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2 **Sensitivity of tanaid *Apseudes latreillei* (Milne-Edwards) populations to**
3 **sewage pollution.**

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10 **Abstract.**

11 *Apseudes latreillei* (Milne-Edwards) is a common and abundant tanaid in soft-
12 bottom communities from waters off East Atlantic and Mediterranean coasts. Its
13 sensitivity to pollution is not clear despite being an abundant and widely
14 distributed crustacean, since it has been reported as both a tolerant and
15 sensitive species. This paper tests the sensitivity of *Apseudes latreillei* to
16 sewage discharges in fine sand communities along the Castellon coast (W.
17 Mediterranean). We analysed variation in tanaid populations between sites at
18 varying distances from sewage outfalls with respect to population density, size
19 distribution, sex ratio and their correlation with different abiotic factors of waste
20 water and sediment. Results showed clearly that *Apseudes latreillei* populations
21 were affected by the presence of sewage outfalls, to such an extent that
22 sewage disposal outlets produced a decrease in population density and
23 changes in size spectra.

Keywords: *Apseudes latreillei*; Tanaidacea; Peracarida; Benthic indicators; sewage; soft-bottoms; Mediterranean.

1. Introduction

Apseudes latreillei (Milne-Edwards) is an abundant and common tanaid in Mediterranean and Easter Atlantic coastal waters. It inhabits sandy and muddy bottoms between 0 and 138 meters (Bakalem et al., 2009; Bouchet and Sauriau, 2008; De Juan et al., 2007; Guerra-García et al., 2003; Le Hir and Hily, 2005; Lourido et al., 2008; Marín-Guirao et al., 2005; Moreira et al., 2008; Sanz, 1992), though its presence was also reported in sediments of *Posidonia oceanica* meadows (Como et al., 2008) or in hard-bottom in the volcanic Nisyros island (Greece) (Conides et al., 1999).

Benthic assemblages, due to their high diversity and abundance, could be essential in trophic guilds and could play a vital role in cycling nutrients and materials between the underlying sediment and the overlying water column (Dauvin et al., 2007). Peracarid crustaceans are an important group in the structuring of benthic assemblages, with a relevant contribution to benthic production (Mancinelli and Rossi, 2002) and represent a significant source of food for other benthic animals and fishes of commercial importance (Dauvin, 1988; Lourido et al., 2008).

Benthic assemblages are often affected by sewage discharges which are released via outfall into shallow subtidal habitats and it is a common source of pollution in coastal marine environments (Koop and Hutchins, 1996; McIntyre, 1995). These discharges may alter the organic content and the biochemical

composition of sediments (Cotano and Villate, 2006) causing changes in these soft-bottom benthic communities. Soft-bottom benthos is frequently used as bio-indicators for marine monitoring because of its rapid response to anthropogenic and natural stress (Dauer et al., 2000; del Pilar Ruso et al., 2007; Gray et al., 2002; Pearson and Rosenberg, 1978).

Crustaceans are usually more sensitive to pollution than other marine taxa (Dauvin and Ruellet, 2007; Rand and Petrocelli, 1985). Its distribution and abundance inhabiting marine sediments are influenced by a number of abiotic factors, such as sediment composition (De Grave, 1999; Parker, 1984) and organic content (Robertson et al., 1989). Their abundance and species diversity may be used as indicators of environmental conditions (Conradi and López-González, 2001; Corbera and Cardell, 1995; Dauvin and Ruellet, 2007; Gómez-Gesteira and Dauvin, 2000; Guerra-García and García-Gómez, 2005; Marques and Bellan-Santini, 1987). Therefore it is important to know the extent of their sensitivity to different pollution sources in order to monitor environmental quality or use them as biological indicators. Despite being an abundant and widely distributed crustacean species, *Apseudes latreillei* response to pollution is not clear, since it has sometimes been reported as a tolerant species (de Juan et al., 2007; Grall and Glémarec, 1997; Marín-Guirao et al., 2005) and by others as a sensitive species (Bouchet and Sauriau, 2008; Sanz-Lázaro and Marin, 2006). It is necessary to clarify its response to pollution in order to avoid mistakes when using it as a biological indicator in environmental assessing.

This paper aims to test the sensitivity of *Apseudes latreillei* to sewage in fine sand communities, characteristic of shallow sublittoral soft-bottoms in the north-

western Mediterranean Sea (Cardell et al., 1999; Pérès and Picard, 1964; Sardá et al., 1996; Sardá et al., 2000). These communities are common off the coast of Castellon (NE Spain), and several municipal treatment plants dump treated water into this community. In this study we examined the possible impact of five sewage outfall sites on the *Apseudes latreillei* (Milne-Edwards) populations along the Castellon coast. We analysed changes in population dynamic parameters such as abundance, size distribution, sex ratio and their correlation with characteristics such as sediment and waste water dumped.

2. Material and methods

The study area is located off the Castellon Coast (NE Spain); five locations affected by sewage outfalls along 40 km of coast were analyzed (Figure 1). The minimum distance between outfalls is 7.62 km and the maximum 11.03 km. These outfalls correspond to the villages of Vinaroz (location I), Benicarló (location II), Peñíscola (location III), Alcossebre (location IV) and Torreblanca (location V). The mean length of the pipelines is 2,138 m and the mean sewage outfall depth is 14.8 m. Waste water treatment plants from locations I, II, III and IV utilise a pre-treatment process, which includes an automated mechanically raked screen, a sand catcher and a grease trap. Secondary treatment was only implemented in location V, consisting of biological treatment of activated sludge with biological aerated filters. The study area has a constant water depth, homogeneous bottom sediment and uniform benthic communities. This homogeneous area with an established pollution gradient represents an ideal

site for investigating links between macrofaunal assemblages and the effect of contaminants (de-la-Ossa-Carretero et al., 2009).

For each location, three distances from the discharge (0, 200 and 1,000 m) were sampled, establishing two stations for each distance, with two orientations (north and south) keeping a constant depth (figure 1). Samples were collected in summer, coinciding with the highest rate of sewage disposal, during July of 2004, 2005, 2006 and 2007, at a depth of approximately 15 m. Four Van Veen grab samples (400 cm²) were obtained at each station. Three samples of them were sieved through a 0.5 mm screen, and preserved in 10% formalin to count total infaunal abundance; sorting invertebrate individuals and putting *Apseudes latreillei* individuals aside. The other sample was used to characterize the sediment. Characteristics of the sediment, data of flow and water quality of sewage disposal (suspended solids, BOD (biologic oxygen demand), COD (chemical oxygen demand), phosphates, nitrates and turbidity) were previously included in de la Ossa Carrretero et al. (2008) and de-la-Ossa-Carrretero et al. (2009).

Apseudes latreillei individuals were counted and measured (anterior-posterior length) using the image manager software Leica IM50 after being photographed through a Leica Mz. 9.5 stereomicroscope. Males were differentiated from females and juveniles, observing chelipeds development, more robust in males and with a tooth on the inner margins of the propodus and dactylus (Sanz, 1992) (figure 2). Ovigerous females with presence of eggs or embryos were also counted.

Steam-Leaf Plot of length values of previously identified individual males were obtained in order to obtain a boundary length between immature and mature individuals (lower hinge in Steam-Leaf Plot was considered).

Abundances of *Apseudes latreillei* were examined using 3-factor analyses of variance (ANOVA) with distance and location as fixed factors and year as random factor. Prior to ANOVA, the homogeneity of variance was tested using Cochran's test. Data were $\sqrt{X+1}$ transformed when variances were significantly different. SNK test (Student-Newman-Keuls) was used to determine which samples were implicated in the differences.

Size frequency distributions were grouped and plotted into 11 size classes using a 0.75 mm total length class interval. Since it is not possible to use analysis of variance with the Tanaidacea length data because there were significant differences in sample size, we used a non-parametric Kruskal Wallis test to compare length medians between varying distances from outlet for each location and year. In order to exclude the influence of recruitment, it was decided not to consider measurements of juveniles.

Kruskal Wallis test was also used to determine differences in the percentage of males and in the presence of eggs or embryos in females between distances in each location.

Correlation between mature size, immature, mature and total abundances and wastewater and sediment descriptors were determined using Pearson Correlation, testing probability of each correlation coefficient with Bonferroni test. Waste water descriptors employed included flow, suspended solids, BOD,

COD, phosphates, nitrates and turbidity, and granulometric analysis, potential redox, organic matter and pH were used as sediment descriptors.

3. Results

A total of 47,654 specimens were collected belonging to different invertebrate taxa (Amphipoda, Bivalvia, Cephalochordata, Copepoda, Cumacea, Decapoda, Echinoidea, Gasteropoda, Holoturoidea, Isopoda, Mysidacea, Nematoda, Nemertea, Ostracoda, Ophiuroidea, Polychaeta, Pycnogonida, Scaphopoda, Sipunculida, and Tanaidacea). Of these specimens, 6,006 individuals were identified as *Apseudes latreillei*; representing 12.60% of total macroinvertebrate community abundance. The highest population density detected was 1,033.33 individuals/m², in a station situated at 1,000 m from the outfall of location II.

ANOVA detected significant differences in *Apseudes latreillei* abundances for the interactions between the pairs of factors distance x year and location x year. Moreover, marginal differences were also detected for the interaction distance x location (table 1). SNK results (table 2) showed that marginal differences for interaction distance x location, were due to decrease of abundance in stations closer to outfall at all locations except in location V (figure 3). These decreases produced differences between location V and the others. Differences for interaction distance x year were due to a decrease of abundance in year 2007 (figure 3) which produced not significant differences among distances this year, meanwhile significant decrease in stations located at the outfall was detected in years 2004, 2005 and 2006. Finally, despite the fact that location V usually obtained higher abundances than the others, in years 2005 and 2006 this trend

165 was not so marked, producing significant differences for the interaction location
166 x year.

167 The mean length of 5,957 specimens measured was 3.58 mm, with a range
168 between 0.8 mm and 8.9 mm. The boundary length between immature and
169 mature specimens was established at 3.7mm (lower hinge in Steam-Leaf Plot of
170 values of length of previously differentiated males based in the sexual
171 dimorphism). Based on this boundary length individuals were classified in 616
172 males, 1,796 females and 3,545 immature. The mean length of males was 5.22
173 mm and the greatest length was 8.6 mm, whereas the mean length of females
174 was 4.84 mm and the greatest length was 8.4 mm. Finally the mean length in
175 immature samples was 2.66 mm and the lowest 0.8 mm.

176 The results of the Kruskal-Wallis test showed significant differences with
177 distance in mean length of mature individuals. In this way in location I, years
178 2004 and 2007, and in location II, year 2004, the mean length of mature
179 individuals was lower in stations closer to the outfall (figure 4), where individuals
180 over 4.5mm in location I and over 6.5 mm in location II disappeared (figure 5) .
181 Moreover, individuals over 5.25 mm disappeared in year 2006 in stations at 0 m
182 from outfall in location IV and V, though significant differences in distance in
183 mean length of mature individuals were not detected in these cases.

184 On the other hand, higher abundances of individuals over 5.25 mm were
185 obtained in stations closer to the outfall in year 2007 (figure 5) producing a
186 higher mean length of mature individuals.

Regarding juveniles, the abundance of the smallest class size decreased or even disappeared in stations closer to the outfall in location I, for years 2005 and 2006, in location II, in location III and in location IV, for years 2005 and 2007 (figure 5).

Finally, almost all *Apseudes* disappeared in stations closer to the outfall in location I, year 2006, in location II, years 2005, 2006 and 2007 and in location III, years 2006 and 2007 (figure 5).

The mean sex ratio (females: males) of samples obtained at different distances to the outfall was similar, being 1:0.32 at stations closer to the outfall; whereas at 200 m to outfall was 1:0.35 and at 1,000m to outfall was 1:0.37. However, Kruskal-Wallis test showed significant differences in the percentage of males between distances for location IV and for location II. Plot showed a decrease of male percentage approaching the outfall in both locations (figure 6).

The percentage of females with eggs or embryos did not show any changes due to the outfalls. Kruskal-Wallis test did not show significant differences between distances for any location (table 3).

Pearson correlation coefficient and Bonferroni probabilities reported that there were significant correlations between abundance values and several abiotic factors; however most of the Pearson coefficients were low (table 4).

With regard to waste water flow data, all coefficients showed significant negative correlations with total abundance values. Suspended solids obtained the highest correlation with phosphates, COD and flow. For immature abundances, the highest Pearson coefficient was also obtained in suspended

solids, phosphates and COD whereas pH, BOD and nitrates did not show any significant correlation. Pearson coefficients for mature abundance values were higher than for immature abundances, and suspended solids obtained the highest values, although high Pearson coefficients were also obtained in nitrates and COD. Although length values showed negative significant correlations with water disposal pH; Pearson coefficients were lower.

On the other hand, percentage of coarse sand in sediment showed a significant and negative correlation with total and mature abundance values. Potential redox of sediment also showed a significant and positive correlation with abundance values and matures length pH values showed a negative correlation. These Pearson coefficients proved to be weaker than with waste water data.

4. Discussion

Despite the fact that *Apseudes latreillei* has sometimes been described as a species tolerant to disturbance (Borja et al., 2000; Marín-Guirao et al., 2005; Simboura and Reizopoulou, 2007), it did not respond to a source of sewage pollution with an increase of densities. Our results showed clearly that presence of sewage outfalls adversely affected *Apseudes latreillei* populations. The presence of a sewage disposal outlet produced a decrease in population densities, indeed values as low as zero were recorded in some of the sampling. However, this effect was reduced if waste water was treated to a high level prior to being dumped as we observed at the only one where biological treatment of activated sludge took place, while the others only undergo pre-treatment.

Apseudes latreillei was included in a list of tolerant species (GIII in AMBI, Borja et al., 2000; GII in BENTIX Simboura and Reizopoulou, 2007), using biotic indexes based on the classification of species (or groups of species) in several ecological groups representing specific levels of sensitivity to disturbance. It was also reported as being tolerant to metal contamination caused by old mining activities in Marín-Guirao et al. (2005), and was reported in high density in Ceuta harbour (Guerra-García et al., 2003). However, *Apseudes latreillei* individuals were also obtained in areas with little human activity that had been classified, using previously cited index, as having good or high ecological status, in muddy sands in S. Evvoilos coast (Aegean Sea, Greece) (Simboura and Reizopoulou, 2007) and in fine sand communities in Bou Ismail Bay, on the Algerian coast (Bakalem et al., 2009).

Moreira et al. (2008) and Lourido et al. (2008) found remarkable increases in numbers of individuals in sandy sediments in Galician rías (NW Spain), which are not apparently related to organic input. *Apseudes latreillei* was also reported as being a non tolerant species in other studies. Bouchet and Sauriau (2008) studying influence of oyster culture in Pertuis Charentais (SW France) found tanaidacea individuals in the reference station. In the same way in Sanz-Lázaro and Marin (2006), *Apseudes latreillei* was one of the best represented taxa in the reference station and in the impacted station following fish-farm abatement.

Regarding length distribution, whilst all size classes showed sensitivity to sewage pollution, differences between locations were detected. As such, larger mature individuals disappeared in outfalls with higher flows, whereas an increase of bigger individuals was detected the last year in the location with the

lowest flow and secondary treatment. Therefore sewage inputs could limit the maximum potential size attainable in species *Apseudes latreillei* since body size is often smaller in stressed environments (Barnes, 2005). Moreover, smaller juvenile individuals seemed to have a high sensitive pattern. *Apseudes latreillei* is a subsuperficial sessile deposit feeder, abundant in the first 5 cm of sediment (de Juan et al., 2007). The length of an individual is an important factor in burrowing; and immature individuals normally live near the surface. If immature individuals were more exposed to sewage pollution a higher mortality would be expected.

Sex ratio showed stronger decrease of the percentage of males in the outfall with the highest flow. Differences in sex ratio could be attributed to the fact that crustaceans show distinct growth rates between sexes (Low, 1978) and this could produce differences in sensitivity to pollution. Another less likely option is a de-masculinisation process (Ford, 2008). Crustacea are by default female and the expression of male secondary characteristics show degrees of plasticity influenced by environmental variables such as light and temperature (Dunn et al., 2005), and even diet (Zupo and Messina, 2007). To date, it is unclear whether widespread sexual disruption of crustaceans is occurring in the wild (Ford, 2008). However, Ford et al. (2004) observed reduced gnathopod sizes in normal male amphipods collected from a field site categorised as contaminated. It is possible that we were detecting a non developed male secondary character (robuster chelipeds), although we do not observed an increase in the females near the outfalls.

Changes in population density showed significant positive correlation with waste water quality. This was consistent with the fact that minor changes were detected in the location with the lowest flow and the only one where biological treatment of activated sludge takes place, and where water quality parameters of sewage disposal showed the best values.

Parameters of sediment that showed correlations with tanaid abundances were potential redox and pH. Both could indicate that *Apseudes latreillei* may be sensitive to hypoxia in sediment, a factor which is reflected by negative potential redox and acidification sediment. In general, hypoxia causes mortality in many invertebrates, and crustaceans are especially sensitive to this lack of oxygen (Gray et al., 2002; Guerra-García and García-Gómez, 2006; Sánchez-Moyano et al., 2002; Sánchez-Moyano and García-Gómez, 1998). Furthermore, oxygen availability could limit the growth of crustacean, suggesting that that size was governed by water oxygen content in the same way as it is in amphipods (Chapelle and Peck, 2004).

Another aspect that must be taken into account is sediment granulometry. *Apseudes latreillei* is abundant in sediments with dominance of fine sands presented in our study area (Bakalem et al., 2009; Bouchet and Sauriau, 2008; Marín-Guirao et al., 2005; Moreira et al., 2008; Lourido et al., 2008). Despite the fact that granulometry of the sediment did not show variability related to the presence of sewage outfalls (de la Ossa Carretero et al., 2008; de-la-Ossa-Carretero et al., 2009) negative correlation of tanaid abundances with coarse sand percentage was detected. Sediment type defines habitats and changes in grain sizes could produce high variations in infaunal species whose distribution

are closely correlated with certain types of sediment (Bishop, 2005; Snelgrove and Butman, 1994). Moreover, peracarids display the ability to search actively for preferred sediments, a factor which may be related to food availability and the ability to burrow (Oakden, 1984; Moreira et al., 2008). However since sediments in the area are homogeneous and no relation is observed between grain size and sewage discharge, this correlation should reflect the variability related with natural punctual changes in sediment size and should not explain the pattern of abundance reduction near the outfalls.

Focusing on our results, *Apseudes latreillei* responded as a sensitive species. We detected a decrease of density in stations closer to outfalls with a higher input of suspended solids, nutrients (nitrates and phosphates) and a possible trend to hypoxia in affected stations. Previous *Apseudes latreillei* reported tolerance could be explained by the fact that organisms are not equally sensitive to all types of anthropogenic disturbance and are likely to respond differently to different types of perturbation (Washington, 1984) and its response to a pollution source could vary. This variability in responses produces a need for testing the tolerance/sensitivity of each species to different pollution sources prior to it being used as an indicator.

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References.

Bakalem, A., Ruellet, T., Dauvin, J.C., 2009. Benthic indices and ecological quality of shallow Algeria fine sand community. *Ecological Indicators* 9, 395-408.

Barnes, D.K.A., 2005. Body and resource size at the land-sea interface. *Marine Biology* 146, 625-645.

Bishop, M.J., 2005. Artificial sampling units: a tool for increasing the sensitivity of tests for impact in soft sediments. *Environmental Monitoring and Assessment* 107, 203–220.

Borja., A., Franco, J., Perez, V., 2000. A marine biotic index to the establish ecology quality of soft-bottom benthos within European estuarine coastal environments. *Marine Pollution Bulletin* 40, 1100-1114.

Bouchet, V.M.P., Sauriau, P.G., 2008. Influence of oyster culture practices and environmental conditions on the ecological status of intertidal mudflats in the

- 350 Pertuis Charentais (SW France): A multi-index approach. Marine Pollution
351 Bulletin 56, 1898-1912.
- 352 Cardell, M.J., Sardà, R., Romero, J., 1999. Spatial changes in sublittoral soft-
353 bottom polychaete assemblages due to river inputs and sewage discharges.
354 Acta Oecologica 20, 343–351.
- 355 Chapelle, G., Peck, L.S., 2004. Amphipod crustacean size spectra: new insights
356 in the relationships between size and oxygen. Oikos 106, 167-175.
- 357 Como, S., Magni, P., Baroli, M., Casu, D., de Falco, G., Floris, A., 2008.
358 Comparative analysis of macrofaunal species richness and composition in
359 *Posidonia oceanica*, *Cymodocea nodosa* and leaf litter beds. Marine Biology
360 153, 1087-1101.
- 361 Conides, A., Bogdanos, C., Diapoulis, A., 1999. Seasonal ecological variations
362 of phyto- and zoobenthic communities in the south of Nisyros Island, Greece.
363 The Environmentalist 19, 109-127.
- 364 Conradi, M., López-González, P.J., 2001. Relationships between environmental
365 variables and the abundance of peracarid fauna in Algeciras Bay (Southern
366 Iberian Peninsula). Ciencias Marinas 27, 481–500.
- 367 Corbera, J., Cardell, M.J., 1995. Cumaceans as indicators of eutrophication on
368 soft bottoms. Scientia Marina 59, 63–69.
- 369 Cotano, U., Villate, F., 2006. Anthropogenic influence on the organic fraction of
370 sediments in two contrasting estuaries: A biochemical approach. Marine
371 Pollution Bulletin 52, 404-414.

- 372 Dauer, D.M., Ranasinghe, J.A., Weisberg, S.B., 2000. Relationships between
373 benthic community condition, water quality, sediment quality, nutrient loads, and
374 land use patterns in Chesapeake Bay. *Estuaries* 23, 80-96.
- 375 Dauvin, J.C., 1988. Rôle du macrobenthos dans l'alimentation des Poissons
376 démersaux vivant sur les fonds de sédiments fins de la Manche occidentale.
377 *Cahiers de Biologie Marine* 29, 445–467.
- 378 Dauvin, J.C., Ruellet, T., 2007. Polychaete/amphipod ratio revisited. *Marine*
379 *Pollution Bulletin* 55, 215-224.
- 380 Dauvin, J.C., Ruellet, T., Desroy, N., Janson, A.L., 2007. The ecology quality
381 status of the Bay of Seine and the Seine estuary: Use of biotic indices. *Marine*
382 *Pollution Bulletin* 55, 241-257.
- 383 De Grave, S., 1999. The influence of sedimentary heterogeneity on within maerl
384 bed differences in infaunal crustacean community. *Estuarine, Coastal and Shelf*
385 *Science* 49, 153–163.
- 386 De Juan, S., Thrush, S.F., Demestre, M., 2007. Functional changes as
387 indicators of trawling disturbance on a benthic community located in a fishing
388 ground (NW Mediterranean Sea). *Marine Ecology Progress Series* 334, 117-
389 129.
- 390 De la Ossa Carretero, J.A., Del Pilar Ruso, Y., Giménez Casaldueiro, F.,
391 Sánchez Lizaso, J.L., 2008. Effect of Sewage Discharge in *Spisula subtruncata*
392 (da Costa 1778) populations. *Archives of Environmental Contamination and*
393 *Toxicology* 54: 226-235.

- 394 De-la-Ossa-Carretero, J.A., Del-Pilar-Ruso, Y., Giménez-Casalduero, F.,
395 Sánchez-Lizaso, J.L., 2009. Testing BOPA index in sewage affected soft-
396 bottom communities in the north-western Mediterranean. Marine Pollution
397 Bulletin 58, 332-340.
- 398 Del Pilar Ruso, Y., De la Ossa Carretero, J.A., Jiménez Casalduero, F.,
399 Sánchez Lizaso, J.L., 2007. Spatial and temporal changes in infaunal
400 communities inhabiting soft-bottoms affected by brine discharge. Marine
401 Environmental Research 64, 492-503.
- 402 Dunn, A., Hogg, J.C., Kelly, A., Hatcher, M.J., 2005. Two cues for sex
403 determination in *Gammarus duebeni*: adaptive variation in environmental sex
404 determination? Limnology and Oceanography 50, 346–353.
- 405 Ford, A.T., Fernandes, T.F., Rider, S.A., Read, P.A., Robinson, C.D., Davies,
406 I.M., 2004. Endocrine disruption in gammaridean amphipod? Field observations
407 of intersexuality and de-masculinisation. Marine Environmental Research
408 58,169–173.
- 409 Ford, A.T., 2008. Can you feminise a crustacean?. Aquatic Toxicology 88, 316-
410 321.
- 411 Gray, J.S., Wu, R.S., Or, Y.Y., 2002. Effects of hypoxia and organic enrichment
412 on the coastal marine environment. Marine Ecology Progress Series 238, 249-
413 279.

- 414 Gomez Gesteira, J.L., Dauvin, J.C., 2000. Amphipods are good bioindicators of
415 the impact of oil spills on soft-bottom macrobenthic communities. *Marine*
416 *Pollution Bulletin* 40, 1017-572.
- 417 Grall, J., Glémarec, M., 1997. Using Biotic Indices to Estimate Macrobenthic
418 Community Perturbations in the Bay of Brest. *Estuarine, Coastal and Shelf*
419 *Science* 44, 43-53
- 420 Guerra-García, J.M., Corzo, J.R., García-Gómez, J.C., 2003. Distribución
421 vertical de la macrofauna en sedimentos contaminados en el interior del puerto
422 de Ceuta. *Boletín del Instituto Español de Oceanografía* 19, 105-121.
- 423 Guerra-García, J.M., García-Gómez, J.C., 2005. Oxygen levels versus
424 chemical pollutants: do they have similar influence on macrofaunal
425 assemblages? A case study in a harbour with two opposing entrances.
426 *Environmental Pollution* 135, 281-291.
- 427 Guerra-García, J.M., García-Gómez, J.C., 2006. Recolonization of defaunated
428 sediments: Fine versus gross sand and dredging versus experimental trays.
429 *Estuarine, Coastal and Shelf Science* 68, 328-342.
- 430 Koop, K., Hutchins, P., 1996. Disposal of sewage to the ocean- a sustainable
431 solution? *Marine Pollution Bulletin* 33, 121–123.
- 432 Le Hir, M., Hily, C., 2005. Macrofaunal diversity and habitat structure in intertidal
433 boulder fields. *Biodiversity and Conservation* 14, 233-250.

- 434 Lourido, A., Moreira, J., Troncoso, J.S., 2008. Assemblages of peracarid
435 crustaceans in subtidal sediments from the Ría de Aldán (Galicia, NW Spain).
436 Helgoland Marine Research 62, 289-301.
- 437 Low, B.S., 1978. Environmental uncertainty and parental strategies of
438 marsupials and placentals. American Naturalist 112, 319–335.
- 439 Mancinelli, G., Rossi, L., 2002. The influence of allochthonous leaf detritus on
440 the occurrence of crustacean detritivores in the soft-bottom macrobenthos of
441 the Po River Delta Area (northwestern Adriatic Sea). Estuarine, Coastal and
442 Shelf Science 54, 849-861.
- 443 Marín-Guirao, L., Cesar, A., Marín, A., Lloret, J., Vita, R., 2005. Establishing the
444 ecological quality status of soft-bottom mining-impacted coastal water bodies in
445 the scope of the Water Framework Directive. Marine Pollution Bulletin 50, 374-
446 387.
- 447 Marques, J.C., Bellan-Santini, D., 1987. Crustacés Amphipodes des côtes du
448 Portugal: faune de l'estuaire du Mira (Alentejo, côte sud-ouest). Cahiers de
449 Biologie Marine 28, 465-480.
- 450 McIntyre, A.D., 1995. Human impact on the oceans: the 1990s and beyond.
451 Marine Pollution Bulletin 31, 147–151.
- 452 Moreira, J., Lourido, A., Troncoso, J.S., 2008. Diversity and distribution of
453 peracarid crustaceans in shallow subtidal soft bottoms at the Ensanada de
454 Baiona (Galicia N.W. Spain). Crustaceana 81, 1069-1079.

- 455 Oakden, J.M., 1984. Feeding and substrate preference in five species of
456 phoxocephalid amphipods from central California. *Journal of Crustacean*
457 *Biology* 4, 233-247.
- 458 Parker, J.G., 1984. The distribution of the subtidal Amphipoda in Belfast Lough
459 in relation to sediment types. *Ophelia*, 23, 119-140.
- 460 Pearson, T.H., Rosenberg, R., 1978. Macrobenthic succession in relation to
461 organic enrichment and pollution of the marine environment. *Oceanography*
462 *Marine Biology. An Annual Review* 16, 229–311.
- 463 Pérès, J.M., Picard, J., 1964. Nouveau manuel de bionomie benthique de la
464 mer Méditerranée. *Recueil des Travaux de la Station Marine d'Endoume* 47,1–
465 138.
- 466 Rand, G., Petrocelli, S., 1985. *Fundamentals of aquatic toxicology*. Hemisphere
467 Corporation, Washington, D.C.
- 468 Robertson, M.R., Hall, S.J., Eleftheriou, A., 1989. Environmental correlates with
469 amphipod distribution in a Scottish sea loch. *Cahiers de Biologie Marine* 30,
470 243-258.
- 471 Sánchez-Moyano, J.E., García-Gómez, J.C., 1998. The arthropod community,
472 especially Crustacea, as a bioindicator in Algeciras Bay (Southern Spain) based
473 on a spatial distribution. *Journal of Coastal Research* 14, 1119–1133.
- 474 Sánchez-Moyano, J.E., García-Adiego, E.M., Estacio, F., García-Gómez, J.C.,
475 2002. Effect of environmental factors on the spatial variation of the epifaunal

- 476 polychaetes of the alga *Halopteris scoparia* in Algeciras Bay (Strait of Gibraltar).
477 Hydrobiologia 470, 133–148.
- 478 Sanz, M.C., 1992. Contribución al estudio biológico de los crustáceos del
479 Mediterráneo occidental. Thesis. Universidad Autónoma de Barcelona.
- 480 Sanz-Lázaro, C., Marín, A., 2006. Benthic recovery during open sea fish
481 farming abatament in Western Mediterranean, Spain. Marine Environmental
482 Research 62, 374-387.
- 483 Sardá, R., Pinedo, S., Martin, D., 1996. Seasonal dynamics of macroinfaunal
484 key speciess inhabiting shallow soft-bottoms in the Bay of Blanes NW
485 Mediterranean. Acta Oecologica 20, 315-326.
- 486 Sardá, R., Pinedo, S., Gremare, A., Taboada, S., 2000. Changes in the
487 dynamics of shallow sandy-bottom assemblages due to sand extraction in the
488 Catalan Western Mediterranean Sea. ICES Journal of Marine Science 57,
489 1446-1453.
- 490 Simboura, N., Reizopoulou, S., 2007. A comparative approach of assessing
491 ecological status in two coastal areas of the Eastern Mediterranean. Ecological
492 Indicators 7, 455-468.
- 493 Snelgrove, P.V.R., Butman, C.A., 1994. Animal–sediment relationships
494 revisited: Cause versus effect. Oceanography and Marine Biology. An Annual
495 Review 32, 111–177.
- 496 Washington, H.G., 1984. Diversity, biotic and similarity indices. A review with
497 special relevance to aquatic ecosystems. Water Research 18, 653-694.

Zupo, V., Messina, P., 2007. How do dietary diatoms cause the sex reversal of the shrimp *Hippolyte inermis* Leach (Crustacea, Decapoda). Marine Biology 151, 907–917.

Table 1. Results of ANOVA for abundance (individuals/m²) of *Apseudes latreelli* for the factors distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007) RES = Residual, df: degrees of freedom, MS= mean square, F of each factor =MS factor/MS residual because all the factors are orthogonal

Source	df	MS	F	P
Distance	2	4110,472	11,1	0,0096
Location	4	628,6924	4,31	0,0216
Year	3	1558,668	20,97	0
Distance x location	8	150,1405	2,25	0,0594
Distance x year	6	370,2303	4,98	0,0274
Location x year	12	145,7796	1,96	0,0001
Distance x location x year	24	66,6982	0,9	0,606
RES	300	74,3237		
TOTAL	359			

Table 2. Summary of the results of SNK post-hoc test for the interactions distance x location, distance x year and location x year.

Factors considered distance (0, 200, 1000 m), location (I, II, III, IV and V) and year (2004, 2005, 2006, 2007). * Significant at $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$

Interaction	Factors	Level	SNK
Distancexlocation	dis(loc)	loc 1	** 0<200 m, ** 0<1000m
		loc 2	** 0<200 m, ** 0<1000m
		loc 3	** 0<200 m, ** 0<1000m
		loc 4	** 0<200 m, ** 0<1000m
Distancexyear	loc(dis)	0 m	** 2<5, **3<5, **4<5, **1<5, **2<4
	dis(year)	2004	**0<1000, *0<200
		2005	** 0<1000, **0<200
		2006	** 0<1000, **0<200
Locationxyear	loc(year)	2004	**1<5, *1<4, *2<5, *3<5
		2005	** 1<5
		2007	** 2<5, *1>2, *3<5, *4<5

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Table 3. Mean $\pm$ standard error of percentage of females of *Apseudes latreillei* with eggs or embryos at each location (I to V) and distance (0, 200 and 1000 m). Kruskal-Wallis Test Statistic and p values for factor distance were indicated.

Location	Mean $\pm$ SEM of % presence of eggs or embryos in females			K-W ^P
	0 m	200 m	1000 m	
I	28.98 ± 10.73	12.95 ± 5.96	22.34 ± 7.92	0.638 ^{0.897}
II	14.29 ± 9.22	33.21 ± 8.35	12.30 ± 5.44	4.704 ^{0.095}
III	29.17 ± 12.89	19.84 ± 6.77	8.87 ± 3.36	1.998 ^{0.369}
IV	7.46 ± 5.37	12.25 ± 5.20	12.42 ± 3.70	3.981 ^{0.137}
V	3.34 ± 1.31	11.98 ± 3.76	8.67 ± 4.89	2.466 ^{0.291}

Table 4. Pearson correlation, total abundance, immature abundance, mature abundance and matures length values with studied factors and Bonferroni probabilities
 ns no significant difference.* Significant at $p < 0.05$.** $p < 0.01$.*** $p < 0.001$.

		Abundance			Matures length
		Total	Immatures	Matures	
Water disposal	pH	-0.235*	-0.179 ^{ns}	-0.283**	-0.264***
	Flow (m ³ /month)	-0.300**	-0.244*	-0.329**	-0.022 ^{ns}
	S.S. (mg/l)	-0.377***	-0.288**	-0.453***	-0.008 ^{ns}
	BOD (mg/l)	-0.269*	-0.194 ^{ns}	-0.350***	-0.000 ^{ns}
	COD (mg/l)	-0.334***	-0.251**	-0.412***	-0.013 ^{ns}
	P (mg/l)	-0.349***	-0.275**	-0.354***	-0.087 ^{ns}
	Ni (mg/l)	-0.251*	-0.167 ^{ns}	-0.403***	-0.063 ^{ns}
	Turb. (NTU)	-0.295**	-0.214*	-0.380***	-0.008 ^{ns}
Sediment	% Mud	0.035 ^{ns}	0.070 ^{ns}	-0.036 ^{ns}	-0.016 ^{ns}
	% Fine Sand	0.076 ^{ns}	0.088 ^{ns}	0.025 ^{ns}	0.019 ^{ns} *
	% Medium sand	-0.059 ^{ns}	-0.098 ^{ns}	0.028 ^{ns}	-0.011 ^{ns}
	% Coarse sand	-0.126*	-0.101 ^{ns}	-0.113*	0.017 ^{ns}
	% Gravel	0.073 ^{ns}	0.069 ^{ns}	0.049 ^{ns}	-0.031 ^{ns}
	Pot. Redox (mV)	0.193***	0.163***	0.159**	0.044*
	% org mat	0.035 ^{ns}	0.008 ^{ns}	0.064 ^{ns}	-0.042 ^{ns}
	pH	-0.171**	-0.180**	0.087 ^{ns}	-0.036 ^{ns}

Figure 1. Study area. Location of the five pipelines and the sampling stations.

Figure 2. *Apseudes latreillei* individuals. Differentiation between male, female, and immature individuals.

Figure 3. Mean and standard error of *Apseudes latreillei* abundances at each location (I to V), year (2004, 2005, 2006 and 2007) and distance (0, 200 and 1000 m).

Figure 4. Mean length and results of Kruskal-Wallis Test for factor distance at each location (I to V) and year (2004, 2005, 2006 and 2007) of mature individuals. The boxes indicate the 25th percentile, median, and 75th percentile, whiskers extend from the 10th to the 90th percentile, while dots indicate the 5th and 95th percentile values.

Figure 5. Size distribution of *Apseudes latreillei* for each location (I to V), year (2004, 2005, 2006 and 2007) and distance (0, 200 and 1000 m). All bars are plotted at the midpoint of the 0.75 mm class intervals.

Figure 6. Percentage of males and females of *Apseudes latreillei* population at each location (I to V) and distance (0, 200 and 1000 m). Kruskal-Wallis Test Statistic and p values for factor distance were indicated.

Figure 1

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