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Response of amphipod assemblages to desalination brine discharge: impact and recovery

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1 **Response of amphipod assemblages to desalination brine discharge:**

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9 **Abstract.**

10 Desalination has become an important industry whose dense, high-salinity effluent has

11 an impact on marine communities. Without adequate dilution, brine remains on the

12 bottom increasing bottom salinity and affecting benthic communities. Amphipods

13 showed high sensitivity to increased salinity produced by desalination brine discharge.

14 A decrease in abundance and diversity of amphipods was detected at the station

15 closest to the outfall, where salinity values reached 53. This salinity was later reduced

16 by including a diffuser at the end of the pipeline. Six months after diffuser installation,

17 amphipod abundance increased. During the first stage of this recovery, species such

18 as *Photis longipes* recovered their abundance, others such as *Microdeutopus*

19 *versiculatus* displayed opportunistic patterns, while others needed more time for

20 recovery, e.g. *Harpinia pectinata*. These differences may be dependent on the

21 organism living habits.

22 **Keywords:** Amphipods, brine discharge, benthos, Mediterranean Sea, recovery,

23 recolonization

24 **1. Introduction**

25 Industrial and urban development in coastal areas has led to an increase in human

26 activities: harbours, wastewater discharge, fish-farming, dredging, oil-wells, shipping

27 etc. These are clearly a potential source of pollution for the coastal marine

28 environment. Such impact induces changes in marine communities due to new

29 physicochemical conditions: decreased hydrodynamic conditions, organic enrichment,

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30 presence of pollutants, hypoxia etc. (Borja et al., 2010). Moreover, new kinds of
31 human activities have been established in recent years in these coastal areas including
32 desalination which has become an important growing industry due to concern over
33 local water scarcity and challenges in meeting future water demand (NRC, 2008). Its
34 main impact on marine communities is caused by the discharge of an effluent with very
35 high salinity. The production of water for public use from seawater leaves behind a
36 concentrated salt solution, 'brine', that can have up to twice the salt content compared
37 to the ambient seawater (Younos, 2005). Without proper dilution, a plume of high
38 salinity discharge may spread out for a considerable distance beyond the mixing zone
39 and harm the ecosystem (Einav et al., 2002). This effluent remains on the sea bottom
40 because of its high density, affecting benthic communities (Lattemann and Höpner,
41 2003). It can cause osmotic pressure changes in cells, leading to mortalities in
42 organisms that are not adapted to these high salinities (Sanchez-Lizaso et al., 2008).
43 The impact may be reduced through dilution of the effluent, either using diffusers or by
44 flushing with normal seawater added to the flow (Fernandez-Torquemada et al., 2009).
45 Such measures facilitate the mixture of the effluent with the surrounding water (Loya-
46 Fernandez et al., 2012), reducing the increase in salinity and its impact on the benthic
47 community (Del-Pilar-Ruso et al., 2015).

48 Among groups that form part of benthic communities, the order Amphipoda is more
49 sensitive to pollution than other organisms (Gomez Gesteira and Dauvin, 2000; Dauvin
50 and Ruellet, 2007). Several studies have reported the response of amphipod
51 assemblages to the impact of oil spills, harbours, wastewater outfalls or fish cages
52 (Dauvin, 1987, 1998; Nipper et al., 1989; Swartz et al., 1994; Ingole et al., 2009; de-la-
53 Ossa-Carretero et al., 2012; Fernandez-Gonzalez et al., 2013). These impacts are
54 detectable as severe changes in the assemblages due to their high sensitivity, with a
55 general decrease in amphipod abundance and diversity when pollution increases
56 (Bellan-Santini, 1980; Conlan, 1994; de-la-Ossa-Carretero et al., 2012, 2015).
57 However, amphipods are capable of inhabiting sediments with different physico-
58 chemical characteristics (Reish, 1993; Thomas, 1993; Gomez Gesteira and Dauvin,
59 2000) and they show various feeding modes and life strategies (Bellan-Santini et al.,
60 1998). These attributes result in differences in sensitivity, habitat requirements or
61 dispersion capabilities among amphipod species (Thomas, 1993; King et al., 2006).
62 Although most Amphipoda are sensitive to different kinds of pollution (Gomez Gesteira
63 and Dauvin, 2000; Dauvin and Ruellet, 2007), several studies have concluded that

some species are more tolerant than others, resulting in changes in amphipod assemblage composition according to the degree of pollution (Bellan-Santini, 1980; de-la-Ossa-Carretero et al., 2012).

The effect of brine on seagrass and its meadows (Fernández-Torquemada and Sánchez-Lizaso, 2005; Gacia et al., 2007), other benthic communities (Del-Pilar-Ruso et al., 2007; Raventos et al., 2006; Riera et al., 2012) and polychaete assemblages (Del-Pilar-Ruso et al., 2008, 2009, 2015) has previously been analysed. However, the impact on amphipod assemblages was only recently reported (de-la-Ossa-Carretero et al., 2015). The main objective of the present research was to determine their response to a brine discharge and assess their recovery succession after application of mitigation measures. Although amphipod sensitivity to this impact is expected, differences among species are hypothesised because of differences in their living habits (biological traits), such as feeding strategies and burrowing behaviour.

2. Materials and methods

2.1. Study site and sampling stations

The San Pedro desalination plants (SE Spain) discharge their effluent by means of a 5 km pipeline at approx. 33 m depth. These desalination plants began operations in 2006 and produce an effluent flow at full capacity of around 150,000 m^3/day characterized by its high salinity (around 70). This discharge caused a salinity increase from 2006 to 2010, reaching bottom salinities of 53 close to outfall. However, in May 2010, the construction of a diffuser at the end of the pipeline to facilitate the mixture of the effluent mitigated this increase to values close to natural salinities (Del-Pilar-Ruso et al., 2015).

The present study is based on the results of the environmental monitoring programme at San Pedro desalination plants, carried out from 2005 to 2014. A benthos survey was performed by establishing three transects perpendicular to the coast: one within the discharge area, following the pipeline, (Transect I) and two control transects 2 km to the north and to the south (Transects N and S). Four distances were sampled at each transect (1, 2, 3 and 4). The distances between 1 and 2 and between 2 and 3 were 250 m. The distance between 4 and 2 was 1 km (Fig. 1).

2.2. Sample collection and processing

95   Samples were collected during 18 sampling campaigns from 2005 to 2014. The first

96   campaign consisted of a previous study before desalination plant activity, campaigns 2

97   to 10 were performed before installing the diffuser, and 11 to 18 afterwards. Four Van

98   Veen grab samples (grab area 400 cm^2) were taken at each station. Three samples

99   were sieved through a 0.5 mm screen, and preserved in 10% formalin. Amphipods

100   were identified using the Mediterranean amphipod fauna key by Bellan-Santini et al.

101   (1982, 1989, 1993, 1998), except for several families that required consulting specific

102   literature (Conradi and López-González, 1995; Krapp-Schickel, 2000; d'Udekem d'Acoz

103   and Vader, 2005; Krapp-Schickel and Sorbe, 2006; White, 2011; Guerra-García et al.,

104   2013). The taxonomy was validated using the European Register of Marine Species for

105   amphipods introduced by Bellan-Santini and Costello (2001)

106   (http://www.marbef.org/data/erms.php, consulted on 12 December 2014). Another Van

107   Veen grab sample was used to characterize the sediment. Grain size was assessed by

108   standard sieve fractionation (Holme and McIntyre, 1984). Redox potential and pH were

109   measured using a CRISON 507 pH meter and organic content of dry sediment was

110   estimated as the weight loss after ashing. Bottom salinity was obtained by means of a

111   RBR XR-420-CTD (conductivity, temperature and depth) logger.

112   **2.3. Data analysis**

113   Data were analysed using the software package PRIMER 6 (Clarke and Gorley, 2006)

114   with the PERMANOVA add-on (Anderson et al., 2008a). Formal tests were done using

115   permutational analysis of variance (PERMANOVA, Anderson, 2001) for either

116   univariate or multivariate data with 9999 permutations of residuals under a reduced

117   model. Analysis was done using a design with five factors in which treatment (impact

118   and control), distance (1, 2, 3, and 4) and period (previous, before diffuser installation,

119   after installation) were fixed factors and transect (I, N and S) and time (T1-T18) were

120   random factors nested in treatment and period respectively.

121   Univariate data, diversity and total abundance, were examined by PERMANOVA based

122   on Euclidean distance. The diversity of each sample was determined using the

123   Shannon Wiener index (H' (log_e). Univariate data were square root transformed prior to

124   the analyses, to avoid heterogeneity of dispersions (Anderson, 2001). Multivariate

125   analyses examining the structure of amphipod assemblages as a whole were done on

126   the basis of the Bray–Curtis similarity coefficient, using abundance values of different

127   amphipod species. Beforehand, the values were dispersion-weighted in order to reduce

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128 'noise' produced by species with an erratic distribution, whose abundance indicates a

129 great variance among replicates (Clarke et al., 2006). A dummy variable was included

130 to minimize error caused by an excess of zeros in this analysis and reduce the weight

131 given to the dominant species (Clarke et al., 2006).

132 Non-metric multi-dimensional scaling (MDS) ordination was used to visualize

133 relationships between control samples and stations close to discharge, on the basis of

134 the Bray–Curtis similarity coefficient. Similarity percentage analysis (SIMPER) of

135 abundances was used to determine the species with a higher percentage contribution

136 involved in segregation of affected stations. The BEST procedure with BIOENV

137 algorithm was applied to link the benthic community with physicochemical parameters,

138 specifically to determine which parameter was most correlated with amphipod

139 assemblage changes among sampled stations.

140 In order to study the variation in feeding behaviour in the food-web structure, trophic

141 groups were assigned to each species according to the criteria of Guerra-Garcia et al.

142 (2014): detritivorous (>90% detritus in the digestive tract); detritivorous-herbivorous

143 (90-50% detritus and >10% algae), detritivorous-carnivorous (90-50% detritus and

144 >10% prey), and carnivorous-omnivorous (90-50% prey and >10% detritus). Variation

145 in percentages of different burrowing behaviours was also studied. Species were

146 classified into three types: domicolous (species that build tubes), fossorial (species that

147 burrow using their periopods) and interstitial (species that live in the interstices

148 between grains of sand). The classification for each species was obtained from the

149 current bibliography (see references in de-la-Ossa-Carretero et al., 2012).

150 PERMANOVA was used to test differences in abundance percentages of groups from

151 both classifications. The appropriate transformation for analysis of this kind of data is

152 the arc-sin of the square-root of the proportion.

153 **3. Results**

154 **3.1. Abundance and diversity**

155 A total of 5,566 individuals of 87 species, belonging to 57 genera and 26 families, were

156 identified. Among these species, *Photis longipes* was the most abundant and

157 contributed to 13.7% of total abundance, followed by *Harpinia pectinata* (10.5%) and

158   *Ampelisca typica* (9.9%). These species were also the most common: *A. typica* was

159   present in 43.20% of the samples, *P. longipes* in 42.4% and *H. pectinata* in 29.47%.

160   The highest abundance (2,406 individuals/m^2) was obtained at the station closest to

161   discharge during the first sampling campaign after installing the diffuser (T11). The

162   diversity index ranged from 0 to 2.55, reaching its highest value at station I3 during

163   T16. Significant differences were detected for abundance and diversity in interaction

164   among the factors: distance x treatment x period (Table 1). This interaction indicated

165   that the station closest to the outfall (I2) had significantly fewer numbers of individuals

166   and lower diversity than others during the period before installation of the diffuser,

167   when salinity reached its highest values. Zero abundance was recorded there in most

168   of the sampling campaigns from T2 to T10. A lesser decrease in abundance and

169   diversity with respect to control stations was also detected at stations I1 and I3 during

170   some sampling campaigns in the activity period in which increased salinity was

171   detected (Fig. 2 and 3).

172   **3.2. Distribution pattern**

173   There were also significant differences in the structure of amphipod assemblages in

174   interaction of the factors distance, treatment and period (Table 2). Pair-wise tests of

175   interaction reflected that significant differences were detected between periods before

176   and after diffuser installation, due to variations between the station close to the outlet

177   (I2) and other stations of the same transect during the period before installation of the

178   diffuser.

179   A two-dimensional MDS plot showed the pattern for the station closest to the outfall.

180   This station I2 became separated from the others during the sampling campaigns

181   before installation of the diffuser (Fig. 4), so different groups were established on the

182   basis of this dissimilarity. Station I2 was highly dissimilar during T2, T3 and T8, due to

183   the low amphipod abundance registered, so these sampling campaigns were grouped

184   in "Activity 1". Group "Activity 2" included the station close to discharge during other

185   sampling campaigns of the period before diffuser installation (T4-T7 and T9), while the

186   group "Activity 3" was formed by the last sampling campaign (T10) before the

187   implementation of this mitigation measure. Dissimilarity of stations close to discharge

188   with control stations was lower after diffuser deployment (T10-T13); these sampling

189   campaigns made up the group "Recovery 1". While the last sampling campaigns (T14-

190   T18) were grouped in "Recovery 2" and found the lowest dissimilarity with control
191   stations. The segregation of station I2 was mainly related to bottom salinity (Spearman
192   correlation coefficient: 0.482), due to the higher salinity values obtained from T2 to T9
193   at stations close to discharge (Fig. 4).

194   Among amphipods contributing to the dissimilarity between the groups established in
195   the MDS plot, most species disappeared at the station close to the outfall during pre-
196   diffuser sampling campaigns (Table 3). Only some individuals of *Ampelisca diadema*,
197   *A. typica* and *P. longipes* were found during this period at this station. After diffuser
198   installation, *Leptocheirus pectinatus*, *Megamphopus cornutus*, *Ampelisca* spp., *P.*
199   *longipes, Siphonoecetes kroyeranus* and *Medicorophium annulatum* recovered their
200   abundance. Species such as *Medicorophium runcicorne*, *Microdeutopus versiculatus* or
201   *Pseudolirius kroyeri* showed a peak in abundance after the deployment of the diffuser,
202   though their abundances decreased during the last sampling campaigns. Finally,
203   *Harpinia pectinita* only recovered its abundance during the last period.

3.3. Feeding and burrowing behaviour

205   Regarding trophic groups, detritivorous species were more abundant at most of the
206   stations (Fig. 5). Only during some sampling campaigns, carnivorous-omnivorous
207   species were more abundant at stations I1, I2 and I3. The dominance of detritivores
208   was especially marked at the station closest to the outfall (I2) before diffuser
209   deployment, because carnivorous-omnivorous species were only found at this station
210   in sampling campaigns previous to activity and after diffuser installation.
211   PERMANOVA detected significant differences in the interaction of distance x transect x
212   time in the detritivorous and carnivorous-omnivorous percentages (Table 4). Significant
213   differences were detected in the interaction period x transect and distance x transect in
214   the detritivorous-carnivorous percentage. The post-hoc test identified significant
215   differences between distances in the discharge transect as well as control transects.

216   Regarding burrowing behaviour, domicolous species were more abundant at most of
217   the stations, although fossorial species also presented high percentages at some
218   stations during some sampling campaigns (Fig. 6). These fossorial species were not
219   present at station I2 during the pre-diffuser period, when domicolous species were
220   dominant. A significant difference in the interaction of distance x treatment x period was
221   detected in the percentage of domicolous species, due to their increase at the station

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222 close to the outfall and changes between activity periods at other stations (Table 5). In
223 the case of interstitial and fossorial species, significant differences in the interaction of
224 distance x transect x time were detected. These differences were due to changes in
225 distances in the discharge transect as well as control transects, both before and after
226 diffuser installation.

227 **4. Discussion**

228 **4.1. Sensitivity of amphipods to brine discharge**

229 Mortality of amphipods at the station close to high-salinity brine outfalls indicates their
230 sensitivity to this impact. Before deployment of the diffuser, this discharge led to salinity
231 values between 40 and 53 at the closest station to the outfall. This salinity increase
232 resulted in a decrease in abundance, diversity and indeed the absence of amphipods
233 at this station. Salinity raises lead to amphipods undergoing an osmotic stress that
234 disturbs their osmoregulation. They have to expend additional energy to maintain the
235 haemolymph osmolality and their rates of other physiological processes decrease
236 significantly (Harris and Aladin, 1997). So that when salinity increases above a critical
237 point the individuals die (Hart et al., 1991).

238 However, a lower increase in salinity would not have such a strong effect, as happened
239 at other stations (I1 or I3) where salinity did not reach such high values. Although
240 amphipods showed high sensitivity to the increase in salinity produced by concentrated
241 effluent, they may tolerate a broader range of salinity than other osmoconformer
242 organisms. Osmoconformer organisms such as echinoderms, that are not able to
243 regulate their osmotic pressure, can tolerate only a narrow increase in salinity (around
244 0.3 to 0.4 above maximum natural salinities) (Fernandez-Torquemada et al., 2013).
245 While other organisms, such as Nematodes, may benefit from increased salinities (Del-
246 Pilar-Ruso et al., 2007) and polychaetes as a class showed different sensitivity levels
247 to brine impact, depending on the family (Del-Pilar-Ruso et al., 2008).

248 Presence of *Ampelisca diadema*, *A. typica* or *P. longipes* at the station closest to the
249 outfall during the higher salinity period could indicate certain tolerance of these species
250 to increased salinity. Some species of amphipods are considered euryhaline (Martins
251 et al., 2002), mainly adapted to life in estuaries. In fact, the genus *Ampelisca* has been
252 considered well-adapted to environmental stress (Lowe and Thompson, 1997; Ingole et

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253   al., 2009). However, the presence of these individuals may be due to immigrant
254   specimens coming from nearby stations the brine did not reach; these species are
255   abundant, since we only collected some specimens. More studies are necessary
256   before attributing a euryhaline character to these species.

257   **4.2. Recovery succession**

258   The diffuser added at the end of the discharge pipeline facilitated mixing of the effluent
259   with the surrounding water, resulting in lower salinity and a smaller area of influence
260   (Loya-Fernandez et al., 2012). This measure led to an increase in abundance and
261   diversity of amphipods at the station closest to the outfall, as happened with
262   Polychaeta (Del-Pilar-Ruso et al., 2015). Recovery after brine discharge appears to be
263   relatively rapid, since just six months after diffuser installation an increase in amphipod
264   abundance was detected at the station close to the outfall. Benthic recovery processes
265   depend on the type of stress (Johnson and Frid, 1995; Karakassis et al., 1999; Gray et
266   al., 2002). The time required for amphipod assemblage recovery after an oil-spill can
267   surpass 10 years (Dauvin, 1998; Gomez-Gesteira and Dauvin, 2000). In this way,
268   recovery time in areas previously affected by a rise in salinity is more similar to
269   restoration after physical disturbances that do not leave a "legacy" stressor such as
270   persistent contaminants (Borja et al., 2010). According to Poggiale and Dauvin (2001)
271   recolonization depends on dispersal of individuals from other sites, but when the
272   pollution level is high the immigrant individuals cannot survive. Recolonization after
273   other impacts requires a decreased level of disturbance: organic degradation, reduction
274   of nutrient load, recovery from persistent pollutants etc. (Borja et al., 2010). In the case
275   of the brine discharge, effective effluent mixing produced a rapid return to previous
276   salinity levels, allowing colonization of specimens from nearby sites unaffected by the
277   salinity rise, since the initial impact of brine discharge was confined to a small area
278   (Del-Pilar-Ruso et al., 2015).

279   While assessing a recovery process, it is important to draw a distinction between re-
280   colonization, which is the settlement of new recruits through immigration of adults from
281   outside the area, and restoration, which can be considered as the return of community
282   structure to the previous state (Boyd et al., 2003). In our study, we consider that this
283   first recolonization corresponded to the period until the end of year 2012, while
284   restoration occurred during the last sampling campaigns. During the recolonization
285   process, an increase in the overall abundance and the number of species was detected

286 during the early stages. Among the species that contribute to such recolonization is *P.*
287 *longipes,* abundant at other stations not affected by brine. Lacking pelagic larvae,
288 amphipods recolonize through dispersal and colonization from other populations
289 unaffected by pollution, which act as a reservoir (Poggiale and Dauvin, 2001). This
290 indicates that the adult replacement through the water column is highly important, since
291 amphipods can colonize after arriving as adults transported by currents or as active
292 swimmers (Díaz-Castañeda et al., 1993; Guerra-García and García-Gómez, 2006).
293 Other species, e.g. *Microdeutopus versiculatus* or *Medicorophium runcicorne*, could
294 show opportunistic behaviour, since despite not being abundant at other stations their
295 abundance at the outfall station increased markedly just after diffuser installation, and
296 decrease during the last period of restoration.

297 Finally, species such as *Harpinia pectinata* only recovered their abundance in the last
298 period. These differences among species may be due to their sensitivity level; e.g.
299 while tolerance of the Corophiidae family was previously attributed to other kinds of
300 pollution (Lowe and Thompson, 1997; de-la-Ossa-Carretero et al., 2012),
301 phoxocephalid amphipods appear to be especially sensitive to pollution, normally
302 avoiding contaminated sediments (Okladen et al., 1984). Other aspects that could
303 affect the recovery rate are species distribution or demographic strategies, while
304 species with two generations per year favour rapid colonization more than others;
305 insularity in species distribution could delay their capacity for recolonization (Dauvin,
306 1987).

307 **4.3. Effect of living habit in sensitivity**

308 We observed certain advantages of detritivorous and domicolous species in tolerating
309 and recolonizing stations affected by brine discharge. The specific response of an
310 amphipod species to an impact can depend on the organism living habits, such as
311 feeding strategy and burrowing behaviour (Simpson and King, 2005; King et al., 2006).
312 Detritus is the main food item of most amphipod species (Guerra-García et al., 2014)
313 and of those in the first stages of recovery (Smith and Shackley, 2006; Munari and
314 Mistri, 2014), since it provides a plentiful food source in these bottoms. Other feeding
315 strategies are restricted by the availability of the food source, as occurs in carnivorous
316 species, whose prey may have a limited abundance after the brine impact.

317 Regarding burrowing behaviour, King et al., (2006) reported less sensitivity in tube-
318 dwelling than in epibenthic amphipods. Several reasons could explain why amphipods
319 with different burrowing behaviour respond in different ways (Anderson et al., 2008b).
320 The interstitial water showed a greater increase in salinity than the water column, given
321 the fact that turnover in pore water is lower than in the water column (Huettel et al.,
322 1996; Gacia et al., 2007). Tube-builders are more isolated than free-burrowing species
323 and the tube construction may reduce interstitial water contact with this species. Under
324 high salinity conditions, the amount of energy absorbed by amphipods decreased; this
325 response involves hypoventilation that reduces water flow through their gills and thus
326 decreases ion uptake (Suyan et al., 2013). Domicolous amphipods are able to control
327 salinity by pumping water down into their tubes to facilitate regulation, whereas
328 fossorial and interstitial species depend on interstitial water and their capacity to
329 regulate interchange in gills is lower, being more vulnerable to increases in salinity.

330 **5. Conclusions**

331 Species of amphipods showed sensitivity to abrupt changes in salinity produced by
332 brine discharges from the desalination plants. An increase in salinity above 40 induced
333 mortalities in amphipod assemblages. However, mitigation measures led to a relatively
334 rapid recovery. During the early stages of recovery, an increase in amphipod
335 abundance was detected at the station closest to the outfall. This recolonization was
336 due to a peak in species with opportunistic behaviour, whose abundance soon
337 decreased. It was also aided by adult immigration through the water column of species
338 abundant at other stations. Finally, other species only recovered their abundance
339 during the last stages of the study. This variable recovery capacity could be due to
340 burrowing and feeding behaviour; indeed we observed a certain tendency of
341 detritivorous and domicolous species to tolerate and recolonize sites affected by brine
342 discharge.

343 **Acknowledgements**

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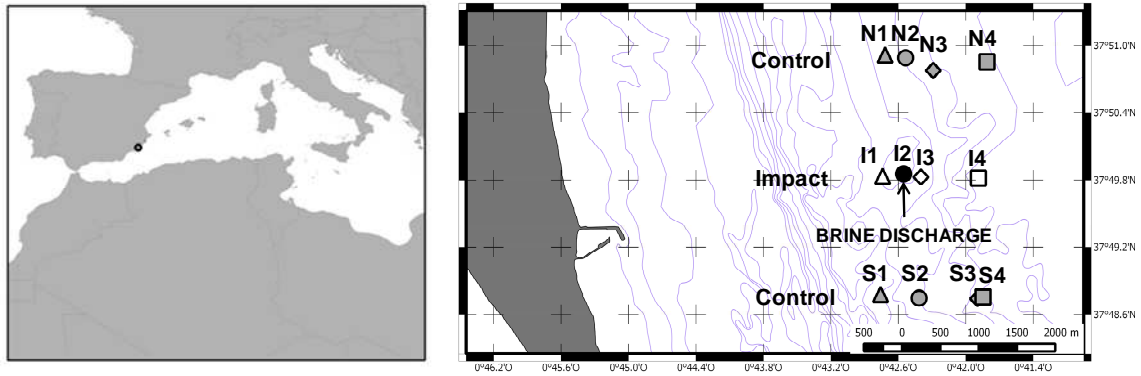557
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575 Figure 1. Studied area showing the sampling stations around the brine discharge.
576 Three perpendicular transects to the coast were established: Transect N, I and S. Four
577 distances were sampled at each transect (1, 2, 3 and 4). Control stations corresponded
578 to transects N (N1, N2, N3 and N4) and S (S1, S2, S3 and S4) while impact stations
579 corresponded to transect I (I1, I2, I3 and I4).

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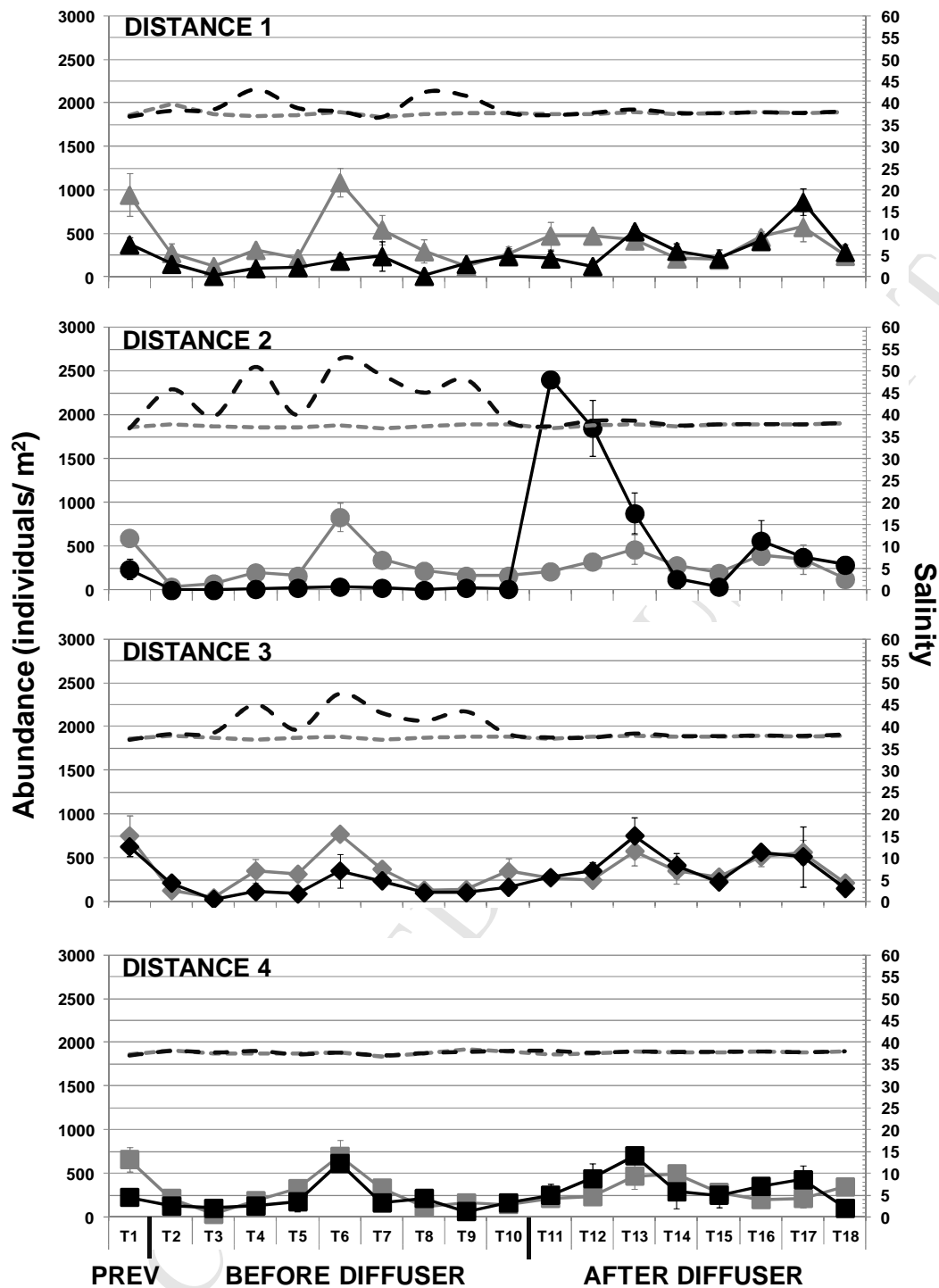
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Figure 2. Mean and standard error of abundance (symbols) and mean salinity (discontinuous line) at each treatment (control: grey, impact: black), distance (1, 2, 3, 4) and sampling campaign. Prev.: previous study before desalination plant activity. Before diffuser: period before addition of the diffuser. After diffuser: period after installation of diffuser.
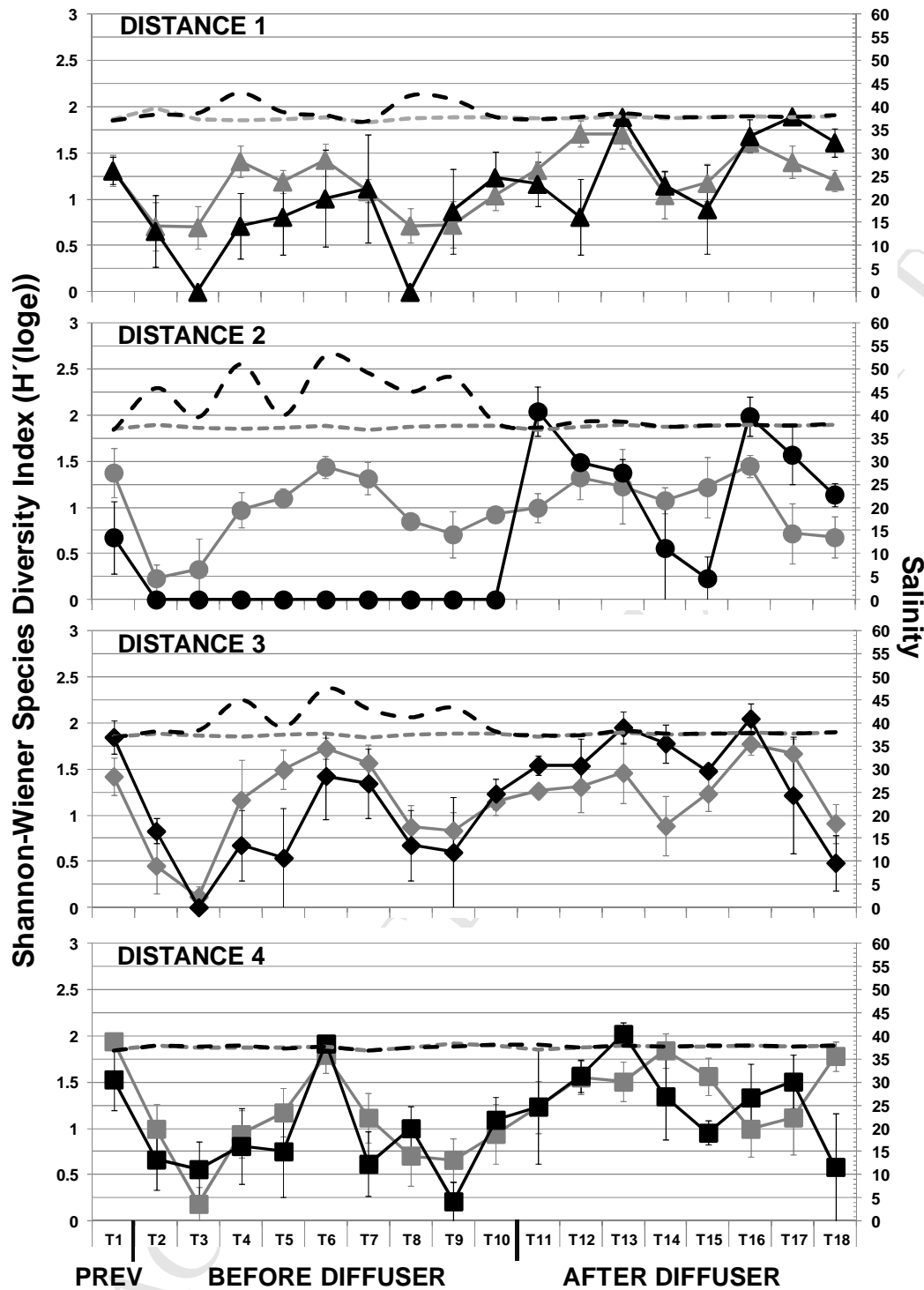
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Figure 3. Mean and standard error of Shannon-Wiener Diversity Index (log based e) (symbols) and mean salinity (discontinuous line) at each treatment (control: grey, impact: black), distance (1, 2, 3, 4) and sampling campaign. Prev.: previous study before desalination plant activity. Before diffuser: period before installation of the diffuser. After diffuser: period after diffuser installation.

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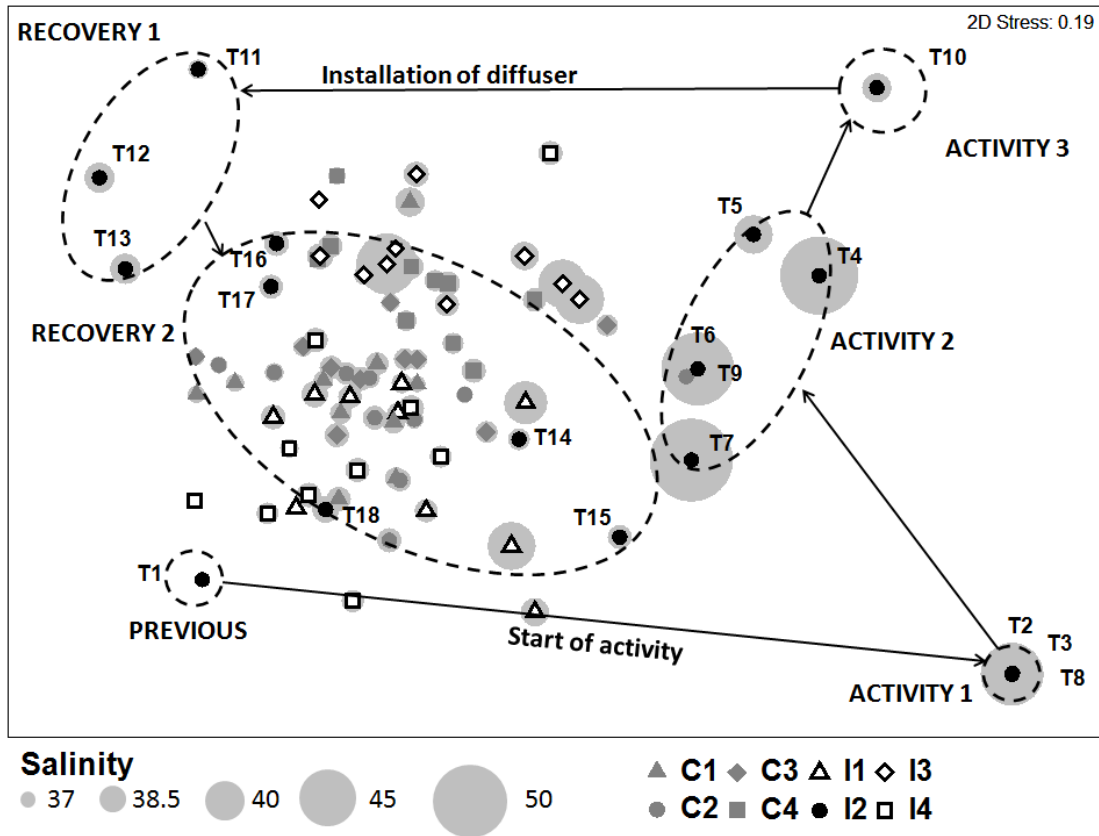
Figure 4. MDS ordination of amphipod abundance and associated stress value of each treatment (Impact and Control), distance (1, 2, 3 and 4) and sampling campaign at Impact 2 station (T1-T18), Bubble plot correlating Amphipod assemblage and mean salinity values. Groups established for SIMPER analysis on the basis of dissimilarity of station I2 are indicated and labelled.

CONTROL 1

IMPACT 1

CONTROL 2

IMPACT 2

CONTROL 3

IMPACT 3

CONTROL 4

IMPACT 4

T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12 T13 T14 T15 T16 T17 T18

PREV   BEFORE DIFFUSER   AFTER DIFFUSER   PREV   BEFORE DIFFUSER   AFTER DIFFUSER

●— detritivorous   ○— detritivorous-herbivorous

◆— detritivorous-carnivorous   ○-- carnivorous-omnivorous
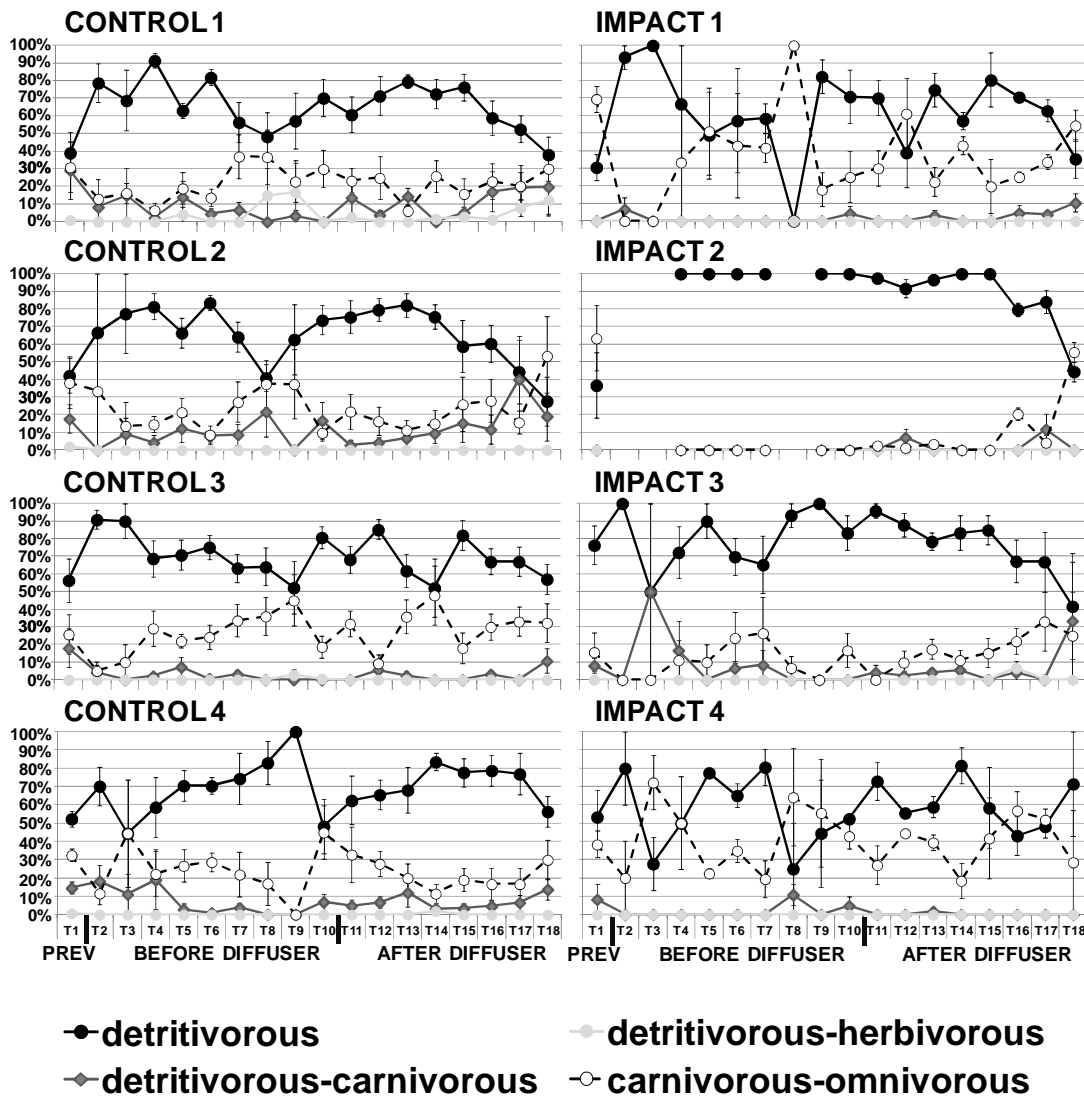
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614   Figure 5. Percentage of Amphipoda individuals for each trophic group at each

615   treatment (control and impact), distance (1, 2, 3 and 4) and sampling campaign. Prev.:

616   previous study before desalination plant activity. Before diffuser: period before

617   installation of the diffuser. After diffuser: period after diffuser installation.

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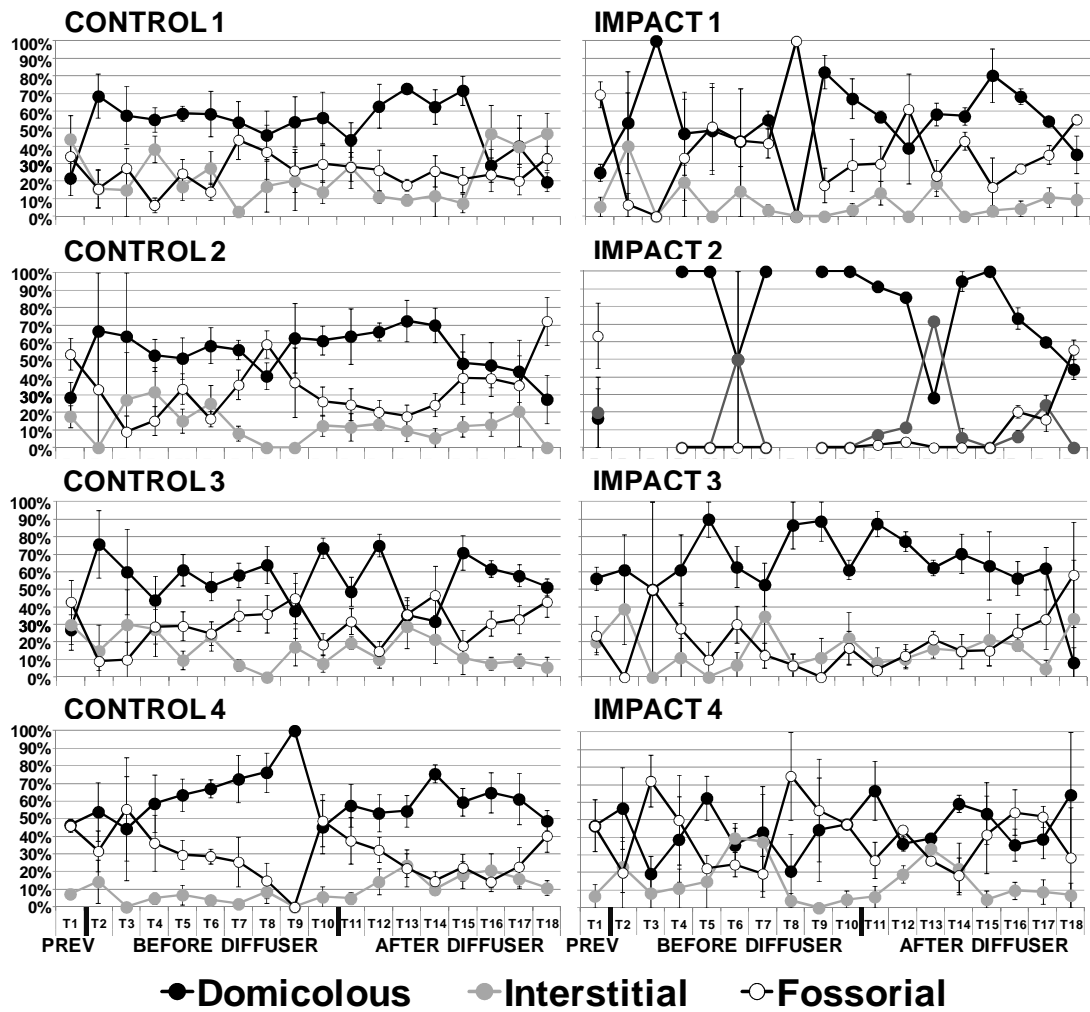
Figure 6. Percentage of Amphipoda individuals for each type of burrowing behaviour in each treatment, distance and sampling campaign. Prev.: previous study before desalination plant activity. Activity: period before installing the diffuser. Mitigation measure: period after installing diffuser.

631 Table 1. Results of PERMANOVA based on Euclidean distance resemblance of
632 abundance and Shannon-Wiener diversity data for the factors distance (Di) , treatment
633 (Treat), transect (Trans), period (Per) and time (Ti), df: degrees of freedom, MS: mean
634 squares, Ps-F: Pseudo-F of each factor. P (perm): permutation P value.

		Abundance			Shannon-Wiener diversity		
	df	MS	Pseudo-F	P(perm)	MS	Pseudo-F	P(perm)
Di	3	149.77	1.105	0.4369	1.1881	5.0635	**0.0035**
Treat	1	787.43	3.6318	0.0638	1.1738	2.0556	0.1943
Trans(Treat)	1	132.9	1.1523	0.2923	0.37948	2.2945	0.1549
Per	2	3712.1	4.5594	**0.0154**	11.563	7.6123	**0.0015**
Ti(Per)	15	551.35	4.7804	**0.0058**	1.2317	7.4471	**0.0009**
DixTreat	3	54.094	0.61322	0.7991	0.50429	2.2745	0.0729
DixPer	6	243.45	2.447	**0.0169**	0.27993	1.3304	0.256
TreatxPer	2	1219.5	3.3059	**0.0424**	1.9499	3.6402	**0.0277**
DixTrans(Treat)	3	119.18	1.4728	0.2328	0.0845	0.35671	0.7458
DixTi(Per)	45	89.588	1.1072	0.3778	0.19689	0.83042	0.7267
TreatxTi(Per)	15	115.68	1.003	0.5061	0.27201	1.6447	0.1595
Trans(Treat)xPer	2	288.11	2.498	0.121	0.30907	1.8688	0.1941
DixTreat xPer	6	295.86	2.6173	**0.0114**	0.73842	2.2522	**0.0291**
Trans(Treat)xTi(Per)	15	115.33	3.7619	**0.0001**	0.16539	1.2037	0.2728
DixTreatxTi(Per)	45	100.99	1.2481	0.2329	0.24139	1.0181	0.4779
DixTran(Treat)xPer	6	42.965	0.53099	0.7576	0.19175	0.80875	0.5373
DixTran(Treat)xTi(Per)	45	80.916	2.6393	**0.0001**	0.23709	1.7255	**0.0043**
Res	432	30.659			0.1374		
Total	647						

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642 Table 2. Results of PERMANOVA based on the Bray–Curtis dissimilarities of the

643 multivariate data set for the factors distance (Di) , treatment (Treat), transect (Trans),

644 period (Per) and time (Ti), df: degrees of freedom, MS: mean squares, Ps-F: Pseudo-

645 F of each factor. P (perm): permutation P value.

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	df	MS	Pseudo-F	P(perm)
Di	3	12444	1.3139	0.0843
Treat	1	12865	0.3580	0.9957
Trans(Treat)	1	49239	7.8944	**0.002**
Per	2	25353	1.3841	0.1117
Ti(Per)	15	9731.8	1.5603	**0.012**
Dix Treat	3	11774	1.2636	0.1154
DixPer	6	8174.1	1.4814	**0.0011**
Treat xPer	2	8666.4	0.8662	0.7226
Dix Trans (Treat)	3	8483.3	1.9815	**0.0079**
DixTi(Per)	45	4245.9	0.9918	0.549
TreatxTi(Per)	15	4114.2	0.6596	0.9983
Trans(Treat)xPer	2	13092	2.0991	**0.016**
DixTreatxPer	6	6912.5	1.3350	**0.0109**
Trans(Treat)xTi(Per)	15	6237.2	2.1915	**0.0001**
DixTreatxTi(Per)	45	4222.9	0.9864	0.5754
DixTran(Treat)xPer	6	4161.8	0.9721	0.5335
DixTran(Treat)xTi(Per)	45	4281.2	1.5042	**0.0001**
Res	432	2846.1		
Total	**647**			

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651 Table 3. Average abundance (individuals/m^2) and average dissimilarity of Amphipod

652 species contributing to a dissimilarity of 70% in controls and stations close to the

653 discharge (previous, activity 1, activity 2, activity 3, recovery 1 and recovery 2). .

Species	CONTROL	PREVIOUS T1		ACTIVITY 1 T2-T3, T8		ACTIVITY 2 T3-T7, T9		ACTIVITY 3 T10		RECOVERY 1 T11-T14		RECOVERY 2 T15-T19	
	Av. Ab.	Av.Ab.	Av.Di.	Av.Ab.	Av.Di.	Av.Ab	Av.Di.	Av.Ab.	Av.Di.	Av.Ab.	Av.Di.	Av.Ab.	Av.Di.
Ampelisca diadema (Costa, 1853)	7.5	0.0	1.4	0.0	2.6	0.0	2.3	12.5	3.7	16.7	1.0	5.0	1.6
Ampelisca tenuicornis Liljeborg, 1855	19.1	0.0	3.6	0.0	6.4	0.0	5.9	0.0	6.1	25.1	1.3	25.1	4.0
Ampelisca typica (Bate, 1856)	36.4	0.0	6.9	0.0	12.5	10.0	8.5	0.0	11.8	25.1	1.8	35.1	4.5
Harpinia ala Karaman, 1987	17.2	0.0	2.6	0.0	4.3	0.0	4.0	0.0	4.1	0.0	0.9	0.0	2.6
Harpinia pectinata Sars, 1891	52.0	112.8	13.9	0.0	14.5	0.0	13.4	0.0	13.8	0.0	2.8	30.1	10.4
Leptocheirus mariae Karaman, 1973	9.4	0.0	1.3	0.0	2.2	0.0	2.0	0.0	2.1	0.0	0.4	2.5	1.5
Leptocheirus pectinatus (Norman, 1869)	12.7	50.1	8.2	0.0	4.1	0.0	3.8	0.0	3.9	62.7	3.1	5.0	2.5
Leucothoe incisa (Robertson, 1892)	10.0	0.0	1.8	0.0	3.1	0.0	2.9	0.0	3.0	8.4	0.8	0.0	1.9
Medicorophium annulatum (Chevreux, 1908)	4.6	0.0	0.9	0.0	1.5	0.0	1.4	0.0	1.4	16.7	1.0	30.1	4.5
Medicorophium runcicorne (Della Valle, 1893)	4.0	0.0	0.7	0.0	1.1	0.0	1.1	0.0	1.1	108.6	4.6	2.5	1.0
Megamphopus cornutus Norman, 1869	7.5	0.0	1.3	0.0	2.1	0.0	2.0	0.0	2.1	37.6	1.6	10.0	2.1
Microdeutopus versiculatus (Bate, 1856)	3.8	0.0	0.6	0.0	1.0	0.0	0.9	0.0	0.9	271.5	9.9	0.0	0.6
Perioculodes aequimanus (Korssman, 1880)	5.9	12.5	2.0	0.0	1.9	0.0	1.8	0.0	1.8	4.2	0.4	0.0	1.1
Photis longipes (Della Valle, 1893)	42.8	0.0	5.8	0.0	9.6	12.5	6.8	0.0	9.2	472.0	18.9	32.6	5.2
Pseudolirius kroyeri (Haller, 1897)	23.2	0.0	3.4	0.0	5.6	0.0	5.2	0.0	5.3	217.2	15.6	15.0	4.2
Siphonoecetes kroyeranus Bate, 1856	4.8	12.5	2.3	0.0	1.6	0.0	1.5	0.0	1.5	25.1	1.3	7.5	1.8

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663 Table 4. Results of PERMANOVA based on Euclidean distance resemblance of

664 frequency of detritivorous, detritivorous-herbivorous, detritivorous-carnivorous and

665 carnivorous-omnivorous species for the factors distance (Di) , treatment (Treat),

666 transect (Trans), period (Per)   and time (Ti), df: degrees of freedom, MS: mean

667 squares, Ps-F: Pseudo-F of each factor. P (perm): permutation P value.

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	df	Detritivorous		Detritivorous-herbivorous		Detritivorous-carnivorous		Carnivorous-omnivorous	
		MS	Ps-F [p]	MS	Ps-F [p]	MS	Ps-F [p]	MS	Ps-F [p]
Di	3	1569.9	$2.78^{0.038}$	24.5	$0.61^{0.792}$	25.6	$0.12^{0.999}$	1578.7	$0.62^{0.802}$
Treat	1	408.4	$0.81^{0.606}$	107.7	$0.92^{0.544}$	3448.2	$0.29^{0.927}$	973.3	$0.10^{0.996}$
Trans(Treat)	1	1147.8	$1.33^{0.2565}$	151.2	$3.10^{0.117}$	13517.0	$\mathbf{35.27^{0.001}}$	18888.0	$\mathbf{21.99^{0.004}}$
Per	2	4892.5	$3.19^{0.059}$	4.1	$2.26^{0.131}$	1002.4	$0.78^{0.627}$	2564.4	$\mathbf{3.98^{0.027}}$
Ti(Per)	15	1388.3	$1.80^{0.154}$	16.0	$0.36^{0.948}$	274.3	$0.81^{0.637}$	681.6	$0.91^{0.570}$
DixTreat	3	1278.0	$2.54^{0.062}$	37.2	$0.74^{0.700}$	682.8	$0.45^{0.913}$	2418.2	$0.90^{0.566}$
DixPer	6	736.1	$1.70^{0.112}$	8.9	$1.54^{0.173}$	157.8	$0.70^{0.780}$	761.5	$1.55^{0.159}$
TreatxPer	2	58.0	$1.06^{0.453}$	7.2	$1.81^{0.207}$	497.5	$0.53^{0.806}$	600.4	$2.75^{0.076}$
DixTrans(Treat)	3	283.1	$0.37^{0.712}$	88.0	$2.23^{0.131}$	1919.4	$\mathbf{8.23^{0.005}}$	2894.5	$\mathbf{5.72^{0.009}}$
DixTi(Per)	45	534.0	$0.70^{0.839}$	19.3	$0.51^{0.956}$	207.6	$0.95^{0.529}$	543.2	$1.15^{0.354}$
TreatxTi(Per)	15	421.3	$0.54^{0.857}$	23.0	$0.51^{0.862}$	99.2	$0.29^{0.978}$	354.8	$0.47^{0.918}$
Trans(Treat)xPer	2	359.6	$0.44^{0.616}$	3.6	$0.10^{0.881}$	1654.3	$\mathbf{4.50^{0.037}}$	125.6	$0.16^{0.833}$
DixTreatxPer	6	352.9	$1.30^{0.275}$	12.9	$1.81^{0.106}$	40.6	$0.54^{0.908}$	469.7	$1.46^{0.194}$
Trans(Treat)xTi(Per)	15	821.7	$\mathbf{2.26^{0.004}}$	46.2	$1.37^{0.182}$	357.9	$\mathbf{2.21^{0.006}}$	802.3	$\mathbf{2.67^{0.001}}$
DixTreatxTi(Per)	42	507.7	$0.66^{0.881}$	17.6	$0.46^{0.974}$	143.1	$0.65^{0.862}$	397.5	$0.84^{0.690}$
DixTran(Treat)xPer	6	343.5	$0.44^{0.801}$	9.7	$0.27^{0.901}$	343.3	$1.52^{0.191}$	243.0	$0.51^{0.758}$
DixTran(Treat)xTi(Per)	44	784.2	$\mathbf{2.16^{0.000}}$	38.3	$1.14^{0.321}$	222.6	$1.37^{0.073}$	483.2	$\mathbf{1.61^{0.013}}$
Res	380	363.8		33.6		162.1		300.2	
Total	591								

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676 Table 5. Results of PERMANOVA based on Euclidean distance resemblance of
677 frequency of domicolous, fossorial and interstitial species for the factors distance (Di) ,
678 treatment (Treat), transect (Trans), period (Per)   and time (Ti), df: degrees of freedom,
679 MS: mean squares, Ps-F: Pseudo-F of each factor. P (perm): permutation P value.

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	df	Domicolous		Fossorial		Interstitial	
		MS	Ps-F [p]	MS	Ps-F [p]	MS	Ps-F [p]
Di	3	469.1	$1.32^{0.314}$	728.7	$0.73^{0.711}$	732.8	$0.90^{0.572}$
Treat	1	564.2	$0.41^{0.850}$	21.0	$0.11^{0.995}$	1842.9	$2.51^{0.139}$
Trans(Treat)	1	2981.0	$3.60^{0.084}$	5969.2	$\mathbf{9.11^{0.011}}$	341.6	$0.67^{0.414}$
Per	2	5987.0	$1.41^{0.300}$	4049.0	$\mathbf{3.42^{0.044}}$	1405.8	$0.50^{0.833}$
Ti(Per)	15	1204.7	$1.64^{0.1885}$	1153.7	$1.97^{0.133}$	940.7	$1.94^{0.117}$
DixTreat	3	1228.7	$1.63^{0.1885}$	2225.4	$1.53^{0.214}$	1256.6	$1.30^{0.3227}$
DixPer	6	803.1	$2.61^{\mathbf{0.0221}}$	694.1	$1.61^{0.141}$	222.1	$1.25^{0.302}$
TreatxPer	2	9.3	$0.18^{0.989}$	261.2	$1.17^{0.401}$	398.4	$0.25^{0.979}$
DixTrans(Treat)	3	686.9	$0.64^{0.506}$	1442.8	$2.05^{0.133}$	1058.9	$1.90^{0.1531}$
DixTi(Per)	45	463.8	$0.46^{0.99}$	526.4	$0.80^{0.73}$	383.5	$0.73^{0.8477}$
TreatxTi(Per)	15	467.5	$0.63^{0.807}$	536.9	$0.91^{0.576}$	547.1	$1.12^{0.4152}$
Trans(Treat)xPer	2	4108.3	$5.13^{\mathbf{0.032}}$	158.7	$0.27^{0.747}$	3231.7	$\mathbf{6.19^{0.0132}}$
DixTreatxPer	6	975.8	$\mathbf{2.15^{0.050}}$	392.7	$1.33^{0.249}$	398.9	$1.68^{0.1145}$
Trans(Treat)xTi(Per)	15	779.5	$\mathbf{2.11^{0.010}}$	613.8	$\mathbf{1.77^{0.039}}$	507.9	$1.66^{0.0609}$
DixTreatxTi(Per)	42	688.0	$0.68^{0.86}$	474.1	$0.72^{0.822}$	345.0	$0.65^{0.9086}$
DixTran(Treat)xPer	6	215.6	$0.21^{0.955}$	309.3	$0.46^{0.790}$	197.1	$0.37^{0.8806}$
DixTran(Treat)xTi(Per)	44	1043.3	$\mathbf{2.83^{0.000}}$	677.5	$\mathbf{1.95^{0.001}}$	538.0	$\mathbf{1.76^{0.0035}}$
Res	380	368.8		347.3		305.8	
Total	591						

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