Soft–bottom sipunculans from San Pedro del Pinatar (Western Mediterranean): influence of anthropogenic impacts and sediment characteristics on their distribution

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Abstract

Soft–bottom sipunculans from San Pedro del Pinatar (Western Mediterranean): influence of anthropogenic impacts and sediment characteristics on their distribution.— We analysed the distribution of soft bottom sipunculans from San Pedro del Pinatar (Western Mediterranean). This study was carried out from December 2005 to June 2010, sampling with biannual periodicity (June and December). Physical and chemical parameters of the sediment were analysed (granulometry, organic matter content, pH, bottom salinity and shelter availability). Nine different species and subspecies were identified, belonging to five families. *Aspidosiphon muelleri muelleri* was the dominant species, accumulating 89.06% of the total abundance of sipunculans. Higher sipunculan abundances were correlated with stations of higher percentage of coarse sand, empty mollusc shells and empty tubes of the serpulid polychaete *Ditrupa arietina,* where some of the recorded species live. Sediment characteristics played the main role controlling the sipunculans distribution. Anthropogenic impacts could be indirectly affecting their distribution, changing the sediment characteristics.

Key words: Sipuncula, *Aspidosiphon muelleri*, Mediterranean, Anthropogenic impact, Soft–bottom.

Resumen

Sipuncúlidos de fondos blandos de San Pedro del Pinatar (Mediterráneo occidental): influencia de los impactos antropogénicos y las características del sedimento sobre su distribución.— Se analizó la distribución de los sipuncúlidos de fondos blandos de San Pedro del Pinatar (Mediterráneo occidental). Este estudio se llevó a cabo entre diciembre de 2005 y junio de 2010, muestreando con periodicidad semestral (junio y diciembre). Se analizaron parámetros físicos y químicos del sedimento (granulometría, contenido de materia orgánica, pH, salinidad de fondo y disponibilidad de refugio). Nueve especies y subespecies diferentes fueron identificadas, pertenecientes a cinco familias. *Aspidosiphon muelleri muelleri* fue la especie dominante, acumulando el 89,06% de la abundancia total de sipuncúlidos. Las mayores abundancias de sipuncúlidos se correlacionaron con las estaciones con mayores porcentajes de arena gruesa, conchas de moluscos vacías y tubos vacíos del poliqueto serpúlido *Ditrupa arietina,* donde viven algunas de las especies registradas. Las características del sedimento jugaron el papel principal en el control de la distribución de sipuncúlidos. Los impactos antropogénicos podrían estar afectando indirectamente su distribución, cambiando las características del sedimento.

Palabras clave: Sipuncúlidos, *Aspidosiphon muelleri*, Mediterráneo, Impacto antropogénico, Fondos blandos.

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Introduction

The phylum Sipuncula is composed of about 150 species and subspecies (Cutler, 1994). Sipunculans are exclusively marine benthic organisms. Almost 75% of the sipunculan species are concentrated in shallow waters (< 200 m), and half of them are confined to the upper photic zone of the shelf (Murina, 1984). The phylum Sipuncula has been overlooked and was barely studied for many years. In the Mediterranean Sea, its distribution has been studied widely since the 1990s (Saiz–Salinas, 1993; Murina et al., 1999; Pancucci–Papadopoulou et al., 1999; Açik et al., 2005; Açik, 2007, 2008a, 2008b, 2009). Most of these studies, however, deal with the Eastern Mediterranean and only Saiz–Salinas gives concise information about sipunculans in the Western Mediterranean (Saiz–Salinas, 1982, 1986, 1993; Saiz–Salinas & Murina, 1982; Saiz–Salinas & Villafranca–Urchegui, 1990).

Most sipunculan worms are deposit feeders and many of them live in soft substrata. Soft–bottom sipunculans live buried inside the sediment and they obtain a substantial part of their food through the ingestion of sediment. Some species of sipunculans have been described as important bioturbators in soft sediments (Murina, 1984; Kędra & Wlodarska–Kowalczuk, 2008; Shields & Kędra, 2009), playing a main role in benthic ecosystems. It is well known that some of these species often find shelter inside the empty shells of certain molluscs or empty tubes of polychaetes (Gibbs, 1985; Troncoso & Urgorri, 1992; Saiz–Salinas, 1993; Murina et al., 1999; Troncoso et al., 2000; Açik et al., 2005; Schulze, 2005; Wanninger et al., 2005), usually empty tubes of the serpulid *Ditrupa arietina* (Solís–Weiss, 1982; Morton & Salvador, 2009) and other cavities inside hard structures buried into the sediment. Those facts promote a strong relation between sipunculans and characteristics of the sediment such as granulometry, pH, organic matter content and shelter availability.

The aim of this study was to analyse the distribution of sipunculan worms in this area and assess the possible effect of abiotic factors of the sediment or anthropogenic impacts on their distribution.

Material and methods

Study area and sampling design

The area studied is located near San Pedro del Pinatar coast (SE Spain). Different types of anthropogenic disturbances merge in this area: a sewage outfall, a brine discharge and fish farm cages (fig. 1). We compared a grid of 12 stations covering the influence area of the three anthropogenic impacts. We sampled twice a year (December and June) over a five–year period, from December 2005 to June 2010. We established three transects (A, B and C), separated by approximately 2 km. Four stations at each transect were established (1, 2, 3 and 4), separated from 250 to 500 m according to depth. The distance between C3 and C4 was shorter due to sampling problems. The initial sampling point for C3 was not soft–bottom and was relocated. Four samples of each station were taken using a Van Veen grab and covering a surface area of 0.04 m^2 for each sample.

The sewage outfall has been in place for decades and produces a flow of 5,000 m^3 per day in winter and 20,000 m^3 per day in summer, with wastewater secondary treatment (Del–Pilar–Ruso et al., 2009). The sewage discharge point is located between stations A1 and B1. The desalination plant began operations in January 2006 with a discharge of 80,000 m^3 per day, but in October 2006 the production was doubled with the start up of a new plant. The discharge takes place through a shared outfall at 33 m depth over soft sediment, and it is located at the B2 station. The brine presents a high salinity, ranging between 60–68 psu. In March 2010, a diffuser was connected to the end of the pipeline, and this has notably reduced the concentration of brine 10 m away from the discharge point (from 68 to 40 psu, unpublished data). A complete description about how the brine plume flows along the sea bottom can be found in Fernández–Torquemada et al. (2009). The fish farm complex has operated since 1998, with an annual production of 6,197 tons of blue fin tuna (*Thunnus thynnus*), sea bream (*Spaurus aurata*), meagre (*Argyrosomus regius*) and sea bass (*Dicentrarchus labrax*) (Ruiz et al., 2010). Some of the fish farm cages are less than 200 m away from stations C3 and C4.

Laboratory analysis

Three samples were used for the faunistic analysis, and in June 2010 one of these samples was also used to count the total shelters available in each station. The samples used for the faunistic analysis were sieved through a 0.5 mm mesh screen to separate the macrofauna and they were fixed in 10% buffered formalin. Later the fauna was preserved in ethanol 70% and sorted in different taxa. Sipunculans were identified to the species level through analysis of their internal and external anatomy using a binocular scope and observing typical structures with taxonomic value, such as presence and shape of hooks and papillaes, using a microscope. Length and thickness of the trunk of each specimen were taken.

The fourth sample was used for the sediment characterization (granulometric analysis, pH and amount of organic matter). We measured the pH of the sediment immediately after collection, using a pH–meter Crisom with a sensor 52–00. Furthermore, a sub–sample of approximately 30 g of the shallow layer of the sediment was separated to subsequently obtain the percentage of organic matter. We determined the organic matter content from the sub–samples as weight loss on ignition after 4 hr at 400ºC inside the muffle furnace. We carried out the granulometric analysis following the methodology described by Buchanan (1984), sorting the samples into six categories: gravel, coarse sand, medium sand, fine sand and silt and clays. Depth and bottom salinity were recorded using a CTD sensor (RBR–XR–420/620).

We calculated the shelter availability from one of the samples used for the fauna analysis in June 2010.

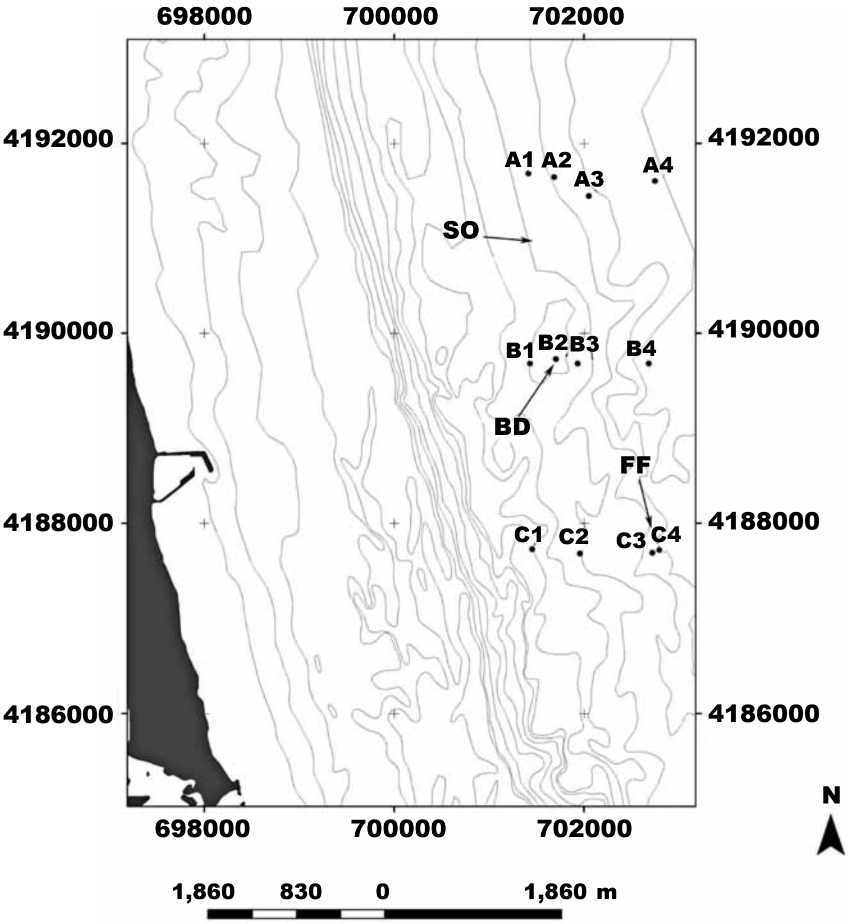

Fig. 1. Map of the study area showing the sampling stations: SO. Sewage outfall; BD. Brine discharge; FF. Fish farm cages. (Adapted from Del–Pilar–Ruso et al., 2009).

Fig. 1. Mapa del área de estudio mostrando las estaciones de muestreo: SO. Emisario de aguas residuales: BD. Vertido de salmuera; FF. Jaulas de piscifactoría. (Adaptado de Del–Pilar–Ruso et al., 2009).

We took three sub–samples and we counted the shelters in each sub–sample to calculate average of shelters/m^2. Available shelters, able to be used by sipunculids according to observations since 2005, were sorted into three categories according to their shape: spiral shape shells (gastropod shells such as *Turritella* sp.), cylindrical shape (scaphopod shells and serpulid tubes), and undefined shape (pieces of shell or calcareous debris among others). We also sorted the shelters into three size classes: S1, S2 and S3, corresponding to the length ranges < 3.5 mm, 3.5–7.0 mm and > 7.0 mm respectively.

Statistical analysis

Univariate and multivariate techniques were used to detect possible changes in the distribution of sipunculan species, and to define their possible relation both with the abiotic factors of the sediment and with the different anthropogenic impacts. Pearson product–moment correlation coefficient (r) was used to detect a possible linear correlation between abundance of sipunculans, species richness and abiotic factors of the sediment (granulometry, pH, organic matter content, and salinity). Pearson coefficient was also used to detect a possible linear dependence relation between shelter availability and sipunculans abundance.

Multivariate analysis is considered a sensitive tool for detecting changes in the structure of marine faunal community (Clarke & Warwick, 1994). Multivariate analysis of data was carried out using the PRIMER statistical package. We used the square root of abundance of sipunculans, and Bray–Curtis similarity coefficient was chosen to calculate the triangular similarity matrix. From this similarity matrix, non–metric multidimensional scaling techniques (nMDS) were applied.

Table 1. Total abundance for each species and for each station: N. Total individuals.

Tabla 1. Abundancia total para cada especie y para cada estación: N. Total de individuos.

	Stations													
	A1	A2	A3	A4	B1	B2	B3	B4	C1	C2	C3	C4	N	%
Aspidosiphon muelleri muelleri														
	9	7	9	35	9	1	8	42	5	385	138	60	708	89.06
Phascolion cf. caupo														
	0	0	0	2	0	0	3	1	1	5	21	6	39	4.91
Thysanocardia procera														
	0	1	2	4	4	0	1	4	0	2	1	3	22	2.77
Onchnesoma steenstruppi steenstruppi														
	0	0	1	0	0	0	0	0	0	0	3	1	5	0.63
Aspidosiphon muelleri kowalevski														
	1	0	2	0	0	0	0	0	0	0	0	0	3	0.38
Phascolion strombus														
	0	0	0	0	0	0	0	1	0	1	1	0	3	0.38
Phascolosoma granulatum														
	0	0	0	0	0	0	0	0	1	1	0	0	2	0.25
Sipunculus nudus														
	0	0	0	0	0	0	1	0	0	0	0	0	1	0.13
Golfingia vulgaris vulgaris														
	0	0	1	0	0	0	0	0	0	0	0	0	1	0.13
Unidentified														
	0	0	2	1	0	0	1	2	3	0	0	2	11	1.38
N	10	8	17	42	13	1	14	50	10	394	164	72	795	100
%	1.26	1.00	2.14	5.28	1.64	0.13	1.76	6.29	1.26	49.56	20.63	9.06	100	—

Table 2. N. Number of individuals; TL. Average trunk length (mean ± SE); MaxTL. Maximum trunk length; MinTL. Minimum trunk length; TW. Trunk width; Shelter. % individuals inhabiting some kind of shelter (empty shells and empty tubes, among others).

Tabla 2. N. Número de individuos; TL. Longitud promedio del tronco (media ± EE); MaxTL. Longitud máxima del tronco; MinTL. Longitud mínima del tronco; TW. Anchura del tronco; Shelter. % de individuos viviendo en algún tipo de refugio (conchas y tubos vacíos entre otros).

	N	TL (mm)	MaxTL (mm)	MinTL (mm)	TW (mm)	Shelter
Aspidosiphon muelleri muelleri	708	4.95 ± 0.01	22.0	0.5	0.63 ± 0.00	94.17
Phascolion cf. caupo	39	3.29 ± 0.44	12.0	0.5	0.74 ± 0.11	81.82
Thysanocardia procera	22	3.87 ± 0.57	11.0	1.0	0.87 ± 0.13	0.00
Onchnesoma steenstruppi steenstruppi	5	1.50 ± 0.22	2.0	1.0	0.60 ± 0.10	0.00
Phascolion strombus strombus	3	1.50 ± 0.29	2.0	1.0	0.10 ± 0.00	66.67
Aspidosiphon muelleri kovalevskii	3	4.83 ± 2,12	9.0	2.0	0.83 ± 0.17	33.33
Phascolosoma granulatum	2	3.00 ± 1.41	4.0	2.0	0.75 ± 0.35	0.00
Sipunculus nudus	1	13.00	—	—	3.00	0.00
Golfingia vulgaris vulgaris	1	1.50	—	—	0.50	0.00

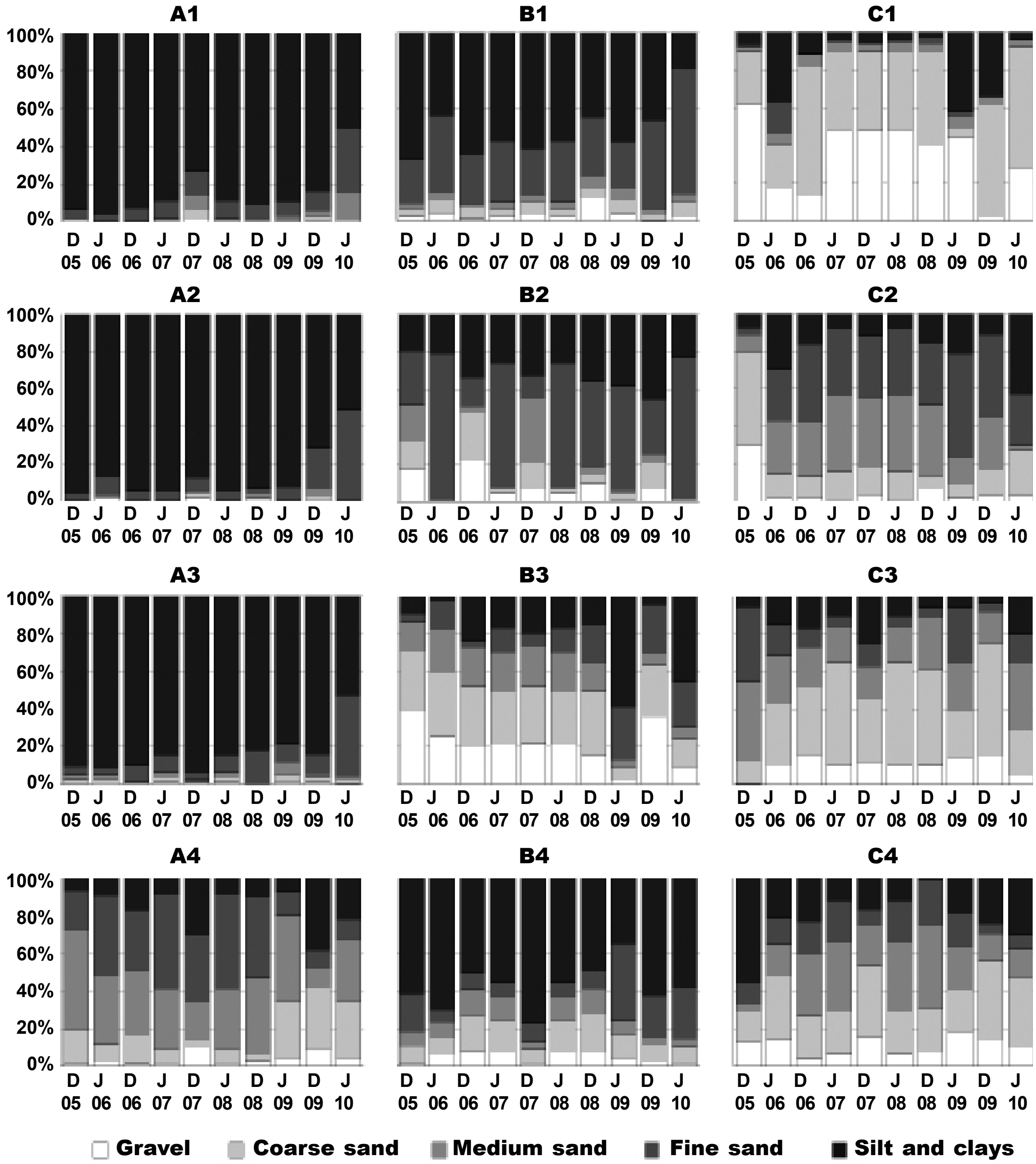

Fig. 2. Granulometric characteristics at each station from 2005 to 2010: J. June; D. December.

Fig. 2. Caracteristicas granulométricas para cada estación desde 2005 hasta 2010: J. Junio; D. Diciembre.

Results

A total of 360 benthic samples, from December 2005 to June 2010, were collected for the fauna analysis. We analysed 795 specimens, finding 9 different species (table 1), belonging to the families: Sipunculidae (1 sp.), Golfingiidae (2 spp.), Phascolionidae (3 spp.), Phascolosomatidae (1 sp.) and Aspidosiphonidae (2 spp.). The dominant species was *Aspidosiphon (Aspidosiphon) muelleri muelleri* (Diesing, 1851), with 708 individuals (89.06% of the total abundance). Eleven specimens (1.38%) could not be identified due to their small size or their state of deterioration. According to the different stations, higher abundances

Table 3. Depth (m), organic matter (OM, %), pH and bottom salinity (psu) at the different stations, and dates sampled: S. Station and depth; Y. Year; M. Month; BS. Bottom salinity.

S	Y	M	OM	pH	BS	S	Y	M
A1	2005	Dec	9.10	6.98	37.5	B1	2005	Dec
33.5 m	2006	Jun	13.13	7.51	38.0	32.8 m	2006	Jun
	2006	Dec	3.19	7.69	37.5		2006	Dec
	2007	Jun	4.58	7.46	38.0		2007	Jun
	2007	Dec	2.13	7.38	37.2		2007	Dec
	2008	Jun	5.11	7.75	37.6		2008	Jun
	2008	Dec	6.48	7.79	37.6		2008	Dec
	2009	Jun	5.2	7.38	37.4		2009	Jun
	2009	Dec	5.00	7.62	37.8		2009	Dec
	2010	Jun	7.59	7.13	37.9		2010	Jun
A2	2005	Dec	12.61	6.80	37.5	B2	2005	Dec
34.1 m	2006	Jun	7.35	7.54	37.5	33.6 m	2006	Jun
	2006	Dec	3.52	7.71	37.5		2006	Dec
	2007	Jun	13.23	7.39	37.0		2007	Jun
	2007	Dec	3.52	7.59	37.2		2007	Dec
	2008	Jun	10.58	7.66	37.2		2008	Jun
	2008	Dec	5.46	7.66	37.4		2008	Dec
	2009	Jun	5.92	7.22	37.3		2009	Jun
	2009	Dec	4.26	7.46	37.8		2009	Dec
	2010	Jun	8.20	7.14	37.8		2010	Jun
A3	2005	Dec	6.47	7.04	37.5	B3	2005	Dec
35.6 m	2006	Jun	15.85	7.04	37.5	33.7 m	2006	Jun
	2006	Dec	3.62	7.82	38.0		2006	Dec
	2007	Jun	13.27	7.48	37.0		2007	Jun
	2007	Dec	3.48	7.48	36.8		2007	Dec
	2008	Jun	10.04	7.73	37.5		2008	Jun
	2008	Dec	4.41	7.89	37.4		2008	Dec
	2009	Jun	4.56	7.43	37.4		2009	Jun
	2009	Dec	4.23	7.44	37.6		2009	Dec
	2010	Jun	5.05	7.19	37.7		2010	Jun
A4	2005	Dec	2.55	7.34	37.5	B4	2005	Dec
36.8 m	2006	Jun	6.97	7.70	37.5	37.2 m	2006	Jun
	2006	Dec	1.72	7.85	37.5		2006	Dec
	2007	Jun	1.41	7.80	37.0		2007	Jun
	2007	Dec	3.29	7.84	36.8		2007	Dec
	2008	Jun	0.98	7.77	37.8		2008	Jun
	2008	Dec	2.26	7.71	37.5		2008	Dec
	2009	Jun	0.95	7.83	37.4		2009	Jun
	2009	Dec	3.09	7.71	38.7		2009	Dec
	2010	Jun	4.82	7.28	38.1		2010	Jun

Tabla 3. Profundidad (m), materia orgánica (OM, %), pH y salinidad del fondo (ups) para las diferentes estaciones y fechas muestreadas: S. Estación y profundidad; Y. Año; M. Mes; BS. Salinidad del fondo.

OM	pH	BS	S	Y	M	OM	pH	BS
18.43	7.29	37.5	C1	2005	Dec	4.94	7.5	38.0
11.06	7.29	38.0	32.6 m	2006	Jun	4.93	6.8	38.0
2.98	7.88	37.5		2006	Dec	1.28	7.9	38.0
8.95	7.43	37.5		2007	Jun	3.37	7.7	37.0
2.27	7.76	37.2		2007	Dec	1.92	7.8	37.6
7.80	7.80	38.1		2008	Jun	1.29	7.8	37.9
4.39	7.65	38.3		2008	Dec	2.18	7.9	37.7
2.16	7.27	39.4		2009	Jun	1.99	7.4	37.4
3.23	7.39	41.6		2009	Dec	1.69	7.6	37.7
8.38	7.19	37.7		2010	Jun	2.30	7.5	37.5
9.15	7.47	37.5	C2	2005	Dec	4.81	7.3	38.0
3.82	7.47	42.0	33.4 m	2006	Jun	9.67	7.4	37.5
1.85	7.35	39.0		2006	Dec	1.77	7.7	38.0
4.54	7.20	45.0		2007	Jun	1.27	7.8	37.0
1.55	7.80	46.0		2007	Dec	1.79	7.6	37.6
3.60	7.83	52.9		2008	Jun	1.84	7.7	37.7
1.92	7.70	44.4		2008	Dec	1.92	7.8	37.7
4.99	7.55	45.1		2009	Jun	1.01	7.7	37.4
2.46	7.42	48.1		2009	Dec	1.54	7.7	37.7
4.68	7.19	38.4		2010	Jun	2.86	7.6	37.6
3.91	7.12	37.5	C3	2005	Dec	1.89	7.4	38.0
14.50	7.63	50.0	34.9 m	2006	Jun	9.05	7.5	38.0
1.62	7.65	41.5		2006	Dec	1.89	7.8	37.5
2.56	7.52	42.0		2007	Jun	4.66	7.4	37.0
1.99	7.45	41.4		2007	Dec	1.66	7.8	37.6
2.37	7.60	47.5		2008	Jun	4.27	7.7	37.8
1.62	7.80	43.1		2008	Dec	1.62	7.8	37.6
1.55	7.40	41.3		2009	Jun	2.00	7.7	37.5
2.15	7.77	43.4		2009	Dec	2.80	7.7	37.6
4.70	7.23	38.2		2010	Jun	2.80	7.2	37.7
6.61	7.06	37.5	C4	2005	Dec	14.14	7.3	38.0
19.96	7.86	38.5	35.6 m	2006	Jun	6.59	7.6	37.5
2.16	7.74	38.0		2006	Dec	1.87	7.8	37.5
3.48	7.43	37.5		2007	Jun	2.95	7.5	37.0
2.34	7.38	38.8		2007	Dec	1.29	7.7	37.6
4.50	7.71	37.6		2008	Jun	3.41	7.7	37.7
4.69	7.82	37.9		2008	Dec	1.60	7.7	37.6
6.76	7.42	37.5		2009	Jun	2.45	7.6	37.4
3.96	7.74	37.8		2009	Dec	1.94	7.5	37.8
7.79	7.12	38.0		2010	Jun	3.42	7.5	37.8

Table 4. Pearson correlation coefficient (r) of the most abundant species *vs.* sediment characteristics: OM. Organic matter (%); D. Depth; S. Salinity; G. Gravel (%); CS. Coarse sand (%); MS. Medium sand (%); FS. Fine sand (%); SC. Silt and clays (%) (* *p < 0.001)*.

*Tabla 4. Coeficiente de correlación de Pearson (r) de las especies más abundantes en relación a las características del sedimento: OM. Materia orgánica (%); D. Profundidad; S. Salinidad; G. Gravas (%); CS. Arena gruesa (%); MS. Arena media (%); FS. Arena fina (%); SC. Limos y arcillas (%) (** p *< 0,001).*

	OM	pH	D	S	G	CS	MS	FS	SC
Aspidosiphon muelleri muelleri	–0.200*	0.192*	–0.030	–0,131	–0.120	0.041	0.497***	0.170	–0.287***
Phascolion cf. caupo	–0.100	0.054	–0.025	–0.057	0.012	0.262**	0.280***	–0.041	–0.245**
Thysanocardia procera	–0.071	0.127	0.214*	–0.116	–0.07	–0.039	–0.053	0.027	0.054

Table 5. Pearson correlation coefficient (r) of *Aspidosiphon (A.) muelleri muelleri vs.* shelters availability: S1 < 0.5 mm; S2 = 0.5–1.0 mm; S3 ≥ 1.00 mm; *** *p* < 0.001.

Tabla 5. Coeficiente de correlación de Pearson (r) de Aspidosiphon (A.) muelleri muelleri *en relación a la disponibilidad de refugio: S1 < 0,5 mm; S2 = 0,5–1,0 mm; S3 ≥ 1,00 mm; ****p *< 0,001.*

	Cylindrical shape			Spiral shape			Undefined shape		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
A. muelleri muelleri	0.593***	0.844***	0.802***	–0.042	–0.308	–0.268	–0.150	0.086	—

were found in C2, C3 and C4, with 49.56%, 20.63% and 9.06% of the total individuals respectively. On the other hand, lower contributions, with less than 2% of the individuals for each station, were recorded in B3 (1.76%), B1 (1.64%), C1 (1.26%), A1 (1.26%), A2 (1.00%) and B2 (0.13%). Four species inhabited empty mollusc shells, empty tubes of *Ditrupa arietina* or other less common shelters (calcareous debris or crevices in chunks of rock) (table 2). The remaining species were found bare in the sediment.

The granulometry of the sediment was heterogeneous among the different sites studied (fig. 2). We detected high levels of silt and clays in the stations close to the sewage discharge (A1, A2, A3 and B1). Despite the variation over seasons and years, higher values of organic matter content were usually found in these stations. In the southern stations (C1, C2, C3 and C4), the granulometric analysis showed lower values of the finest fraction, and a more equitable distribution of the grain size, with higher values of gravel and sand, and decrease of silt and clays. A similar granulometric pattern was observed in B2, the brine discharge point.

The bottom salinity records showed how the station B2 and, to a lesser extent B1 and B3, were affected by the brine discharge (table 3), reaching maximum salinity values of 52.9 psu (VI 2008; B2). While the desalination plant was working (XII 2006–VI 2010) the salinity average in B1, B2 and B3 was 38.4 ± 0.5 psu (mean ± SE), 44.6 ± 1.5 psu and 43.2 ± 0.9 psu respectively.

We analysed the correlation between the three most abundant species and several characteristics of the sediment (table 4). The number of specimens of the remaining species was too low (< 20 individuals) to analyse a possible correlation. *A. muelleri muelleri* was positively correlated with the percentage of medium sands (r = 0.497, *p* = 0.000) and pH (r = 0.192, *p* = 0.018), whereas it was negatively correlated with the percentage of organic matter (r = –0.200, *p* = 0.014) and the percentage of silt and clays (r = –0.287, *p* = 0.001). *A. muelleri muelleri* was also correlated with the availability of shelter, showing a strong positive correlation with the three different sizes corresponding to the cylindrical shape (table 5). *Phascolion. cf. caupo* showed a positive correlation, with the percentage of coarse sand and the percentage of medium sand (r = 0.262 and r = 0.280 respectively both *p* < 0.01). *T. procera* only showed significant correlation with the depth (r = 0.214, *p* < 0.05).

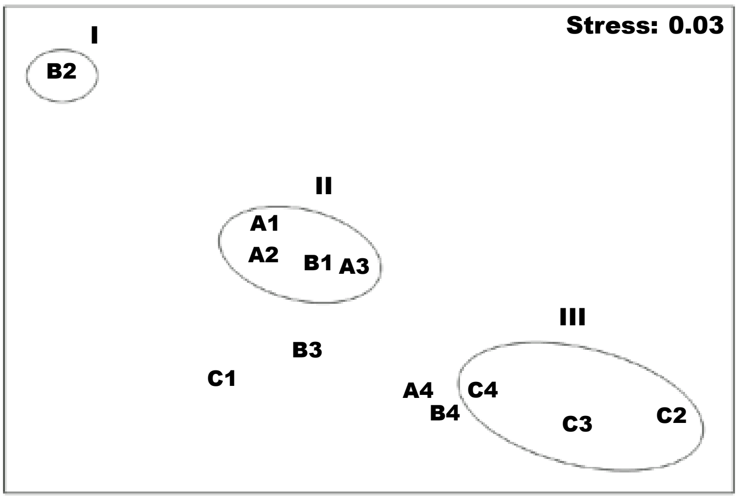

Fig. 3. MDS plot using Bray–Curtis similarity indices from square root of average abundances of sipunculan species from 2005 to 2010. Group I. Station matching with the brine discharge point; Group II. Stations closest to the sewage spill; Group III. Stations closest to fish farm cages.

Fig. 3. Gráfico MDS usando índices de similaridad de Bray–Curtis a partir de la raíz cuadrada de las abundancias medias de sipuncúlidos desde 2005 hasta 2010. Grupo I. Estación coincidente con el punto de vertido de la salmuera; Grupo II. Estaciones cercanas al emisario de aguas residuales; Grupo III. Estaciones cercanas a las jaulas de piscifactoría.

Stations nearest the different impacts were grouped by the nMDS procedure (fig. 3). Group I consisted only of B2 station, which matched with the brine discharge point. The second group marked (group II) was made up of the closest stations to the sewage outfall (A1, A2, A3 and B1), the muddiest stations, with a higher fraction of silt and clays. Group III was made up of the stations near the fish farms cages (C2, C3 and C4). In addition, these stations also had the highest percentages in coarser fractions of the sediment.

Discussion

Sipunculans have a worldwide distribution because of their high tolerance to a wide range of temperatures and depths, inhabiting different habitats ranging from shallow waters to the abyssal zone (Cutler, 1965, 1977; Murina, 1984). However, the sensitivity of the phylum to environmental changes has barely been investigated, perhaps because there is a lack of specialists for its identification throughout the world (Pancucci–Papadopoulou et al., 1999) and their abundances are usually low, although on some occasions they can become dominant species (Klaoudatos et al., 2006; Solís–Weiss, 1982). *A. muelleri muelleri* was the prevalent species at the sites studied. It was found mainly inside empty shells or tubes, and this shelter was rarely shared with another individual or another kind of organism, such as nematodes, bivalves or polychaetes. This behaviour has been described previously (Gage, 1968; Murina, 1984; Solís–Weiss, 1982). In our case, the shared shelter always had a cylindrical shape (almost always *D. arietina* tubes).

The Pearson correlation coefficient showed some correlation between sediment characteristics and abundance of sipunculan worms. We found a negative correlation between *A. muelleri muelleri*, *P. cf. caupo* and a high proportion of the fine fraction of the sediment. Solís–Weiss (1982) described how the abundance of *A. muelleri* can decrease in muddy sediments. These two species also have a good positive correlation with medium grain size sand. The strongest correlation was established with the availability of shelters with cylindrical shape, particularly with the size classes S2 and S3, and there was no correlation with spiral shells. The reason is probably that spiral shells, usually *Turritella* sp., were found in abundance in the muddy stations. These merged factors make it difficult to be conclusive establishing a relation between abundances of sipunculans and shelter availability. On the other hand, species that do not usually inhabit empty shells, such as *T. procera*, did not show any trend toward muddy sediments or toward a particular grain size. *T. procera* has been recorded in different types of sediment and several different habitats, even parasiting the polychaete *Aphrodite aculeata* (Stephen & Edmonds, 1972 in Saiz–Salinas, 1993).

Group II formed in the MDS includes the stations nearest to the sewage outfall. These stations are characterized by their high sedimentation load and their muddy bottom, resulting in a decline in abundance of *A. muelleri muelleri*.

There are limited studies about the response of sipunculans to a salinity increase (Oglesby, 1982), and lack of information about the effect that a sudden fluctuation in salinity, either temporary or constant, could have over the survival of these animals and their behaviour.

Some species studied seem to be osmoconformers, with limited power of ion regulation, during an event of salinity change (Adolph, 1936; Oglesby, 1982; Ferraris et al., 1994; Chew et al., 1994). However, studies are limited to only few particular species and do not explain how a salinity change could influence over their behaviour or their long–term survival ability within these altered conditions. Only *Phascolosoma arcuatum* (Gray, 1828) has been described as an active ion regulator with good response and resistance to sudden fluctuations in salinity (Chew et al., 1994), making it capable of survival in mangroves with freshwater inputs.

The nMDS separates the B2 station (the brine discharge point) far away from each other. Although we did not find any sipunculan in the discharge area until 2010, after the diffuser was installed and the salinity concentrations near the discharge point dropped drastically, a direct relation with the brine discharge is difficult to establish. Salinity could be affecting sipunculans assemblage but the absence of individuals at the discharge point before the implementation of the desalination plant and the heterogeneity of the bottoms studied do not allow results to be conclusive. In addition, only one sipunculan appeared in June 2010, and its presence can be considered anecdotic. Additional studies are necessary, including field and laboratory experiments, to accurately determine the effect that salinity fluctuations could have on the sipunculan distribution.

Group III established by the nMDS procedure (stations C2, C3 and C4) is characterized by its higher sipunculan abundance. These three stations are the closest to fish cages. A decrease in abundance of *A. muelleri* from fish farm bottoms has been reported (Klaoudatos et al., 2006) but muddy sites in fish farms are more likely to be identified as impacted than coarse sediment sites (Papageorgiou et al., 2010). Stations C2, C3 and C4 are distinguished by their low percentage of silt and clays and high percentages of medium sand, which was the fraction best correlated with *A. muelleri muelleri* abundance. This area also presented the highest amount of scaphopod shells and *Ditrupa arietina* tubes.

The main factor explaining the distribution of sipunculans in San Pedro del Pinatar seems to be the abiotic characteristics of the bottom, especially the granulometry of the sediment. It seems that anthropogenic impacts could indirectly play a role by changing the sediment characteristics, as is probable in the case of sewage discharge that is increasing the silt and clay fractions at the closest stations.

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References

Açik, S., 2007. Observations on the population characteristics of *Apionsoma (Apionsoma) misakianum* (Sipuncula: Phascolosomatidae), a new species for the Mediterranean fauna. *Scientia Marina*, 71(3): 571–577.

– 2008a. Occurrence of the alien species *Aspidosiphon (Aspidosiphon) elegans* (Sipuncula) on the Levantine and Aegean coasts of Turkey. *Turkish Journal of Zoology*, 32(4): 443–448.

– 2008b. Sipunculans along the Aegean coast of Turkey. *Zootaxa*, 1852: 21–36.

– 2009. Soft–bottom sipunculans in Izmir Bay (Aegean Sea, eastern Mediterranean). *Zootaxa*, 2136: 40–48.

Açik, S., Murina, G. V., Çinar, M. E. & Ergen, Z., 2005. Sipunculans from the coast of northern Cyprus (eastern Mediterranean Sea). *Zootaxa*, 1077: 1–23.

Adolph, E. F., 1936. Differential permeability to water and osmotic exchanges in the marine worm *Phascolosoma*. *Journal of Cellular and Comparative Physiology*, 9(1): 117–135.

Buchanan, J. B., 1984. Sediment analysis. In: *Methods for the Study of Marine Benthos*, 2nd edition: 41–46 (N. A. Holme & A. D. McIntyre, Eds.). Blackwell Scientific Publications, Oxford.

Chew, S. F., Peng, K. W., Low, W. P. & Ip, Y. K., 1994. Differences in the responses between tissues of the body wall and the internal organs of *Phascolosoma arcuatum* (Sipuncula) to changes in salinity. *Comparative Biochemistry and Physiology*, 107A: 141–147.

Clarke, K. R. & Warwick, R. M., 1994. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*. Plymouth Marine Laboratory, Plymouth.

Cutler, E. B., 1965. Sipunculids of Madagascar. Extrait des Cahiers ORSTOM. *Oceanographie*, 3(4): 51–63.

– 1994. *The Sipuncula: Their Systematics, Biology, and Evolution*. Cornell Univ. Press, Ithaca, New York.

– 1977. The bathyal and abyssal Sipuncula. *Galathea Report*, 14: 135–156.

Del–Pilar–Ruso, Y., De–la–Ossa–Carretero, J. A., Loya–Fernández, A., Ferrero–Vicente, L. M., Giménez–Casalduero, F. & Sánchez–Lizaso, J. L., 2009. Assessment of soft–bottom Polychaeta assemblage affected by a spatial confluence of impacts: sewage and brine discharges. *Marine Pollution Bulletin*, 58: 765–786.

Fernández–Torquemada, Y., Gónzalez–Correa, J. M., Loya, A., Ferrero, L. M., Díaz–Valdés, M. & Sánchez–Lizaso, J. L., 2009. Dispersion of brine discharge from seawater reverse osmosis desalination plants. *Desalination and Water Treatment*,

5: 137–145.
Ferraris, J. D., Fauchald, K. & Kensley, B., 1994. Physiological responses to fluctuation in temperature or salinity in invertebrates. Adaptions of *Alpheus viridari* (Decapoda, Crustacea), *Terebellides parva* (Polychaeta) and *Golfingia cylindrata* (Sipunculida) to the mangrove habitat. *Marine Biology*, 120: 397–406.
Gage, J., 1968. The mode of life of *Mysella cuneata*, a bivalve 'commensal' with *Phascolion strombi* (Sipunculoidea). *Canadian Journal of Zoology*, 46(5): 919–934.
Gibbs, P. E., 1985. On the Genus *Phascolion* (Sipuncula) with Particular Reference to the North–East Atlantic Species. *Journal of the Marine Biological Association of the United Kingdom*, 65: 311–323.
Kędra, M. & Wlodarska–Kowalczuk, M., 2008. Distribution and diversity of sipunculan fauna in high Arctic fjords (west Svalbard). *Polar Biology*, 31: 1181–1190.
Klaoudatos, S. D., Klaoudatos, D. S., Smith, J., Bogdanos, K. & Papageorgiou, E., 2006. Assessment of site specific benthic impact of floating cage farming in the eastern Hios island, Eastern Aegean Sea, Greece. *Journal of Experimental Marine Biology and Ecology*, 338: 96–111.
Morton, B. & Salvador, A., 2009. The biology of the zoning subtidal polychaetes *Ditrupa arietina* (Serpulidae) in the Açores, Portugal, with a description of the life history of its tube. *Açoreana,* Suplemento, 6: 145–156.
Murina, G. V. V., 1984. Ecology of Sipuncula. *Marine Ecology Progress Series*, 17: 1–7.
Murina, G. V. V., Pancucci–Papadopoulou, M. A. & Zenetos, A., 1999. The phylum Sipuncula in the eastern Mediterranean: composition, ecology, zoogeography. *Journal of the Marine Biological Association of the UK*, 79: 821–830.
Oglesby, L. C., 1982. Salt and water balance in the sipunculan *Phascolopsis gouldi*: Is any animal a 'simple osmometer'? *Comparative Biochemistry and Physiology*, 71A: 363–368.
Pancucci–Papadopoulou, M. A., Murina, G. V. V. & Zenetos, A., 1999. The Phylum Sipuncula in the Mediterranean Sea. In: *Monographs on marine sciences, Athens:* 1–109. National Centre for marine Research, Athens.
Papageorgiou, N., Kalantzi, I. & Karakassis, I., 2010. Effects of fish farming on the biological and geochemical properties of muddy and sandy sediments in the Mediterranean Sea. *Marine Environmental Research*, 69: 326–336.
Ruiz, J. M., Marco–Méndez, C. & Sánchez–Lizaso, J. L., 2010. Remote influence of off–shore fish farm waste on Mediterranean seagrass (*Posidonia oceanica*) meadows. *Marine Environmental Research*, 69(3): 118–126.
Saiz–Salinas, J. I., 1982. Nuevos datos sobre los sipuncúlidos de costas españolas y de mares adyacentes. *Actas II Simposio Ibérico Estudios Bentos Marino, III:* 193–201.
– 1986. Los gusanos sipuncúlidos (Sipuncula) de los fondos litorales y circalitorales de las costas de la Península Ibérica, Islas Baleares, Canarias y mares adyacentes. *Monografías del Instituto Español de Oceanografía*, 1: 1–84.
– 1993. Sipuncula. In: *Fauna Ibérica*, vol. 4: 1–200 (M. A. Ramos et al.., Eds.). Museo Nacional de Ciencias Naturales–CSIC, Madrid.
Saiz–Salinas, J. I. & Murina, G. V., 1982. Lista de especies de sipuncúlidos de las costas ibéricas y de mares adyacentes. *Actas II Simposio Ibérico Estudios Bentos Marino, III:* 203–212.
Saiz–Salinas, J. I. & Villafranca–Urchegui, L., 1990 Sipuncula from the Alboran Sea and Ibero–Moroccan Bay. *Journal of Natural History*, 24: 1143–1177.
Schulze, A., 2005. Sipuncula (Peanut Worms) from Bocas del Toro, Panama. *Caribbean Journal of Science*, 41(3): 523–527.
Shields, M. A. & Kędra, M., 2009. A deep burrowing sipunculan of ecological and geochemical importance. *Deep–Sea Research I*, 56: 2057–2064.
Solís–Weiss, V., 1982. Estudio de las poblaciones macrobénticas en áreas contaminadas de la bahía de Marsella, Francia. *Anuario Instituto. Ciencias del Mar y Limnología. UNAM.*, 9(1): 1–18.
Stephen, A. C. & Edmonds, S. J., 1972. *The Phyla Sipuncula and Echiura.* Trustees of the British Museum (Natural History), London.
Troncoso, J. S. & Urgorri, V., 1992. Asociación de *Tellimya phascolionis* (Dautzenberg *et* Fischer, 1925) (Bivalvia, Montacutidae) con el sipuncúlido *Phascolion strombi* (Montagu, 1804) en la Ría de Ares y Betanzos (Galicia, NO de España). *Boletín de la Real Sociedad Española de Historia Natural (Sección Biológica),* 88(1–4): 189–194.
Troncoso, N., Moreira, J. & Troncoso, J. S., 2000. *Tellimya phascolionis* (Dautzenberg & Fischer, 1925) (Bivalvia, Montacutidae) and other fauna associated with the sipunculid *Phascolion strombi* (Montagu, 1804) in the Ría de Aldán (Galicia, NW Península Ibérica). *Argonauta*, XIV(1): 59–66.
Wanninger, A., Koop, D., Bromham, L., Noonan, E. & Degnan, B. M., 2005. Nervous and muscle system development in *Phascolion strombus* (Sipuncula). *Development, Genes and Evolution*, 215: 509–518.