Mediterranean Marine Science

Vol 17, No 3 (2016)

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doi: 10.12681/mms.1712

To cite this article:

ANTIT, M., DAOULATLI, A., URRA, J., RUEDA, J. L., GOFAS, S., & SALAS, C. (2016). Seasonality and trophic diversity in molluscan assemblages from the Bay of Tunis (southern Mediterranean Sea). *Mediterranean Marine Science*, *17*(3), 692–707. https://doi.org/10.12681/mms.1712

Research Article

Mediterranean Marine Science
Indexed in WoS (Web of Science, ISI Thomson) and SCOPUS
The journal is available on line at http://www.medit-mar-sc.net
DOI: http://dx.doi.org/10.12681/mms.1712

Seasonality and trophic diversity in molluscan assemblages from the Bay of Tunis (southern Mediterranean Sea)

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Handling Editor: Argyro Zenetos

Received: 17 March 2016; Accepted: 13 June 2016; Published on line: 26 September 2016

Abstract

Soft bottom molluscan assemblages from the Bay of Tunis have been studied in order to analyse their seasonality and trophic diversity in relation to environmental variables. A total of 147 species of molluscs was identified, with gastropods displaying the highest species richness and bivalves the highest abundances, and including five non-indigenous species such as the dominant bryozoan grazer *Polycerella emertoni*. Carnivorous and scavenger gastropods were among the most frequent species, reflecting a diverse benthic community. Seasonal changes were significant, being more acute at 3-4 m than at 10-15 m depth, and were correlated mainly with seawater temperature and percentage of organic matter in the sediment. The high affluence of tourists in summer was coincident with high decreases in species richness and abundance of molluscs, together with a strong siltation of the sediment. Nevertheless, most trophic groups persisted and the trophic diversity was relatively high. Significant relationships were found between the index of trophic diversity and Shannon-Wiener diversity and evenness indices, suggesting that the identity of the species with its particular trophic trait, together with the balanced distribution of the individuals among the species would be the drivers for the maintenance of the molluscan food web under environmental stress. The abundance of *P. emertoni* altered the trophic structure of the molluscan assemblage, bringing the ectoparasite trophic group to an unusual peak of dominance. Soft bottom molluscan assemblages of the Bay of Tunis should be taken into account in monitoring programs for anthropogenic impacts and for non-indigenous species trends throughout the Mediterranean basin.

Keywords: Molluscs, temporal dynamics, trophic groups, anthropogenic, non-indigenous species, Mediterranean.

Introduction

Coastal zones constitute the natural interface between land and ocean and, in spite of representing a small proportion of all marine areas, are valuable ecosystems due to their remarkable productivity and diversity (Archambault & Bourget, 1996). Nevertheless, coastal zones are usually under intense anthropogenic pressure and their benthic communities are widely used as ecological indicators of the marine ecosystem (Pearson & Rosenberg, 1978; Bilyard, 1987; Dauer *et al.*, 2000). Detailed knowledge on the composition/structure and dynamics of benthic communities is important for understanding the functioning of ecosystems and for detecting disturbances due to human activity and climatic changes. Information on alterations of ecosystem functioning (such as trophism) is found in studies regarding functional diversity (e.g. number of taxa per trait category), and they include reductions in species richness which are further predicted to increase due to the unstoppable human impact in coastal areas (Törnroos *et al.,* 2015).

Molluscs, together with polychaetes and crustaceans, are an important component of benthic communities, contributing extensively to the local biodiversity of habitats, such as seagrass meadows (Hemminga & Duarte, 2000; Luque & Templado, 2004), hard bottoms (Gabriele *et al.,* 1999; Ballesteros, 2006; Casellato & Stefanon, 2008) and soft bottoms (Koulouri *et al.,* 2006; Lourido *et al.,* 2006; Urra *et al.,* 2011). In the Mediterranean Sea, Coll *et al.* (2010) recorded 2113 molluscan species that represent 20.9% of all invertebrates known to occur in this basin. Therefore, studies concerning molluscan assemblages can be considered as representative for understanding the ecological status and dynamics of benthic communities in coastal areas.

The geographic situation of Tunisia, between the Eastern and Western Mediterranean basins, and the strategic position of the Bay of Tunis itself, makes it an important monitoring site for Mediterranean alien species in relation to global warming (Antit *et al.,* 2011; Zenetos *et al.,* 2012; Galil *et al.,* 2014). Maritime traffic, including calling at the important and busy harbour of La Goulette, is an important pathway of introduction of non-indigenous species in the Mediterranean Sea (Katsanevakis *et al.,* 2014) and elsewhere (Arenas *et al.,* 2006). Most of the ca. 1000 non-indigenous species for the Mediterranean Sea have settled on shallow rocky shores or vegetated habitats, or in lagoons with aquaculture activity (Zenetos *et al.,* 2012), but few studies have been focused on soft bottoms as receptors for alien species, and/or their impact on the soft-bottom native species and on the food web in an ecosystem context.

Tourism is an important economic activity in the Mediterranean, with one third of the international tourism occurring here (Reizopoulou & Zenetos, 2005). The affluence of tourists in summer increases the local populations, making the existing infrastructures and facilities insufficient, and so affecting the quality of the marine environment and benthic communities (e.g. Glemarec & Hily, 1981; Anderlini & Wear, 1992). Shifts in infaunal communities driven by anthropogenic and natural disturbances may lead to functional changes and cause dramatic changes in the performance of the ecosystems (Thrush *et al.,* 2006; Kristensen *et al.,* 2014), but this has been poorly studied in some Mediterranean areas.

Despite the importance of Tunis as a faunistic crossroad between the Eastern and Western Mediterranean basins, studies concerning the soft-bottom macrofauna of the Bay of Tunis are still scarce (Zghal & Bouaziz-Azzouna, 1982; Ayari & Afli, 2003; Zaabi & Afli, 2005, 2006). In order to provide detailed information that can be used as a baseline for future evaluation of anthropogenic and non-indigenous species impacts on benthic fauna in the bay of Tunis, a spatial-temporal study and trophic analysis of the sublittoral soft bottom molluscan assemblages was carried out at La Goulette (Bay of Tunis). The main aims of the present research were: (1) to analyse the spatial-temporal dynamics of molluscan assemblages in relation to environmental variables; (2) to know the trophic diversity of molluscan assemblages and its variations in relations to environmental changes; (3) to check the role of soft bottoms as receptor of non-indigenous species. The starting hypothesis of this study are that (I) the changes of the environmental factors, possibly triggered by the affluence of tourists in summer and the vicinity of the Khereddine Channel, connecting with the Lake of Tunis, affect the composition and structure of assemblages, as well as the food web; (II) considering the location of La Goulette in the Mediterranean context and close to an important harbour, sublittoral soft bottoms might receive non-indigenous species, which would affect the structure and food web of the molluscan assemblages.

Materials and Methods

Faunistic samples

This study was conducted off La Goulette, a touristic resort in the Bay of Tunis (Fig. 1). The study area is located near the harbour of La Goulette, with an important maritime traffic, and the Khereddine Channel that communicates with the Lake of Tunis. The bay is dominated by soft bottoms, with patches of the seagrasses *Cymodocea nodosa* and *Posidonia oceanica* close to artificial rocky reefs. Two sampling stations located at 3-4m depth (36°49.1’N, 10°18.9’E) and at 10-15m depth (36°49.1’N, 10°23.2’E) were selected for covering different depths within the sublittoral level (Fig. 1).

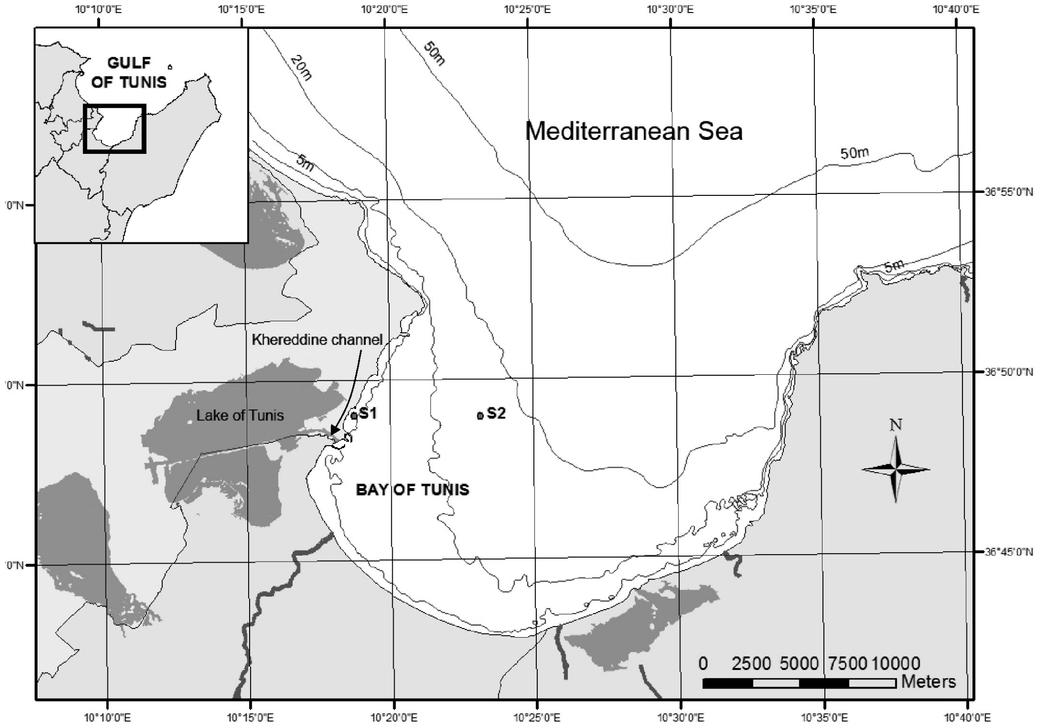

Fig. 1: Location of the sampling sites in the bay of Tunis.

Monthly samples were collected from March 2009 to March 2010, in order to analyse the temporal variation of molluscan assemblages at both depths and the spatial relationships among them. Samples were collected using a small rock dredge (72 cm width, 30 cm height and 3 mm mesh size) that was towed for 5 minutes, with each haul covering ca. 112 m^2. In spite of the mesh size, large amounts of fine fractions were collected which stretched the net and therefore clogged the mesh size, so the underestimation of the abundance of juveniles was minimal. Three replicates per station and month were generally taken, except in May and July 2009 at 10-15 m, with a total of 65 faunistic samples. This methodology has already been used successfully in studies of soft bottom molluscan assemblages (Rueda & Salas, 2003b; Urra *et al.,* 2011). In the laboratory, faunistic samples were sieved over mesh sizes down to 0.5 mm, then stored in 70% ethanol. Live-taken molluscs were separated, identified to species level when possible and their individuals counted. Images of most of the species are also posted on the World Register of Marine Species <www.marinespecies.org>.

Environmental variables

Sediment samples were collected for studying the grain size distribution and to estimate the percentage of organic matter at each sampling station and sampling time. These samples were collected using a metal container and were frozen at -20°C until laboratory processing for avoiding organic matter alterations. Monthly samples of sediment of ca. 400 g were dried at 100°C during 24 hours, after which they were washed over a 0.063 mm sieve, in order to separate the mud fraction (silt and clay) that was then computed as the difference in dry weight before and after the wash. Then, the dried sediment was sieved over a column of sieves (6.30, 4, 2, 1, 0.5, 0.25 and 0.125 mm), and the fractions retained on each sieve were weighted. Buchanan's classification (Buchanan, 1984) was used to characterize the type of sediment. The percentage of organic matter in samples of dry sediment (three monthly replicates of 20 g per station) was obtained from the weight loss on ignition at 560ºC for 1 h (Luczac *et al.,* 1997).

Seawater temperature was measured at the time of sampling. In addition, water samples for measuring chlorophyll *a* and salinity were taken in order to study the relationships between these variables and the seasonal changes of the molluscan assemblages throughout the year. Two replicates of 1 l of seawater (at ca. 1 m depth) were monthly collected and transported in darkness at low temperature to the laboratory for salinity determination, using a WTW Cond 315i/SET salinometer, and for chlorophyll *a* determination, for which filtration through Whatman GF/C glass filters was carried out. The pigments of the retained cells were then extracted using 100% acetone for 12h in cool and dark conditions. The solution was measured using a spectrophotometer at wavelengths of 630, 647, 664 and 750 nm. The chlorophyll *a* concentrations were obtained using the equation proposed by Jeffrey and Humphrey (1975).

Data analysis

Molluscan species were characterized according to their abundance (N: total number of individuals collected), Dominance index (D%: percentage of individuals of a particular species /total individuals collected in the sample), and Frequency index (F%: percentage of monthly samples in which a particular species is present) (Glémarec, 1964). The composition and structure of the molluscan assemblages were characterized by the abundance (N), species richness (S), Shannon-Wiener diversity (H') (Magurran, 1988) and evenness index (J) (Pielou, 1966) that were obtained using the software PRIMER v.6 (Clarke & Warwick, 1994).

The functional diversity of the molluscan assemblage is expressed here using trophic categories (following Rueda *et al.,* 2009) instead of species for calculating the Shannon-Wiener diversity index. Danovaro *et al.* (2004), following Heip (1985, 1998), proposed an Index of Trophic Diversity ($ITD = \sum q_i^2$), in which q is the trophic group and *ITD* ranges from 0 (high trophic diversity) to 1 (low trophic diversity). In this equation, each trophic category is treated as "species". Here, the inverse index (1-ITD) was used in order to give more intuitive values. This index is similar to Simpson's index, in that the dominant species are given the greatest weight. This index was calculated for each monthly sample at both depths in order to examine trophic changes throughout the year.

For the functional approach, we have considered the trophic categories following Rueda *et al.* (2009, and references therein), viz.: (CA) *carnivores*, feeding on other mobile organisms; (SC) *scavengers*, feeding on dead organism remains; (DE) *detritivores* (or deposit feeders), feeding on sedimentary organic particles; (EC) *ectoparasites* and *specialized carnivores*, feeding on much larger sessile/mobile organisms or colonies on which they can live; (F) *filter feeders*, capturing seston particles with their gills and/or with mucous strings; (AG) *macroalgae grazers*; (SG) *seagrass grazers* (*herbivores*) feeding directly on seagrass tissues; (MG) *microalgae or periphyton grazers*, feeding on microalgae (e.g. diatoms); and (SY) *symbiont-bearing species*, in which symbiont bacteria play an important role for obtaining a complementary food source.

Two-factor analyses of variance (ANOVAs) were carried out for testing statistical differences in the abundance, species richness, diversity and evenness indexes according to depth and seasonality after verifying normality (Kolmogorov–Smirnov) and homogeneity of variances (Levene). If data did not meet the ANOVA assumptions (i.e. were non-normally distributed) data were transformed using the appropriate transformation method. Pearson correlation analyses were performed for testing the relationships between the Index of Trophic Diversity (as 1-ITD) and ecological indices (species richness, Shannon-Wiener diversity and evenness index), as well as with percentage of organic matter in sediment (the variables expressed in percentages were previously transformed by log (x + 1), and then all of them were normalized), in order to elucidate which one displayed a higher influence on the functional (trophic) diversity of the assemblage. These statistical procedures were performed using the software SPSS.

Multivariate methods, such as CLUSTER and nMDS, using the Bray-Curtis similarity index (Bray & Curtis, 1957), were applied to both qualitative (presence/absence) and quantitative data (fourth-root transformed), for identifying seasonal similarities/dissimilarities of the assemblages. To test for differences between the studied assemblages, a distance-based permutational multivariate analysis of variance (PERMANOVA [Anderson, 2001]) was used. This analysis included 2 fixed and orthogonal factors, depth (2 levels: 3-4m vs. 10-15m) and season (4 levels: spring, summer, autumn, winter), with a total of 65 samples and 2-3 replicate samples per depth and season. The analysis was based on Bray-Curtis similarity on previously fourth-root transformed quantitative data, with permutation of residuals under a reduced model. Further pairwise comparisons were carried out since the interaction Depth×Season was significant. The SIMPER procedure was used to identify those species that contributed to the similarity and dissimilarity between these same groups of samples. Multivariate analysis, PERMANOVA and SIMPER analyses were carried out using routines included in the PRIMER6 + software package (Clarke & Warwick, 1994).

Canonical correspondence analysis (using the CANOCO v4.5; Ter Braak, 1986) were carried out for studying the relationships between molluscan assemblages and environmental variables (seawater temperature, percentage of organic matter, concentration of chlorophyll *a* and salinity), in order to detect those variables that explained most variance in the assemblages. All variables expressed in percentages were previously transformed by $\log_{10}$ (x+1).

Results

Environmental variables

Seawater temperature and salinity displayed maxima in spring and summer and minima in autumn and winter, but conversely chlorophyll a displayed maxima in summer and autumn and minima in winter and spring (Table 1). The sediment at 3-4 m depth was composed of fine sand and at 10-15 m depth of very fine sand, with a higher silt and clay content at deeper stations and maxima in August 2009, when 57.0% of silt and clay was found in the sediment (Table 1). The percentage of organic matter increased with depth, and it displayed a very acute monthly variation that ranged between 2.7% in May 2009 and 1.1% in February 2010 at 3-4 m depth, and between 3.8% in August and 1.4% in November2009 at 10-15 m depth (Table 1).

Composition and structure of the molluscan assemblages

A total of 121 species of molluscs were identified from 11780 individuals collected at 3-4 m depth. Gastropods dominated in species number, with 67 species, followed by bivalves (52 spp.) and scaphopods (2 spp.). Regarding abundance, bivalves were dominant with 7501 individuals followed by gastropods (4098 ind.) and scaphopods (181 ind.). The four most frequently occurring species (F%=

Table 1. Monthly environmental data from the sampling sites off La Goulette. The sediment was classified by the mean grain size (Q_{50}). Muddy bottom $Q_{50}<0.063$; very fine sand: $0.125>Q_{50}>0.063$; fine sand: $0.25>Q_{50}>0.125$.

Month	T	Salinity	Chlorophyll a	Grain Size (Q_{50})		% Silt and Clay		% Organic Matter	
	(°C)	(psu)	(µg/l)	3-4 m	10-15 m	3-4 m	10-15 m	3-4 m	10-15 m
March 09	20	37	0.74	$0.25>Q_{50}>0.125$	$0.25>Q_{50}>0.125$	1.9	3.7	1.89	2.53
April 09	21	36.2	0.26	$0.25>Q_{50}>0.125$	$0.125>Q_{50}>0.063$	1.4	44.6	1.23	3.27
May 09	25	36.7	0.40	$0.125>Q_{50}>0.063$	$0.125>Q_{50}>0.063$	42.2	32.5	2.67	2.89
June 09	27.8	37	0.71	$0.25>Q_{50}>0.125$	$0.125>Q_{50}>0.063$	3.8	43.1	1.29	3.73
July 09	28	37.3	2.77	$0.25>Q_{50}>0.125$	-	0.7	-	1.37	-
August 09	27	37.8	2.33	$0.25>Q_{50}>0.125$	$Q_{50}<0.063$	2.4	57.0	2.66	3.78
September 09	28	37.4	2.11	$0.125>Q_{50}>0.063$	$0.25>Q_{50}>0.125$	31.2	7.2	1.43	-
October 09	19.2	37.5	1.70	$0.25>Q_{50}>0.125$	$0.125>Q_{50}>0.063$	2.1	21.8	1.09	1.70
November 09	17	37.2	2.11	$0.25>Q_{50}>0.125$	$0.125>Q_{50}>0.063$	2.4	35.9	1.21	1.36
December 09	15	37	<0.01	$0.25>Q_{50}>0.125$	$0.125>Q_{50}>0.063$	3.8	44.5	2.45	3.10
January 10	13.5	36.8	0.67	$0.125>Q_{50}>0.063$	$0.125>Q_{50}>0.063$	19.8	21.5	1.86	1.84
February 10	8	36.6	1.73	-	$0.125>Q_{50}>0.063$	-	28.7	1.08	3.07
March 10	20	36.8	1.42	$0.125>Q_{50}>0.063$	$0.125>Q_{50}>0.063$	31.7	31.9	1.89	3.10

100%) were the carnivorous gastropods *Neverita josephinia*, *Hexaplex trunculus*, the scavenger gastropod *Nassarius mutabilis* and the filter-feeding bivalve *Dosinia lupinus*. The most abundant species were the filter-feeding bivalves *Musculus subpictus*, *Dosinia lupinus*, *Corbula gibba* and *Mactra stultorum*, which together contributed with 41.6% of all the individuals collected at this depth (Table 2). The non-indigenous ectoparasitic nudibranch *Polycerella emertoni* showed high abundance values (6.1% of all the individuals), but it was only collected in high numbers in winter. The mytilid *M. subpictus* (12.7% of all the individuals) followed a similar pattern, with abundance peaks in winter and spring (Fig. 2).

A similar pattern was found at 10-15 m depth, where a total of 12386 molluscs were collected, belonging to 113 species. Gastropods displayed the highest species richness with 65 species, followed by bivalves (45 spp.) and scaphopods (3 spp.). Bivalves dominated quantitatively with 7654 individuals, followed by gastropods (4412 ind.) and scaphopods (320 ind.). The four most frequent species (F% = 100%) were the carnivorous gastropods *N. josephinia* and *Fusiturris undatiruga*, and the scavenger gastropods *Nassarius mutabilis* and *N. pygmaeus*. The most abundant species were the filter-feeding bivalve *Corbula gibba* (19.0% of all the individuals collected at this depth) and *M. subpictus* (11.8%). Among the dominant species, *P. emertoni* represented 5.3% of the total abundance at this depth (Table 2).

When considering both depths together, the molluscan assemblages comprised a total of 147 species, from which 63.2% were shared between them, 21.1% were found exclusively at 3-4 m depth and 15.6% at 10-15 m depth. Among the shared species, the non-indigenous ectoparasitic nudibranch *P. emertoni* was dominant at both sampling depths in winter (Table 2). This species seems to be well adapted to the environmental conditions of the area, where specimens spawning egg masses were also observed. Other four non-indigenous species have been collected in these soft bottoms, but with low abundances, such as *Pinctada radiata* (juveniles), *Fulvia fragilis*, *Arcuatula senhousia* and a juvenile resembling *Anadara transversa.*

The monthly dynamics of the composition and structure of the molluscan assemblages at different depths are displayed in Figure 3. Overall, seasonal changes were more acute at shallow sampling stations (3-4 m depth) than at deeper ones (10-15 m depth), and the number of species and individuals were lower in summer. Species richness (S) values were significantly different according to depth and season (Two-factor ANOVA: factor Depth, F= 14.3; factor Season, F= 15.8, p< 0.001 in both cases) and there was a statistically significant interaction between the effects of both factors on S (F= 2.8, p< 0.05) (Fig. 3A), with high values in spring (40 spp. at 3-4 m depth and 46 spp. at 10-15 m depth) and a strong decrease in summer and autumn (8 spp. respectively). Abundance (N) values displayed significant differences according to depth and season (Two-factor ANOVA: factor Depth, F= 7.5; factor Season, F= 14.7, p< 0.05 in both cases). At 3-4 m depth N showed the maxima in January 2010 (ca. 850±154 ind.) and the minima in November 2009 (ca. 23±20 ind.) (Fig. 3B), whereas at 10-15 m depth the maxima was in June 2009 (988±137 ind.) and the

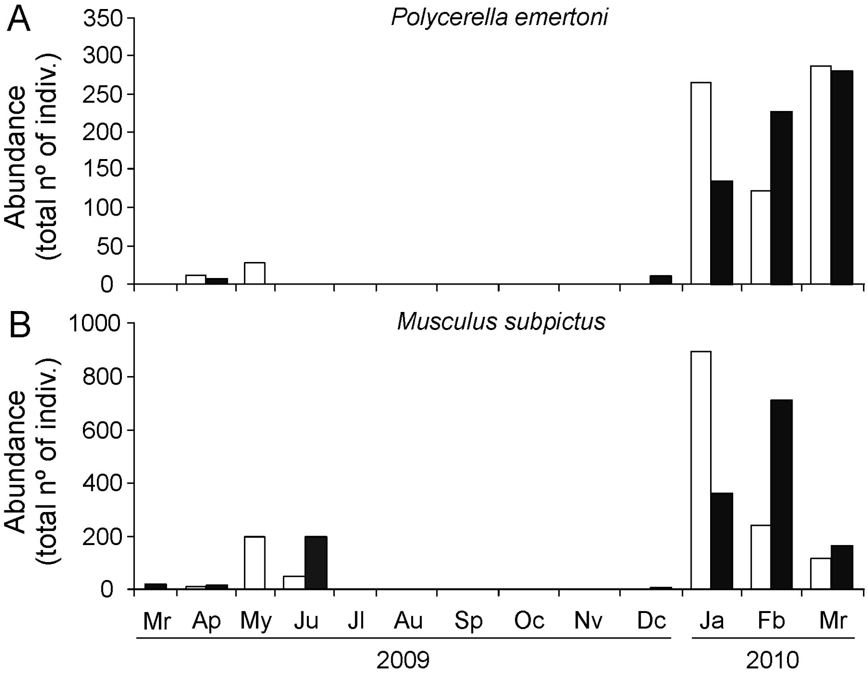

Fig. 2: Monthly abundance of (A) *Polycerella emertoni* and (B) *Musculus subpictus* at 3-4 m depth (solid bars) and 10-15 m depth (empty bars).

Table 2. List of the 20 most abundant and frequent molluscan species over the whole study period (March 2009 to March 2010) in soft bottom at 3-4 m depth and 10-15 m depth off La Goulette (Bay of Tunis). N: total number of specimens; D%: dominance index in %; TG: trophic group (CA: carnivore; SC: scavenger; DE: detritivore; EC: ectoparasite; FI: Filter feeders (plankton and seston feeders); MG: microalgae or periphyton grazers); F%: Frequency of live specimens, considering monthly samples with three pooled replicates.

3-4 m depth molluscan assemblage

Species	N	D%	TG	Species	F% (m)	TG
Musculus subpictus (Cantraine, 1835)	1496	12.7	FI	*Neverita josephinia* Risso, 1826	100.0	CA
Dosinia lupinus (Linné, 1758)	1455	12.3	FI	*Hexaplex trunculus* (Linné, 1758)	100.0	CA
Corbula gibba (Olivi, 1792)	1007	8.5	FI	*Nassarius mutabilis* (Linné, 1758)	100.0	SC
Mactra stultorum (Linné, 1758)	946	8.0	FI	*Dosinia lupinus* (Linné, 1758)	100.0	FI
Polycerella emertoni Verrill 1881	713	6.0	EC	*Fustiaria rubescens* (Deshayes, 1825)	91.7	DE
Donax venustus Poli, 1795	462	3.9	FI	*Bolinus brandaris* (Linné, 1758)	83.3	CA
Nassarius mutabilis (Linné, 1758)	460	3.9	SC	*Nassarius pygmaeus* (Lamarck, 1822)	83.3	SC
Bittium reticulatum (da Costa, 1778)	452	3.8	DE	*Fusinus syracusanus* (Linné, 1758)	83.3	CA
Hexaplex trunculus (Linné, 1758)	383	3.2	CA	*Donax venustus* Poli, 1795	83.3	FI
Parvicardium exiguum (Gmelin, 1791)	329	2.8	FI	*Pandora inaequivalvis* (Linné, 1758)	83.3	FI
Pusillina inconspicua (Alder, 1844)	326	2.8	MG	*Pusillina* sp.	75.0	MG
Tellina nitida Poli, 1791	319	2.7	DE	*Bittium reticulatum* (da Costa, 1778)	75.0	DE
Pusillina sp.	313	2.7	MG	*Mactra stultorum* (Linné, 1758)	75.0	FI
Nassarius pygmaeus (Lamarck, 1822)	304	2.6	SC	*Tellina distorta* Poli, 1791	75.0	DE
Chamelea gallina (Linné, 1758)	296	2.5	FI	*Tellina nitida* Poli, 1791	75.0	DE
Nucula nitidosa Winckworth, 1930	197	1.7	DE	*Corbula gibba* (Olivi, 1792)	75.0	FI
Neverita josephinia Risso, 1826	191	1.6	CA	*Antalis vulgaris* (da Costa, 1778)	75.0	DE
Fustiaria rubescens (Deshayes, 1825)	108	0.9	DE	*Mangelia unifasciata* Deshayes, 1835	66.7	CA
Columbella rustica (Linné, 1758)	106	0.9	MG	*Bela zonata* (Locard, 1892)	66.7	CA
Acanthocardia paucicostata (Sowerby G.B. II, 1841)	105	0.9	FI	*Parvicardium exiguum* (Gmelin, 1791)	66.7	FI

10-15 m depth molluscan assemblage

Species	N	D%	TG	Species	F% (m)	TG
Corbula gibba (Olivi, 1792)	2353	19.0	FI	*Neverita josephinia* Risso, 1826	100.0	CA
Musculus subpictus (Cantraine, 1835)	1460	11.8	FI	*Nassarius mutabilis* (Linné, 1758)	100.0	SC
Bittium reticulatum (da Costa, 1778)	723	5.8	DE	*Nassarius pygmaeus* (Lamarck, 1822)	100.0	SC
Dosinia lupinus (Linné, 1758)	711	5.7	FI	*Fusiturris undatiruga* (Bivona Ant., 1838)	100.0	CA
Abra nitida (Müller O.F., 1776)	659	5.3	DE	*Nucula nitidosa* Winckworth, 1930	100.0	DE
Polycerella emertoni Verrill 1881	657	5.3	EC	*Abra nitida* (Müller O.F., 1776)	100.0	DE
Nucula nitidosa Winckworth, 1930	578	4.7	DE	*Pitar rudis* (Poli, 1795)	100.0	FI
Nassarius mutabilis (Linné, 1758)	538	4.3	SC	*Dosinia lupinus* (Linné, 1758)	100.0	FI
Nassarius pygmaeus (Lamarck, 1822)	469	3.8	SC	*Corbula gibba* (Olivi, 1792)	100.0	FI
Tellina nitida Poli, 1791	339	2.7	DE	*Fustiaria rubescens* (Deshayes, 1825)	100.0	DE
Pusillina inconspicua (Alder, 1844)	326	2.6	MG	*Bittium reticulatum* (da Costa, 1778)	90.0	DE
Hexaplex trunculus (Linné, 1758)	320	2.5	CA	*Hexaplex trunculus* (Linné, 1758)	90.0	CA
Abra alba (Wood W., 1802)	295	2.4	DE	*Odostomia conoidea* (Brocchi, 1814)	90.0	EC
Fustiaria rubescens (Deshayes, 1825)	217	1.7	DE	*Tellina nitida* Poli, 1791	90.0	DE
Tellina distorta Poli, 1791	172	1.4	DE	*Abra alba* (Wood W., 1802)	90.0	DE
Aequipecten opercularis (Linné, 1758)	146	1.2	FI	*Pandora inaequivalvis* (Linné, 1758)	90.0	FI
Phaxas pellucidus (Pennant, 1777)	133	1.1	FI	*Antalis vulgaris* (da Costa, 1778)	90.0	DE
Pharus legumen (Linné, 1758)	120	1.0	FI	*Bolinus brandaris* (Linné, 1758)	80.0	CA
Neverita josephinia Risso, 1826	103	0.8	CA	*Acanthocardia paucicostata* (Sowerby G.B. II, 1841)	80.0	FI
Antalis vulgaris (da Costa, 1778)	102	0.8	DE	*Tellina distorta* Poli, 1791	80.0	DE

minima in November 2009 (87±45 ind.) (Fig. 3B,). The evenness index displayed significant changes according to season (Two-factor ANOVA: factor Depth, F= 0.4, p= 0.5; factor Season, F= 9.4, p< 0.001), with maxima in November and minima in June in both depths (Fig. 3C). Finally, the Shannon-Wiener diversity (H´) index displayed significant differences according to depth and season (Two-factor ANOVA: factor Depth, F= 4.9, p< 0.05; factor Season, F= 6.3, p< 0.001), with higher H´values at 3-4 m than at 10-15 m (Fig. 3D).

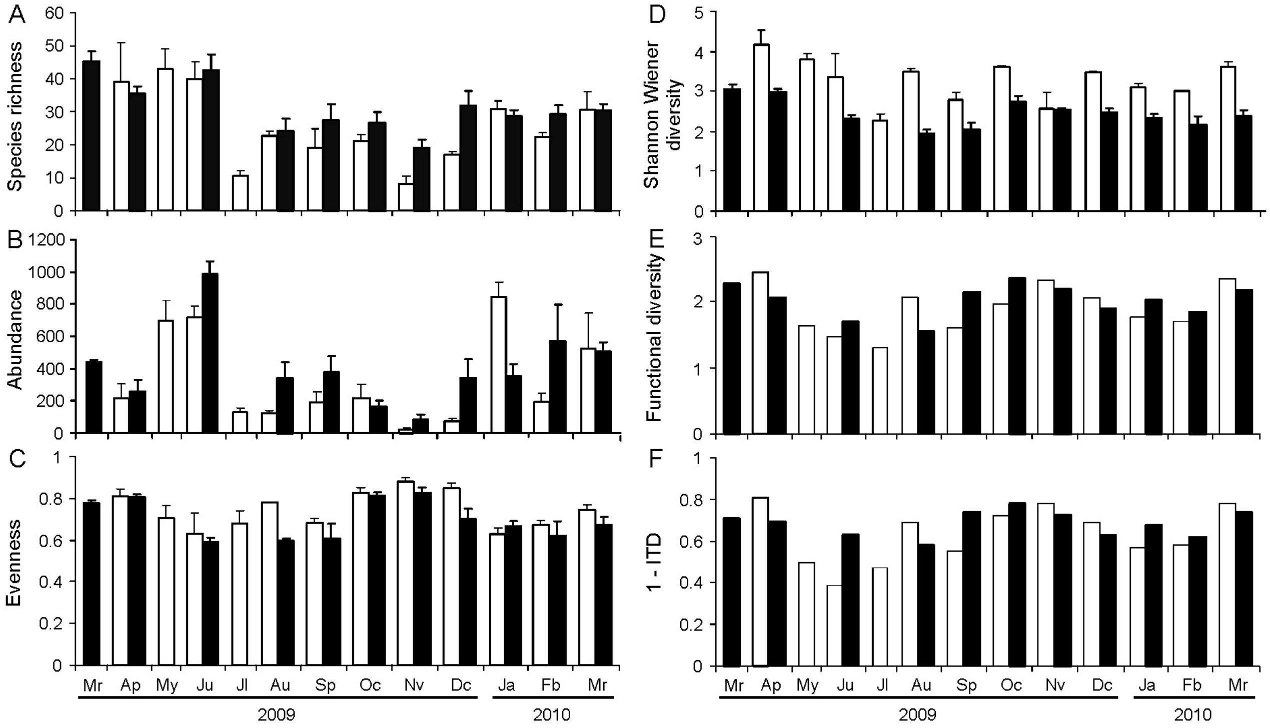

Fig. 3: Monthly dynamics of the soft bottom molluscan assemblages at 3-4 m depth (solid bars) and 10-15 m depth (empty bars). (A) species richness, (B) abundance, (C) evenness, (D) Shannon-Wiener diversity index, (E) Functional Shannon-Wiener diversity index (using trophic groups as "taxa"), (F) Index of Trophic Diversity (expressed as 1-ITD).

Functional (Trophic) diversity

Filter feeding molluscs displayed high species richness and abundance values throughout the year at both depths, with a higher dominance at 3-4 m depth (29.5% of the species and 61.5% of the individuals at this depth) than at 10-15m depth (Fig. 4). On the other hand, a higher number of detritivores was observed at 10-15 m depth (25% of the individuals) than at 3-4 m depth. The latter would be related with increase in muddy sand content and percentage of organic matter at higher depths (Table 1, Fig. 4).

An important increase of detritivores was recorded at both depths from mid-summer to mid-autumn, especially in August, when an acute increase of percentage of organic matter and loss of some trophic groups were observed at 10-15 m depth (Fig.5). The number of detritivores decreased in winter, whereas filter feeders increased. The presence of herbivores and micrograzers was noted during most of the year, particularly at 3-4 m depth (Fig. 5A). Carnivores and scavengers were also well represented throughout the year at both depths, with higher species richness and abundance values at 3-4 m depth (Fig. 5). The trophic groups increased their numbers in autumn, during the fall of seagrass leaves. It is remarkable the good representation of ectoparasites found during this study (Figs. 4, 5), including the non-indigenous *P. emertoni* that feeds on bryozoans. Most ectoparasites belong to Eulimidae and Pyramidellidae, the former feeding on echinoderms and the latter feeding on different groups of invertebrates, mostly annelids and molluscs.

The values of the index of trophic diversity (1-ITD) follow a rather similar pattern compared to the Shannon-Wiener diversity. A significant relationship was obtained

Table 3. Results of the canonical correspondence analysis for the molluscan assemblages inhabiting sedimentary soft bottoms off La Goulette (Bay of Tunis).

Intraset values	3-4m depth		10-15m depth	
	Axis 1	Axis 2	Axis 1	Axis 2
%OM	0.371	-0.075	-0.399	-0.639
Chl *a*	-0.104	-0.658	0.167	0.462
Salinity	-0.233	-0.874	-0.345	0.572
Temperature	-0.797	-0.066	-0.792	0.009
Species-environment correl	0.949	0.914	0.844	0.866
Cumulative % variance of species data	10.6	16.2	8.5	14.6
Cumulative % variance of species-environment relation	43.1	65.8	40.8	70.0

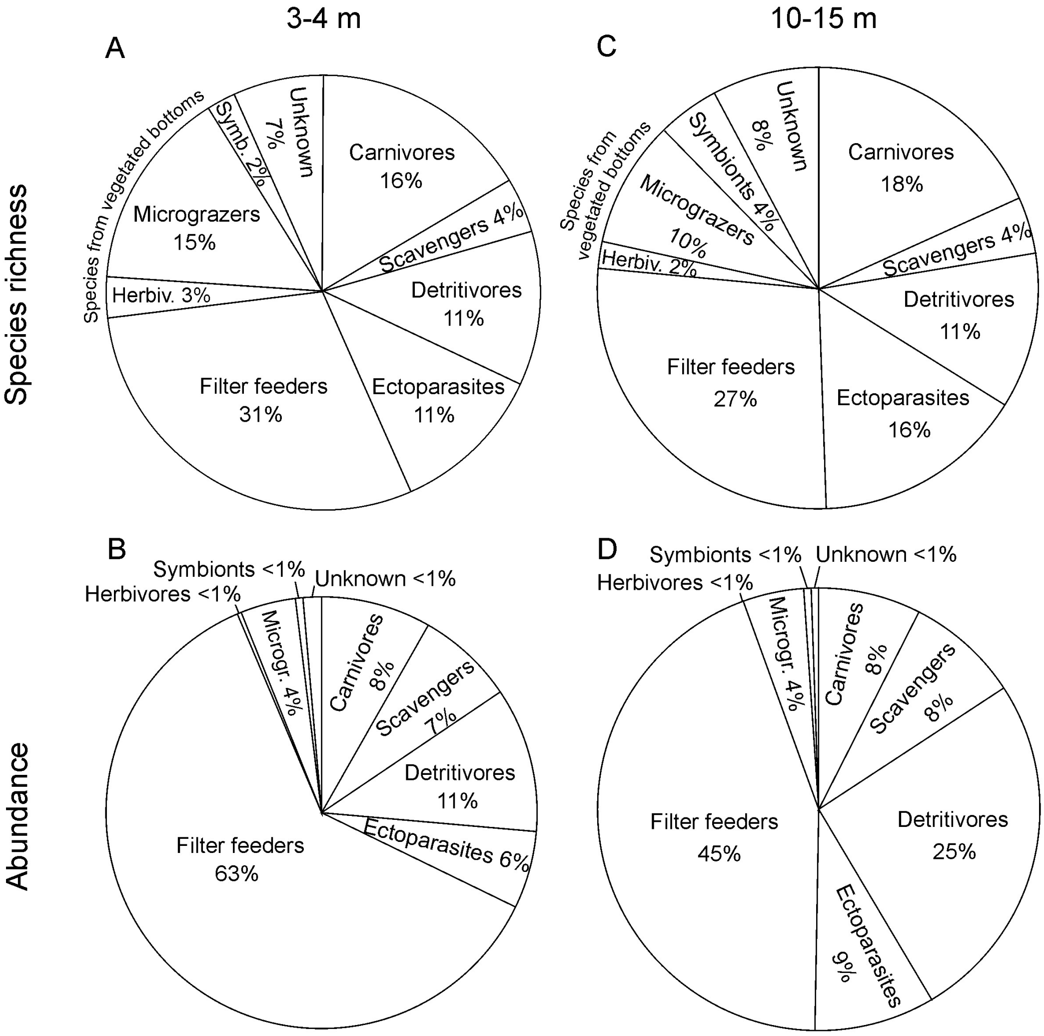

***Fig. 4**:* Distribution of the species and individuals by the trophic groups considering all samples. (A) Number of species and (B) Abundance by trophic groups from 3-4 m depth. (C) Number of species and (D) abundance by trophic group from 10-15 m depth.

between both (Fig. 6A). A significant relationship was also observed between the index of trophic diversity and evenness (Fig. 6B). Nevertheless, no relationship was found between species richness and the index of trophic diversity (Fig. 6C). Finally, no relationship was observed between the index of trophic diversity and the percentage of organic matter in the sediment (Fig. 6D).

Affinity between samples

The PERMANOVA test revealed significant differences in the composition and structure of the molluscs assemblages between depths (PERMANOVA: F= 7.12, $p< 0.001$, df =1) and seasons (PERMANOVA: F= 6.93, $p< 0.001$, df =3). Pairwise comparisons revealed that differences among assemblages (i.e. depths) were significant in spring, summer and autumn ($p< 0.05$), but in winter the studied molluscs assemblages displayed non-significant differences among them ($p> 0.05$); and for both assemblages, seasonal differences were consistently significant ($p< 0.005$) with the exception of the group summer-autumn ($p> 0.05$). Spatial differences were related to the lower presence (1) at 3-4 m depth of *Corbula gibba*, *Musculus subpictus* and *Abra nitida*, and (2) at 10-15 m depth of *Donax venustus*, *Chamelea gallina* and *Parvicardium exiguum*, among others (average dissimilarity= 62.6%).. Similar results were obtained when PERMANOVA test was carried out with presence/absence transformed data.

The highest seasonal differences were observed between summer and winter for the 3-4 m assemblage (Pairwise comparisons: t= 2.82, $p< 0.001$; average dissimilarity= 72.2%), and between autumn and winter for the 10-15 m assemblage (Pair-wise comparisons: t= 2.26, $p< 0.001$; average dissimilarity= 58.3%). The species that contributed mostly to these differences were the filter-feeder *M. subpictus* and the non-indigenous ectoparasite *Polycerella emertoni*, due to their high abundance values in winter and their absence in summer and autumn. The dissimilarities values between season groups observed for the 3-4 m assemblage were higher (range: 60.5%-72.2%) that those observed for the 10-15 m assemblage (range: 50.5%-58.3%). In the case of the groups summer–autumn, spring-

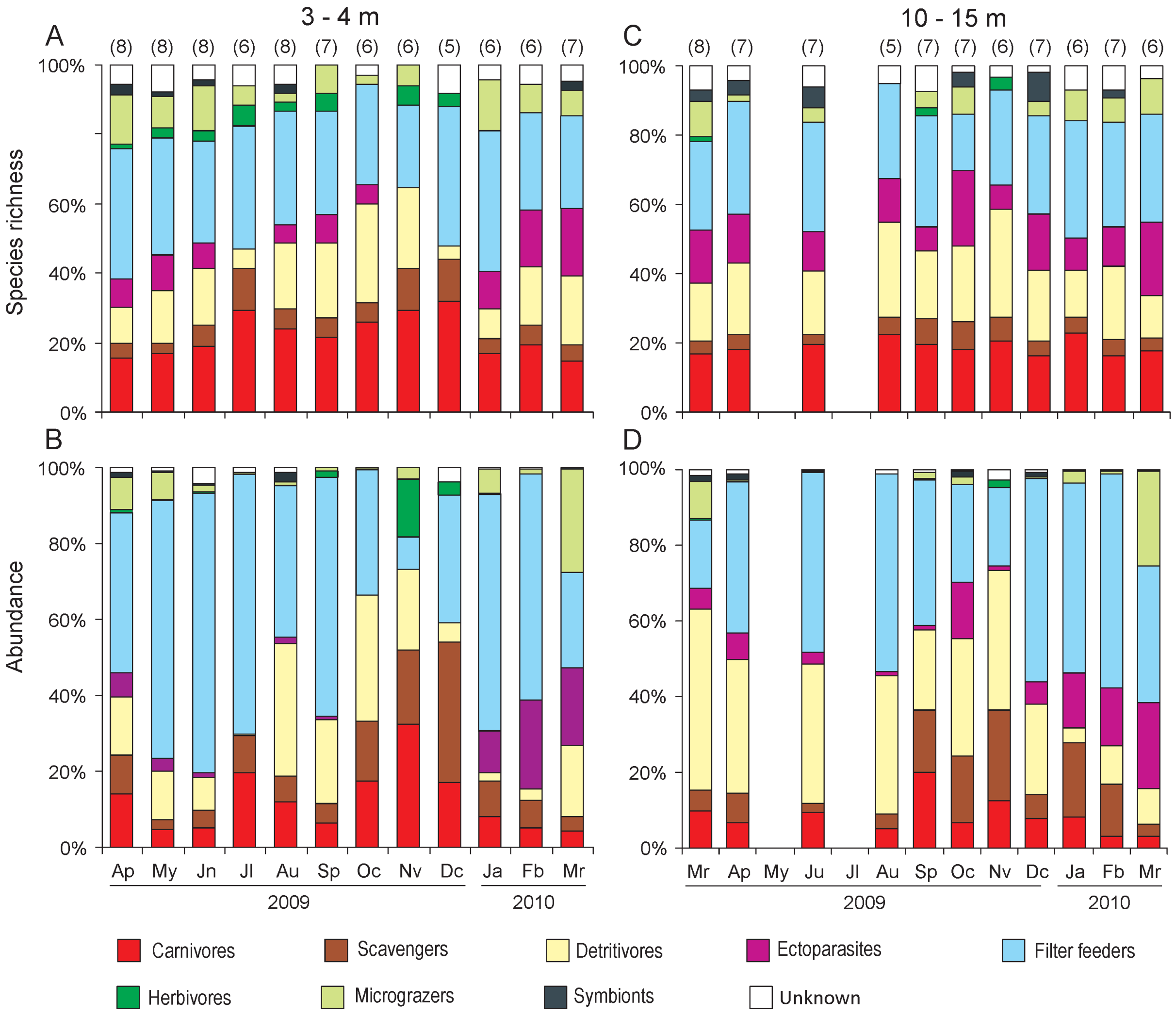

Fig. 5: Monthly changes in species richness and abundance of molluscs by trophic group in samples collected off La Goulette at 3-4 m depth (A, B) and 10-15 m depth (C, D) respectively. Numbers on bars correspond to the number of trophic groups in each monthly sample.

autumn and spring-summer these dissimilarities were due to a higher number of species with low percentages of contribution, being mostly filter feeding bivalves. Most of the qualitative and quantitative summer samples from 3-4 m form a group apart from other seasonal samples (Fig.7). It is interesting to point that these summer samples were collected during the tourist season in La Goulette, with high affluence of people in the beach.

Relationship between molluscan assemblages and environmental variables

The forward selection of canonical correspondence analysis (CCA) for the molluscan assemblage studied at 3-4 m depth selected seawater temperature (T), percentage of organic matter (%OM) in sediment and salinity (S) as the environmental variables explaining most of the temporal variance in the species data ($p< 0.005$). Axes I and II were the most important in CCA ordination, accumulating 16.2% of the species variance and 65.8% of species-environment variance. Temperature showed a strong negative correlation with axis I, whereas %OM was the only studied variable with a positive correlation. On the other hand, both salinity and concentration of chlorophyll a (Chl *a*) showed a strong negative correlation with axis II. Thus, sampling stations appeared distributed from the left to the right of axis I following an increase in %OM and a decrease in temperature (Fig. 8A).

At 10-15m depth, Temperature and %OM were also the variables explaining most of the variance in the data ($p< 0.005$). Figure 8B shows the samples ordination obtained by CCA for the first two axes. The first axis is related mainly to temperature, while the second axis is correlated preferentially to %OM. Axes I and II were the most important in CCA ordination, accumulating 14.6% of the species variance and 70% of the species-environment variance. In this case, salinity and chlorophyll *a* showed a positive correlation with axis II. The Monte Carlo test was significant for all canonical axes ($p< 0.001$) in both depth samples.

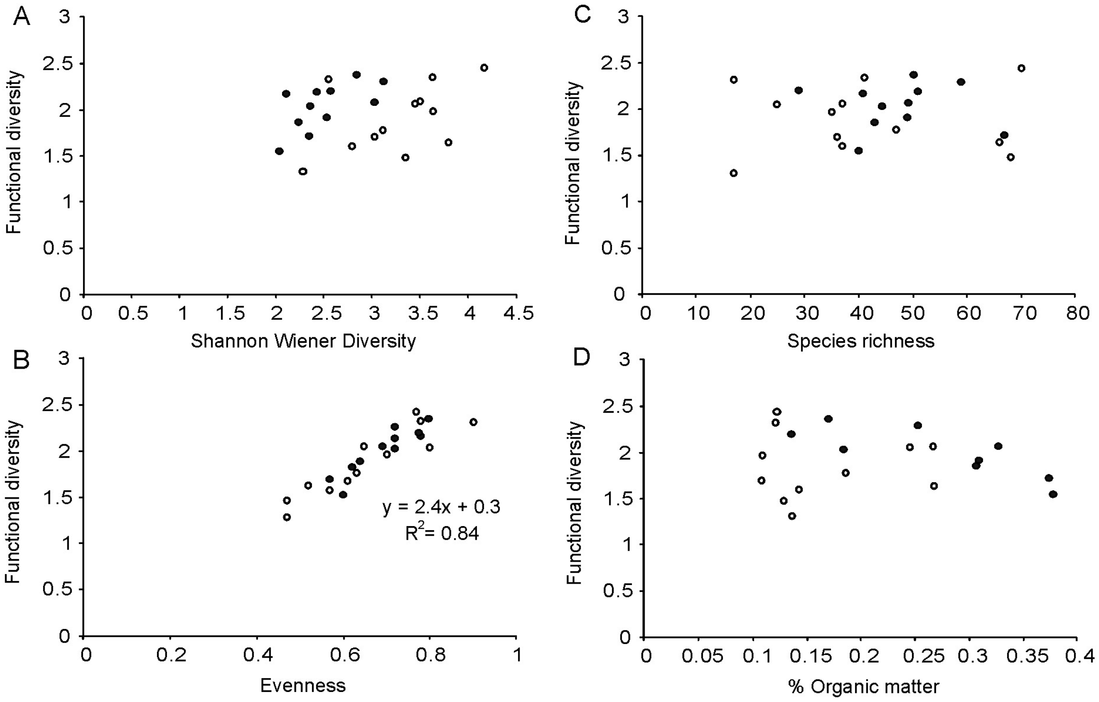

***Fig. 6**:* Relationships between monthly values of the Index of Trophic Diversity and monthly values of (A) Shannon-Wiener diversity index, (B) evenness, (C) species richness and (D) percentage of organic matter in the sediment. Empty symbols: samples from 3-4 m depth. Solid symbols: samples from 10-15 m depth.

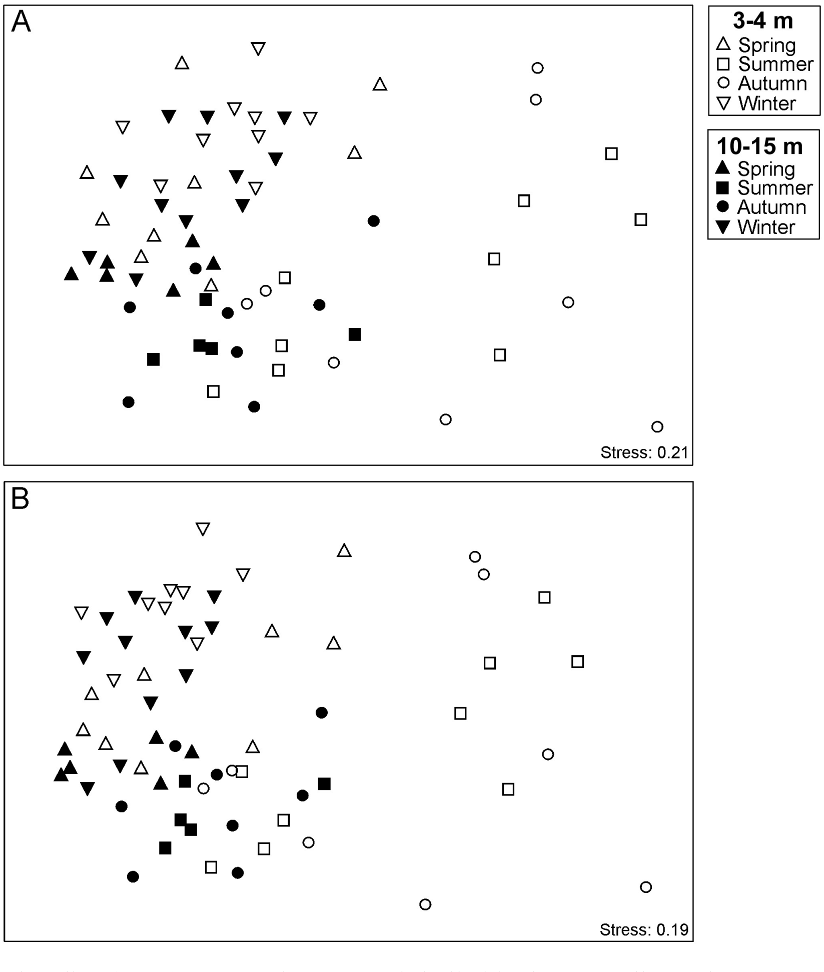

***Fig. 7**:* Multi-dimensional scaling (MDS) representing seasonal similarities between all samples. (A) MDS using qualitative data (presence/absence). (B) MDS using quantitative data transformed to fourth root.

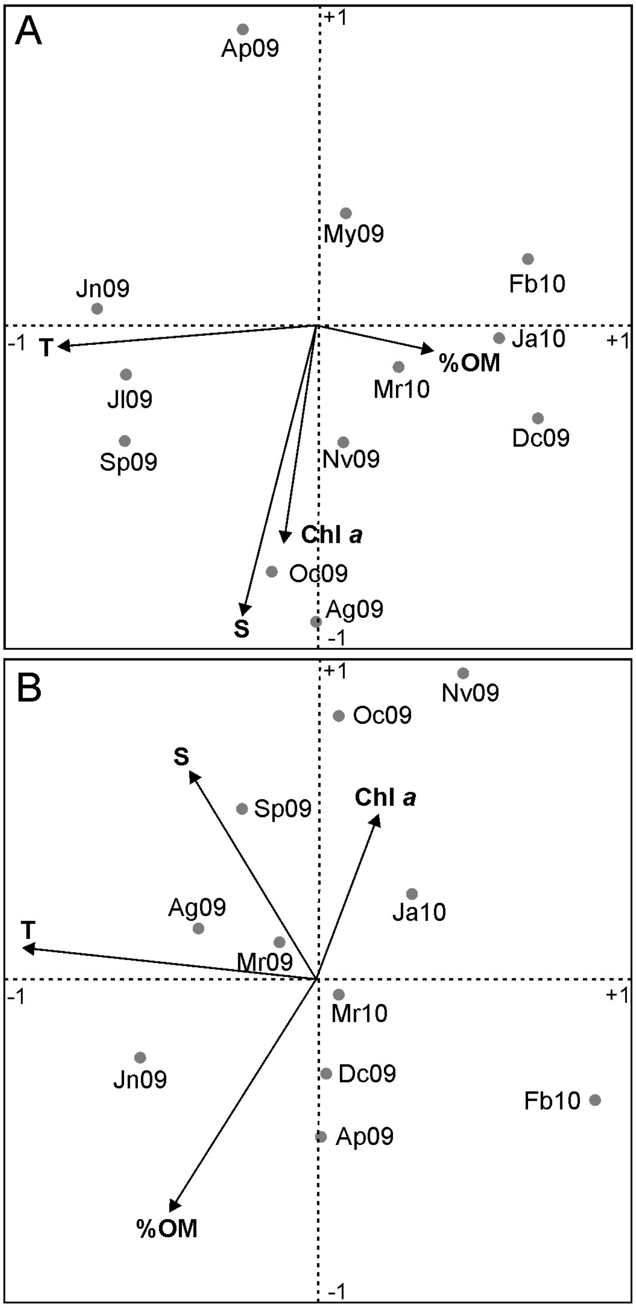

Fig. 8: Canonical correspondence analysis (CCA) representation of the monthly samples collected at (A) 3-4 m depth and (B) 10-15 m depth off La Goulette (Bay of Tunis) with respect to axes I and II. The samples are displayed by points and the environmental variables by arrows. T, seawater temperature; Chl *a*, concentration of Chlorophyll *a*; %OM, percentage of organic matter; Ja, January; Fb, February; Mr, March; Ap, April; My, May; Jn, June; Jl, July; Ag, August; Sp, September; Oc, October; Nv, November; Dc, December.

Discussion

The soft bottoms of La Goulette (Bay of Tunis) harbour a high biodiversity of molluscs, with 147 species identified so far, which represents a higher species richness than that found in adjacent photophilous algal stands (Antit *et al.,* 2013). This contrasts with observations made in other regions when comparing neighbouring bottoms with and without vegetation (Currás *et al.,* 1993; Connoll, 1994; Turner & Kendall, 1999). The species richness values reported here are even higher than those reported in soft bottoms studied in other European coastal areas (e.g. Koulouri *et al.,* 2006; Lourido *et al.,* 2006), and this could be favoured by the presence of different adjacent habitats such as seagrass meadows (*C. nodosa* at shallow depths and *P. oceanica* occurring deeper) and artificial hard bottoms with dense algal turfs. The number of species could be enhanced because our study takes into account small individuals down to 0.5 mm, including many species of small gastropods (e.g. Pyramidellidae) which may be missed in other studies where material is sieved at 1mm. In spite of the presence of 93 shared species, the molluscan assemblages studied at 3-4 m and 10-15 m depth are significantly different. The main driving factor seems to be related to the sediment composition, which is considered the dominant factor in structuring soft bottom benthic communities (Dauer *et al.,* 2008; Dauvin *et al.,* 2004; Glémarec & Hily, 1981), with a higher muddy component at deeper bottoms.

Among the species found in this study that could be associated with adjacent habitats were *Smaragdia viridis*, an herbivorous gastropod feeding on seagrass leaves (Rueda & Salas, 2007; Rueda *et al.,* 2011), and some micrograzer gastropods that are associated with vegetated or rocky habitats such as *Tricolia* spp., *Gibbula* spp. or *Rissoa* spp. (Antit & Azzouna, 2012). Structurally complex environments increase species diversity by providing more niches and different ways of exploiting environmental resources (Kerr & Packer, 1997; Gingold *et al.,* 2010), commonly referred as the "habitat heterogeneity hypothesis" (MacArthur & Wilson, 1967). The influence of heterogeneous habitats on the increase of local biodiversity has been indicated in other Mediterranean areas, such as the Alboran Sea (Urra *et al.,* 2011, 2013a).

Five non-indigenous mollusc species have been found in the studied soft bottoms, some of them reaching high abundance values. The most dominant among them is the nudibranch *P. emertoni*, originally described from the western Atlantic and associated with the bryozoan *Amathia verticillata*, which has also been found in the Mediterranean Sea (Zenetos *et al.,* 2004; Antit *et al.,* 2011; Zenetos *et al.,* 2012; Galil & Gevili, 2014). In the western Atlantic and Caribbean Sea, A. *verticillata* inhabits different coastal habitats (e.g. seagrass meadows, mangroves, oyster reefs and rocky shores), with colonies growing well from April through late September and dying when water temperatures decreases (autumn-winter), although fragments can remain alive until spring, when growth restarts (Winston 1995). This species is actually recognized as an invasive species in the fouling community worldwide (Amat & Tempera, 2009). The presence of *P. emertoni* in the Bay of Tunis could be related to the proximity of La Goulette harbour, through which the species could have entered in the area, and the presence of seagrasses where the bryozoan can be attached (Antit *et al.,* 2011). The high abundance of individuals of *P. emertoni* in soft bottoms in winter would be linked with the loss of leaves in the seagrass meadows and the death of the bryozoan due to low seawater temperatures, whereas the presence of few individuals in April and May would be related to recruitment events. Overall, the abundance of ectoparasites in the mollusc trophic structure is low; the high percentage found in winter in La Goulette is due to the abundance of *P. emertoni*, which became a dominant species at both depths in this season, disturbing the usual distribution of individuals among trophic groups in the food web. The bivalve *Fulvia fragilis* is another non-indigenous species reported for the Mediterranean Sea (Zenetos *et al.,* 2012) that has been found in the studied soft bottoms, although in this case in low numbers. A spatial-temporal monitoring in the bay of Tunis would be necessary in order to check the evolution and potential impact of these and the other non-indigenous species on the native community. Recent observations have confirmed the presence in the soft bottom of La Goulette (with high abundance) of *Bursatella leachii*, another non-indigenous nudibranch in the Mediterranean.

The molluscan assemblages inhabiting soft bottoms of La Goulette were dominated by few species (<20 species with dominance values higher than 1%), most of them being filter feeding and detritivore bivalves, but also carnivorous or scavenger gastropods whose presence reflects a rich soft bottom benthic community, on which they feed. The presence at shallow depths of the dominant carnivorous gastropod *Fusiturris undatiruga*, usually inhabiting deeper muddy bottoms (Gofas *et al.,* 2011), would be related to the unusually high content in mud and organic matter due to the nearby harbour of La Goulette and the Khereddine Channel. The presence of juveniles of this species and its frequency throughout the study period confirms its successful settlement at these depths. The dominance of *Musculus subpictus*, a species normally associated with compound ascidians that are common in vegetated bottoms, in winter and spring would be related with two seasonal events, the loss of seagrasses leaves in autumn and its posterior transport to adjacent soft bottom by waves during winter, and its recruitment in spring, when the development of seagrass leaves occur.

According to Petchey & Gaston (2006) functional diversity is the range of functions that are performed by organisms in a system. One possible approach would be the selection of food items among different guilds (Simberloff & Dayan, 1991). Highly controlled manipulative experiments have revealed significant and positive effects of increased diversity on ecosystem functioning, but uncertainties in experimental design have made it difficult to determine whether such effects are related to the number of species or to effects associated with species identity and density (Ieno *et al.,* 2006). Communities with high diversity and evenness would be more resilient because any one of the species having similar functional traits could buffer the system against the loss of other species. Some authors have proposed the "insurance hypothesis" to explain this phenomenon (Yachi & Loreau, 1999). In this situation, species identity -and particular species traits- are the driving force stabilizing the system rather than species richness per se. Our data are in agreement with those of Ieno (2006) and Yachi & Loreau (1999) because the index of trophic diversity is positively correlated with the Shannon-Wiener and evenness indices. However, there is no relationship with species richness (Figure 6). The latter observation suggests that the identity of the species with its particular trophic trait, together with the balanced distribution of the individuals among the species, would be the drivers for the maintenance of the molluscan food web under environmental stress, such as during the peak of tourists in summer. In this line, Törnroos *et al.,* (2015) found that decreases in taxonomic richness involve decline in functional properties of benthos, but the functional richness may still remain comparatively high even at low taxon richness.

Moore *et al.,* (2004) considered that a detritus based food web results in a more diverse, stable and structured community. No positive relationship was found between the percentage of organic matter in the sediment and the number of trophic groups. The regular input of detritus from the Khereddine Chanel in winter and spring (rainy season), the loss of seagrass leaves in autumn, and the increase of sewage in summer, could possibly favour the maintenance of a saturated detritus-based food web and a high trophic diversity of the molluscan assemblage.

The seasonality observed in the structure of the molluscan assemblages at both depths is mainly driven by the monthly variation of the seawater temperature and the percentage of organic matter in the sediment, being more acute at 3-4 m depth. Seasonality is a common feature in vegetated-bottom assemblages (Rueda *et al.*, 2003a, 2008; Urra *et al.,* 2013b), but not so common in soft-bottom assemblages (Urra *et al.,* 2013a). In this case, most of these changes were related to few dominant species that were abundant only in some periods, such as *M. subpictus* and the non-indigenous *P. emertoni* in winter, which are associated with vegetated habitats, or to the strong reduction of the species richness in summer. A maximum of abundance and species richness values have been found in colder regions at the end of the summer and in autumn (Ibañez & Dauvin, 1988; Gestoso *et al.,* 2007; Moreira *et al.,* 2010), as a result of recruitment events occurring in spring and summer. In the Mediterranean, maxima are observed usually in spring and summer (Koulouri *et al.,* 2006; Rueda *et al.,* 2001; Sardà *et al.,* 1999), in agreement with our own observations at La Goulette. The decline of the abundance and species richness observed in summer could be related to environmental factors, such as the high seawater temperatures and a usual increment in sewage during the touristic season, as it has been reported in other Mediterranean coastal areas (Reizopoulou & Zenetos, 2005).

In summary, the molluscan assemblages inhabiting soft bottoms off La Goulette (Bay of Tunis) display a high species richness (147 species), which is enhanced by the heterogeneity of adjacent habitats (e.g. seagrass meadows, algal turfs, rocky outcrops), being dominated by filter-feeder and detritivorous bivalves. Main environmental factors influencing the seasonality of assemblages are seawater temperature and percentage of organic matter, followed by salinity. The affluence of tourists is coincident with the decrease of species and their abundance, nevertheless the food web and trophic diversity remains rather stable throughout the study period, which could be due to redundancy of the species in each trophic category and to the balanced distribution of individuals among the different species of the assemblage. The strategic location of the study area in the context of the Mediterranean Sea and the nearby presence of an important harbour seem to be behind the presence of five non-indigenous molluscan species in the studied soft bottoms, including the dominant *P. emertoni*. Information from sediment and benthic community monitoring provides a detailed baseline for rational and sustainable management of natural resources, and also a reliable way to assess the health of the marine environment (Bellan-Santini *et al.,* 1994). This study must be considered as a baseline for future monitoring programs on the coastal management in the bay of Tunis, where important human, industrial and maritime traffic pressures occurs at the sublittoral zone.

Acknowledgements

We would like to express our sincere gratitude to Dr. Atf Azzouna for the help provided in the laboratory procedures, and to the colleagues from the Department of Geology and Ecology of the University of Málaga for their assistance in the sediment analyses. Part of this study has been funded by the project B/9430/07 of the "Agencia Española de Cooperación Internacional".

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