

# ORIGINAL ARTICLE

# Amphipoda assemblages in a disturbed area (Alicante, Spain, Western Mediterranean)

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#### Keywords

Amphipoda; benthos; Iberian Peninsula; Mediterranean Sea; pollution.

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### Abstract

The distribution and composition of Amphipoda assemblages were analysed off the coasts of Alicante (Spain, Western Mediterranean), a disturbed area affected by several co-occurring anthropogenic impacts. Although differences among sampled stations were mainly related to natural parameters, anthropogenic activities were linked with changes in amphipod assemblages. Expansion of the Port of Alicante, a sewage outfall and a high salinity brine discharge could be causing the disappearance of amphipods at stations closer to these disturbances. However, the completion of port enlargement works and mitigatory dilution of the brine discharge has led to the recovery of the amphipod assemblage. Among the natural parameters, depth determines the distribution of some of the species. While Siphonoecetes sabatieri was abundant at shallow stations, Ampelisca spp., Photis longipes, Pseudolirius kroyeri, Apherusa chiereghinii and Phtisica marina were more abundant at deeper stations. Grain size and percentage of organic matter also influenced amphipod distribution, resulting in changes in species composition and in the relative percentages of different trophic groups. Species such as Ampelisca brevicornis, Perioculodes longimanus, Urothoe hesperiae and Urothoe elegans were more abundant at stations with a high content of fine sand. Carnivorous species, mainly of the Oedicerotidae family, were more abundant at those stations with a low organic matter content, while detritivorous species were more abundant at stations with a higher mud content. Among 62 identified species, three were reported for the first time from the Spanish Mediterranean coast, two species were recorded for the second time and a new species of Siphonoecetes was found, Siphonoecetes (Centraloecetes) bulborostrum. These results confirm the need for further data on amphipods from the Mediterranean Spanish coast.

#### Introduction

Among groups that comprise benthic communities, amphipods are considered one of the fundamental components of marine bottom communities (Marques & Belllan-Santini 1993). They are numerically dominant, ecologically and trophically important, and exhibit a high degree of niche specificity, inhabiting sediments with different physico-chemical characteristics over a wide distribution (Reish 1993; Thomas 1993; Gomez Gesteira & Dauvin 2000; Dauvin & Ruellet 2007). These characteristics suggest that they play major roles in the ecology of these habitats (Conlan 1994). For this reason, knowledge of their ecology and distribution will aid in understanding the structure and functioning of benthic communities. To elucidate the processes that determine Amphipoda distribution, various studies have been conducted in different habitats and areas: soft bottoms of shallow coastal areas (Carvalho *et al.* 2012), rocky shores (Jimeno & Turón 1995), estuaries (Cunha *et al.* 2000), deep sea bottoms (Cartes & Sorbe 1999), *Posidonia oceanica* meadows (Zakhama-Sraieb *et al.* 2011; Sturaro *et al.* 2014), marine caves (Navarro-Barranco *et al.* 2012), *etc.* All this research has established that Amphipoda distribution is determined by several highly correlated parameters.

Depth is considered one of the major physical factors that affects Amphipoda distribution (Carvalho et al. 2012). However, along the depth profile other factors vary progressively, such as the type of substrate or food availability. The type of substrate determines species distribution. While some species can settle on several substrates, other species show active selection: a specific type particle size or the presence of other species that provide a structural habitat and may offer protection or potential food items, e.g. bryozoans, seagrasses and seaweeds (Jimeno & Turón 1995; Conradi & López-González 1999; Vázquez-Luis et al. 2009). Trophic resource availability is also crucial for species distribution (Zakhama-Sraieb et al. 2011; Carvalho et al. 2012; Sturaro et al. 2014). Although amphipods are principally deposit feeders, they can show preferences for other trophic resources such as epiphytic algae or metazoans and feed selectively on discrete particles of organic matter (Barnard 1962; Guerra-García et al. 2014). Consequently, their distribution is sensitive to changes in the flux of particles and organic matter deposition (Cartes & Sorbe 1999), as well as the availability of other preferred feeding sources.

Besides these natural parameters, anthropogenic disturbances can greatly influence Amphipoda distribution. Amphipods are considered more sensitive to polluted sediments than other benthic organisms (Gomez Gesteira & Dauvin 2000; Dauvin & Ruellet 2007). This sensitivity results in a general decrease in amphipod abundance and diversity when pollution increases (Bellan-Santini 1980; Conlan 1994). However, despite it being established that most amphipod species are sensitive to different types of pollution (Dauvin, 1987,1998; Gomez Gesteira & Dauvin 2000; Dauvin & Ruellet 2007), several studies have concluded that some species are more tolerant than others, resulting in changes in amphipod composition related to the degree of pollution (Bellan-Santini 1980; de-la-Ossa-Carretero et al. 2012). Anthropic activities can therefore be just as important as natural parameters in determining Amphipoda distribution.

The coast of Alicante (Southeast Spain) comprises a small area where several human activities have cooccurred: the presence of an important, recently enlarged commercial port, a sewage outfall and brine discharge from a desalination plant (Del-Pilar-Ruso 2011). Several studies have reported the response of amphipod assemblages in areas adjacent to harbors or wastewater outfalls (Nipper *et al.* 1989; Swartz *et al.* 1994; Ingole *et al.* 2009; de-la-Ossa-Carretero et al. 2012), to the extent that their sensitivity to the contaminated sediments (Riba et al. 2003; Ramos-Gómez et al. 2009) and hypoxia (Gray et al. 2002) generated by both activities is generally accepted. However, the response of amphipod assemblages to highsalinity discharges has scarcely been studied, apart from some work examining the resultant changes in benthic communities and Polychaeta assemblages (Del-Pilar-Ruso et al. 2007, 2008). Research into amphipod species on the Mediterranean coast of the Iberian Peninsula has intensified in recent years (Guerra-García et al. 2009; Vázquez-Luis et al. 2009; de-la-Ossa-Carretero et al. 2010; Guerra-García & Izquierdo 2010; Navarro-Barranco et al. 2012 Fernandez-Gonzalez et al. 2013). Indeed, a recent study in this area reported several new species records and one species new to science (de-la-Ossa-Carretero et al. 2010). These results emphasize the need for studies on the distribution of amphipods along this coast.

The main objective of this study was to gain greater knowledge about the distribution of amphipod assemblages in disturbed areas where various impacts converge. The distribution of the order Amphipoda should be related to both natural variability and human activities. Monitoring amphipod assemblages along the Alicante coast is an effective strategy to understand and predict the effects of human activities on this sensitive group and clarify their distribution and taxonomic aspects.

## **Material and Methods**

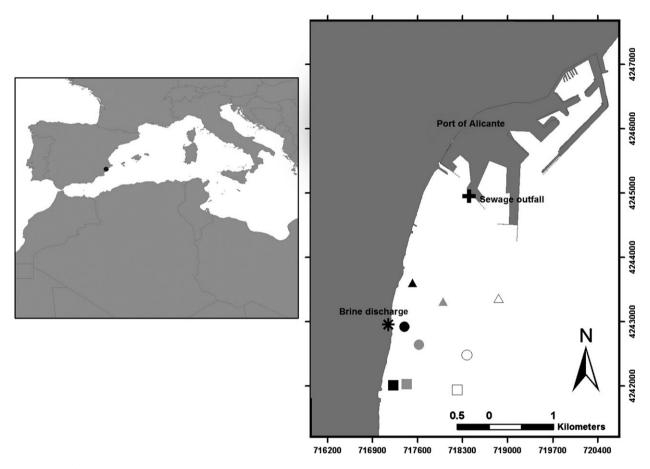
## Study area

The present study is based on the results of a benthos survey of the Alicante coast carried out in summer and winter from 2005 to 2012 (Western Mediterranean Sea) (Del-Pilar-Ruso et al. 2007, 2008, 2014). Three transects 400 m apart were established (A, B and C) with three stations along each transect at 4, 10 and 15 m depth (Fig. 1). Predominantly a zone of soft bottoms, the study area is mainly composed of fine sand and mud. Shallower stations were usually characterized by fine sand, although a higher content of gravel, and medium and coarse sands were detected in transect B. Deeper stations were covered by dead Posidonia rhizomes and typified by finer sediments. Sediment was composed of fine sand and mud at stations 10 m deep, with some medium and coarse sand content along transects A and B; with a higher percentage of mud at 15 m depth. The percentage of organic matter was also related to depth, being lower at shallower stations and higher at 15 m depth (Table 1; Del-Pilar-Ruso et al. 2007, 2008, 2014).

These sediments correspond to a disturbed area where several anthropogenic impacts have converged for

Transect	Depth	% gravel	% coarse sand	% medium sand	% fine sand	% mud	% organic matter
A	4	1.4	1.7	2.8	82.7	11.5	1.9
В	4	8.5	9.6	21.1	46.1	14.7	2.3
С	4	2.2	3.2	7.4	77.3	9.9	1.4
А	10	14.7	10.6	6.6	23.5	44.6	6.3
В	10	9.7	12.3	6.3	32.6	39.1	3.8
С	10	2.7	2.9	2.3	65.0	27.0	3.0
А	15	5.7	4.3	4.0	33.7	52.4	5.4
В	15	8.8	6.6	5.7	28.3	50.6	7.4
С	15	7.0	6.6	5.1	36.1	45.3	6.9

Table 1. Transect, depth (m) and physical characteristics of sediment at each station.



**Fig. 1.** Map of study area. Symbols indicate sampling stations ( $\blacktriangle$ , A 4 m,  $\blacktriangle$  A 10 m,  $\Delta$  A 15 m; B B 4 m,  $\blacktriangle$  B 10 m,  $\bigcirc$  B 15 m;  $\blacksquare$  C 4 m,  $\blacktriangle$  C 10 m,  $\square$  C 15 m), \*brine discharge and + sewage outfall position. UTM Zone 30N.

decades: extensive dock facilities, a sewage outfall and a brine discharge (Del-Pilar-Ruso 2011; Fig. 1). The Port of Alicante is about 2 km to the north of the studied area. A new dock to the south of the original harbor was built from 2005 to 2007. After these enlargement works, the Port area now covers more than 1,000,000  $m^2$  where around 1000 ships, 300,000 passengers and more than 2,000,000 tons of bulk cargo transit per year (Autoridad Portuaria de Alicante 2012). Rincon de León sewage out-

fall is south of the Port and closer to the northern margin of the sampled stations. An average wastewater flow of more than 75,000 m<sup>3</sup> · day<sup>-1</sup> is discharged through a pipeline at 7 m depth, after a secondary biological treatment at the wastewater plant through activated sludge. Alicante desalination plant discharges effluent in a coastal zone close to transect B. This desalination plant began operations in September 2003. Initially, it had a discharge of 65,000 m<sup>3</sup> · day<sup>-1</sup> characterized by its high salinity (68 psu); however, in 2006, a pre-discharge dilution process was introduced to reduce its salinity.

### Sample collection and processing

Sampling was carried out by SCUBA divers. Three replicates were collected at each station using a square box and sieved through a 0.5-mm mesh screen in order to sort the amphipods present. Amphipods were mainly identified using the Mediterranean amphipod fauna key established by Bellan-Santini *et al.* (1982, 1989, 1993, 1998), except for several families that were identified using specific literature (Conradi & López-González 1995; Krapp-Schickel 2000; D'Udekem d'Acoz & Vader 2005; Krapp-Schickel & Sorbe 2006; White 2011; Guerra-García *et al.* 2013). The taxonomy was validated using the European Register of Marine Species reference for amphipods introduced by Bellan-Santini & Costello (2001) (http:// www.marbef.org/data/erms.php, consulted on 12 August 2013).

#### Data analysis

The Shannon–Wiener diversity index, which is based on abundance of species, was calculated. An analysis of variance (ANOVA), with transect and depth as fixed factors and year and season as random factors, was used in order to test differences in this diversity index. Prior to ANO-VA, the homogeneity of variance was tested using Cochran's test. The Student–Newman–Keuls test was used to determine which samples were involved in the differences.

Non-parametric multivariate techniques were used to compare the abundances of the different amphipod species present at each station. These multivariate analyses were performed using the PRIMER v. 6 statistical package (Clarke & Warwick 2001). Triangular similarity matrices were calculated through the Bray-Curtis similarity co-efficient using abundance values. Beforehand, the values were dispersion-weighted to reduce 'noise' produced by species with an erratic distribution and whose abundance indicates a great variance between replicates (Clarke et al. 2006). Graphical representation of the multivariate patterns of amphipod assemblages was obtained by non-metric multidimensional scaling (nMDS). PERMANOVA (permutational analysis of variance) was used to test spatial differences and quantify the temporal variability, establishing four factors: transect and depth as fixed factors, and year and season as random factors.

In order to study variation in the food web structure among the stations, trophic groups were assigned to each species according to Guerra-García *et al.* (2014) results: detritivorous (>90% detritus in the digestive tract); detritivorous-herbivorous (90–50% detritus and >10% algae); detritivorous-carnivorous (90–50% detritus and >10% prey); carnivorous-omnivorous (90–50% prey and >10% detritus). The Shannon–Wiener diversity index based on the abundance of trophic groups was also calculated. An ANOVA with transect and depth as fixed factors and year and season as random factors was used to test differences in this index.

Finally, in order to link benthic communities to sediment parameters, BEST and canonical correspondence analysis (CCA) procedures were applied. The BEST procedure was used to determine the parameter combination most correlated with amphipod assemblage changes among the sampled stations. BEST was performed using the PRIMER v. 6 statistical package (Clarke & Warwick 2001). CCA was used to identify the relationships among the spatial distribution patterns of amphipods and environmental gradients. CCA was conducted using the software CANOCO (Ter Braak & Šmilauer 1998). The output is displayed as a biplot, in which the plotted points for stations can be related to environmental gradients represented as arrows. The strength of the correlation of an environmental variable is reflected in the length of the arrow, and its association is reflected in the acuteness of the angle with the axis. Thus, the relationships among stations and environmental variables can be displayed on one plot.

## Results

A total of 3385 individuals of 62 species, belonging to 41 genera and 24 families, was identified (Table 2). Among them, a new species, *Siphonoecetes bulborostrum* (de-la-Ossa-Carretero & Martí 2014), was found; *Megamphopus longicornis, Elasmopus pectenicrus* and *Hippomedon ambiguous* were reported for the first time from the Spanish Mediterranean coast; and *Ampelisca spinipes* and *Urothoe hesperiae* were recorded for the second time. This study therefore confirms the presence of the latter two species along this coast.

Among all collected species and considering their frequency of appearance, 14.5% were very common, 11.3% rather common, 29% common, 19.4% uncommon and 25.8% rare at the sampled stations (Table 2). Some species were relatively abundant and very common, such as *Siphonoecetes sabatieri*, *Ampelisca typica*, *Photis longipes* and *S. bulborostrum*. Other species were abundant, but less frequent, such as *Gammarella fucicola*, *U. hesperiae*, *Pariambus typicus* and *Maera grossimana*, as they were only collected at some stations. Others were very common, but had low abundances: *A. spinipes*, *Perioculodes longimanus*, *Microprotopus maculatus* and *Leucothoe incisa*.

Table	2.	Amphipoda	species	identified	in	the	present s	tudy.
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Family	Species	Mean density	Frequency	Stations
Ampeliscidae	Ampelisca brevicornis (Costa, 1853)	45.6	15.3 (RC)	4 m (A, C), 10 m (B, C)
	Ampelisca diadema (Costa, 1853)	24.0	8.3 (C)	10 m (A, B, C), 15 m (A, B)
	Ampelisca spinipes (Boeck, 1861)**	46.5	27.8 (VC)	10 m (A, B, C) 15 m (A, B, C)
	Ampelisca tenuicornis (Liljeborg, 1855)	43.1	4.9 (C)	4 m (A), 10 m (A, B, C) 15 m (A, B)
	Ampelisca typica (Bate, 1856)	57.5	28.5 (VC)	10 m (A, B, C), 15 m (A, B, C)
Amphilochidae	Amphilochus brunneus (Della Valle, 1893)	25.6	7.6 (C)	10 m (A, B), 15 m (A, B)
	Apolochus neapolitanus (Della Valle, 1893	19.8	0.7 (R)	15 m (B)
Amphithoidae	Amphithoe ramondi (Audouin, 1826)	19.8	6.3 (C)	4 m (C), 10 m (A, B), 15 m (A, C)
Aoridae	Aora gracilis (Bate, 1857)	27.2	5.6 (C)	10 m (A, B, C), 15 m (A)
	Aora spinicornis (Afonso, 1976)	24.7	4.9 (C)	10 m (A, C), 15 m (A, B)
	Lembos websteri Bate, 1857	43.5	4.9 (C)	10 m (A), 15 m (A, C)
	Microdeutopus stationis Della Valle, 1893	34.6	2.1 (UC)	10 m (A, C), 15 m (A)
Calliopiidae	Apherusa chiereghinii Giordani-Soika, 1949	55.4	15.3(RC)	10 m (A, B, C), 15 m (A, B, C)
Atylidae	Atylus guttatus (Costa, 1851)	27.7	2.8 (UC)	10 m (C), 15 m (A, C)
, ttyndde	Atylus vedlomensis (Bate & Westwood, 1862)	26.3	3.5 (C)	10 m (A), 15 m (B, C)
	Atylus massiliensis (Bellan-Santini, 1975)	32.0	18.8(RC)	4 m (A, B, C), 10 m (A, B, C), 15 m
		52.0	10.0(IIC)	(A, B, C)
	Nototropis swammerdamei (Milne-Edwards, 1830)	19.8	0.7 (R)	4 m (A)
Bathyporeiidae	Bathyporeia guilliamsoniana (Bate, 1857)	34.6	2.8 (UC)	4 m (A, C), 15 m (A)
Caprellidae	Pariambus typicus (Krøyer, 1844)	88.9	0.7 (R)	15 m (A)
	Phtisica marina (Slabber, 1769)	43.6	18.8 (RC)	4 m (C), 10 m (A, B, C), 15 m (A, B, C)
	Pseudolirius kroyeri (Haller, 1897)	287.8	18.1 (RC)	4 m (A, B, C), 10 m (A, B, C), 15 m (A, B, C)
	Caprella scaura Templeton, 1836	19.8	0.7 (R)	4 m (B)
Iphimediidae	Iphimedia minuta G.O. Sars, 1882	39.5	0.7 (R)	15 m (A)
Corophiidae	Leptocheirus bispinosus Norman, 1908	19.8	0.7 (R)	15 m (C)
	Monocorophium acherusicum (Costa, 1853)	27.7	11.1 (RC)	4 m (A, B, C), 10 m (A, B, C)
	Monocorophium sextonae (Crawford, 1937)	24.7	2.8 (UC)	10 m (A, B, C)
Cyproideidae	Peltocoxa marioni Catta, 1875	19.8	2.1 (UC)	10 m (C), 15 m (A)
Dexaminidae	Dexamine spinosa (Montagu, 1813)	32.5	11.8 (RC)	4 m (A, B), 10 m (A, B, C), 15 m (A, B, C)
	Dexamine spiniventris (Costa, 1853)	19.8	2.1 (UC)	10 m (A), 15 m (B)
Ischyroceridae	Ericthonius punctatus (Bate, 1857)	46.3	20.1 (VC)	4 m (A, B), 10 m (A, B), 15 m (A, B)
iscrigiocenuae	Jassa marmorata Holmes, 1905	19.8	0.7 (R)	10 m (C)
	Siphonoecetes sabatieri (de Rouville, 1894)	104.2	26.4 (VC)	4 m (A, B, C), 10 m (B, C)
	Siphonoecetes subarier (de Rouvine, 1894) Siphonoecetes bulborostrum (de-la-Ossa-Carretero & Marti, 2014)***	73.4	20.4 (VC) 22.9 (VC)	10 m (A, B, C), 15 m (A, B, C)
Leucothoidae	Leucothoe incisa (Robertson, 1892)	28.0	28.5 (VC)	4 m (A, B, C), 10 m (B, C), 15 m (A, B, C)
	Leucothoe spinicarpa (Abildgaard, 1789)	21.7	5.6 (C)	10 m (A, B), 15 m (A, B)
Lysianassidae	Hippomedon massiliensis (Bellan-Santini, 1965)	31.0	6.9 (C)	4 m (B, C), 10 m (B, C)
Lysianassiaac	Hippomedon ambiguus (Ruffo, 1946)*	23.7	3.5 (C)	4 m (B, C), 10 m (C)
	Lepidepecreum longicornis (Bate & Westwood, 1862)	19.8	2.8 (UC)	4 m (C), 15 m (A)
	Orchomenella nana (Kroyer, 1846)	19.8	0.7 (R)	10 m (C)
	Orchomene humilis (Costa, 1853)			
Megaluropidae	Megaluropus massiliensis (Ledoyer, 1976)	19.8 22.8	0.7 (R) 8.3 (C)	15 m (C) 4 m (A, B, C), 10 m (B, C)
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Melitidae	Maera grossimana (Montagu, 1808)	79.0	3.5 (C)	10 m (A, C)
	Maera inaequipes (Costa, 1857)	44.4	2.8 (UC)	10 m (A, B)
	Elasmopus pectenicrus (Bate, 1862)*	19.8	0.7 (R)	15 m (C)
	Elasmopus pocillamus (Bate, 1862)	19.8	0.7 (R)	10 m (C)
Microprotopidae	<i>Microprotopus maculatus</i> Norman, 1867	35.6	23.6 (VC)	4 m (A, B, C), 10 m (A, B, C), 15 m (A, B, C)
Nuuanuidae	Gammarella fucicola (Leach, 1814)	202.1	5.6 (C)	4 m (A, B),10 m (A, C), 15 m (A)

## Table 2. Continued

Family	Species	Mean density	Frequency	Stations
Oedicerotidae	Deflexilodes acutipes (Ledoyer, 1983)	19.8	3.5 (C)	10 m (B), 15 m (A, C)
	Monoculodes carinatus (Bate, 1857)	19.8	0.7 (R)	15 m (C)
	Perioculodes aequimanus (Korssman, 1880)	26.3	3.5 (C)	10 m (A, B), 15 m (A)
	Perioculodes longimanus (Bate & Westwood, 1868)	38.8	23.6 (VC)	4 m (A, B, C), 10 m (A, B, C), 15 m (A, B, C)
	Synchelidium haplocheles (Grube, 1864)	19.8	1.4 (UC)	4 m (B), 10 m (A)
	Synchelidium maculatum (Stebbing, 1906)	19.8	4.2 (C)	4 m (A, B, C), 15 m (B)
	Synchelidium longidigitatum Ruffo, 1947	39.5	0.7 (R)	10 m (C)
Photidae	Megamphopus cornutus (Norman, 1869)	32.1	5.6 (C)	10 m (A, B), 15 m (A, B)
	Megamphopus longicornis (Chevreux, 1911)*	47.4	2.1 (UC)	10 m (A), 15 m (C)
	Photis longipes (Della Valle, 1893)	70.4	22.9 (VC)	4 m (C), 10 m (A, B, C), 15 m (A, B, C)
Phoxocephalidae	Harpinia crenulata (Boeck, 1871)	19.8	1.4 (UC)	15 m (A)
Stenothoidae	Stenothoe monoculoides (Montagu, 1815)	19.8	0.7 (R)	4 m (A)
Urothoidae	Urothoe elegans (Costa, 1853)	19.8	1.4 (UC)	4 m (B, C)
	Urothoe hesperiae (Conradi, López-González & Bellan- Santini, 1986)**	118.5	0.7 (R)	4 m (C),
	Urothoe pulchella (Costa, 1853)	19.8	0.7 (R)	15 m (A)

Asterisks indicate new records (\*), second records (\*\*) and a new species (\*\*\*) for the Spanish Mediterranean coast. Mean density, individuals  $\cdot m^{-2}$ . Frequency, % of samples in which each species was found.

VC = very common; RC = rather common; C = common; UC = uncommon; R = rare.

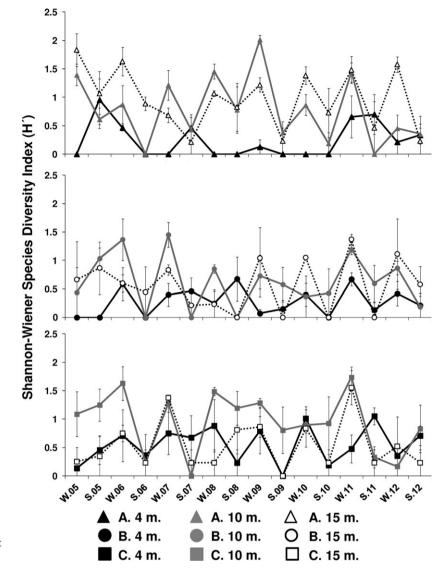
Table 3. Results of the analysis of variance for the Shannon–Wiener diversity index based on species abundance for the following factor	rs: tran-
sect (A, B, C), depth (4, 10 and 15 m), year (2005–2013) and season (winter and summer).	

source	df	SS	MS	F	Р
Transect	2	3.1919	1.5959	0	0
Depth	2	14.5936	7.2968	0	0
Year	7	2.8435	0.4062	0.33	0.9184
Season	1	21.4718	21.4718	17.27	0.0043
Transect $\times$ depth	4	7.4674	1.8669	0	0
Transect × year	14	4.0062	0.2862	1.19	0.3748
Transect × season	2	0.2344	0.1172	0.49	0.6243
Depth $\times$ year	14	5.6919	0.4066	0.72	0.7279
Depth $\times$ season	2	7.7611	3.8806	6.86	0.0084
Year × season	7	8.7033	1.2433	6.15	0
Transect $\times$ depth $\times$ year	28	7.9894	0.2853	0.9	0.6072
Transect $\times$ depth $\times$ season	4	1.8371	0.4593	1.45	0.2436
Transect $\times$ year $\times$ season	14	3.3674	0.2405	1.19	0.2826
Depth $\times$ year $\times$ season	14	7.9235	0.566	2.8	0.0006
Transect $\times$ depth $\times$ year $\times$ season	28	8.8628	0.3165	1.57	0.038
Residual	288	58.2422	0.2022		
Total	431	164.1877			

SS = sum of squares; MS = mean squares; F ratio of each factor; P = P-value.

The Shannon–Wiener diversity index reached values of 0 to 2.01. The highest values were detected at the 10m-deep station in transect A, whereas null values were mainly obtained at the shallower stations of transects A and B. Significant differences were detected in the interaction among the four factors (Table 3). These significant differences were mainly due to a decrease in diversity at shallower stations of transect A and B with respect to transect C and the deeper stations in all transects, where higher diversity values were obtained (Fig. 2).

nMDS showed that shallow stations of transects A and B were segregated with respect to the others during some sampling campaigns (Fig. 3a,b). These stations were highly dissimilar during these campaigns due to the low amphipod abundance registered. Most of these stations did not record any amphipods during these sampling



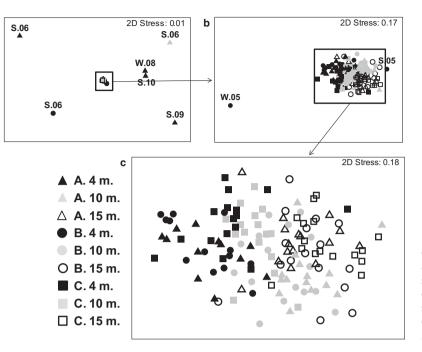
**Fig. 2.** Mean and SE of Shannon–Wiener diversity index based on species abundance at each station and in each sampling campaign.

campaigns; only one specimen each of *Synchelidium maculatum* and *Dexamine spinosa* was collected at B1 during 2005, in winter and summer, respectively. These segregated stations with null abundances were excluded from the PERMANOVA and the remaining analyses, as the absence of amphipods produced undefined resemblances among these stations. With respect to the other stations, segregation according to depth was observed, although some individual stations did not follow this pattern (Fig. 3c).

PERMANOVA showed significant differences in the interactions of the factors considered (Table 4 ). Pairwise tests of interaction detected significant differences for the depth factor in most sampling campaigns due to variations among the shallow stations and those at 10 and 15 m depths. This difference among depths appeared

in all transects, but particularly in transect A. Significant differences among transects were mainly detected among shallow stations in transect C compared with A and B, whereas differences among transects were usually not significant when comparing the deeper stations.

Species distributions reflected the multivariate analysis results (Table 5). Siphonoecetes sabatieri was the most abundant species at shallow stations whereas another member of the same genus, S. bulborostrum, was more abundant at deeper stations. The genus Ampelisca was more abundant at deeper stations, as were Photis longipes, Pseudolirius kroyeri, Apherusa chiereghinii and Phtisica marina, species that were not collected at 4 m. Among Ampelisca species, A. typica was abundant at 15 m, whereas A. spinipes was abundant at 10 m in transects A and B, and Ampelisca brevicornis was abundant at 10 m а



**Fig. 3.** Non-metric multidimensional scaling ordination of amphipod abundance and associated stress values of (a): all stations and sampling campaigns; (b): excluding A1 (summer 2006, winter 2008, summer 2009, summer 2010), A2 (summer 2006) and B1 (summer 2006); (c): excluding B1 (winter 2005).

**Table 4.** Results of permutational analysis of variance (PERMANOVA) based on the Bray–Curtis dissimilarities of the multivariate data set for the following factors: transect (A, B, C), depth (4, 10 and 15 m), year (2005–2013) and season (winter and summer).

source	Df	SS	MS	ps-F	P (perm)
Transect	2	35,059	17,530	2.4244	0.0007
Depth	2	85,365	42,683	3.4037	0.0005
Year	7	52,912	7558.9	1.3473	0.0671
Season	1	13,138	13,138	2.5481	0.0294
Transect × depth	4	54,451	13,613	1.9532	0.0002
Transect × year	14	73,370	5240.7	1.0962	0.2357
Transect × season	2	8244.1	4122.1	0.91281	0.5806
Depth $\times$ year	14	79,787	5699.1	0.99847	0.5113
Depth $\times$ season	2	17,645	8822.5	1.6576	0.0422
Year × season	7	40,044	5720.5	2.2449	0.0001
Transect $\times$ depth $\times$ year	28	1.65E+05	5908.5	1.3505	0.0011
Transect $\times$ depth $\times$ season	4	13,978	3494.4	0.81306	0.8252
Transect × year × season	14	67,538	4824.2	1.8932	0.0001
Depth $\times$ year $\times$ season	14	80,355	5739.6	2.2524	0.0001
Transect $\times$ depth $\times$ year $\times$ season	22	95,136	4324.4	1.697	0.0001
Residual	223	5.68E+05	2548.2		
Total	360	1.60E+06			

SS = sum of squares; MS = mean squares; ps-F = pseudo-F ratio of each factor; P (perm) = permutation P-value.

in transect C. Other species were occasionally detected, such as *G. fucicola* at 10 m in transect A, and *P. longim-anus* at 4 and 10 m in transect C.

The detritivorous trophic group was dominant at all stations. However, the percentage of carnivores was higher at the 4 and 10 m stations along transect C (Fig. 4). These differences were reflected in the diversity index according to trophic groups in such a way that higher values were usually obtained in transect C at 4

and 10 m depth, while higher values were obtained in transect B at 10 m and in transect A at 15 m during some sampling campaigns. ANOVA detected significant differences in the interaction among the four factors (Table 6), as a significant increase in diversity values was detected in transect C at 4 and 10 m, while diversity was significantly higher in transect A at 15 m (Fig. 5).

These species distributions were related to the percentage of organic matter and mud, a parameter that attained

**Table 5.** Mean densities (individuals  $\cdot$  m<sup>-2</sup>) of the most abundant Amphipoda species at each station, except for the stations excluded in the non-metric multidimensional scaling analysis (Fig. 3b and c).

Depth	4 m			10 m			15 m		
Transect	A	В	С	A	В	С	A	В	С
Ampelisca brevicornis	2.74	0.00	3.70	0.00	4.12	30.04	0.00	0.00	0.00
Ampelisca spinipes	0.00	0.00	0.00	36.43	12.76	1.65	2.88	7.00	3.70
Ampelisca typica	0.00	0.51	0.00	2.63	0.41	0.41	28.81	31.28	26.75
Aoridae female	5.49	2.53	0.41	25.02	11.93	19.34	29.22	8.64	9.47
Apherusa chiereghinii	0.00	0.00	0.00	13.17	8.64	3.70	9.47	4.94	2.47
Atylus massiliensis	5.49	7.09	3.70	0.88	0.41	3.70	4.94	0.41	0.41
Ericthonius punctatus	4.94	2.53	0.41	4.83	2.88	14.40	3.70	1.23	0.41
Gammarella fucicola	0.55	7.09	0.00	46.53	0.00	0.41	4.53	0.00	0.00
Leucothoe incisa	0.55	1.01	2.47	0.00	4.53	10.70	4.53	2.47	2.47
Microprotopus maculatus	8.78	2.53	8.23	3.07	3.29	6.58	2.06	5.76	0.41
Monocorophium acherusicum	1.65	6.58	1.23	0.44	0.82	2.47	0.00	0.00	0.00
Perioculodes longimanus	0.55	0.51	20.99	0.44	1.65	15.64	3.29	1.65	0.82
Photis longipes	0.00	0.00	0.41	5.71	9.88	8.23	8.64	15.23	43.62
Phtisica marina	0.00	0.00	0.41	10.53	2.88	1.65	12.76	2.88	7.41
Pseudolirius kroyeri	1.10	1.01	0.82	8.78	0.82	1.23	40.33	103.70	107.00
Siphonoecetes sabatieri	24.14	96.74	17.70	0.00	29.63	13.17	0.00	0.00	0.00
Siphonoecetes bulborostrum	0.00	0.00	0.00	44.33	4.94	0.41	9.88	5.35	17.28

Table 6. Results of the analysis of variance for the Shannon–Wiener diversity index based on abundance of trophic groups for the following factors: transect (A, B, C), depth (4, 10 and 15 m), year (2005–2013) and season (winter and summer).

Source	df	SS	MS F		P (perm)	
Transect	2	127.5815	63.7908	0.00	0.0000	
Depth	2	32.0160	16.0080	0.00	0.0000	
Year	7	79.8386	11.4055	0.61	0.7381	
Season	1	96.9606	96.9606	5.15	0.0576	
Transect × depth	4	299.4138	74.8535	0.00	0.0000	
Transect × year	14	171.0482	12.2177	1.16	0.3944	
Transect × season	2	0.3622	0.1811	0.02	0.9830	
Depth × year	14	128.5728	9.1838	0.96	0.5317	
Depth × season	2	30.1614	15.0807	1.57	0.2420	
Year × season	7	131.8820	18.8403	3.27	0.0024	
Transect $ imes$ depth $ imes$ year	28	248.1225	8.8615	0.89	0.6252	
Transect $\times$ depth $\times$ season	4	18.2900	4.5725	0.46	0.7666	
Transect × year × season	14	147.8361	10.5597	1.83	0.0340	
Depth × year × season	14	134.2520	9.5894	1.66	0.0628	
Transect $\times$ depth $\times$ year $\times$ season	28	280.2605	10.0093	1.74	0.0141	
Residual	288	1661.0178	5.7674			
Total	431	3587.6160				

SS = sum of squares; MS = mean squares; F = F ratio for each factor; P = P-value.

the highest Spearman correlation (Rho: 0.344 and 0.238). CCA showed that both parameters were related, as the deeper stations had higher organic matter percentages and muddier sediments (Fig. 6; Table 1). Other granulometry classes were related to changes among transects: fine sand content was related to amphipod assemblages of the stations at 4 and 10 m along transect C, whereas coarser grain sizes were related to changes at stations at 4 and 10 m depth in transect B.

## Discussion

Amphipoda assemblages along the coast near Alicante showed spatial differences in diversity and composition among the sampled stations. These differences were related to both anthropogenic disturbance and environmental parameters. Diversity values decreased at shallower stations along transects close to human activities: the harbor, and sewage and brine discharges. Thus,

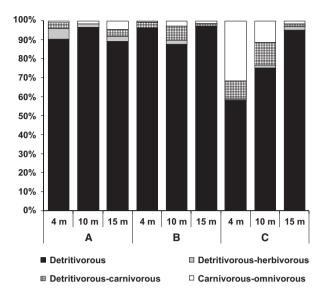


Fig. 4. Percentage of Amphipoda individuals for each trophic group at each station.

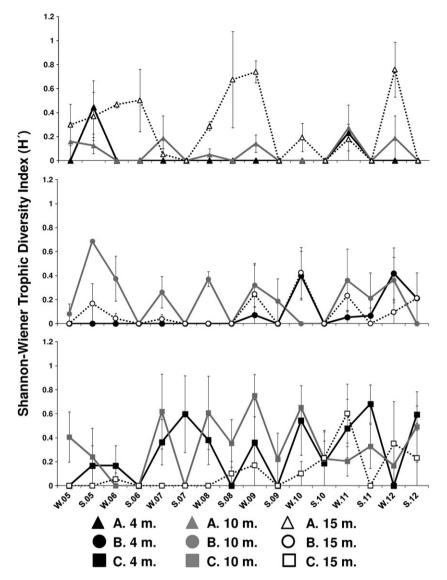
absence of amphipods was indeed detected during some sampling campaigns. The expansion of Alicante docks, together with sewage discharge, could have caused the absence of amphipods at the shallowest station along transect A. Harbor expansion works entail dredging, release and dispersion of accumulated contaminants and changes in water circulation (Riera et al. 2011), while sewage disposal can produce physical and chemical changes in sediments (Abessa et al. 2005). These activities result in changes in benthic communities, the proliferation of opportunistic species and lower abundances of sensitive species (Saiz-Salinas & Urkiaga-Alberdi 1999; Martínez-Lladó et al. 2007 Callier et al. 2009; Riera et al. 2011). The dispersion of contaminants has previously been shown to result in massive mortalities of amphipods (Riera et al. 2011a), due to the widely demonstrated sensitivity of this taxon to this type of pollution (Dauvin 1982; Nikitik & Robinson 2003). Moreover, the presence of sewage discharge may reinforce this impact in Alicante as the harbor has low levels of hydrodynamic mixing and exchange rates of sewage effluent. Poorly flushed waters could facilitate hypoxia in the bottom water due to inputs of sewage pollution (Xu et al. 2011) and amphipods show a general sensitivity to this (de-la-Ossa-Carretero et al. 2012).

However, some Amphipoda showed relatively high abundances in the transect close to the harbor and sewage discharge. Although most amphipods are considered more sensitive to polluted sediments than other benthic organisms (Gomez Gesteira & Dauvin 2000; Dauvin & Ruellet 2007), not all species showed the same level of sensitivity. Species such as *Gammarella fucicola*, *Ampelisca*  spinipes, Siphonoecetes bulborostrum and Pseudolirius kroyeri were in fact abundant in the transect closest to the harbor and sewage discharge. Some tolerance to pollution was previously attributed to *G. fucicola* (Glémarec & Hily 1981; Borja *et al.* 2000), while *Ampelisca* and *P. kroyeri* were previously collected at the edge of fish farm facilities, showing a moderate positive response (Fernandez-Gonzalez *et al.* 2013).

An absence of amphipods was also noted at the station closest to the brine discharge before its mitigatory dilution. Only one specimen was collected in both winter and summer 2005 at this station, Synchelidium haplocheles and Dexamine spinosa, respectively. The effect of brine discharge on the benthic communities and Polychaeta assemblage was previously monitored by Del-Pilar-Ruso et al. (2007, 2008) for the years 2004 and 2005. During this period salinity values exceeded 39 psu at this station and such an abrupt change of salinity could induce this decrease in abundance of amphipods. So, as with oil or organic pollution, amphipods appear to show high sensitivity to increased salt concentration produced by this kind of discharge. However, the dilution process established in 2006 mitigated the rise in salinity and may be favoring the recovery of the amphipod assemblage. Amphipod abundance at the station closest to the brine discharge has increased since the establishment of brine dilution, with an assemblage highly dominated by Siphonoecetes sabatieri settling there. This shallow station is affected by hydrodynamism due to waves, wind and storm events, in addition to brine flow, and its sediment was composed of a higher content of medium and coarse sand in comparison with deeper stations. These characteristics may have favored the dominance of this species as Siphonoecetes spp. are known for unstable population dynamics that are correlated with the nature of the sediment (Bigot et al. 2006).

By contrast, natural parameters were also highly related to assemblage structure, such as depth or sediment. Depth was a determining factor along this section of the Alicante coast, as amphipod composition at shallower stations contrasted with the deepest stations. Some species were even depth specific. *Apherusa chiereghinii, A. spinipes* and *S. bulborostrum* were only collected at 10 and 15 m depth, whereas *S. sabiateri*, *Ampelisca brevicornis* and *Monocorophium acherusicum* were not found at 15 m. Some species were almost entirely restricted to shallower stations with high fine sand content, such as *A. brevicornis, Perioculodes longimanus, Urothoe hesperiae* and *Urothoe elegans.* 

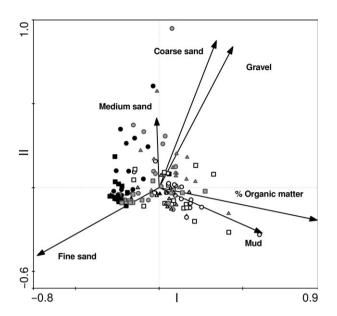
Depth has previously been reported as an important factor for crustacean distribution (Cacabelos *et al.* 2010; Carvalho *et al.* 2012). It is linked to determinant factors for benthos distribution, such as hydrodynamic conditions



**Fig. 5.** Mean and SE of Shannon–Wiener diversity index based on abundance of trophic groups at each station and in each sampling campaign.

and substrate stability (Cunha et al., 1999). Along a depth gradient, species are distributed according to physical parameters such as sediment composition and biological factors such as food supply (Carvalho et al. 2012). Increasing depth off Alicante is correlated with changes in grain size and organic matter content. Grain size is usually established as a decisive factor in crustacean distribution (Robertson et al. 1989; Marques & Bellan-Santini 1993) and is especially so in this study area, where it is related to a change in the habitat. Deeper stations were covered by dead rhizomes of Posidonia, an organicenriched environment that allowed higher abundances of detritus-feeding amphipods, such as Ampelisca typica, A. spinipes and Aoridae (Guerra-García et al. 2014), as well as those associated directly with this seagrass leaflitter, such as G. fucicola (Lepoint et al. 2006). The importance of such litter to amphipod assemblage structure has previously been reported (Sanchez-Jerez *et al.* 2000). It includes various phytoplankton, other invertebrates such as meiofauna, microscopic benthic food such as fungi, bacteria, micro- and macroalgae, and the detritus itself (Darnell 1967).

A high percentage of detritivores was also detected at shallower stations, which is logical as detritus is the main food item of most amphipod species (Guerra-García *et al.* 2014), *e.g. S. sabiateri*, a detritivorous domicolus species dominant at the shallow stations of transects A and B. However, at the stations at 4 and 10 m depth in transect C, which had lower percentages of organic matter, species that also feed on prey were more abundant and diversity based on trophic groups was higher compared with those in transects A and B. The distributions of these carnivorous species might also be explained by their burrowing behavior. Most of them are fossorial, species that burrow



**Fig. 6.** Results of correspondence analysis biplot. Points correspond to stations ( $\blacktriangle$ , A 4 m,  $\blacktriangle$  A 10 m,  $\Delta$  A 15 m; O B 4 m,  $\clubsuit$  B 10 m, O B 15 m;  $\blacksquare$  C 4 m,  $\blacktriangle$  C 10 m,  $\Box$  C 15 m). Arrows indicate sediment characteristics. Axes I and II had eigenvalues of 0.520 and 0.286, respectively.

using their periopods. *Posidonia* meadow bottoms can be too compacted for this and so a more loose meadow could be more suitable for this burrowing behavior. Therefore, species were established at each station according to both sediment composition and food supply, parameters that are inter-related.

With regard to the taxonomic composition of Amphipoda, the present paper confirms the need to gain more information about this order along the Spanish Mediterranean coast. We sampled a relatively accessible area of around 1.5 km<sup>2</sup> covered by fine sand, mud and Posidonia dead rhyzomes, common habitats in the Mediterranean Sea, and new records and an undescribed species were found. Previous studies in nearby areas reported several new records and other new species of the genus Medicorophium (de-la-Ossa-Carretero et al. 2010; Myers et al. 2010). Among the species for which our records are the first for this area, Megamphopus longicornis, Elasmopus pectenicrus and Hippomedon ambiguus were previously reported in the Western and Eastern Mediterranean; the first two are also found in the Atlantic Ocean (France and Iberian Peninsula), while the latter is a Mediterranean endemic (Bellan-Santini et al. 1998). The species recorded here for the second time in this area, A. spinipes and U. hesperiae, were previously reported in Algeciras Bay (Strait of Gibraltar; Conradi & López-González 1999); A. spinipes has a wide distribution (Bellan-Santini et al. 1998) while U. hesperiae was first reported

An important finding is the presence of Caprella scaura at the shallow station of transect B. It is a species native to the Western Indian Ocean first cited from Mauritius and later reported from several regions of the world, including the Spanish Mediterranean (Martínez & Adarraga 2008; Guerra-García et al. 2011; Fernandez-Gonzalez et al. 2013; Ros et al. 2013). The introduction of this species into Mediterranean waters seems to be due to the traffic of yachts (Martínez & Adarraga 2008). It is associated with hard substrate in artificial enclosed habitats, but has never before been reported in natural habitats. This species has adapted to unexposed environments, given its affinity for fouling communities in habitats with limited hydrodynamism, sheltered from wave action (Ros et al. 2013). The presence of C. scaura in the study area appears to be sporadic as only one specimen was detected, at the shallow station of transect B; its presence could be incidental or could report the possibility of dispersion of C. scaura to a natural area. Further studies that confirm its presence in nearby enclosed areas, the Port of Alicante and in natural habitats more suitable for this species, would clarify the dispersion of C. scaura in this area.

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