



Does increased connectivity with the Mediterranean Sea improve the ecological status of the macroinvertebrates in the lagoon of Boughrara (SW Mediterranean)?

I. Khedhri^{1,2}, H. Djabou¹ and A. Afli¹

¹ Laboratoire de Biodiversité et Biotechnologies Marines, Institut National des Sciences et Technologies de la Mer, 28 rue du 2 mars 1934, 2025 Salammbô – Tunisie

² Corresponding author, E-mail: ines.khedhri@hotmail.fr

Keywords: Benthic macroinvertebrates, Ecological status, Lagoon of Boughrara, Temporal changes, Trophic structure.

Abstract: The lagoon of Boughrara is an almost closed system, with limited hydrological connections with the sea. Over the past several decades, the lagoon has been exposed to increased pollution due to growing human activities in the surrounding area. From 2004–2007, the channel “El-Kantra” connecting the lagoon to the sea has been extended with increased mixing with marine waters. In this work, we monitored the ecosystem of the lagoon after its rapid exposure to development and associated pollution. Two marine surveys were carried out in winter 2010 and then in winter 2013; the sediment was sampled and the principal physical and chemical parameters were measured at 13 stations. Results show that aspects of the environmental quality of the lagoon have improved from 2010 to 2013, most likely due to the extension of the channel whereby lagoon waters are better mixed resulting in improved physico-chemical conditions over this timeframe. Nevertheless, the study of the macrobenthic community did not show any improvement. On the contrary, the species richness and the abundance of the macrofauna have decreased, and some tolerant species such as bivalves (*Abra alba* and *Cerastoderma edule*) have increased in abundance. This demonstrates continued impacts on the lagoon ecosystem.

Abbreviations: AFNOR—Association Française de Normalisation; DGPA—Direction Générale de la Pêche et de l’Aquaculture; WFD – European Water Framework;

Introduction

By their geographical location between the sea and mainland, coastal lagoons are the most productive and complex marine ecosystems (Nixon 1982). They have been subjected to increasing human activities which lead to environmental degradation and to an overexploitation of natural resources. The urban, industrial, agricultural/touristic development and human activities exert high pressure on lagoon environments (Bresler et al. 2003, Magni 2003, Daby 2006, Huang et al. 2007, Rao et al. 2007). Globally, coastal lagoons are characterized by restricted water exchange, shallow depths and high biological productivity resulting in excessive rate of organic matter due to the nutrient inputs which can induce unbalance in the trophic organization in these isolated coastal areas (Nixon 1995, EEA 1999).

Several studies have been undertaken to understand the functioning of lagoon ecosystems and evaluate their ecological status. However, most studies were limited to physico-chemical approaches only to measure the impact of disturbances on marine systems (Dauer 1993, Carvalho et al. 2006). Ecologists have noted that biological elements are important to establish the real ecological status (Afli et al. 2008b, Birk et al. 2012) and assess the biological integrity of marine systems (Dauvin 2007). Like many Mediterranean lagoons, the lagoon of Boughrara which is the largest lagoon in Tunisia is a vulnerable environment with limited access to open sea and

subjected to negative effects of aquaculture activities, fishing ports, sewage outfalls and industrial wastes (Romdhane et al. 1998, Ben Aoun et al. 2007).

During 2004–2007, the channel of “El-Kantra”, which connects the lagoon to the open sea, underwent an extension of 160 m length and 5 m height to allow the passage of fishing and pleasure boats and the exchange of about 6.9 million m³ of water a day instead of 0.8 million m³ previously (DGPA 2001).

Within the European Water Framework Directive (WFD), benthic invertebrates are one of the suggested biological quality elements (Borja et al. 2009, Pinto et al. 2009) because these organisms are generally considered as potentially powerful indicators of marine ecosystem health (Warwick 1986, Dauvin 1993, Blanchet et al. 2008), due to their large capacity to indicate stresses that have occurred locally and over a period of time (Patrício et al. 2009). Also, they live at the sediment-water interface which makes them excellent integrators of changes in both systems (Dauvin 1993).

This work aims to (1) study the ecological quality of the Boughrara lagoon, at the same season (winter) in 2010 and 2013, after the extension of the channel “El-Kantra” by analyzing the main physico-chemical parameters of the seawater/sediment, (2) study the response of the benthic macro-invertebrate community to the new environmental conditions by using structure parameters and ecological indices and (3) pri-

oritize the main factors governing the organization of the benthic macro-invertebrate community in the Boughrara lagoon.

Material and methods

Study site

The lagoon of Boughrara (500 km²), located on the southeastern coast of Tunisia, is bounded on the North by the Djerba Island and on the other sides by the mainland (Fig. 1). It is connected with the gulf of Gabes by the channel of Ajim in the North-West of the lagoon (2.2 km width), and the channel “El-Kantra” which is a narrow connection in the East of around 12.5 m wide at the middle of the Roman road connecting the Djerba Island to the continent. In 2004, the channel “El-Kantra” underwent an extension that provide an exchange of about 6.9 millions m³ of water a day instead of 0.8 million m³ previously (DGPA 2001, Romdhane 2001, Tlili et al. 2008). The average depth of the lagoon is about 4 m with a maximum of 16 m in the center. The air temperature has a very important role in the lagoon of Boughrara because it has a strong influence on the water temperature (Zaouali 1971, Jedoui 1980). The seasonal variation of the water surface temperature is wide in the lagoon of Boughrara; it is on average around 24.7°C in summer and 11.2°C in winter, with an increasing North-South gradient (Ben Aoun et al. 2007). The salinity is very high especially in summer with an average value up to 42 PSU (Ben Aoun et al. 2007) and 43 PSU (Guetat et al. 2012). The lagoon of Boughrara is currently considered as a fragile and vulnerable environment due to increasing natural and anthropogenic stressors. Indeed, besides the variable hydrodynamic/climatic conditions, the lagoon is impacted by increasing and harmful anthropogenic disturbances, due to the demographic/industrial surrounding development (Daly-Yahia et al. 1994, Ben Khemis 2000, Ben

Rejeb-Jenhani and Romdhane 2002). The main sources of disturbance in the lagoon of Boughrara are the input of waste waters discharged by aquaculture farms on both banks of the lagoon (Turki and Hamza 2001), the traffic/fishing activities in the harbours of Jorf, Ajim, Boughrara and Hassi Jalleba, the entry of marine waters loaded with phosphorus coming from the gulf of Gabes (Bejaoui et al. 2004) and the low water exchange between the lagoon and the open sea.

Sampling and laboratory procedures

Two marine surveys were carried out in winter (February 2010 and January 2013), and 13 stations facing main disturbance sources were sampled (Table 1). The temperature, salinity and dissolved oxygen were measured in the field at the water surface by a Multi-parameter 340i/SET (WTW). Water samples were also taken at the surface, and were preserved at -4°C in an icebox to determine later the content in main nutrients (NO₂⁻, NO₃⁻, NH₄⁺ and PO₄³⁻) (Strickland and Parsons 1965). To measure chlorophyll *a* contents, part of collected waters was immediately filtered with a device of filtration type Wattman, and the filtering membrane was then placed in a spin-dried tube which was then preserved in cool and dark conditions (Rodier et al. 1996). The study of the physico-chemical parameters has an important role, since they control the functioning of the lagoon ecosystem and can lead to changes in the macrofauna community after the extension of the “El-Kantra” channel (Khedhri et al. 2015). The granulometry was analysed after drying during 48 hours at 60°C, then washing through a 63 µm, drying again at 60°C and sieving through an AFNOR (Association Française de Normalisation) succession meshes. The granulometric structure was then estimated as the percentage (in weight) of each sediment fraction (Buchanan 1971).

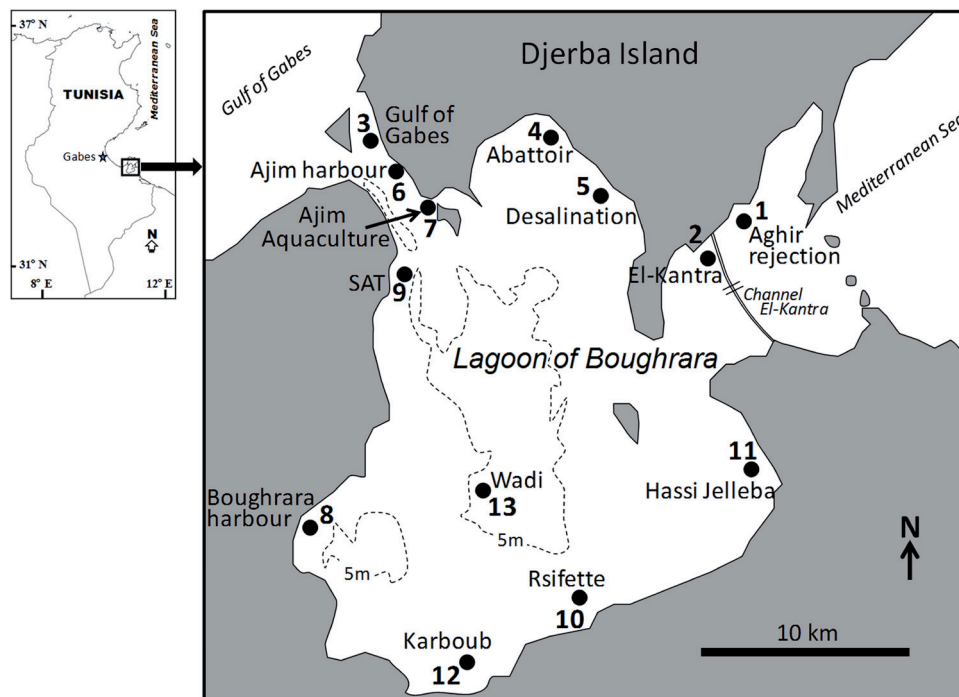


Figure 1. Map of the study site showing the location of the sampling stations (1 to 13).

Table 1. Characteristics of sampled stations.

Numeric codes	Stations	Latitude	Longitude	Depth (m)	% mud (<63µm)
1	Aghir rejection	10.5552° N	33.4130°E	0.2	7.79
2	El-Kantra	10.5532°N	33.3933° E	2.3	31.81
3	Gulf of Gabes	10.4416°N	33.4317° E	1.1	4.07
4	Abattoir	10.5000°N	33.4400° E	0.2	2.82
5	Desalination	10.5035°N	33.3427° E	0.2	1.87
6	Ajim harbour	10.4437°N	33.4259° E	1.0	4.11
7	Ajim aquaculture	10.4542°N	33.4155° E	1.0	0.83
8	Boughrara harbour	10.4129°N	33.3221° E	3.1	11.25
9	SAT	10.1421°N	33.3945° E	0.2	10.55
10	Rsiffette	10.5452°N	33.3130° E	0.3	2.75
11	Hassi Jalleba	10.5623°N	33.3455° E	0.2	4.00
12	Karboub	10.5244°N	33.3059° E	0.3	1.01
13	Wadi	10.4221°N	33.3216° E	10.5	10.45

Sediment samples for benthic macrofauna studies were taken by scuba diving using a quadrat (10 cm depth), and three replicates were carried out at each station making a total sampling surface of 0.75 m². Collected sediments of benthic macrofauna were sorted out aboard, through a 1 mm mesh sieve, and preserved in a 7% formaldehyde/seawater solution. In the laboratory, the contents in main nutrients (NO₂⁻, NO₃⁻, NH₄⁺ and PO₄³⁻) were determined with a device type Bran-Luebbe Autoanalyseur 3 (Tréguer and Le Corre 1975). The concentration in chlorophyll *a* was determined according to Lorenzen and Jeffrey (1980). The macrofauna samples were washed with freshwater on a square mesh of 1 mm a side, and the animals collected were preserved with diluted alcohol (70%) before being identified, for most of them, up to species level.

Data analysis

The main structural parameters of the benthic macrofauna determined at each station are the species richness *S* (number of species), the abundance *A* (number of individuals/m²), the Shannon index *H'* (Shannon and Weaver 1963) and Pielou's evenness *J'* (Pielou 1966). Calculations were performed using PRIMER v6 package (Clarke and Gorley 2006). Identified species were classified into trophic groups according to Fauchald and Jumars (1979) and notably modified by Grall and Glémarec (1997), Hily and Bouteille (1999), Afli and Glémarec (2000), Pranovi et al. (2000) and Afli et al. (2008a) as follows:

- Carnivores (C), predatory animals (i.e., mobile polychaetes, sea-anenomes).
- Detritus feeders (DF), feeding on particulate organic matter, essentially vegetable detritus (mainly amphipods and tanaids).
- Suspension-feeders (SF), feeding on suspended food in the water column (e.g., most bivalves).

- Micrograzers (µG), feeding on benthic microalgae, bacteria and detritus (essentially polyplacophores and gastropods).
- Selective deposit feeders (SDF), feeding on organic particles settled on the sediment, e.g., most sedentary polychaetes and some bivalves and crustaceans.

Three currently available univariate biotic indices, namely AMBI (Borja et al. 2000) and BENTIX (Simboura and Zenetos 2002) (Table 2) were used to qualify the ecological status within a five-class scale of pollution. They are calculated on the basis of the relative proportions of the 5 ecological groups (I. sensitive species; II. indifferent species, III. tolerant species, IV. second-order opportunistic species and V. first order opportunistic species) established initially by Glémarec and Hily (1981). The normality of data was assumed, statistically significant differences in the numerical values of abiotic variables and biotic indices between 2010 and 2013 were tested through the paired Student t-tests using the software STATISTICA8. Standard deviation (SD) was also calculated to evaluate the dispersion of the recorded values.

To assemble the similar stations on the biotic/abiotic parameters and characterize them by the principal variables, Principal Component Analysis (PCA) was carried out using the XLSTAT software (version 2014; XLSTAT; Addinsoft) on data organized in a rectangular matrix where stations occupy the columns and the biotic/abiotic parameters occupy the lines.

Results

Figure 2 shows the differences in the main physico-chemical parameters in the lagoon of Boughrara between winter 2010 and winter 2013. On average, the water temperature shows a slight decrease (18.7 to 16.9°C), and the salinity, dissolved oxygen and chlorophyll *a* show slight increase (42.6-43.8, 8.3-9.9 mg.l⁻¹ and 2.4-2.8 µg.l⁻¹, respectively), but none of these differences were significant (-2.010 < *t* < 1.231 and 0.056 < *P* < 0.632). Nevertheless, stations sheltered from cur-

Table 2. Characteristics of the biotic indices used to qualify the ecological status using benthic invertebrates (modified respectively from Borja et al. (2000), Simboura and Zentos (2002) and Labruno et al. (2006)). Eg_{*i*}: ecological group *i* in AMBI, GS: sensitive species in BENTIX, GT: tolerant species in BENTIX, n_{*i*}: number of individuals of species *i*, N: total number of individuals and S: number of species.

Biotic index	Algorithm	Index value	Ecological status
AMBI	$(0 \times EG_I + 1.5 \times EG_{II} + EG_{III} + 4.5 \times EG_{IV} + 6 \times EG_V) / 100$	0.0-1.2	High
		1.2-3.3	Good
		3.3-4.3	Moderate
		4.3-5.5	Poor
		5.5-7.0	Bad
BENTIX	$(6 \times GS + 2 \times GT) / 100$ where GS = EG _I + EG _{II} and GT = EG _{III} + EG _{IV} + EG _V	4.5-6.0	High
		3.5-4.5	Good
		2.5-3.5	Moderate
		2.0-2.5	Poor
		0	Bad
H'	$-\sum_{i=1}^S \frac{n_i}{N} \log_2 \left(\frac{n_i}{N} \right)$	> 4	High
		3-4	Good
		2-3	Moderate
		1-2	Poor
		< 1	Bad

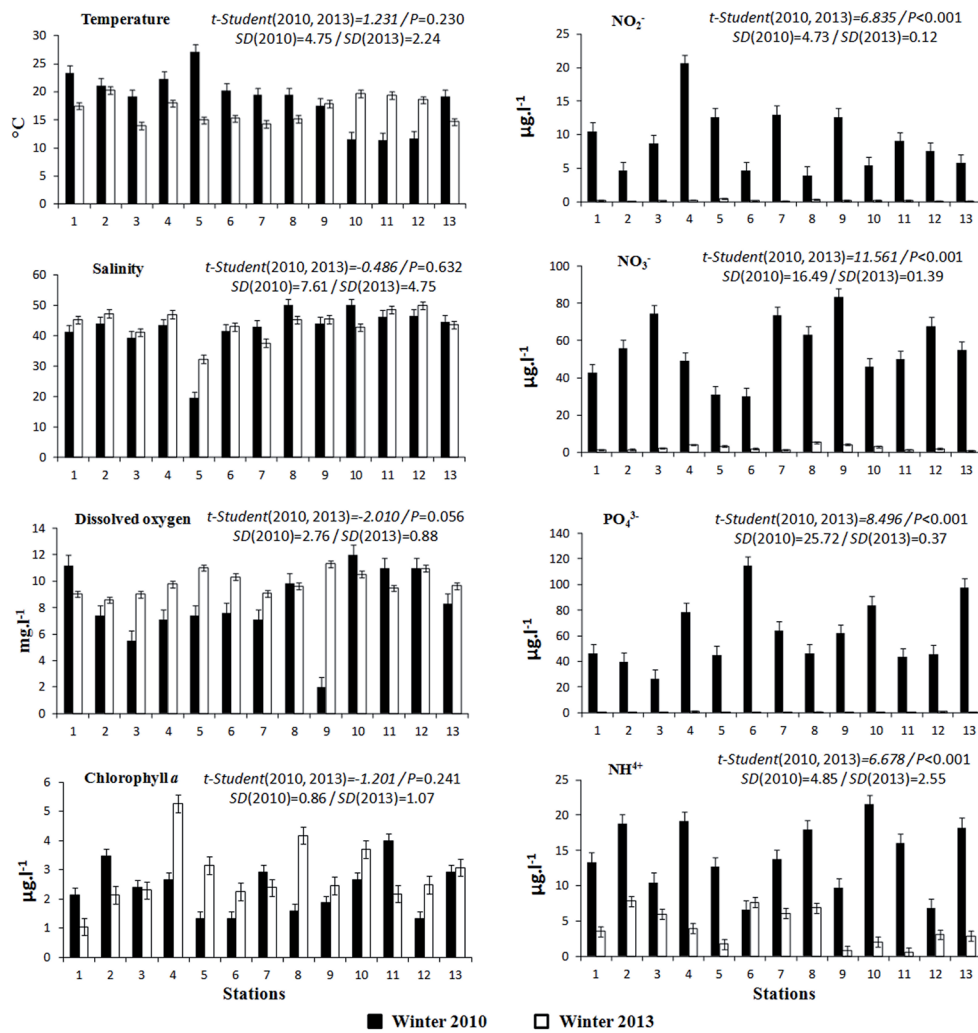


Figure 2. Spatial-temporal variations of main physico-chemical parameters in the Boughrara lagoon. NO₂⁻, nitrites, NO₃⁻, nitrates, PO₄³⁻, phosphates, NH₄⁺, ammonium, P, P-values of the Student test and SD, Standard deviation.

Table 3. List of principal species collected in the lagoon of Boughrara during winter 2010 and winter 2013. White: <1 ind.m⁻², pale gray: 1-30 ind.m⁻², dark gray: 30-100 ind.m⁻², black: ≥ 100 ind.m⁻².

Species	Winter 2010	Winter 2013
<i>Abra alba</i> (W. Wood, 1802)		
<i>Acanthocardia spinosa</i> (Lightfoot, 1786)		
<i>Alitta succinea</i> (Leuckart, 1847)		
<i>Antalis panorma</i> (Chenu, 1843)		
<i>Antalis vulgaris</i> (da Costa, 1778)		
<i>Bittium depauperatum</i> Watson, 1897		
<i>Bittium incile</i> Watson, 1897		
<i>Bittium latreilli</i> (Payraudeau, 1826)		
<i>Bittium reticulatum</i> (da Costa, 1778)		
<i>Calliostoma zizyphinum</i> (Linnaeus, 1758)		
<i>Carcinus aestuarii</i> Nardo, 1847		
<i>Cerastoderma edule</i> (Linnaeus, 1758)		
<i>Cerastoderma glaucum</i> (Bruguière, 1789)		
<i>Cerithium scabridum</i> Philippi, 1848		
<i>Chthamalus stellatus</i> (Poli, 1791)		
<i>Conus ventricosus</i> Gmelin, 1791		
<i>Corbula gibba</i> (Olivi, 1792)		
<i>Cymodoce truncata</i> Leach, 1814		
<i>Echinogammarus</i> sp.		
<i>Euclymene droebachiensis</i> (Sars, 1872)		
<i>Euthria cornea</i> (Linnaeus, 1758)		
<i>Gastrana fragilis</i> (Linnaeus, 1758)		
<i>Gibbula albida</i> (Gmelin, 1791)		
<i>Gibbula ricketti</i> (Payraudeau, 1826)		
<i>Glycera alba</i> (O.F. Müller, 1776)		
<i>Glycera tridactyla</i> Schmarra, 1861		
<i>Haminoea navicula</i> (da Costa, 1778)		
<i>Hexaplex trunculus</i> (Linnaeus, 1758)		
<i>Holothuria tubulosa</i> Gmelin, 1791		
<i>Loripes lucinalis</i> (Lamarck, 1818)		
<i>Metapenaeus monoceros</i> (Fabricius, 1798)		
<i>Mytilaster minimus</i> (Poli, 1795)		
<i>Naineris setosa</i> (Verrill, 1900)		
<i>Nassarius granum</i> (Lamarck, 1822)		
<i>Nassarius mutabilis</i> (Linnaeus, 1758)		
<i>Nassarius pygmaeus</i> (Lamarck, 1822)		
<i>Nassarius reticulatus</i> (Linnaeus, 1758)		
<i>Nassarius</i> unidentified		
<i>Necallianassa truncata</i> (Giard & Bonnier, 1890)		
<i>Neverita josephina</i> Risso, 1826		
<i>Nucula nucleus</i> (Linnaeus, 1758)		
<i>Paramysis (Longidentia) noveli</i> Labat, 1953		
<i>Paranemonia cinerea</i> (Contarini, 1844)		
<i>Perinereis cultrifera</i> (Grube, 1840)		
<i>Phylo foetida adjimensis</i> (Fauvel, 1924)		
<i>Pinctada imbricata radiata</i> (Leach, 1814)		
<i>Pisania striata</i> (Gmelin, 1791)		
<i>Polititapes aureus</i> (Gmelin, 1791)		
<i>Rissoa paradoxa</i> (Monterosato, 1884)		
<i>Ruditapes decussatus</i> (Linnaeus, 1758)		
<i>Serpulidae</i> unidentified		
<i>Smaragdia viridis</i> (Linnaeus, 1758)		
<i>Sphaeroma serratum</i> (Fabricius, 1787)		
<i>Theridium vulgatum</i> (Bruguière, 1792)		
<i>Thracia phaseolina</i> (Lamarck, 1818)		
<i>Tricolia tenuis</i> (Michaud, 1829)		
<i>Trivia monacha</i> (da Costa, 1778)		

rents (stations 10, 11 and 12) appear to be remarkably different from the other stations, mainly for temperature and dissolved oxygen. Average of pH ranged from 29.6 in station 5 in autumn and 50 at station 12 in winter 2013.

Nutrients (NO₂⁻, NO₃⁻, PO₄³⁻ and NH₄⁺) show a significant temporal decrease ($6.678 < t < 11.561$ and $P < 0.001$), respectively 9.17-0.20 µg.l⁻¹, 55.53-2.46 µg.l⁻¹, 61.07-0.44 µg.l⁻¹ and 14.23-4.07 µg.l⁻¹. The standard deviation (SD) is

higher in 2010 compared with 2013 for temperature, salinity and dissolved oxygen and lower for nutrients and chlorophyll *a*.

In total, 45 species were collected in the lagoon of Boughrara in winter 2010 and 31 species in winter 2013. The temporal tracking for both winter seasons in 2010 and 2013 allowed to highlight the presence of 29 species in 2010 but absence in 2013, mainly molluscs (40 species in 2010 and 17 species in 2013). However, about 15 species (among the 31 species) were recently collected in 2013 and absent in 2010, mainly polychaetes (2 species in 2010 and 7 species in 2013) and crustaceans (3 species in 2010 and 5 species in 2013) (Table 3). It should be noted that at the station 13, no organisms were collected in winter 2013. The abundance (*A*) and species richness (*S*) show a significant decrease ($t = 3.981$ and $P < 0.001$ for *A*, and $t = 3.540$ and $P = 0.002$ for *S*) respectively from 684 to 147 ind.m⁻² and from 14 to 7 species (per station) on average (Fig. 3). Contrarily, the Shannon index (*H'*) and evenness (*J'*) show a significant increase ($t = -6.063$ and $P < 0.001$ for *H'*, and $t = -3.041$ and $P = 0.006$ for *J'*) respectively from 0.12 to 1.47 bits.ind⁻¹ and from 0.27 to 0.51 on average, and an important increase for the standard deviation.

In both 2010 and 2013, the distribution of trophic groups differs from one station to another (Fig. 4a). But, on average, the macrobenthic community is dominated in 2010 by SDF (50.2%), followed by DF (15.4%), µG (12.7%), SF (11.9%) and C (9.8%). In 2013, the µG dominates with 43%, SDF declines to 23% and DF to 3.5% in favour of SF that passes to around 25.11%. It should be noted that sheltered stations (stations 10, 11 and 12) show in 2010 a similar trophic structure largely dominated by SDF.

The distribution of the ecological groups (Fig. 4b) shows that sensitive species (ecological group I) dominate widely in winter 2010 at all stations, except at stations 9 and 13 which were co-dominated with respectively ecological group II and ecological group III. In 2013, sensitive species still dominate generally, nevertheless the other groups (II, III and IV) are more present, and reach over half at stations 2, 6 and 9. The Shannon index *H'* classifies all the station to be in a bad ecological status in 2010, and to be in a moderate (stations 9, 11 and 12), poor and bad (stations 1 and 2) ecological status in 2013 (Table 4). However, biotic indices based on ecological groups (AMBI and BENTIX) classify all sampled stations whether in 2010 or 2013 to be in a high/good ecological status.

Axes 1-2 of Principal Component Analysis (PCA) accumulate around 55% of eigenvalues (Fig. 5). Samples of winter 2013 form a consolidated group characterized by dissolved oxygen only. However, samples of winter 2010 show a clearly wider dispersion and are characterized by the other biotic/abiotic parameters including all trophic groups.

Discussion

Physico-chemical parameters

The sediment observed in the lagoon of Boughrara is fine and is constituted mainly of sand and mud (Table 1), which

Table 4. Calculated values of used biotic indices at each sampled station and their position on environmental quality scale.

Indices	Years	Stations												
		1	2	3	4	5	6	7	8	9	10	11	12	13
H'	2010	0.15	0.10	0.11	0.08	0.15	0.10	0.12	0.11	0.16	0.13	0.06	0.14	0.15
	2013	0	0.67	1.51	1.46	1.84	1.58	1.29	1.98	2.45	1.76	2.21	2.32	azoic
AMBI	2010	0.15	0.71	0.32	0	0.16	0.37	0.90	0.65	0.21	0.15	0.30	0.42	0.50
	2013	0	1.50	0.64	0.50	0	1	0	1.33	0.66	0.90	0.18	0.45	azoic
BENTIX	2010	6	5.57	6	6	6	6	5.46	5.50	6	6	6	5.42	5.33
	2013	6	4	6	5.33	6	6	6	4.66	5.55	5.20	6	5.60	azoic
High	Good	Moderate			Poor		Bad							

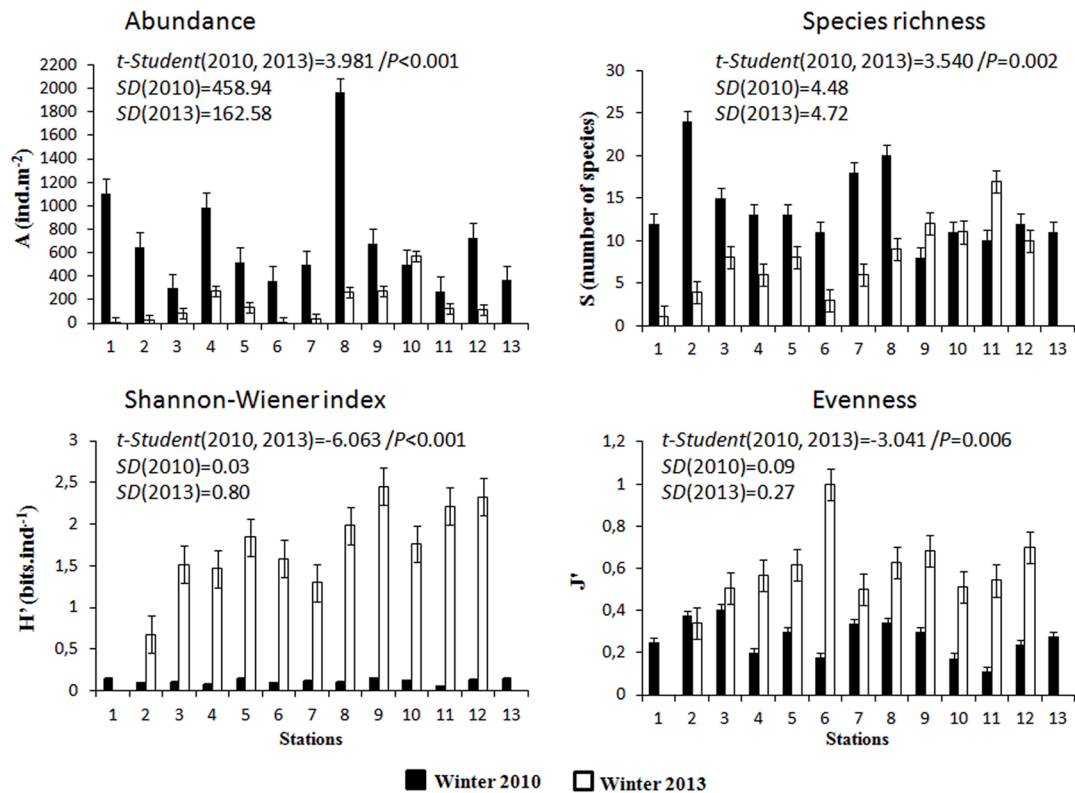


Figure 3. Spatial-temporal variations of macrofauna synthetic parameters in the Boughrara lagoon. *P*, *P*-values of the Student *t* test and SD, Standard deviation.

confirms results observed by Daly-Yahia (1993). Thus, the paramount role of the sedimentary texture that structures benthic communities is minimized in this study, due to the homogeneity of the sediment. Before its development in 2004-2007, the lagoon of Boughrara was very productive due to its warm/stagnant waters and the high nutrient contents which increase the phytoplankton production and lead often to eutrophic conditions (Hamza and El Abed 1994, Daly-Yahia and Kefi-Daly-Yahia 2003). Studies carried out 2 years after this development show that the lagoon is still at risk of eutrophication, although conditions have improved significantly (Guetat et al. 2012). In this study, values recorded confirm the continued improvement of the physico-chemical conditions of the lagoon, downward trend of the nutrients and upward trend of the dissolved oxygen. Nevertheless, nutrients are still very high in 2010 at all the stations and despite a great decline in 2013 which can reach

at some stations (stations 5 and 8) 28/18 times respectively for nitrites/nitrates, some recorded values ($< 1 \mu\text{g/l}$, Fig. 2) remain at the threshold of detection (CEAEQ 2007).

The standard deviation (SD) shows interesting results. It is higher in 2010 compared with 2013 for the temperature, salinity and dissolved oxygen. This means that values measured in 2010 are relatively more spatially heterogeneous, which is clearly visible in the PCA. In fact, the extension of the channel has induced an acceleration of the marine currents and a higher water exchange between the lagoon and the open sea. This has led to a general mixing of the lagoon waters, which attenuates with time the physico-chemical conditions, except probably sheltered areas (stations 10, 11 and 12) where this improvement does not seem to be well pronounced.

Compared with the surrounding sea, the salinity of the lagoon of Boughrara is still high during 2010 and 2013, ex-

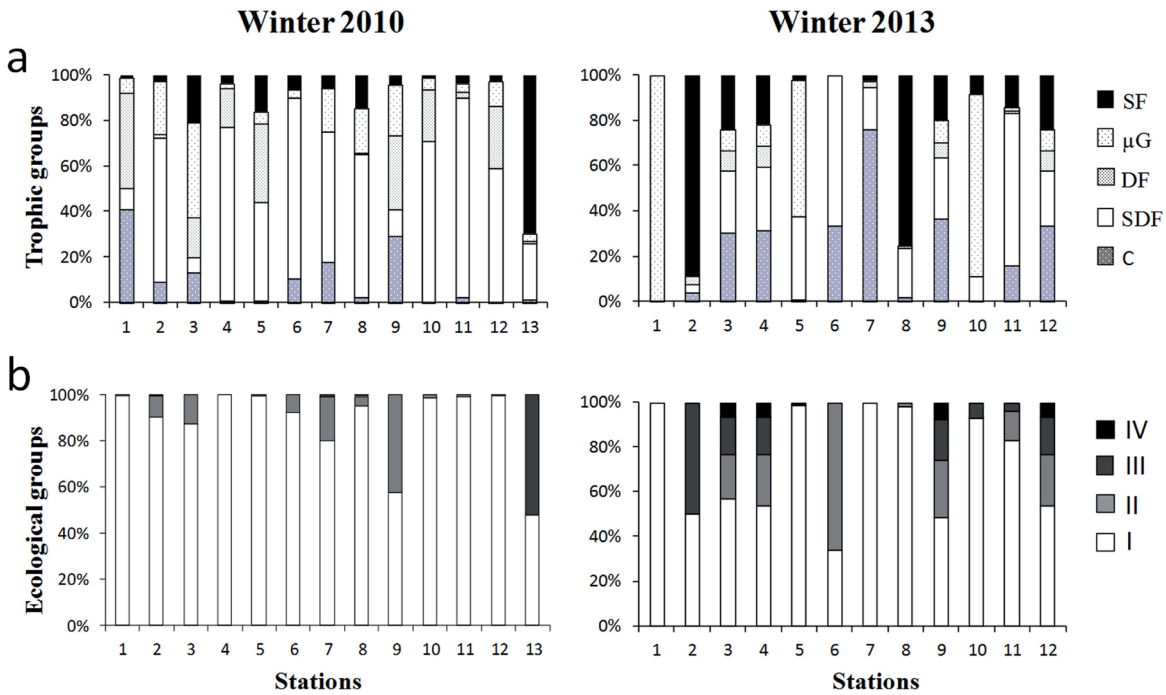
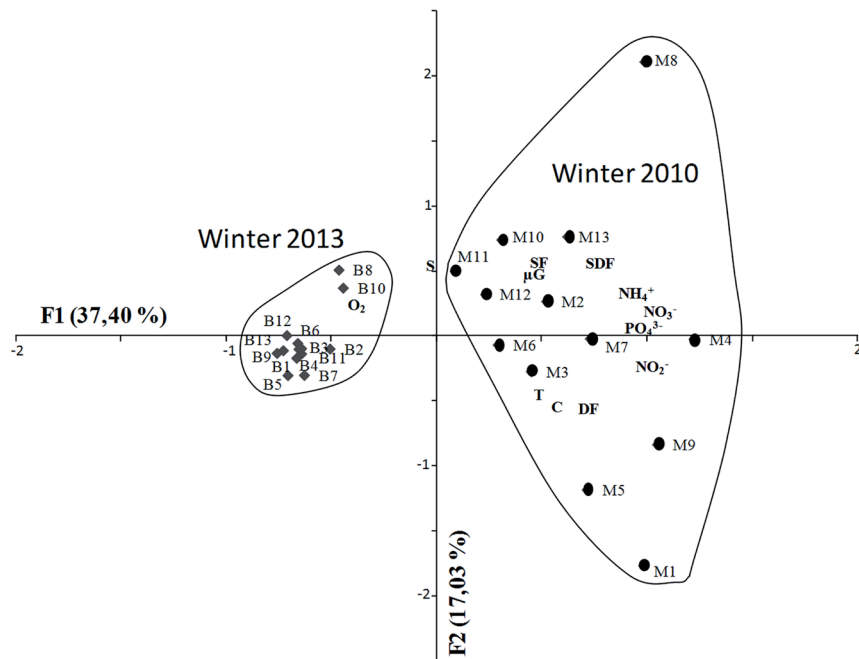


Figure 4. Spatial-temporal variations of (a) trophic groups and (b) ecological groups (I to IV) of the benthic macrofauna in the Boughrara lagoon, C: carnivores, SDF: selective deposit-feeders, DF: detritus-feeders, μG: micrograzers and SF: suspension-feeders, I: sensitive species, II: indifferent species, III: tolerant species, IV: second-order opportunistic species and V: first order opportunistic species.

Figure 5. Results of Principal Component Analysis carried out on biotic/abiotic parameters at stations sampled in winter 2010 (M1-M13) and winter 2013 (B1-B13). C, carnivores, SDF, selective deposit-feeders, DF, detritus-feeders, μG, micrograzers, SF, suspension-feeders, T, temperature, S, salinity, O₂, dissolved oxygen, NO₃⁻, nitrates, NO₂⁻, nitrites, NH₄⁺, ammonium and PO₄³⁻, phosphates.



cept at the station of desalination (station 5) where the salinity is clearly lower due to discharges of desalinated waters. At this location, hot freshwater from subterranean sources is discharged after desalination, which could explain the higher temperature and lower salinity levels at this station.

The dissolved oxygen has been also improved, especially at station 9 near an aquaculture farm where concentrations

went from 2 mg.l⁻¹ in 2010 to 11.31 mg.l⁻¹ in 2013. This seems to stimulate the phytoplankton growth, as shown by the development in 2013 of the chlorophyll *a* at some stations.

In comparison with other Mediterranean lagoons, nutrient values measured in 2013 in the lagoon of Boughrara are comparable to those obtained by Bejaoui et al. (2010)

in the lagoon of Bizerte (400 km to the North); nitrites and phosphates were always lower than $1 \mu\text{g.l}^{-1}$, nitrates around $0.50\text{--}1.46 \text{ mg.l}^{-1}$ and ammonium around $1.47\text{--}2.91 \text{ mg.l}^{-1}$. Elsewhere in the Mediterranean, values registered in 2010 in the lagoon of Boughrara are comparable with those measured in Varano lagoon, and become in 2013 comparable with the Orbetello lagoon (Specchiulli et al. 2008).

Macro-benthic community

Data collected in this study show that the structure and the organization of the macro-benthic assemblage in the lagoon of Boughrara has undergone significant changes during the period 2010–2013. Although molluscs still remain the principal group despite their decrease, polychaetes such as *Euclymene droebachiensis*, *Glycera tridactyle* and *Naineris setosa* (newly recorded by Khedhri et al. 2014) have greatly increased followed by crustaceans including the amphipod *Echinogammarus* sp. and the decapod *Carcinus aestuarii*. This codominance of molluscs/polychaetes/crustaceans is common in Mediterranean lagoons, such as Bizerte (Afli et al. 2008b) and El-Bibans (Zaouali and Baeten 1985) in Tunisia, that of Biguglia in Corsica (Clanzig 1991), Muggia in Italy (Solis-Weiss et al. 2004) and Thau in France (Gangnery et al. 2003). Similar results have been also observed between 1986/7 and 2003/4, but in a deteriorating environment, the Swan-Canning Estuary in Australia (Wildsmith et al. 2011); the densities and number of species of molluscs, and especially of crustaceans, which are particularly susceptible to environmental stress, declined, while the more tolerant polychaetes increased.

The abundance and species richness have remarkably decreased at all sampled stations during the period 2010–2013, especially at lagoonal-marine stations (stations 1, 2, 3, 6 and 7) more confronted with the marine currents and undergoing influences of both the marine and lagoon environment (Khedhri et al. 2015) and at station 13 in the middle of the lagoon. Contrariwise, stations sheltered from currents (stations 9, 10, 11 and 12) show a relatively moderate decrease. Thus, the abundance and species richness in 2013 was clearly lower compared to several other Mediterranean lagoons such as the lagoon of Bizerte (Afli et al. 2008b), the lagoon of Ghar El-Melh, the southern lagoon of Tunis (Afli et al. 2009b) and the lagoon of Smir (Chaouti and Bayed 2005). Contrary to the species richness and abundance, the Shannon index (H') and evenness (J') show a significant overall increase which means that the community in 2013 was less dominated by a small number of species as in 2010. However, 2 lagoonal-marine stations (stations 1 and 2) and the station in the middle of the lagoon (station 13) show an important decrease, and the station 3 a slight increase.

In terms of trophic diversity, the benthic macrofauna of the lagoon of Boughrara show important changes inducing a more balanced trophic structure. The order of the trophic groups has changed, their dominance has undergone significant changes; SDF halved and DF reduced to a quarter in favor of the other trophic groups, mainly μG , SF and C. But these changes have affected the relative proportions and not

abundances, and the question that should be asked here is not “why μG and SF have increased” but more importantly “why SDF and DF have declined...”. On another level, the trophic groups were grouped on the PCA with the samples of 2010, because the data used were expressed in abundances which are clearly higher in 2010, and not in percentages. Actually, currents induced by the extension of the channel seem to have an interesting role in these changes, since they can affect the production/distribution of the trophic resources (Khedhri et al. 2015). Thus, the situation where SDF and DF benefit from the deposits on the organic matter funds induced by the deceleration of the marine currents has changed in 2013, and marine currents become stronger and more charged in organic matter that benefits SF and μG . The PCA shows, once again, that the general mixing of the lagoon waters due to the extension of the channel has led also to important changes within the benthic community. Indeed, in 2013 the stations are not scattered as in 2010, which means that there is a general homogenization of the ecosystem conditions and the benthic community. Nevertheless, the lagoon of Boughrara seems to be more balanced trophically, especially in 2013, compared with the other Mediterranean lagoons, as the lagoon of Bizerte dominated widely by carnivores (Afli et al. 2008a), the southern lagoon of Tunis dominated by micrograzers, the lagoon of Ghar El-Melh dominated by selective deposit feeders (Afli et al. 2009b) and the lagoon of Smir dominated by detritus feeders (Chaouti and Bayed 2011).

The analysis of the ecological groups shows that sensitive species are dominant in both 2010 and 2013, but the tolerant/opportunistic species were a larger proportion of the community in 2013. As for trophic groups, the question raised here does not concern the dominance of tolerant/opportunistic species at a moment when it is believed that the environmental conditions are improved, because their abundance remains relatively low. But the real question is why the sensitive species do not develop more in the areas which seem favorable to their presence. As answer to this question, it seems that sensitive species have declined due to hydrodynamic/trophic reasons, and not to increasing pollution as suggests the dominance of ecological groups. Indeed, these sensitive species appear to be disturbed by water currents charged in sediment particles that can block their filtration structures (Aloui-Bejaoui and Afli 2012), and may also limit deposits of organic matter which is their main trophic resource. However, this does not change the general status (high) of the lagoon given by biotic indices AMBI and BENTIX which are more consistent in classifying the stations both in 2010 than in 2013. Nevertheless, the change in H' was greater which indicates bad ecological status at all stations in 2010 and moderate/poor/bad statuses in 2013. Indeed, AMBI and BENTIX are based on ecological groups of pollution sensitive species. However, H' is different, because it is a structure index based on specific dominances without taking into account the ecological affinity of species (Salas et al. 2004, Afli et al. 2009a). Globally, the species richness and the abundance have decreased between the two study periods, the presence of sensitive species remained high, and biotic indices AMBI and BENTIX show good status. Nevertheless, the macrobenthic

community did not show any improvement until now, except the improvement of the evenness and the Shannon index, despite the mixing of the physico-chemical conditions.

In conclusion, the extension of the channel “El-Kantra” in 2004-2007 shows that water quality in the lagoon of Boughrara improved, which has led to beneficial effects on the physico-chemical parameters, which affected the benthic community status. Nevertheless, this lagoon still seems to be impacted by certain environmental/anthropogenic stressors as indicated by the presence of tolerant species such as the bivalves *Abra alba* and *Cerastoderma edule*. The ecosystem will gradually seek a new balance that will ultimately lead to a new structure and organization of biological organisms adapted to the new environmental conditions. This transitional phase could take many years before the community organization reaches a more stable status.

Acknowledgements: This work was undertaken within the framework of the project ‘EBHaR’ financed by the Tunisian Ministry of Higher Education, Scientific Research, Information and Communication Technologies. The authors wish to thank people who contributed to the field and laboratory components of this project.

References

- Affi, A., R. Ayari and M. Brahim. 2008a. Trophic organization of the macro-zoobenthic assemblages within coastal areas subjected to anthropogenic activities. *J. Mar. Biol. Assoc. UK.* 88: 663–674.
- Affi, A., R. Ayari and S. Zaabi. 2008b. Ecological quality of some Tunisian coast and lagoon locations, by using benthic community parameters and biotic indices. *Estuarine Coastal and Shelf Sci.* 80: 269–280.
- Affi, A., F. Boufahja, S. Sadraoui, K. Ben Mustpha, P. Aissa and R. Mrabet. 2009a. Functional organization of the benthic macrofauna in the Bizerte lagoon (Sw Mediterranean Sea), semi-enclosed area subject to strong environmental/ anthropogenic variations. *Cahiers de Biologie Marine* 50: 105–117.
- Affi, A., R. Chakroun, R. Ayari and P. Aissa. 2009b. Seasonal and spatial variability of the community and trophic structure of the benthic macrofauna within Tunisian lagoonal and marine coastal areas (south-western Mediterranean). *J.Coastal Res.* 25: 1198–1209.
- Affi, A. and M. Glémarec. 2000. Fluctuation à long terme des peuplements macrobenthiques de la partie orientale du golfe du Morhiban (Bretagne, France). *Cahiers de Biologie Marine* 41: 67–89.
- Aloui-Béjaoui, N. and A. Affi. 2012. Functional diversity of the macro invertebrate community in the port area of Kerkennah Islands (Tunisia). *Mediterranean Marine Science* 13: 93–102.
- Bejaoui, B., D. Ferjani, N. Zaaboub, A. Chapelle and M. Moussa. 2010. Caractérisation hydrobiologique saisonnière de la lagune de bizerte (Tunisie) *Revue des sciences de l'eau/ J.Water Sci.* 23: 215–232.
- Bejaoui, B., S. Rais and V. Koutitonsky. 2004. Modelisation de la dispersion du Phoshogypse dans le Golfe de Gabes. *Bulletin de l'Institut National des Sciences et Technologies de la Mer de Salammbô Tunisia* 31: 103–109.
- Ben Aoun, Z., F. Farhat, L. Chouba and M.S. Hadj-Ali. 2007. Investigation on possible chemical pollution of the Boughrara lagoon, South of Tunisia, by chemical wastes. *Bulletin de l'Institut. National des Sciences et Technologies de la Mer de Salammbô, Tunisia* 34: 19–127.
- Ben Khemis, R. 2000. Caractérisation environnementale d'un milieu lagunaire dégradé, la lagune de Boughrara. Projet de fin d'études, Institut Nationale Agronomique de Tunis.
- Ben Rejeb-Jenhani, A. and M.S. Romdhane. 2002. Impact des perturbations anthropiques sur l'évolution du phytoplancton de la lagune de Boughrara, (TUNISIE), *Bulletin de l'Institut. National des Sciences et Technologies de la Mer de Salammbô, Tunisia* 29: 65–75.
- Birk, S., W. Bonne, A. Borja, S. Brucet, A. Courrat, S. Poikane, W. van de Bund, N. Zampoukas and D. Hering. 2012. Three hundred ways to assess Europe's surface waters, an almost complete overview of biological methods to implement the Water Framework Directive. *Ecol. Ind.* 18: 31–41.
- Blanchet, H., N. Lavesque, T. Ruellet, J.C. Dauvin, P.G. Sauriau, N. Desroy, C. Desclaux, M. Leconte, G. Bachelet, A.L. Janson, C. Bessineton, S. Duhamel, J. Jourde, S. Mayot, S. Simon and X. de Montaudouin. 2008. Use of biotic indices in semi-enclosed coastal ecosystems and transitional waters habitats – implications for the implementation of the European Water Framework Directive. *Ecol. Ind.* 8: 360–372.
- Borja, A., J. Franco and V. Pérez. 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Poll. Bull.* 40: 1100–1114.
- Borja, A., A. Miles, A. Occhipinti-Ambrogi and T. Berg. 2009. Current status of macroinvertebrate methods used for assessing the quality of European marine waters, implementing the Water Framework Directive. *Hydrobiologia* 633: 181–196.
- Bresler, V., O. Mokady, L. Fishelson, T. Feldstein and A. Abelson. 2003. Marine molluscs in environmental monitoring, II. Experimental exposure to selected pollutants. *Helgoland Marine Research* 57: 206–211.
- Buchanan, J.B. 1971. Measurement of the physical and chemical environment: Sediments In: N.A.Holme and A.D. McIntyre (eds.), *Methods for the Study of Marine Benthos. International Biological Programme Handbook.* N° 16. Blackwell, Oxford.
- Carvalho, S., M.B. Gaspar, A. Moura, C. Vale, P. Antunes, O. Gil, L. Cancela da Fonseca and M. Falcao. 2006. The use of the marine biotic index AMBI in the assessment of the ecological status of the Obidos lagoon (Portugal). *Marine Poll. Bull.* 52: 1414–1424.
- CEAEQ, 2007. Détermination des nitrates et des nitrites dans l'eau; méthode colorimétrique automatisée avec le sulfate d'hydrazine et le N.E.D, MA. 303 – NO3 1.0, Rév. 3, Centre d'expertise en analyse environnementale du Québec, Ministère du Développement durable, de l'Environnement et des Parcs du Québec.
- Chaouti, A. and A. Bayed. 2005. Diversité taxonomique et structure de la macrofaune benthique des substrats meubles de la lagune de Smir. *Travaux de l'Institut Scientifiques Rabat. Série générale* 4 : 33–42.
- Chaouti, A. and A. Bayed. 2011. Structure et organisation trophique du peuplement macrobenthique de la lagune méditerranéenne de Smir (Maroc). *Bulletin de l'Institut Scientifique, Rabat, section Sciences de la Vie* 33 : 1–12.
- Clanizig, S. 1991. Le benthos de la lagune de Biguglia (Corse), tendances évolutives du milieu. *Document du Centre Interdisciplinaire d'Etudes Littorales (C.I.E.L.),* n° 12.
- Clarke, K.R. and R.N. Gorley. 2006. PRIMER v6: User Manual/Tutorial. PRIMER-E Ltd, Plymouth.

- Daby, D. 2006. Coastal pollution and potential biomonitors of metals in Mauritius. *Water, Air and Soil Pollution* 174: 63–91.
- Daly-Yahia, M.N. 1993. Contribution à l'étude du milieu et du zooplancton de la lagune de Boughrara, systématique, biomasse et relations trophiques. Ph.D. Université de Tunis II, Faculté des Sciences de Tunis.
- Daly-Yahia, M.N. and O. Kefi-Daly-Yahia. 2003. Distribution spatio-temporelle et dynamique du zooplancton de la lagune de Boughrara (année 1999-2000). *Revue de la Faculté des Sciences de Bizerte, Tunisie* 2: 74–91.
- Daly-Yahia, M.N., O. Kefi-Daly-Yahia and M.S. Romdhane. 1994. Les phosphates dans la lagune de Bou Ghrara. *Bulletin de l'Institut National des Sciences et Technologies de la Mer de Salammbô, Tunisia*, pp. 42–45.
- Dauer, D.M. 1993. Biological criteria, environmental health and estuarine macrobenthic community structure. *Marine Poll. Bull.* 26: 249–257.
- Dauvin, J.C. 1993. Le benthos, témoin des variations de l'environnement. *Océanis* 19: 25–53.
- Dauvin, J.C. 2007. Paradox of estuarine quality, benthic indicators and indices, consensus or debate for the future. *Marine Poll. Bull.* 55: 271–281.
- DGPA, 2001. Etude de l'amélioration de la qualité de l'eau de la lagune de Bou Ghrara. Phase II – partie I, Etude approfondie de la solution retenue. Direction Générale de la Pêche et de l'Aquaculture.
- EEA, 1999. Environmental indicators, Typology and overview. Technical report No. 25, European Environment Agency.
- Fauchald, K. and P.A. Jumars. 1979. The diet of worms, a study of Polychaete feeding guilds. *Oceanography and Marine Biology Annual Reviews* 17: 173–284.
- Gangnery, A., J.M. Chabirand, F. Lagarde, P. Le Gall, J. Oheix, C. Bacher and D. Buestel. 2003. Growth model of the Pacific oyster, *Crassostrea gigas*, cultured in Thau Lagoon (Méditerranée, France). *Revue. Des Sciences.de l'Eau* 215: 267–290.
- Glémarec, M. and C. Hily. 1981. Perturbations apportées à la macrofaune benthique de la baie de Concarneau par les effluents urbains et portuaires. *Acta Oecol.* 2: 139–150.
- Grall, J. and M. Glémarec. 1997. Biodiversité' des fonds de maërl en Bretagne, approche fonctionnelle et impacts anthropiques. *Vie Milieu* 47: 339–349.
- Guetat, F., F. Sellem, F. Akrouf, M. Brahim, A. Atoui, M.S. Ben Romdhane and M.N.Daly-Yahia. 2012. Etat environnemental de la lagune de Boughrara et ses alentours deux ans après les travaux d'aménagement et d'élargissement du pont d'El Kantara. *Bulletin de l'Institut National des Sciences et Technologies de la Mer de Salammbô, Tunisia*, 39 :149–160
- Hamza, A. and A. El Abed. 1994. Les eaux colorées dans le golfe de Gabès, bilan de sept ans de surveillance. *Bulletin de l'Institut National des Sciences et Technologies de la Mer. Océanographie. Pêche Salammbô* 21: 66–77
- Hily C. and M. Bouteille. 1999. Modifications of the specific diversity and feeding guilds in an intertidal sediment colonized by an eelgrass meadow (*Zostera marina*) (Brittany, France). *Comptes Rendus de l'Académie des Sciences – Section III. Sciences de la vie* 322: 1121–1131.
- Huang, H. J.Y. Wu and J.H. Wu. 2007. Heavy metal monitoring using bivalved shellfish from Zhejiang coastal waters, East China Sea. *Environ. Monit. Assess.* 129: 315–320.
- Jedoui, Y. 1980. Etude hydrologique et sédimentologique d'une lagune en domaine Méditerranéen, la bahiret de Boughrara (Tunisie). Ph.D. Université Bordeaux I.
- Khedhri, I., Djabou H and Affi A 2015. Trophic and functional organization of the benthic macrofauna in the lagoon of Boughrara – Tunisia (SW Mediterranean Sea). *J. Marine Biol. Ass. UK* 95: 647–659.
- Khedhri I, N. Lavesque, P. Bonifacio, H. Djabou and A. Affi. 2014. First record of *Naineris setosa* (Verrill, 1900) (Annelida, Polychaeta, Orbiniidae) in the Western Mediterranean Sea. *BioInvasions Records* 3(2): 83–88.
- Labruno, C., J.M. Amouroux, R. Sarda, E. Dutrieux, S. Thorin, R. Rosenberg and A. Grémare. 2006. Characterization of the ecological quality of the coastal Gulf of Lions (NW Mediterranean). A comparative approach based on three biotic indices. *Marine Poll. Bull.* 52: 34–47.
- Lorenzen, C. and J. Jeffrey. 1980. Determination of chlorophyll in seawater. *Technical Paper in Marine Science (UNESCO)* 35: 574–576.
- Magni, P. 2003. Biological benthic tools as indicators of coastal marine ecosystems health. *Chemistry and Ecology* 19: 363–372.
- Nixon, S.W. 1995. Metal inputs to Narragansett Bay, a history and assessment of recent conditions. Rhode Island Sea Grant, Narragansett, RI.
- Nixon, S.W. 1982. Nutrient dynamics, primary production and fisheries yields of lagoons. In: Proceedings of the International Symposium on coastal lagoons. *Oceanol. Acta* 4: 357–371.
- Patrício, J., J.M. Neto, H. Teixeira, F. Salas and J.C. Marques. 2009. The robustness of ecological indicators to detect long-term changes in the macrobenthos of estuarine systems. *Mar. Environ. Res.* 68: 25–36.
- Pielou, E.C. 1966. The measurement of diversity in different types of biological collections. *J. Theor. Biol.* 13: 131–144.
- Pinto, R. J., Patrício, A. Baeta, B. D. Fath, J. M. Neto and J.C. Marques 2009. Review and evaluation of estuarine biotic indices to assess benthic condition. *Ecol. Indic.* 9: 1–25.
- Puente, A., J.A. Juanes, A. García, C. Álvarez, J.A. Revilla and I. Carranza. 2008. Ecological assessment of soft bottom benthic communities in northern Spanish estuaries. *Ecol. Indic.* 8: 373–388.
- Pranovi, F., D. Curiel, A. Rismondo, M. Marzocchi and M. Scatolin. 2000. Determination of food sources for benthic invertebrates in a salt marsh (Aiguillon Bay, France) by carbon and nitrogen stable isotopes, importance of locally produced sources. *Scientia Marina* 64: 303–388.
- Rao, J.V., P. Kavitha, K. Srikanth, P.K. Usman and T.G. Rao. 2007. Environmental contamination using accumulation of metals in marine sponges, *Sigmadocia fibulata* inhabiting the coastal waters of Gulf of Mannar, India. *Toxicological and Environmental Chemistry* 89: 487–498.
- Rodier, J., C. Bazin, J.P. Broutin, P. Chambon, H. Champsaur and L. Rodi. 1996. L'analyse de l'eau, eaux naturelles, eaux résiduaires, eaux de mer. 8^{ème} édition. Dunod, Paris.
- Romdhane, M.S 2001. Etude de l'amélioration de la qualité des eaux de la lagune de Boughrara. Compartiment Macrofaune benthique. Groupement SCET–Tunisie/Eco-Ressources International.
- Romdhane, M.S., O. Kefi Daly Yahia, A. Ben Rjeb Jenhani and M.N. Daly Yahia. 1998. Ressources piscicoles et proliférations phyto-planctoniques dans les lagunes tunisiennes (Cas de Boughrara et Ghar El Melih). Centenaire de l'INAT 1998. Actes du 1^{er} séminaire International, **Mobilisation, exploitation et conservation des ressources naturelles**. INAT, pp. 511–526.
- Salas, F., J.M. Neto, A. Borja and J.C. Marques. 2004. Evaluation of the applicability of a marine biotic index to characterize the status of estuarine ecosystems, the case of Mondego estuary (Portugal). *Ecol. Ind.* 4: 215–225.

- Shannon, F.P., and W. Weaver. 1963. The mathematical theory of communication. University of Illinois Press, Urbana, IL.
- Simboura, N. and A. Zenetos. 2002. Benthic indicators to use in Ecological Quality classification of Mediterranean soft bottom marine ecosystems, including a new Biotic Index. *Mediterr. Mar. Sci.* 3: 77–111.
- Solis-Weiss, V., F. Aleffi, N. Bettoso, P. Rossin, G. Orel and S. Fonda-Umani. 2004. Effects of industrial and urban pollution on the benthic macrofauna in the Bay of Muggia (industrial port of Trieste, Italy). *Sci. Total Env.* 328: 247–263.
- Specchiulli, A., S. Focardi, M. Renzi, T. Scirocco, L. Cilenti, P. Breber and S. Bastianoni. 2008. Environmental heterogeneity patterns and assessment of trophic levels in two Mediterranean lagoons, Orbetello and Varano, Italy. *Sci. Total Env.* 402: 285–298.
- Strickland, J.D.H. and T.R. Parsons. 1965. A manual of seawater analysis. Bulletin no. 125, 2nd edn. Ottawa, Fisheries Research Board of Canada.
- Tlili, I., M. Ayari and M. Allegue. 2008. Etude pour la construction d'un ouvrage de communication au niveau de la chaussée romaine entre la lagune de Boughrara et la mer méditerranée. Xèmes Journées Nationales Génie Côtier – Génie Civil, 14–16 octobre 2008 Sophia Antipolis.
- Tréguer, P. and P. Le Corre. 1975. Manuel d'analyse des sels nutritifs dans l'eau de mer. Utilisation de l'AutoAnalyser II Technicon. 2nd ed, Univ. Bretagne Occidentale, Laboratoire de Chimie marine, Brest, France.
- Turki, S. and A. Hamza. 2001. Le phytoplancton toxique. Effets des changements globaux sur les écosystèmes marins et les habitats côtiers. Colloque PRICAT 6 RIGEDMER, Sousse, mars 2001.
- Warwick, R.M. 1986. A new method for detecting pollution effects on marine macrobenthic communities. *Mar. Biol.* 92: 557–562.
- Wildsmith, M.D., T.H. Rose, I.C. Potter, R.M. Warwick and K.R. Clarke. 2011. Benthic macroinvertebrates as indicators of environmental deterioration in a large microtidal estuary. *Marine Poll. Bull.* 62: 525–538.
- Zaouali, J. and S. Baeten. 1985. Etude des peuplements animaux macrobenthiques de la zone centrale et du bassin oriental de la mer des Bibans (Tunisie méridionale) par la méthode de l'analyse factorielle des correspondances. *Rapport de la Commission internationale de la Mer Méditerranée* 29: 199–203.
- Zaouali, J. 1971. La mer de Boughrara, les fonds et les peuplements végétaux. *Bulletin de l'Institut Océanographique et de Pêche Salammbô, Tunisia* 2: 229–235.

Received December 15, 2015

Revised April 15, 2016

Accepted May 15, 2016