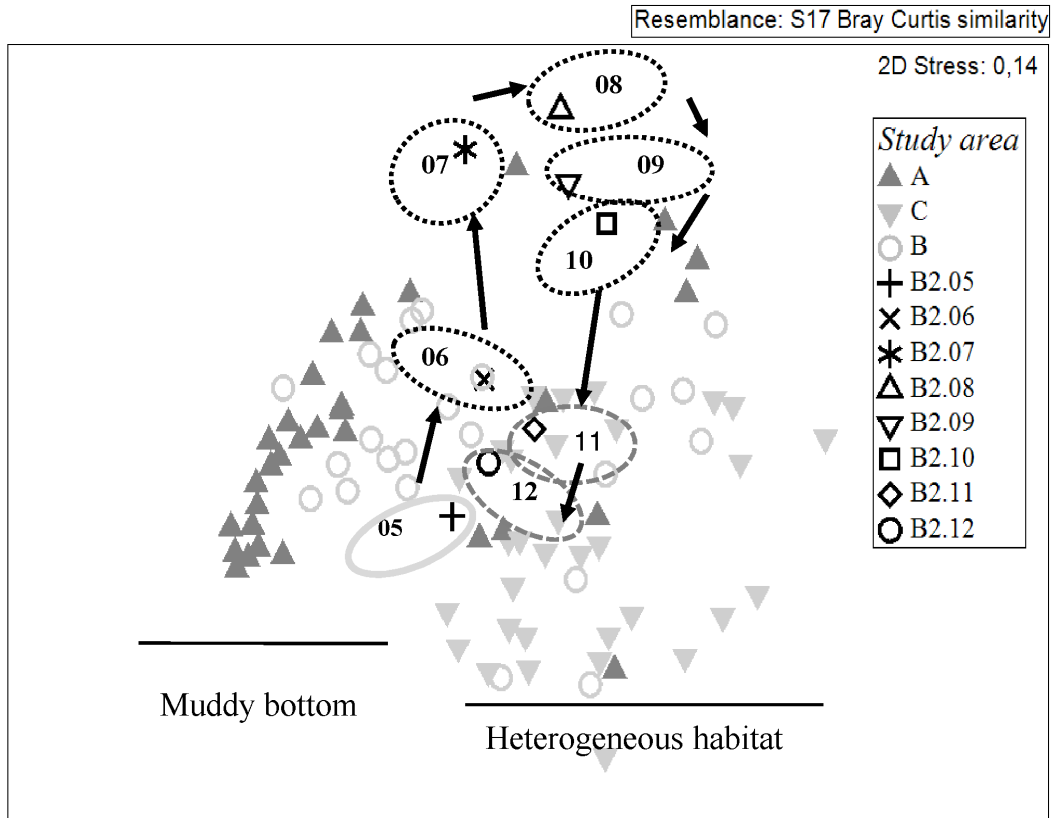


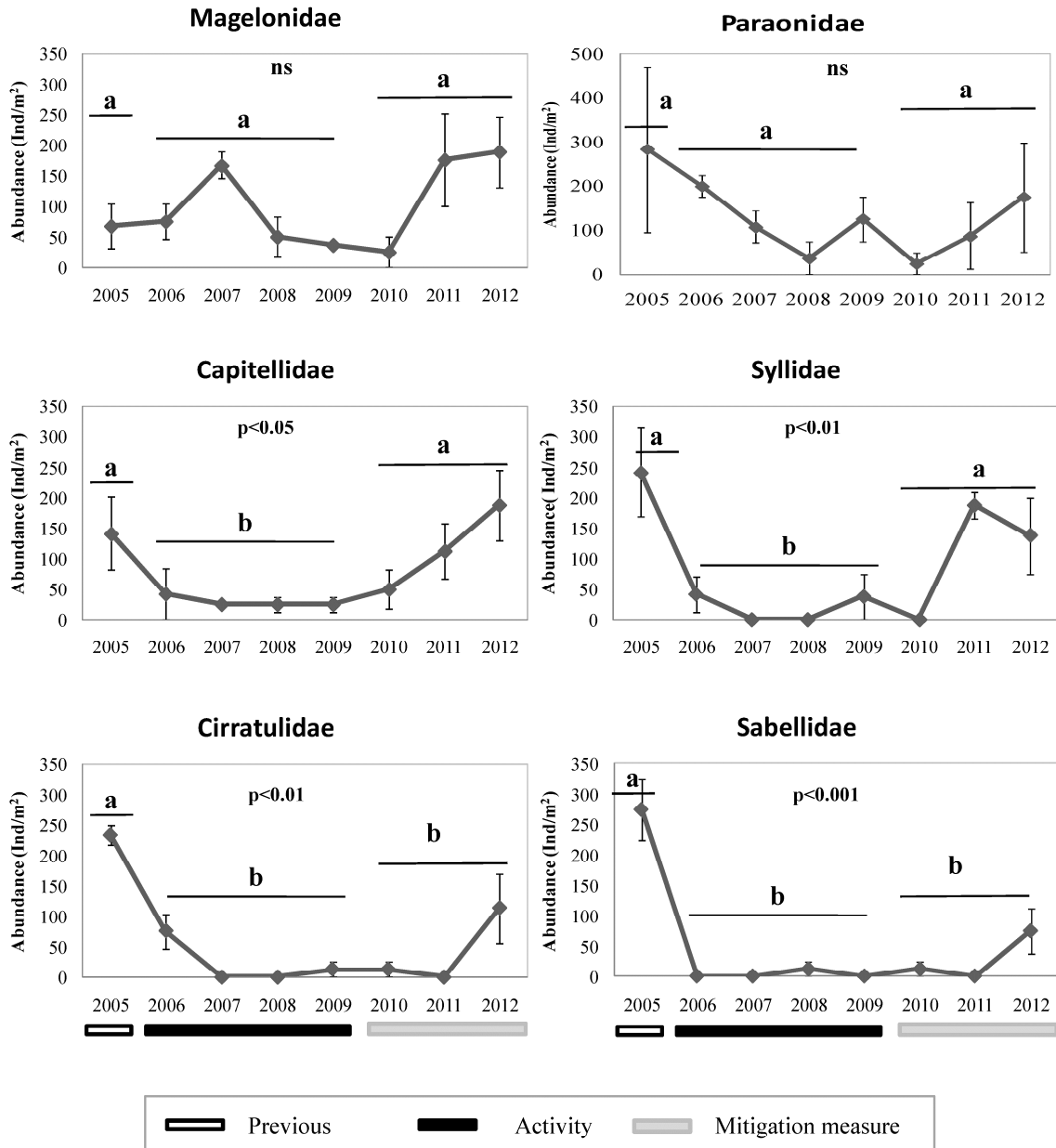
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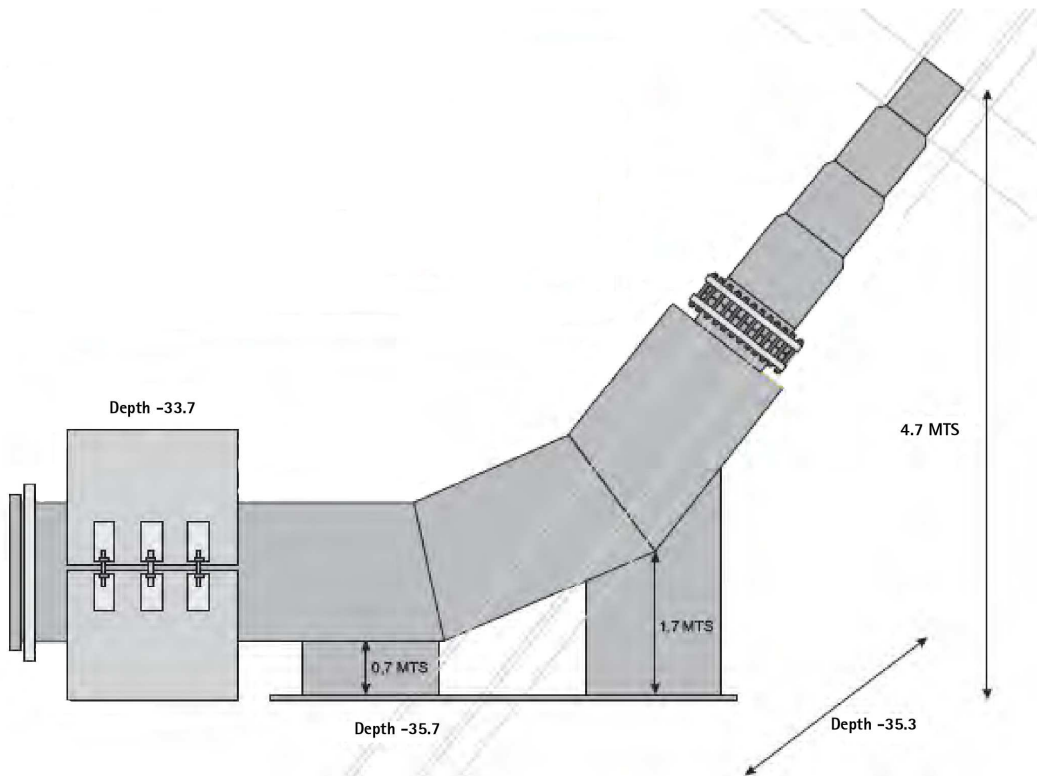


Highlights

- *Effect of brine discharge on benthic community was investigated.
- *A mitigation measure was implemented to reduce the impact of a brine discharge.
- * Recovery on polychaete assemblage parameters was detected after its implementation.
- * The recovery process seems to be relatively rapid.
- *Different recovery rates of polychaete families were detected.

Supplementary data. Summary of the results of SIMPER similarities for muddy and heterogeneous bottoms throughout the sampling periods (2005-2012). (Av. Abund: average abundance, Contrib. %: percentage contribution; Cum. %: cumulative percentage, A.S = average similarity).

<u>Muddy bottom</u>				
A.S: 57.73%				
Families	Av. Abund	Av.Sim	Contrib.%	Cum.%
Paraonidae	671.86	18.53	32.09	32.09
Lumbrineridae	358.18	12.07	20.90	52.99
Magelonidae	261.39	7.93	13.74	66.73
Cirratulidae	175.97	4.07	7.04	73.77
Nephtyidae	110.12	2.85	4.94	78.71
Capitellidae	109.43	2.78	4.81	83.52
Cossuridae	98.44	2.49	4.31	87.84
Paralacydoniidae	69.14	1.64	2.84	90.68
<u>Heterogeneous bottom</u>				
A.S: 45.47%				
Families	Av. Abund	Av. Sim	Contrib.%	Cum.%
Syllidae	262.77	8.57	18.84	18.84
Lumbrineridae	122.99	4.53	9.95	28.79
Paraonidae	120.65	4.31	9.48	38.27
Onuphidae	116.27	4.08	8.98	47.25
Eunicidae	89.04	3.38	7.44	54.68
Capitellidae	72.58	2.63	5.77	60.46
Cirratulidae	82.65	2.50	5.49	65.95
Nephtyidae	79.14	2.32	5.10	71.05
Sabellidae	109.40	1.99	4.38	75.44
Magelonidae	80.13	1.87	4.12	79.56
Dorvilleidae	53.67	1.32	2.91	82.47
Maldanidae	51.20	1.27	2.78	85.25
Paralacydoniidae	36.04	1.02	2.25	87.49
Nereididae	33.97	0.82	1.80	89.29
Ampharetidae	33.98	0.65	1.44	90.73



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