

## Temporal variation of the algae-associated molluscan assemblage of artificial substrata in the Bay of Tunis (Tunisia)

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### Abstract

The molluscan assemblage inhabiting photophilous algae from artificial rocky shores in the Bay of Tunis) was studied from February 2009 to February 2010. Five monthly replicates were taken with a quadrat of 25 × 25 cm. A total of 16105 individuals of molluscs, belonging to 93 species, were collected. Gastropods were the most species-rich group, with 66 species (70.96%), followed by bivalves with 26 species (27.95%). Gastropods were also the most abundant with 11529 individuals (71.60%), followed by bivalves with 4575 individuals (28.41%). Nevertheless, *Mytilaster minimus* was the dominant species overall during the studied period (15.6% of total abundance), followed by *Tricolia miniata* (11.6%), *Bittium reticulatum* (11.5%), *Gibbula racketsi* (10.4%) and *Rissoa similis* (9.8%). The high abundance of juveniles of soft (e.g. *Venerupis aurea*, *Nassarius corniculum*) and hard bottom species (e.g. *M. minimus*, *Fissurella nubecula*) highlight the important role played by this habitat as a nursery for species from nearby habitats. The presence of the alien molluscs *Mitrella psilla* (2.5% dominance) and *Arcuatula senhousia* stresses the importance of algae as a vehicle and/or receptor habitat for alien species. Species richness and abundance displayed significant seasonal changes, with maxima in winter 2009, but the Shannon-Wiener diversity index showed similar values throughout the year. Multivariate analyses also indicated the presence of significant seasonal differences for this molluscan assemblage.

**Keywords:** Molluscs, macroalgae, alien, biodiversity, seasonal dynamics, Africa, Mediterranean Sea.

### Introduction

Photophilous algal bottoms are one of the most widespread types of vegetated habitats in the Mediterranean Sea (Pérès & Picard, 1964; Ballesteros & Pinedo, 2004; Sales *et al.*, 2012). Their presence enhances the bottom complexity and heterogeneity of hard substrates, thus representing an available habitat for many species (Christie *et al.*, 2009). The reduced hydrodynamism within the fronds favours the formation of enclaves of sediment in the cavities of the rocky substratum, thus providing a microhabitat for many species (Littler *et al.*, 1983). Within macroalgal communities, benthic herbivorous invertebrates and fishes are important for the structure and dynamics of shallow rocky sublittoral communities (Andrew & Jones 1990; Bégin *et al.*, 2004; Bussell *et al.*, 2007). Among the rich faunal communities inhabiting algal bottoms, molluscs are one of the best represented and dominant taxa (Poulicek, 1985; Chemello *et al.*, 1997; Milazzo *et al.*, 2000; Costa & Ávila, 2001; Rueda & Salas, 2003), being considered an important food source for higher trophic levels (Pohle *et al.*, 1991; Ruitton *et al.*, 2000; Pasquaud *et al.*, 2010).

The diversity, structure and dynamics of molluscan

assemblages in algae has already been studied in the Mediterranean (Chemello & Russo, 1997; Chemello *et al.*, 1997; Milazzo *et al.*, 2000; Chemello & Milazzo, 2002; Urre *et al.*, 2013b) and also along the Atlantic coasts (Sánchez-Moyano *et al.*, 2000; Costa & Ávila, 2001; Kelaher, 2003; Rueda & Salas, 2003). However, few studies have been carried out along the North African coasts, except for those on the diversity, structure or dynamics of molluscan assemblages on the Moroccan coasts (Menioui, 1992a,b; Bayed & Bazairi, 2008). Tunisia is situated at the threshold between the Eastern and Western basins of the Mediterranean, and the study of its fauna, including molluscs, could be very interesting for checking the influence of climate change on the increase of non-native species, particularly the invasive ones (Stachowicz *et al.*, 2002). Published reports on Tunisian molluscs are either relatively old (e.g. Pallary, 1914), the most recent updates on molluscs being those by Fekih & Gougerot (1974) for the Gulf of Tunis, Zaouali (1978) and Cecalupo *et al.* (2008) for the Gulf of Gabes.

This study is a result of an ongoing sampling programme along the shores of northern Tunisia, to improve knowledge about molluscan fauna, including the smaller and/or taxonomically difficult species. Here we have

studied the molluscan assemblage associated with photophilous algal turfs in the inner Bay of Tunis, at an urban sea resort site near the harbour of La Goulette, with significant human influence during summer (Antit, 2012). The complete list of species was published by Antit & Azzouna (2012), with notes on the taxonomy of some of the species. Illustrations of some specimens used for this study have also been posted on the World Register of Marine Species (WoRMS, Appeltans *et al.*, 2013) under the appropriate species headings.

This paper focuses on the dynamics during an annual cycle, with the aim to assess the influence of biotic (algal biomass, chlorophyll *a*) and abiotic (seawater temperature and salinity) variables on the dynamics of the molluscan assemblage. Our hypotheses are that the macroalgae bottoms of the Bay of La Goulette function as a shelter for the molluscan assemblage and that they undergo certain changes during the year in relation to environmental factors.

## Material and Methods

### Study area

The study was carried out in the Bay of La Goulette (Fig. 1), characterized by a diversified seabed, where seagrasses (*Posidonia oceanica* and *Cymodocea nodosa*), photophilous algal bottoms, natural or artificial rocky substrates and extensive soft bottoms, among other, are found. The whole area is influenced by La Goulette Harbour and the Khéreddine Canal that communicates with the Tunis Lagoon and may drive up to ca. 950,000 m<sup>3</sup> / day of fresh water (Ministère de l'Environnement et de l'Aménagement du Territoire, 2000). Among all these habitats, shallow photophilous algae represent a widespread type of vegetated habitat that grows on the rocky breakwaters constructed for shore protection at La Gou-

lette. This shoreline is located within an urban environment, highly visited in summer as a seaside resort and in which most rocky substrates are artificial.

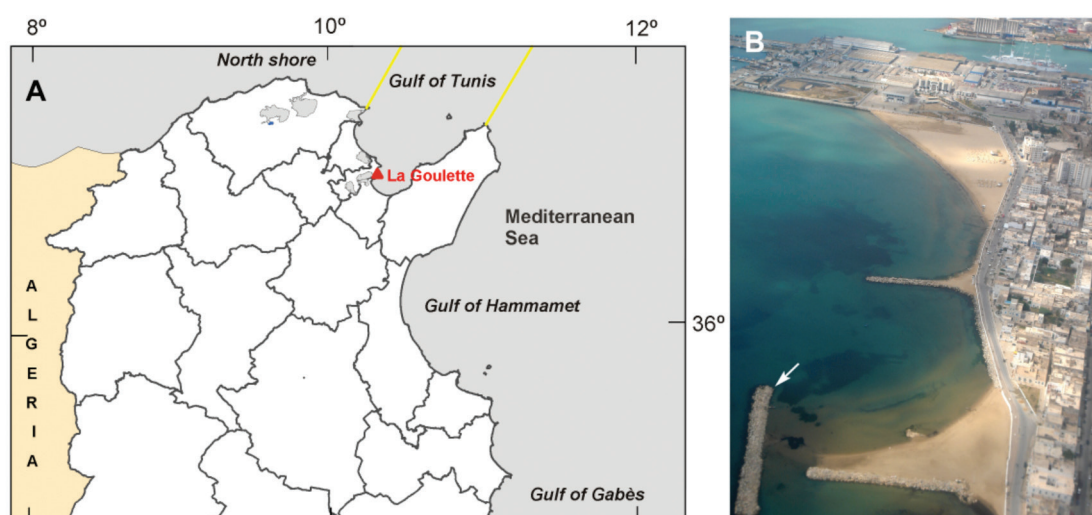
### Sample collection and laboratory procedures

The samples were collected monthly by snorkelling, from February 2009 to February 2010, at a depth of ca. 1-2 m on the rocky breakwater (36°49.15'N - 10°18.60'E). Five monthly replicates of the algae and their associated fauna were taken on the same day by scraping the surface within a 25 x 25 cm frame carefully, as in other similar studies (e.g. Antoniadou & Chintiroglou, 2005; Fraschetti *et al.*, 2005; Balata *et al.*, 2006) and collecting the material in < 0.5 mm mesh bags, resulting in a total of 65 samples.

In the laboratory, every sample was sieved through 10, 5, 2, 1 and 0.5 mm mesh sizes, storing each size fraction in 70% ethanol. Molluscs were separated from the different fractions, each species was identified and their individuals counted under a binocular microscope. Care has been taken to count separately juveniles (half or less the known adult size of the species) and subadults or adults, in order to detect species that inhabit the algae at certain stages of their life cycle only.

### Environmental variables

Seawater temperature was measured *in situ* at the time of sampling, and water samples for measuring the concentration of chlorophyll *a* and salinity were taken, in order to study the relationships of these variables with the molluscan assemblages throughout the year. During each monthly sampling trip, 2 l of sea water were collected at ca. 1 m depth and transported in darkness at low temperature to the laboratory for salinity and chlorophyll *a* determination. The measurements of salinity were made using a WTW Cond 315i/SET salinometer.



**Fig. 1:** Study site. A: map of northern Tunisia showing the position of the site. B: Aerial view, the arrow shows the site where the algal turf was sampled. The dark patches underwater are seagrass meadows.

For the pigment analyses, 1 l of sea water was filtered through Whatman GF/C glass filters (Nr 5, 1.2  $\mu\text{m}$ ). The pigments of the retained cells were then extracted using 100% acetone for 12 hours in cool and dark conditions. The solution was assayed with absorption measurements using a spectrophotometer at wavelengths of 630, 647, 664 and 750 nm. The chlorophyll *a* concentrations were obtained using the equation proposed by Jeffrey & Humphrey (1975). The monthly amount of macroalgae collected was quantified as (1) Volume of fresh algae (V, measured in  $\text{cm}^3$ ): calculated by the displacement of a known volume of water in a graduated flask (Bussett *et al.*, 2007); (2) Dry weight (DW, measured in g): algal weight after drying for 48 hours at 84°C and after removal of the epiphytes and animals (Edgar, 1983). The macroalgae were identified in order to identify the major components of the algal assemblage, although this was not quantified month to month.

### Data analysis

Species identification was carried out using literature and reference collections as detailed in Antit & Azzouna (2012). Each molluscan species was assigned to a trophic group essentially following the categories considered by Rueda *et al.* (2009) and checking previously published papers (Rueda *et al.*, 2009; Gofas *et al.*, 2011; Urra *et al.*, 2013a, 2013b).

These categories included (1) carnivores feeding selectively on other motile organisms, (2) scavengers feeding on remains of dead organisms, (3) detritivores feeding on organic particles contained in the sediment, (4) ectoparasites and specialized carnivores feeding on much larger organisms, both sessile and motile, on which they can live during their life cycle, (5) plankton and seston feeders feeding on suspended organic particles, and (6) microalgae or periphyton grazers feeding on microalgae or on a complex mixture of algae, cyanobacteria and detritus that are attached to algal fronds.

Abundance (N, number of live-taken individuals of a species in a sample), Frequency index (%F, percentage of samples in which a particular species is present) and Dominance index (%D, percentage of individuals of a particular species within the sample) were calculated for each species (Glémarec, 1964). The molluscan assemblage was characterized according to species richness (S), and several ecological indices, such as the Shannon-Wiener diversity index ( $H'$ :  $\log_e$ ) (Krebs, 1989) and the evenness index ( $J'$ ) (Pielou, 1969). These ecological indices were calculated using the PRIMER v.6 software package (Clarke & Warwick, 1994).

One-factor ANOVA (Analysis of Variance) was carried out for testing statistical differences in the values of ecological indices according to season. The analyses were carried out after verifying normality (Kolmogorov-Smirnov) and homogeneity of variances (Levene). Kruskal-

Wallis analyses was applied when data did not display a normal distribution. These statistical procedures were performed using the SPSS software package.

Multivariate analyses, such as group-average sorting classification (CLUSTER) and non-metric multidimensional scaling (n-MDS) ordination, using the Bray-Curtis similarity index, were applied to both qualitative (presence/absence) and quantitative data, in order to identify similarities/dissimilarities in the composition and structure of the molluscan assemblage according to different seasons. A fourth root transformation pre-treatment was applied to the quantitative data in order to minimize the contribution of the most abundant species to the analysis. ANOSIM (Analysis of Similarity) was carried out for statistical comparison of the groups of samples according to the different factors analysed (Clarke & Warwick, 1994). The SIMPER procedure was used to identify those species that most contributed to the similarity and dissimilarity between these same groups of samples. These multivariate analyses were executed using the PRIMER v.6 software package.

The faunistic and environmental ordinations of samples were also analysed using BIOENV (BIOTic and ENVironmental linking) analysis. The BIOENV analysis resulted in different combinations of environmental variables that are highly correlated with the ordination of faunistic samples. These multivariate analyses were executed using the PRIMER v.6 software package.

## Results

### Sea-water variables

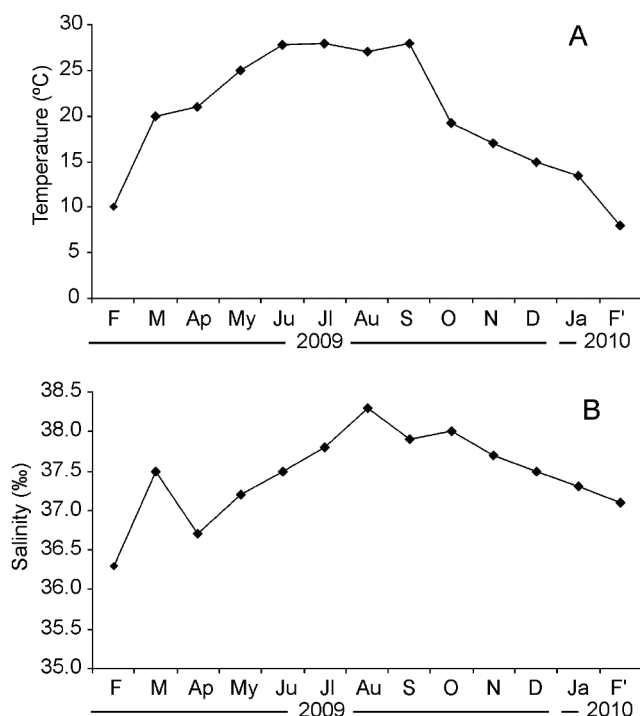
The highest seawater temperature (T) values were recorded in summer and autumn (28 °C in July and September) and the lowest value in winter (8 °C in February 2010) (Fig. 2A). As regards salinity (S), the maximum value was also recorded in summer (37.8 ‰ in August) and the minimum value in winter (35.8 ‰ in February) (Fig. 2B). Chlorophyll *a* concentrations (Chl *a*) fluctuated significantly. The highest recorded values were in summer (2.76; 2.33 and 2.11  $\mu\text{g} / \text{l}$ ), and the lowest in April and March (0.26; 0.40  $\mu\text{g} / \text{l}$ ) (Fig. 3A).

### Macroalgal composition

The most abundant macroalgae species were *Corallina elongata* Ellis & Solander, 1786, *Jania* sp. and *Ulva rigida* C. Agardh, 1823. Other less abundant species were *Gracilaria* sp., *Cladophoropsis membranacea* (Hofman Bang & C. Agardh) Børgesen, 1905 and *Centroceras clavulatum* (C. Agardh) Montagne, 1846.

Mean dry weight of macro-algae (biomass) had a marked peak in March 2009, with low values in summer months (Fig. 3B). The macroalgal volume (V) was quite well correlated with the algal biomass and follows similar temporal dynamics.





**Fig. 2:** Abiotic environmental variables measured at the sampling site during the year of survey. A: Sea surface temperature (°C); B: Salinity.

### Composition and structure of the molluscan assemblage

A total of 16105 molluscs, belonging to 93 species, were collected. Gastropods were the most species-rich group, with 66 species (70.96%), followed by bivalves with 26 species (27.95%) and only one species of polyplacophoran (1.07%). In relation to total abundance, gastropods were also the most numerous with 11529 individuals (71.60%), followed by bivalves with 4575 individuals (28.41%).

Eighteen species displayed dominance values higher than 1% (Table 1), with *Mytilaster minimus* (Poli, 1795) as the top dominant species (15.66% D), followed by *Tricola miniata* (Monterosato, 1884) (11.57%), *Bittium reticulatum* (da Costa, 1778) (11.49%) and *Gibbula racketsi* (Payraudeau, 1826) (10.39%). Ten species were found every month (F=100%, Table 1). Five of them are gastropods, including the alien species *Mitrella psilla* (Duclos, 1846), and five bivalves, including another alien species, namely *Arcuatula senhousia* (Benson in Cantor, 1842). The most frequent species as regards number of replicates were *Bittium reticulatum* and *Mytilaster minimus* and nearly 50% of the species could be considered as rare (F < 12%) (Table 1).

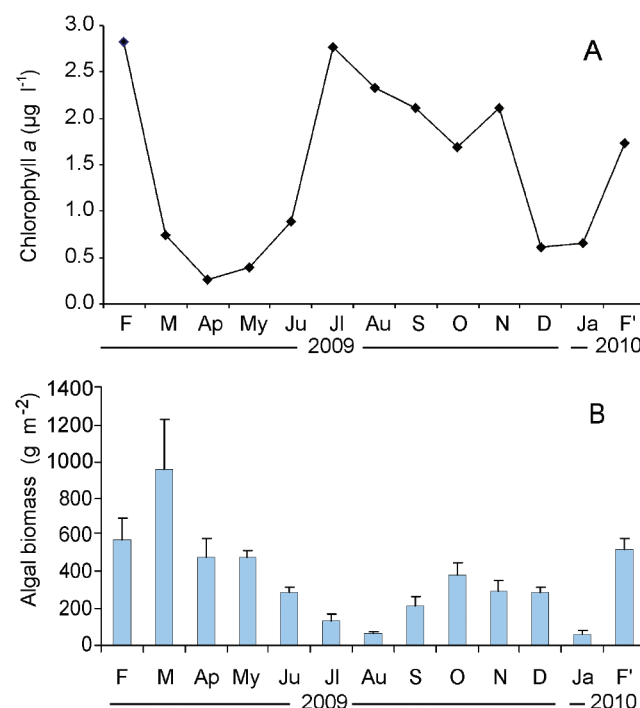
The feeding guild with the highest number of species was that of microalgae and periphyton grazers (Rissoidae and Trochidae), with 31 species, followed by the seston and plankton feeders (26 species), most of them being bi-

valves. The rich faunal assemblage attracts a high number of carnivores, such as the Muricidae, Mangeliidae or Retusidae (gastropods), which together present 10 species in the assemblage. However, if we add to the latter the species of ectoparasites and specialist carnivores (13 species), such as Eulimidae, Pyramidellidae and Cerithiopsidae that feed on other invertebrates, then the carnivores “sensu lato” would be a dominant trophic group in the assemblage after microalgae and periphyton grazers and seston and plankton feeders.

The most abundant feeding guilds were microalgae and periphyton grazers (47%) and seston and plankton feeders (29%), followed in this case by detritivores (14%). The carnivores represented only 7% of total abundance. Regarding micro-habitat preference, the most species-rich and abundant groups were those of epifaunal species associated with macroalgae (48% and 71%, respectively) and hard bottoms (22% in both cases).

### Temporal dynamics

Mean monthly species richness (S) is displayed in Figure 4A as a histogram. The species richness of the molluscan assemblage varies between more than 30 species in March 2009 and ca. 15 species in September 2009 (Figure 4A, histogram). Nevertheless, the species richness (S) scaled to algal biomass shows a different trend (Figure 4A, solid line), with higher richness for molluscs in August 2009 and January 2010, when algal biomass



**Fig. 3:** Biotic environmental variables measured at the sampling site during the year of survey. A: Chlorophyll a (µg/litre). B: Mean algal biomass (from February 2009 to February 2010, five replicates per month). Bars represent standard error.

**Table 1.** List of the 20 most dominant and most frequent species over the whole study period (from February 2009 to February 2010). N: total number of specimens; D%: dominance index in %; TG: trophic group (CA: carnivore; SC: scavenger; DE: detritivore; EC: ectoparasite; FI: plankton and seston feeder; HE: herbivore; MG: micrograzer); F% (m): frequency of live specimens, considering monthly samples with five pooled replicates; F% (r): Frequency considering separately each one of the 65 replicates. Photographs of specimens used in this study are posted on the WoRMS website (Appeltans *et al.*, 2013) under the respective species headings.

Species	N	D%	TG	Species	F% (m)	TG	Species	F% (r)	TG
<i>Mytilaster minimus</i> (Poli, 1795)	2522	15.66	FI	<i>Fissurella nubecula</i> (Linné, 1758)	100.00	MG	<i>Bittium reticulatum</i> (da Costa, 1778)	95.38	DE
<i>Tricolia miniata</i> (Monterosato, 1884)	1863	11.57	MG	<i>Gibbula racketti</i> (Payraudeau, 1826)	100.00	MG	<i>Mytilaster minimus</i> (Poli, 1795)	90.77	FI
<i>Bittium reticulatum</i> (da Costa, 1778)	1851	11.49	DE	<i>Pisinna glabrata</i> (von Mühlfeldt, 1824)	100.00	MG	<i>Venerupis aurea</i> (Gmelin, 1791)	87.69	FI
<i>Gibbula racketti</i> (Payraudeau, 1826)	1674	10.39	MG	<i>Bittium reticulatum</i> (da Costa, 1778)	100.00	DE	<i>Gibbula racketti</i> (Payraudeau, 1826)	87.69	MG
<i>Rissoa similis</i> (Scacchi, 1836)	1574	9.77	MG	<i>Mitrella psilla</i> (Duclos, 1846)	100.00	CA	<i>Pisinna glabrata</i> (von Mühlfeldt, 1824)	86.15	MG
<i>Pisinna glabrata</i> (von Mühlfeldt, 1824)	814	5.05	MG	<i>Mytilaster minimus</i> (Poli, 1795)	100.00	FI	<i>Gregariella petagnae</i> (Scacchi, 1832)	86.15	FI
<i>Venerupis aurea</i> (Gmelin, 1791)	572	3.55	FI	<i>Arcuatula senhousia</i> (Benson, 1842)	100.00	FI	<i>Mitrella psilla</i> (Duclos, 1846)	84.62	CA
<i>Petricola lithophaga</i> (Retzius, 1788)	468	2.91	FI	<i>Gregariella petagnae</i> (Scacchi, 1832)	100.00	FI	<i>Petricola lithophaga</i> (Retzius, 1788)	81.54	FI
<i>Nassarius corniculum</i> (Olivi, 1792)	453	2.81	SC	<i>Venerupis aurea</i> (Gmelin, 1791)	100.00	FI	<i>Fissurella nubecula</i> (Linné, 1758)	80.00	MG
<i>Haminaea orbignyana</i> (de Férussac, 1822)	406	2.52	HE	<i>Petricola lithophaga</i> (Retzius, 1788)	100.00	FI	<i>Haminaea orbignyana</i> (de Férussac, 1822)	66.15	HE
<i>Mitrella psilla</i> (Duclos, 1846)	362	2.25	CA	<i>Alvania montagui</i> (Payraudeau, 1826)	92.31	MG	<i>Alvania montagui</i> (Payraudeau, 1826)	64.62	MG
<i>Cerithium scabridum</i> Philippi, 1848	317	1.97	DE	<i>Gibberula miliaria</i> (Linné, 1758)	92.31	CA	<i>Arcuatula senhousia</i> (Benson, 1842)	61.54	FI
<i>Fissurella nubecula</i> (Linné, 1758)	310	1.92	MG	<i>Irus irus</i> (Linné, 1758)	92.31	FI	<i>Rissoa similis</i> (Scacchi, 1836)	55.38	MG
<i>Alvania montagui</i> (Payraudeau, 1826)	267	1.66	MG	<i>Cerithium scabridum</i> Philippi, 1848	84.62	DE	<i>Cerithium scabridum</i> Philippi, 1848	53.85	DE
<i>Gregariella petagnae</i> (Scacchi, 1832)	267	1.66	FI	<i>Odostomia kromi</i> van Aartsen <i>et al.</i> , 1984	84.62	EC	<i>Tricolia miniata</i> (Monterosato, 1884)	52.31	MG
<i>Musculus costulatus</i> (Risso, 1826)	229	1.42	FI	<i>Haminaea orbignyana</i> (de Férussac, 1822)	84.62	HE	<i>Nassarius corniculum</i> (Olivi, 1792)	49.23	SC
<i>Arcuatula senhousia</i> (Benson, 1842)	195	1.21	FI	<i>Tricolia miniata</i> (Monterosato, 1884)	76.92	MG	<i>Gibberula miliaria</i> (Linné, 1758)	46.15	CA
<i>Columbella rustica</i> (Linné, 1758)	162	1.01	?	<i>Rissoa similis</i> (Scacchi, 1836)	76.92	MG	<i>Irus irus</i> (Linné, 1758)	43.08	FI
<i>Ammonicera fischeriana</i> (Monterosato, 1869)	144	0.89	MG	<i>Ocenebrina edwardsi</i> (Payraudeau, 1826)	76.92	CA	<i>Pusillina philippi</i> (Aradas & Maggiore, 1844)	41.54	MG
<i>Gibberula miliaria</i> (Linné, 1758)	125	0.78	CA	<i>Nassarius corniculum</i> (Olivi, 1792)	76.92	SC	<i>Odostomia kromi</i> van Aartsen <i>et al.</i> , 1984	40.00	EC

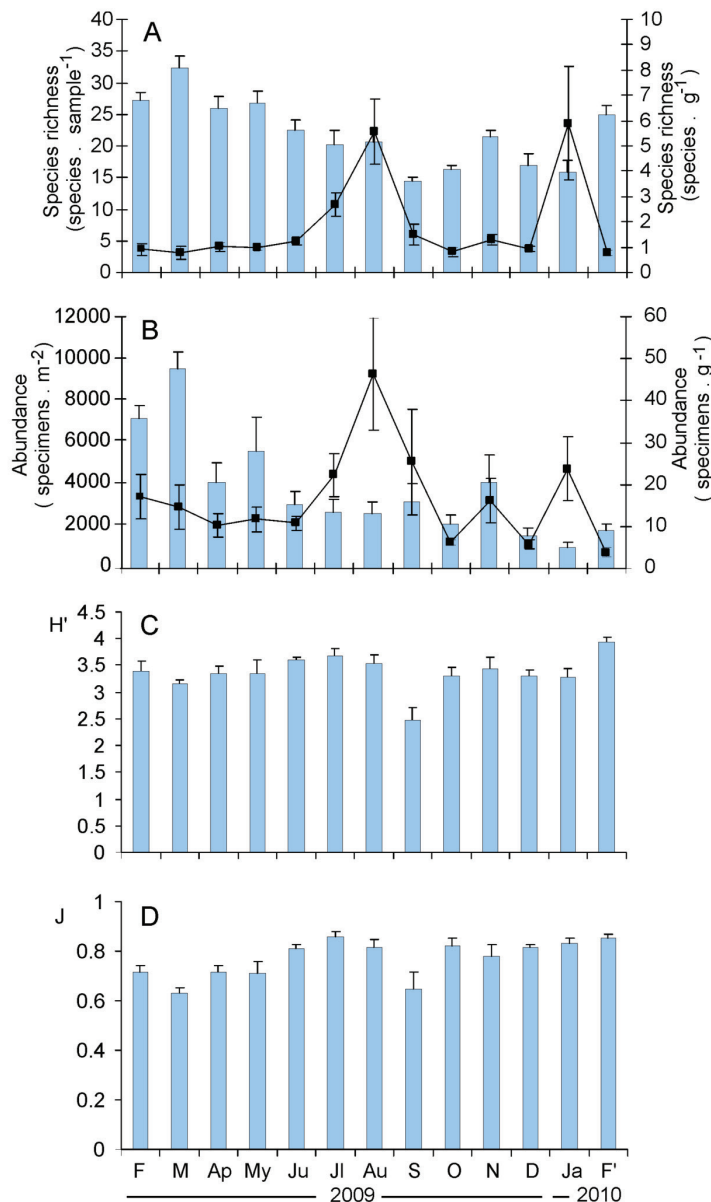
was low. Significant seasonal changes were obtained for the species richness of molluscs (one-factor ANOVA;  $F=14.28$ ;  $p<0.001$ ).

The abundance of molluscs also showed monthly changes (Fig. 4B, histogram), with maxima in March 2009 (ca. 10.000 specimens  $m^{-2}$ ) and minima in January 2009 (ca. 1000 specimens  $m^{-2}$ ). Mean monthly abundance displayed a trend that accompanies mean monthly algal biomass, but mollusc density (per algal biomass) displayed higher values in August 2009, when algal biomass is low (Fig 4B, solid line). Significant seasonal changes in the abundance of molluscs during the year have been

observed (Kruskal-Wallis;  $\chi^2 = 32.36$ ,  $p < 0.001$ ).

The Shannon-Wiener diversity index ( $H'$ ) shows rather high values, around 3 bits, throughout the study year (Fig. 4C), with higher values in February 2010 (3.94 bits) and July (3.68 bits), and lower values in September (2.27 bits). No significant seasonal differences were obtained (Kruskal-Wallis;  $\chi^2 = 4.41$ ,  $p > 0.05$ ).

The evenness ( $J$ ) index shows high values throughout the year (Fig. 4D), with a peak in July (0.85) and low values in March (0.63) and September (0.70). Significant seasonal differences were obtained (one-factor ANOVA;  $F=5.18$ ;  $p<0.01$ ).



**Fig. 4:** Monthly variation of relevant biotic measures and indexes during the study period (from February 2009 to February 2010, five replicates per month), mean values for the five replicates and standard error. A: Species richness expressed as mean number of species per month (histogram, left axis) and as ratio of mean number of species to mean biomass of algae (line, right axis). B: Abundance, expressed as mean number of specimens per month (histogram, left axis) and as ratio of mean number of specimens to mean algal biomass (line, right axis). C: Shannon-Wiener diversity index ( $H'$ ). D: Evenness ( $J$ ).

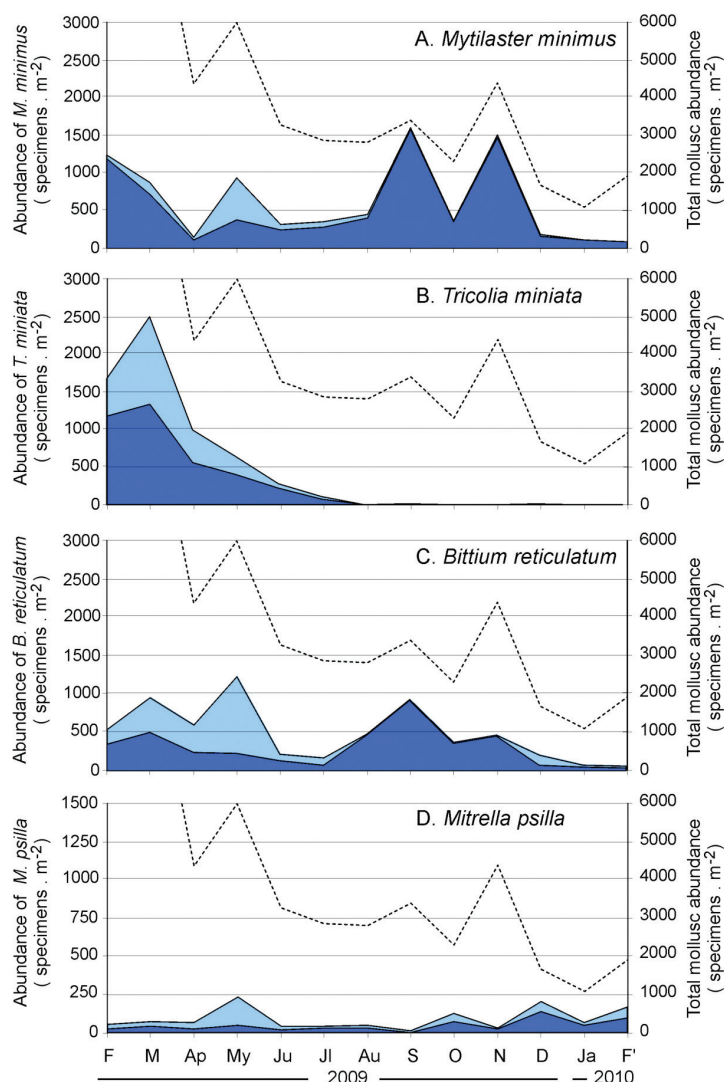
### Dynamics of several dominant species

Several mollusc species have shown important fluctuations of their populations, which in the case of the top dominant species have determined the abundance and structure of the entire molluscan assemblage.

*Mytilaster minimus*. The species was present in the algal turf throughout the year. The monthly dynamics of its population is displayed in figure 5A, where the juveniles are distinguished. The bulk of the population in the algae consists of juveniles, which highlight the use of the macroalgal turf as a nursery ground. Several recruitment peaks were observed in February, September and November 2009, which seem to influence the total abundance of the molluscan assemblage.

*Tricolia miniata*. This species was present in the algal turf only during winter and spring 2009, with a peak in March (Fig. 5B), but the bulk of the population was composed of juveniles. Although the preferred habitat of the species is the algal turfs, its presence was limited to 6 months, until summer when it disappeared. Its winter recruitment was so abundant that it contributed to an increase of the entire molluscan assemblage.

*Bittium reticulatum*. This ubiquitous species was present in the algal turf throughout the year (Fig. 5C), with a recruitment peak at the end of the summer. However, juveniles were present throughout the study year, with a minimum in February 2010.



**Fig. 5:** Temporal dynamics of selected molluscan species in the algal turf at La Goulette. The shaded area represents species abundance (left axis), as total number of specimens per square meter; the darker area referring to juveniles. The dashed line (right axis) represents the total abundance of molluscs in the five replicas of the month.

*Mitrella psilla*. This alien species was also present in the algal turf throughout the study year, with recruitment peaks in the cold months (Fig. 5D). The presence of eggs with juveniles in the algae bottom indicates the establishment of the species in its new area.

### Similarities between samples

Multivariate analysis of qualitative data (presence/absence) based on the Bray-Curtis similarity index showed seasonal groups of samples in n-MDS representation (Fig. 6A). ANOSIM analysis showed significant seasonal differences (one-way ANOSIM,  $R_{\text{ANOSIM}} = 0.37$ ,  $p < 0.001$ ). Multivariate analysis using fourth root transformed (Fig. 6B) and non-transformed abundance data also showed similar seasonal groups of samples in n-MDS representation. Significant global seasonal differences were obtained for non-transformed data (one-way

ANOSIM,  $R_{\text{ANOSIM}} = 0.31$ ,  $p < 0.001$ ) and transformed data (one-way ANOSIM,  $R_{\text{ANOSIM}} = 0.42$ ,  $p < 0.001$ ).

The SIMPER analysis indicated that in winter and spring months, the species contributing most to the difference between seasons are *Tricolia miniata*, *Rissoa similis*, *Ammonicera fischeriana*, *Nassarius corniculum* or *Gibbula racketsi*. In summer the most contributing species are *Cerithium scabridum*, *Columbella rustica*, *Mytilaster minimus*, *Venerupis aurea*, *Arcuatula senhousia* and *Gregariella petagnae*, whereas in autumn they include *Fissurella nubecula*, *Alvania montagui*, *Venerupis aurea*, among others. The highest dissimilarities of SIMPER analysis were obtained between spring and summer (Dissimilarity: 59.62%), spring and autumn (56.95%) and summer and winter months (56.59%). The lowest dissimilarities were obtained between summer and autumn (44.53%), winter and spring (50.16%) and autumn and winter months (51.97%).



### Relationships between molluscan assemblages and environmental variables

Environmental variables from the water column, as well as those of the macroalgae turf were correlated with the molluscan assemblage using BIOENV analysis. The combination of variables that showed the best relationship with the dynamics of the molluscan assemblage was the combination of salinity and algal biomass (Sal-Biom), although sea water temperatures also show a relationship, combined with salinity and biomass (Table 2). Chlorophyll *a* did not show any relationship with the dynamics of the molluscan assemblage.

### Discussion

#### Macroalgae assemblage

The studied macroalgal assemblage was composed of species forming low turfs, the most abundant ones being *Corallina elongata*, *Jania* sp. and *Ulva rigida*. This low diversity algal assemblage is characteristic of eutrophicated environments, which at the studied site could be due to its proximity to La Goulette Harbour and urban pollution during summer months. *Corallina elongata* and *Ulva rigida* were also reported as dominant in the Saronikos Gulf (Greece) near the Piraeus Harbour (Panayotidis *et al.*, 2004). The presence of algal turf on rocky breakwaters, near a much visited beach, makes this habitat heavily impacted by human activities such as trampling during summer months, which seems to cause a drastic reduction of the algal canopy and their associated assemblages around the world (Povey & Keough, 1991; Liddle, 1991; Milazzo *et al.*, 2004). In Greece, Reizopoulou & Zenetos (2005) indicated that seasonal growth of human pressure is an important factor for the decrease of biodiversity in the coastal area. They found a reduction in species number and an increase of opportunistic species dominance near summer resorts, related to decreases of water quality. The reduction of algal biomass during summer in the bay of La Goulette (Fig. 3B) could be partly related to the increase of human population during summer months and their influence on water quality, but also to the high increase of water temperature during summer months, which may cause the disappearance of certain macroalgae (e.g. *Ulva*).

#### Environmental variables

Seawater temperatures show one of the broadest ranges known in the Mediterranean Sea (Fine *et al.*, 2001), with near tropical seawater in summer (28°C in July and September 2009) and a considerable decrease in winter (8°C in February 2010), similar to that found in the northern Adriatic Sea, where the lowest temperatures during the winter months are associated with the increase in coastal flows related to the Po River outflow (Böhm *et al.*, 2003). The low values during the winter months in the study area could also be due to the large input of fresh water through

**Table 2:** BIOENV analyses relating several combinations of abiotic factors to the semi-matrix of faunal similarity using qualitative (presence/absence) and quantitative (transformed to fourth root) data. The higher values correspond to combinations of factors that best explain the faunal data. Tem: sea surface temperature; Sal: salinity; Biom: algal biomass; Chl: chlorophyll *a*.

Variables	Qualitative	Quantitative fourth root
Tem-Sal-Biom	0.31	0.40
Sal-Biom	0.40	0.46
Tem-Sa-Chl-Biom	0.28	0.38
Sal-Chl-Biom	0.33	0.39

the Khéreddine Canal. This range of seawater temperatures is a handicap for many species. On the other hand, salinity also showed a normal distribution, but with two peaks, the first one in March, coincident with a strong increase in seawater temperature, which probably favoured water evaporation. The rainy periods in the area generally occur in November and February, which reduces nearshore salinity. Finally, chlorophyll *a* showed strong fluctuations, with peaks in February, July and November 2009, and higher values during summer and autumn and minimum in spring. This data could be related to the input of nutrients through the Khéreddine Canal during the rainy seasons, as observed in the northern Adriatic Sea also (Böhm *et al.*, 2003), and an increased volume of waste produced by the human population in summer, as found in the eastern Mediterranean Sea (Reizopoulou & Zenetos, 2005).

#### Molluscan assemblages

In spite of the low development of algal turf in the bay of La Goulette, this study shows a rich molluscan assemblage (93 species), comparable to other studies on molluscs associated with *Corallina officinalis* from Australia and South America with less sampled surface (Kelahe *et al.*, 2001, 2007; Kelahe & Castilla, 2005). There are nevertheless molluscan assemblages from Mediterranean sites with higher species richness in different algal turfs, in Lampedusa (Italy) with 176 species in a *Cystoseira* dominated assemblage (Chemello & Russo, 1997), Algeciras and Cabo Pino (Spain) with respectively 98 and 193 species in a *Halopteris* dominated assemblage (Sánchez-Moyano *et al.*, 2000; Urra *et al.*, 2013b) and the Aegean Sea with 184 spp. in photophilous algae (Chintiroglou *et al.*, 2005).

The presence as dominant in the assemblage of juveniles of species characteristic of other neighbouring habitats, such as *Mytilaster minimus*, *Petricola lithophaga* or *Fissurella nubecula* from intertidal and subtidal rocks or *Venerupis aurea* and *Haminoea orbignyana* from soft bottoms (Table 1), highlights the nursery role of the algal turf. According to Woods & Podolsky (2007), photosynthesis performed by macrophytes can induce large changes in the internal oxygen profile of the fertilized



eggs of aquatic animals deposited among the algae as adherent clutches or gelatinous masses. The latter could partly explain the nursery role of algal turfs. Another factor favouring the nursery role is protection against predators. However, this nursery role may also favour some species of *Mitrella* that have been observed feeding on egg masses, which could explain the success of this genus in vegetated bottoms (Rueda *et al.*, 2009). Moreover, protection against wave action offered by the algal turf may represent another factor that is essential for juveniles that do not yet have the ability to attach efficiently to the substrate as they do later on as adults, such as those of some mytilids with recruits (large ones) that are able to move efficiently, using their byssus threads, to nearby located adult beds (Erlandsson *et al.*, 2008). Other species with pelagic larvae, such as the bivalve *Venerupis aurea*, live as adults in neighbouring soft bottoms and their juveniles may not yet be able to bury themselves thus requiring the microhabitat of the algal turf because otherwise they could drift away by wave action, as happens for other soft bottom species (Norkko *et al.*, 2001).

Gastropods are the dominant (qualitatively and quantitatively) group, with *Gibbula racketsi*, *Tricolia miniata*, *Rissoa similis*, *Nassarius cuvierii* and *Nassarius corniculum* as characteristic species. The families best represented in the molluscan assemblage were Rissoidae and Trochidae, which is the same as found in other Mediterranean areas (Poulicek, 1985; Chemello & Russo, 1997; Milazzo *et al.*, 2000). The rissoids, with 13 species, are typical micrograzers inhabiting photophilous algae (Gofas *et al.*, 2011; Costa & Ávila, 2001). The alien exotic species *Mitrella psilla* from West Africa (Antit *et al.*, 2010) has reached a dominant position in the molluscan assemblage, together with a permanent population throughout the year and reproduction in algal fronds (Antit *et al.*, 2011). It would be very interesting to analyse the feeding habit of this species; this would allow a better understanding of its position in the trophic chain and the reasons for its successful colonization of the algal turf. The presence of the bivalve *Arcuatula senhousia*, another alien species, as one of the dominant species in the assemblage highlighted the role of the algae as a collector of alien species in their colonization of new areas.

Among the molluscan species in this assemblage there was a high diversity of feeding guilds, which are related to the temporal presence of species from the different surrounding habitats, such as rocky substrate, seagrasses or soft bottoms. The potential flux of species, either due to the wave action or the active movement of species across habitats, favours a rich faunal assemblage in all of them and increments the feeding guilds in each habitat. The same trend was also found in seagrass beds (Urre *et al.*, 2013a). The most species-rich trophic group in the algal turf was that of microalgae and periphyton grazers, followed by seston and plankton feeders, carnivores *sensu lato*, ectoparasites and specialist carnivores, and scaven-

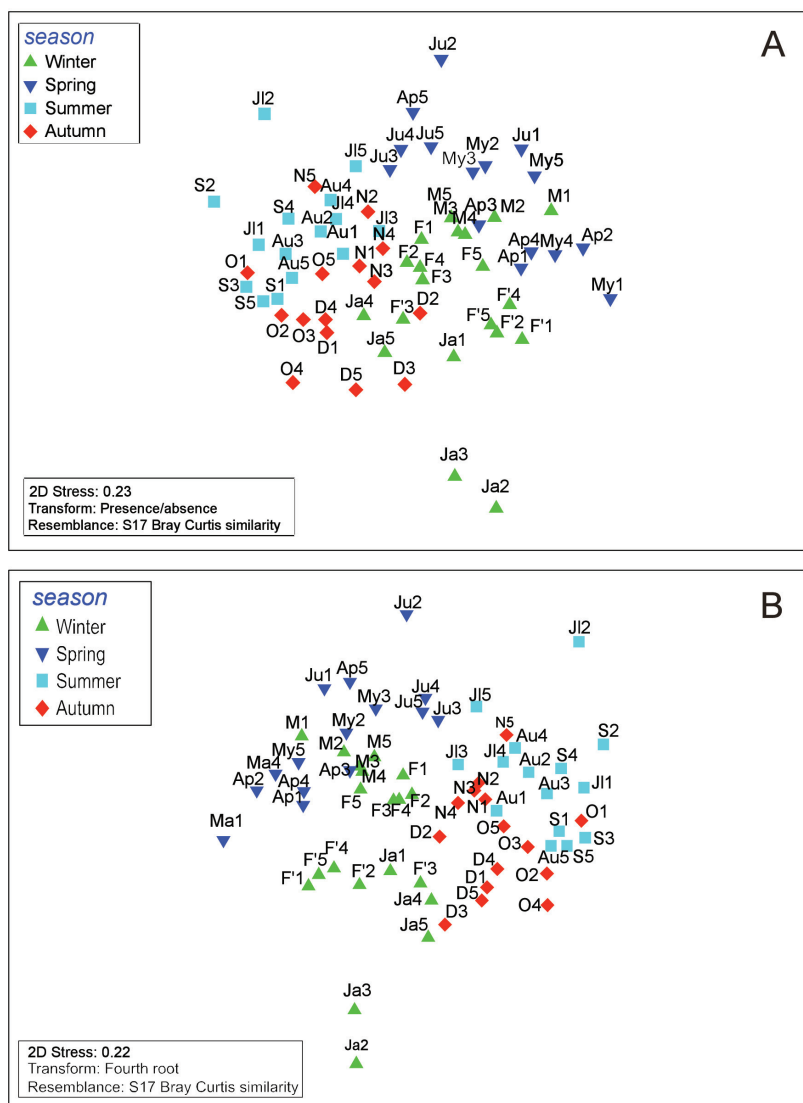
gers. The latter indicates a high mortality and settlement rate in this vegetated habitat where most species have short life cycles, and may also be affected by different negative impacts, such as trampling (Milazzo *et al.*, 2000) and urban pollution (Worm & Lotze, 2006) during summer, and by a strong input of fresh water due to rainy weather in autumn and winter. The trophic structure is similar to that observed in other vegetated habitats, such as seagrasses (Rueda *et al.*, 2009; Urre *et al.*, 2013a).

It is a general rule that degraded environments (at small scale) are more easily colonized by alien species than pristine environments (Fridley *et al.*, 2007). However, many factors can influence invasibility (Beisner *et al.*, 2006; Stachowicz & Byrnes, 2006) including competition for the space and/or resources. Despite the presence of two alien species among the dominant species of the assemblage, the location of the algal turf near the harbour, potentially affected by eutrophication as in the smaller ports of Tunisia (Aloui-Bejaoui & Afli, 2012), is likely to favour invasion by most species. The rich and abundant assemblage, with consecutive settlements of different species throughout the year, which occupy space, and the diversity of feeding guilds, are the elements that probably control the invasion by more alien species (Stachowicz & Byrnes, 2006).

### Temporal dynamics

The molluscan assemblage associated with the algal turfs in the bay of La Goulette displayed a seasonal pattern in its composition and structure, similar to that observed in molluscan assemblages from other macroalgal turfs (Sánchez-Moyano *et al.*, 2000; Rueda & Salas, 2003; Urre *et al.*, 2013b) and seagrasses (Arroyo *et al.*, 2006; Rueda *et al.*, 2008) in southern Spain. In spite of species richness and abundance fluctuations, diversity ( $H'$ ) is relatively high and stable in time, which seems to indicate self-regulation of the molluscan assemblage, with a relatively high index of evenness throughout the year. The presence throughout the year of recruitment peaks, for different species, may influence the entire assemblage and seems to be characteristic of other vegetated habitats (Rueda & Salas, 2003).

The qualitative and quantitative monthly data tend to group by season, as is shown in the MDS (Fig. 6). This corroborates the seasonal pattern of the composition and structure of the assemblage, which according to the BIOENV analysis seem to be somewhat related with seasonal changes in seawater salinity, algae biomass (drastically reduced in summer) and sea water temperatures. However, the seasonal pattern could also be related to other variables not considered in this study, such as wave action, trampling by humans and waste quality, among others, which may influence the species inhabiting this rather degraded site and are likely to tolerate broad variations in environmental factors (e.g. temperature, salinity).



**Fig. 6:** Multi-dimensional scaling representing similarities between all samples during the study period (from February 2009 to February 2010, five replicates per month). A: MDS using qualitative data (presence-absence); B: MDS using quantitative data transformed to fourth root.

In summary, the shallow algal turfs represent a habitat with a rather rich molluscan (and invertebrate in general) assemblage, which is rather stable in time despite the urban and harbour influence, but still displaying some seasonal patterns for particular species and the entire assemblage. A particularly sensitive matter is invasion by alien species, because these habitats constitute a stronghold for many non indigenous species. Thus, the bay of La Goulette in particular and the bay of Tunis in general being located at the border between the Eastern and Western Mediterranean Sea may represent a landmark as regards the advance of alien species within a climate change scenario. Therefore, studies on the benthos of different coastal habitats of Tunisia, especially vegetated ones, should be monitored regularly in order to increase knowledge about the longitudinal dispersion of alien species in the Mediterranean Sea. Finally, we consider that the benthic communities associated with photophilous

algae can be a good indicator of the environmental state of the area in relation to (a) the pollution (Brown *et al.*, 1990; Dhargalkar & Komarpant, 2003) and (b) the introduction and establishment of non indigenous plant and animal species (Antit *et al.*, 2011).

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