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Isopod assemblages in the Bizerte lagoon (SW Mediterranean Sea): composition, structure, and environmental relationships

Marwa KHAMMASSI[1], Jérôme JOURDE[2], Sarra LABIDI[1] and Mohamed Sghaier ACHOURI[1]

[1] Diversité, gestion et conservation des systèmes biologiques, LR18ES06, Faculté des Sciences de Tunis, Université de Tunis El Manar, 2092 Manar II, Tunis, Tunisia
[2] UMR 7266 LIENSs CNRS, La Rochelle Université, 2 rue Olympe de Gouges, 17000 La Rochelle, France

Corresponding author: Marwa khammassi; kh-marwa@hotmail.fr

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Abstract

The spatial and temporal distribution, abundance and structure of isopod assemblages in Bizerte Lagoon (SW Mediterranean Sea) were studied at 22 stations selected from seagrass and macroalgae sampled during four seasons in 2017. A total of 6808 individuals and 17 species of isopods belonging to 5 suborders, 7 families and 12 genera were identified. Six new species were recorded on Tunisian coasts. In addition, and compared to previous studies, nine isopod species were collected for the first time in this lagoon. Analysis of the trophic structure revealed that the majority of stations are strongly dominated by herbivore-deposit feeders (78.3%), followed by deposit-suspension feeders (13.6%) and carnivores (8.01%). Three isopod assemblages were identified (G1, G2 and G3). The distribution of isopod assemblages is influenced by the nature and structure of the substrates. The seasonal variations in the isopod community patterns at 22 stations showed maximum abundance and diversity during spring, whereas the lowest ones were recorded during winter.

Keywords: Isopod diversity; non-indigenous species; habitat; spatio-temporal variations; Bizerte lagoon; Mediterranean Sea.

Introduction

Understanding assemblages of organisms based on the estimation of species abundance and quantitative description of patterns of distribution (Underwood *et al*., 2000) has become one of the important challenges that studies on biogeography currently face (Pereira *et al*., 2006). Borders at an ecological level are often attributed to antagonistic relationships of predation and competition (Hersteinsson & McDonald, 1992) or climatic conditions (Wardell-Johnson & Roberts, 1993). Likewise, food selection and habitat complexity may play a crucial role in the abundance and distribution of marine organisms (Edgar & Robertson, 1992).

Isopods are a group of peracarid crustaceans with a depressed body and seven pairs of pereopods that are generally similar to each other. They display wide morphological diversity and have adapted to all environments (marine, freshwater and terrestrial) (Castelló *et al.,* 2020). More than 6250 marine species (Poore & Bruce, 2012), belonging to 379 genera divided into 37 families, were recorded (Espinosa-Pérez & Hendrickx, 2006; Poore & Bruce, 2012). Furthermore, they are considered one of the most important macrobenthic epifauna associated with sea grasses and macroalgae (Arrontes & Anadón, 1990).

Isopods provide crucial food resources for crabs and fishes, and they show a variety of feeding modes, including herbivores, carnivores, detritus feeders, parasites, browsers and filter feeders (Gudmundsson *et al*., 2000). Therefore, and consequently to their fundamental role in the trophic food web, studies on isopods are important in coastal marine ecosystems (Castelló *et al*., 2020).

For the Mediterranean Sea, isopod studies extend back over many decades, and numerous exotic species have been described (Ulman *et al*., 2017; Castelló *et al*., 2020). Recent studies have demonstrated that aquaculture and boats indeed act as major hubs for the transfer of isopod species and act as effective vectors of spread and continuous expansion of their range (Katsanevakis *et al*., 2013; Marchini *et al*., 2014; Ulman *et al*., 2019).

Along these lines, the isopods of southern Mediterranean lagoons are still relatively unexplored compared to those of northern lagoons (Mancinelli, 2010; Vincenzi *et al*., 2013; Longo & Mancinelli, 2014). Indeed, studies has not been so intensive in the Tunisian lagoon, and the knowledge in this field is still fragmented (e.g., Afli *et al*., 2009; Zaabar *et al*., 2017; Ounifi-Ben Amor *et al*., 2017).

The Bizerte Lagoon is an interesting ecosystem due to its important biodiversity of fauna and flora (Afli *et al*., 2008; Djellouli, 1988). However, there is no complete list of isopods species from the Bizerte lagoon, and little information is available on the benthic isopod in this area (e.g., Zaouali, 1980; Zaabar *et al*., 2017; Khemaissia *et al*., 2017). To fill this gap, the present paper focuses on a major collection of isopods from Bizerte Lagoon during four seasons in 2017. Samples were collected from 22 vegetated sites, with the aim of delimiting the distribution and abundance patterns of benthic isopods associated with macrophytes and seagrasses in the Bizerte Lagoon and improving our taxonomic knowledge of the isopod fauna in this area.

Materials and Methods

Description of the sampling area

The Bizerte lagoon (37.14 372°N/9.78 994°E) is an area of great biogeographical interest due to its location between the Mediterranean Sea and Ichkeul Lake. It is located in northern Tunisia and covers an area of 150 km^2 with an average depth of 11 m (Fig. 1).

The lagoon receives salt water from the Mediterranean Sea through a transition channel and fresh water from Ichkeul Lake and 18 other sources, with the principal tributaries being the Rivers Tinja, Garek, Mrezig, Gueniche and Ben Hassine (Triki *et al*., 2014). The lagoon is subject to the influence of several physical factors strongly fluctuating during the year. During the dry season, inputs of seawater in the lagoon are important, as well as surface water warming, which can lead to water column stratification. However, during the rainy season, strong winds induce a vertical mixture of the water column, rains are heavy and the freshwater flow coming from Ichkeul Lake is important (Sakka Hlaili *et al*., 2003). These very different water masses, with distinctive patterns of temperature and salinity, make this region highly interesting for faunal investigations.

The main important cities bordering the lagoon are Menzel Bourguiba, where a naval port and a metal factory are present, Menzel Jemil and Menzel Abderrahman (Fig. 1). Other industries (an iron and steel plant, a cement factory, and a refinery) are established on its shoreline.

The sites observed in the Bizerte Lagoon have all been exposed to high propagule pressure due to a variety of man-mediated pathways: shellfish farming areas, naval ports and commercial harbours.

Grain size analysis performed by Brahim *et al*. (2013) on Bizerte Lagoon revealed that the northwest and east areas were characterized by sandy and muddy sands, while sandy facies sediment characterized the north and south lagoons.

The vegetation cover at Bizerte Lagoon mainly consists of *Gracilaria* sp.**,** *Ellisolandia elongata* (J. Ellis & Solander) K.R. Hind & G.W. Saunders, 2013, *Ulva lactuca* Linnaeus, 1753, *Hypnea* sp., *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, 1975*, Chaetomorpha linum* (O.F. Müller) Kützing 1845, *Dyctiopteris polypodioides* (A.P. De Candolle) J.V. Lamouroux, 1809, *Codium fragile (Suringar) Hariot, 1889, Dictyota dichotoma* (Hudson) J.V. Lamouroux, 1809 and the seagrasses *Cymodocea nodosa* (Ucria) Ascherson, 1870 and *Zostera noltei* Hornemann,1832 (pers.obs).

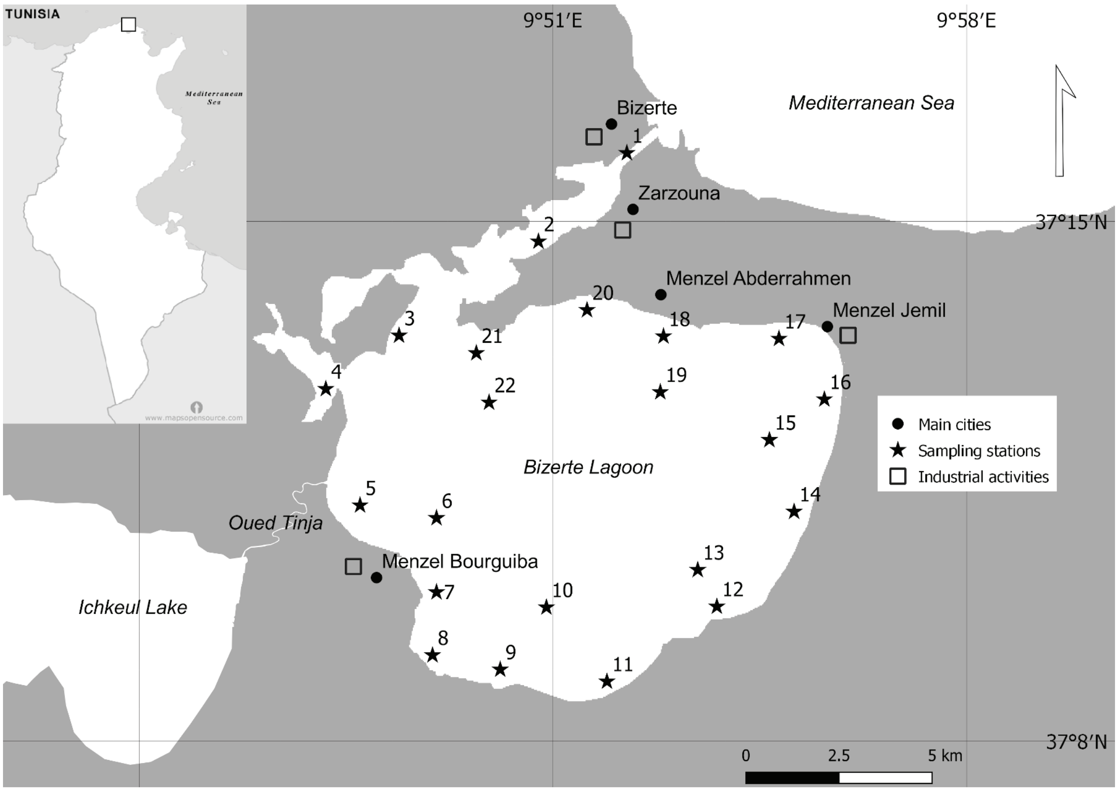

Fig. 1: Map of the Bizerte lagoon with location of the sampling stations.

Sample collection and laboratory procedures

Seagrass and macroalgae were sampled at 22 sites (S1-S22) under different hydrodynamic conditions (Fig. 1). The samples were collected once per season using a quadrat (25x25) at depths between 0.5 and 4 m in January (winter), April (spring), July (summer) and November (autumn) 2017. The stations were also chosen to represent the different macrophyte communities found in the study area.

Water analyses were undertaken on samples collected approximately 16 cm below the surface. Water temperature, the amount of dissolved oxygen, pH and salinity rates were measured using a thermometer (WTW LF 196, SUNTEX, Weilheim, Germany), an oxygen meter (WTW Oxi315i/SET), a pH meter (pH 330i/SET) and a salinometer (WTW Cond 315i), respectively. Three measurements were made for each parameter, from which mean values and standard deviations were calculated. Simultaneously, water depth was recorded with a sonar as well as the position of each station with a GPS (Global Positioning System).

A variety of macroalgae were sampled, amongst which, *Gracilaria* sp., *Ellisolandia Elongata*, *Ulva Lactuca*, *Hypnea* sp., *Cystoseira compressa, Chaetomorpha linum* and *Dyctiopteris polypodioides* were selected as substrates of isopods, since they are among the dominant algae along the Bizerte Lagoon during the study period (pers. observ.). Likewise, the seagrasses *Cymodocea nodosa* and *Zostera noltei* were sampled. At each station, three random replicates were collected by hand to adequately sample macrofaunal diversity. The surface was scraped, and the vegetation was removed after rinsing the algae and seagrasses with fresh water over a 0.5-mm sieve and samples were subsequently preserved in 70% ethanol. The vegetation was stored in a 5% formalin/sea water and then brought to the laboratory.

In the laboratory, all macrofauna were sorted in different taxonomic groups and for this study, only isopods were considered. Mouthparts and appendages were carefully dissected in glycerine with dissecting needles. The material for microscopic examination was kept in a drop of glycerine as the mounting medium and sealed with paraffin

To study the influence of the structure and composition of the macrophyte assemblage on the isopod community throughout the seasons, their dry biomass was obtained after drying for 48 h at 80 °C and after removal of the animals and epiphytes (measured in g) (Edgar, 1983).

For each station and season, a list containing the names of algae present, their dry weights and the abundance of associated isopod species was created.

Identifications were carried out under a stereomicroscope (LEICA MS5), while the appendages were mounted between the blade and lamella and observed under a microscope.

Isopod family, genus and species identifications were performed according to taxonomic references for each group and basic sources written by prominent authors of this field (e.g., Poore & Lew Ton, 1986; Jacobs, 1987; Poore, 2001; Lorenti *et al*., 2009; Vieira *et al*., 2016; Castelló, 2017).

Some specimens, whose identification required confirmation, were shipped to acknowledged experts in the field. All species were checked for current valid nomenclature according to the World Isopoda Database, which is part of the World Register of Marine Species (WoRMS Editorial Board, 2019). For macroalgae species, the cross-validation of species names and authorities was based on AlgaeBase (Guiry & Guiry, 2014).

Total abundance, species richness, density (ind.m^{-2}) and relative abundance (Ra %) were calculated seasonally at each station. A trophic guild analysis was performed and attributed the identified species to three trophic categories, according to the classification schemes available in the literature: deposit-suspension feeders (DsF); herbivores-deposit feeders (HeDF) and carnivores (C) (e.g., Gambi *et al*., 1992; Gudmundsson *et al*., 2000; Orav-Kotta & Kotta, 2004).

Statistical analyses

Environmental parameters

Two-way ANOVA was used to test differences for each ecological parameter independently (salinity, temperature, pH, dissolved oxygen and macrophyte biomass) for stations and seasons. The Tukey honestly significant difference test was performed when ANOVA showed significant differences ($p < 0.05$). Before each ANOVA, a Shapiro–Wilk normality test and a Bartlett test for the homogeneity of variances were applied.

Biological parameters

Two-way ANOVA was used to assess spatiotemporal changes in species richness and total abundance of isopods in the Bizerte Lagoon. Again, before each ANOVA, a Shapiro–Wilk normality test and a Bartlett test for homogeneity of variances were applied. The Tukey honestly significant difference test was performed when ANOVA showed significant differences. These statistical procedures were carried out using the IBM SPSS Statistics 20 software.

Multivariate analysis was performed to study the spatial variability of the isopod assemblage associated with macroalgae and seagrass. A square root transformation was applied to the abundance matrix before calculating the Bray-Curtis similarities using the statistical package Xlstat software (version 1.6). A dendrogram is thus created, with group averages expressed in cluster mode. Then, a non-parametric multidimensional scaling (MDS) ordination, using the Bray-Curtis similarity measure, is applied to the abundance matrix (after square-root transformation), with the objective of examining the spatial variability of the isopod assemblage.

Furthermore, environmental variables that best matched the observed community changes were statistically determined using the BIO-ENV algorithm of the BEST analysis routine. The significance of the correlation coefficients was assessed by a random permutation test. The BEST analyses and permutation tests were performed using the PRIMER v6 software package (Clarke & Gorley, 2006).

Cartography

GIS mapping was applied as a basic analysis tool for the assessment of the spatial distribution of isopods in the Bizerte lagoon. Maps were prepared using the ArcGIS software (Environmental Systems Research Institute). The database structure of the GIS-system included the geographic coordinates of each location as well as the mean abundance and richness of isopod species at each station during the four seasons.

Results

Physical and Chemical Characteristics

Physical and chemical variables of water in sampling area varied depending on rainy or dry season, except for pH, which showed a homogeneous distribution in values over the entire sampling period (Fig.2). The temperature varies between 14°C in winter (S15) and 32.7°C in summer (S8), and marked seasonal variations are recorded (two–way ANOVA; F=17586; $p < 0.0001$; Tukey test ; winter ≠ spring ≠ summer ≠ autumn). Salinity ranges from 36.1 psu in winter (S15) to 40 in summer (S12). Similarly, salinity displays a significant seasonal pattern (two-way ANOVA; F=245.3; $p < 0.0001$; Tukey test; winter≠ spring ≠ summer ≠ autumn). The level of dissolved oxygen is relatively low (1.1mg.L/1) in July (S6) and high (8.42mg.L/1) in January (S18). Also, dissolved oxygen displays a significant seasonal pattern (two–way ANOVA; F=1595; $p < 0.0001$; Tukey test; winter≠ spring ≠ summer ≠ autumn). The pH varies between 7.28 in winter (S7) and 8.93 in summer (S19) (two-way ANOVA; F=2, 25; $p > 0.05$).

Additionally, higher mean temperatures were recorded in summer and spring (31.8 ± 0.4 and 26.2 ± 0.3, respectively), and the lowest values were recorded in winter and autumn (14.8 ± 0.4 and 24.1 ± 0.3, respectively). Similarly, the mean salinity was higher during summer and spring (39.11 ± 0.4 and 38.5± 0.5, respectively), while the lowest values were recorded during winter and autumn (36.8 ± 0.3 and 37.8 ± 0.5). However, dissolved oxygen content showed an obvious seasonal variation from (2.23 ±0.55 mg/L) in rainy season (January) to 7.5±0.55 mg/L in dry season (July). The mean pH value remained stable and close to 8 (i.e. summer: 8.4 ± 0.37; autumn: 8.2 ± 0.49; spring: 8.3 ± 0.37; winter: 8.3 ± 0.37).

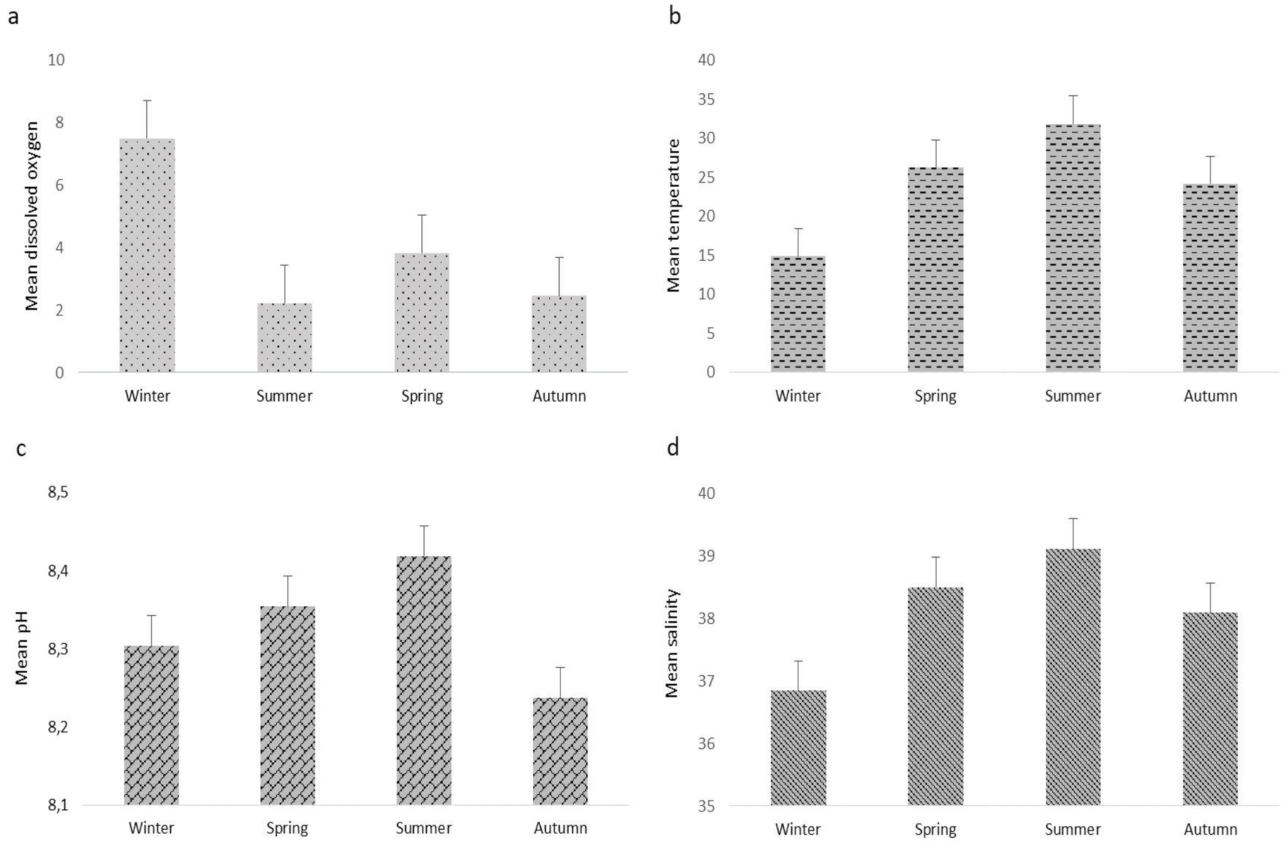

Fig. 2: Seasonal variations of the main physico-chemical parameters in the Bizerte lagoon: (a) dissolved oxygen (mg/L); (b) Temperature (°C); (c) Hydrogen potential; (d) Salinity (psu).

Macrophyte dynamics

Regarding the mean dry biomass of the different algae, the species *Gracilaria* sp., *E. elongata*, *U. lactuca*, *Hypnea* sp., *C. compressa, C. linum* and *D. polyploides* dominated the macroalgal assemblages of the Bizerte lagoon, showing a wide variety of sizes and forms. In addition, *C. nodosa* and *Z. noltei* dominated the meadow assemblages of the lagoon. The structure and the mean dry biomass of these assemblages showed significant seasonal variations; they were significantly higher in spring and summer than in autumn and winter (two-way ANOVA; F=25.57; $p < 0.0001$; Tukey test (winter, autumn ≠ summer, spring).

Composition and relative abundance distribution of the isopod taxocoene

A total of 6808 individuals were collected and identified, resulting in 17 species belonging to 5 suborders, 7 families and 12 genera (Table 1). The suborder Valvifera contributes greatly to the overall richness (50%) and biodiversity (58.62%), with 6 species present in the Bizerte Lagoon. The isopod assemblage is dominated by the family Idoteidae (2992 ind; 2 species), followed by Sphaeromatidae (2267 ind; 5 species) and Arcturidae (940 ind; 4 species). The Sphaeromatidae family was the richest in species with 5 species (Table 1), followed by Arcturidae (4 species), Idoteidae, Paranthuridae and Anthuridae (2 species each), and Holognathidae and Janiridae (1 species each). It is worth noting that approximately 80% of the total individuals were assigned to two families, namely, Idoteidae (43.9%) and Sphaeromatidae (33.27%).

Idotea balthica (Pallas, 1772), *Cymodoce truncata* (Leach, 1814) and *Idotea chelipes* (Pallas, 1766) represented more than 50% of the total number of individuals collected, with values of 36.35%, 9.38% and 7.56%, respectively. This study revealed six new species in Tunisian water: *Astacilla mediterranea* Koehler, 1911, *Astacilla gorgonophila* Monod, 1925, *Astacilla monodi* Tattersall, 1925, *Astacilla axeli* Castelló, 1992, *Mesanthura.* cf. *romulea* Poore & Lew Ton, 1986 and *Apanthura corsica* Amar, 1953. In addition, three other species were recorded for the first time in the Bizerte lagoon: *Paranthura japonica* Richardson, 1909, *Paradella dianae* (Menzies, 1962) and *Cleantis prismatica* (Risso, 1826).

The current list of isopod species shows four non-indigenous species, which account for 20.25% of the total individuals (Table 1): *Paracerceis sculpta* (Holmes, 1904) (7.30%), *P. japonica* (7.05%), *M.* cf. *romulea (*0.60%) and *P. dianae* (5.30%).

Remarks: According to Castelló *et al*. (2020), two species of the genus *Mesanthura* are recorded in the Mediterranean Sea (*Mesanthura. cf.romulea* and *Mesanthura pacoi* Castelló, 2017). In the present study, 45 specimens belonging to the genus *Mesanthura* shared major diagnostic characteristics with *Mesanthura* sp. described from the Salerno and Taranto harbours (Italy) by Lorenti *et al*. (2009) and subsequently identified by Ulman *et al*. (2017) as *M. cf. romulea*. Ulman *et al*. (2017) provided useful morphological details for identification: dorsal pigment spots persistent in alcohol, distinctive granulations especially at dactylus of pereopod 1, basal article margin of maxillipedal palp rectilinear, mandibular palp with six setae on article 3, pereopod 1 characterized by a distally truncate, ridged carpus, a sub-proximal shallow step on the propodus palm and a toothed process at the base of unguis, pleonites 1-5 clearly fused medially but not laterally.

Temporal patterns of density, isopod species and composition

In terms of seasonal variation, the abundance of isopod was significantly different among the four seasons (two-way ANOVA, F = 4.41, DF = 3, $P < 0.005$; Tukey test; (autumn, spring ≠ winter, summer)). The species number and mean density of isopod were higher in April 2017 (spring) (17 species; 1871 ind/m^2) and October 2017 (autumn) (15 species; 1134 ind/m^2) than those in July 2017 (summer) (15 species; 1078 ind/m^2) and January 2017 (winter) (14 species; 710 ind/m^2). Nevertheless, the highest density (8016 ind/m^2) was collected during spring, in the northeast part of the lagoon (S17) while the lowest density (96 ind/m^2) was found along the eastern coasts during summer (S15).

The highest abundance of the families Idoteidae (1382 individuals), Sphaeromatidae (797 individuals), Paranthuridae (293 individuals), Anthuridae (30 individuals), Janiridae (2 individuals) and Holognathidae (23 individuals) was reported in spring. Idoteidae and Holognathidae (328 individuals and 3 individuals respectively) showed the lowest abundance in autumn, while Anthuridae, Paranthuridae and Sphaeromatidae (5 individuals, 18 individuals, and 286 individuals respectively) showed the lowest abundance in winter. In contrast, Arcturidae was the most abundant family in autumn (646 individuals) and the least abundant family in summer (18 individuals) (Fig. 3).

Spatial distribution of the isopod community

In terms of species richness: the northern part of the lagoon (S1) appeared to be more diverse (12 species). This site is located in the vicinity of the commercial harbour showing an important number of NIS such as *P. japonica* (103 indiv), *P. dianae* (38 indiv) and *P. sculpta* (5 indiv.), followed by stations in the northern part which had intermediate richness, followed by stations on the eastern coast which had the lowest richness (2 species) (Fig. 4).

The density varied significantly among stations. The highest mean value (4308 ind/m^2) was recorded at stations located on the northeastern coast of the lagoon, while the lowest value (148 ind/m^2) was found in the southern part of the lagoon (Fig. 5). An analysis of variance (ANOVA) indicated significant differences in species richness (F = 5.70, $P < 0.001$) and abundance among stations (F = 3.22, $P < 0.005$).

Table 1. Isopod species recorded in the Bizerte lagoon during the present study ([a] new record in Bizerte lagoon, [b] new records in Tunisia, * non-indigenous species, %Ra: relative abundance, TG: trophic group, DsF: deposit-suspension feeders, HeDF: herbivores-deposit feeders, C: carnivores).

Suborder	Family	Species	Ra %	TG	Habitat preference
Valvifera	**Arcturidae**	*Astacilla mediterranea* [b] Koehler, 1911	5. 2	DsF	Among algae (*Chaetemorpha linum, Ulva lactuca, Hinksia* sp) and *C. nodosa* meadows
		Astacilla gorgonophila [b] Monod, 1925	2. 7	DsF	Among algae (*Chaetomorpha linum, Ulva lactuca, Hinksia* sp) and *C. nodosa* meadows
		Astacilla monodi [b] Tattersall, 1925	3. 5	DsF	Among algae (*Chaetomorpha linum*) and *C. nodosa*, *Z. noltei* meadows
		Astacilla axeli [b] Castelló, 1992	2. 2	DsF	Among algae (*Chaetomorpha linum, Ulva intestinalis, Hinksia* sp) and *C. nodosa* meadows
	Idoteidae	*Idotea balthica (*Pallas, 1772)	36. 3	HeDF	Among algae (*Gracilaria* sp*, Bryopsis plumosa, Ulva lactuca*)
		*Idotea chelipes (*Pallas, 1766)	7. 5	HeDF	Among algae (*Gracilaria* sp, *Bryopsis plumosa*)
Limnoriidea	**Holognathidae**	*Cleantis prismatica* [a] *(*Risso, 1826)	0. 9	HeDF	Among algae (*Gracilaria* sp)
Cymothoida	**Paranthuridae**	*Paranthura japonica* [a] * Richardson, 1909	7. 05	C	Among algae (*Cystoseira compressa*, *Sargassum vulgare*, *Ellisolandia elongata, Hypnea* sp*)*
		Paranthura nigropunctata (Lucas, 1849)	0. 06	C	Among algae (*Stypocaulon scoparium*)
	Anthuridae	*Mesanthura cf. romulea* [b]* *Poore & Lew-Ton, 1986*	0.6	C	Among algae (*Hinksia* sp) and *C. nodosa* meadows
		Apanthura corsica [b] Amar,1953	0.3	C	Among algae (*Hypnea* sp, and *Ellisolandia elongata*)
Asellota	**Janiridae**	*Jaera (Jaera) hopeana* Costa, 1853	0.02	HeDF	Among algae (*Cladophora rupestris)*
Sphaeromatidea	**Sphaeromatidae**	*Cymodoce truncata* Leach, 1814	9. 3	HeDF	Among algae (*Dictyota dichotoma, Ellisolandia elongata, Ulva lactuca* and *Cystoseira compressa, Dictyopteris polyploïdes)*
		Sphaeroma serratum (Fabricius, 1787)	6. 2	HeDF	Among algae *(Caulerpa prolifera, Ellisolandia elongata, Ulva lactuca* and *Ulva intestinalis)* and *C. nodosa* and *Z. noltei* meadows
		Paracerceis sculpta *(Holmes, 1904)	7. 3	HeDF	Among algae (*Dictyota dichotoma, Ellisolandia elongata, Cystoseira compressa, Dictyopteris polyploïdes)* and *C. nodosa* meadows
		Dynamene edwardsi (Lucas, 1849)	5. 03	HeDF	Among algae (*Dictyota dichotoma, Dictyopteris polyploïdes* and *Sargassum vulgare)*
		Paradella dianae [a] * (Menzies, 1962)	5. 3	HeDF	Among algae *(Cladophora rupestris, Padina pavonica)*

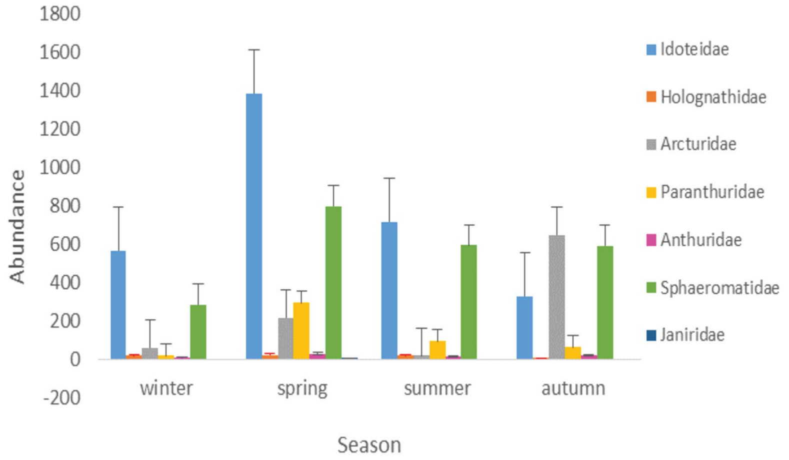

Fig. 3: Seasonal variations of the isopod family composition in the Bizerte Lagoon.

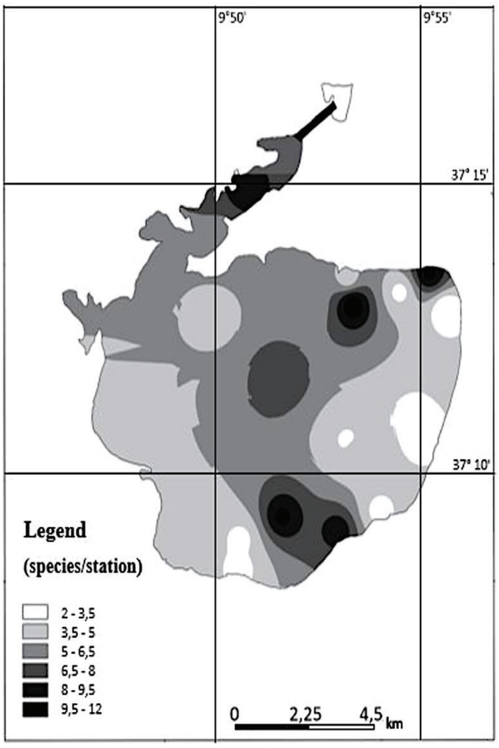

Fig. 4: Spatial distribution of the isopod species (number of species/station) over the whole study in the Bizerte Lagoon.

The dendrogram and MDS ordination based on abundance data allowed us to separate the 22 stations into three main groups (Fig. 6): the first group (GI) was made up of fourteen stations (1, 6-14, 17, 19-22), which were dominated by red and brown macroalgae, particularly the red macroalgae *Gracilaria* sp. This assemblage was composed of 13 species dominated mainly by *I. balthica, I. chelipes, C. truncata* and *D. edwardsi*. This assemblage exhibited the highest mean density (4845 ± 291.25 ind/m^2).

Group G2 was composed of stations 3, 4, 5 and 18 located in the northern part of the lagoon. These stations were dominated by the brown macroalgae such as *C. compressa* and *D. polypodioides* and characterized by low hydrodynamism and the presence of species belonging to the genus *Astacilla* with a mean density of 3316± 206.33 ind/m^2.

Group G3 included stations 2, 10, 15 and 16 and was dominated by the species *Sphaeroma serratum* (Fabricius, 1787). This assemblage had the lowest mean density (2884±120.74 ind/m^2) and stations were characterized by the presence of *C. nodosa* and *Z. noltei* meadows.

Relationships between isopod assemblages, macrophyte and environmental parameters

BIOENV analysis, applied to better explore the relationship between average density of the isopod assemblage and environmental variables, showed that the best match with the observed community temporal changes were temperature, salinity and macrophyte biomass (Spearman ρ = 0.602).

BIOENV analysis was also used to explore the relationship between average density of the isopod assemblage and macrophyte biomass (mean dry biomass of sea-

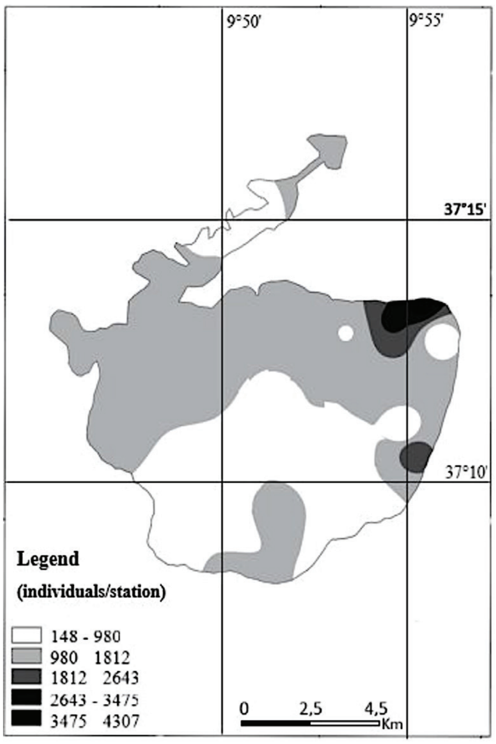

Fig. 5: Spatial distribution of the isopod abundances (number of individuals/station) over the whole study in the Bizerte Lagoon.

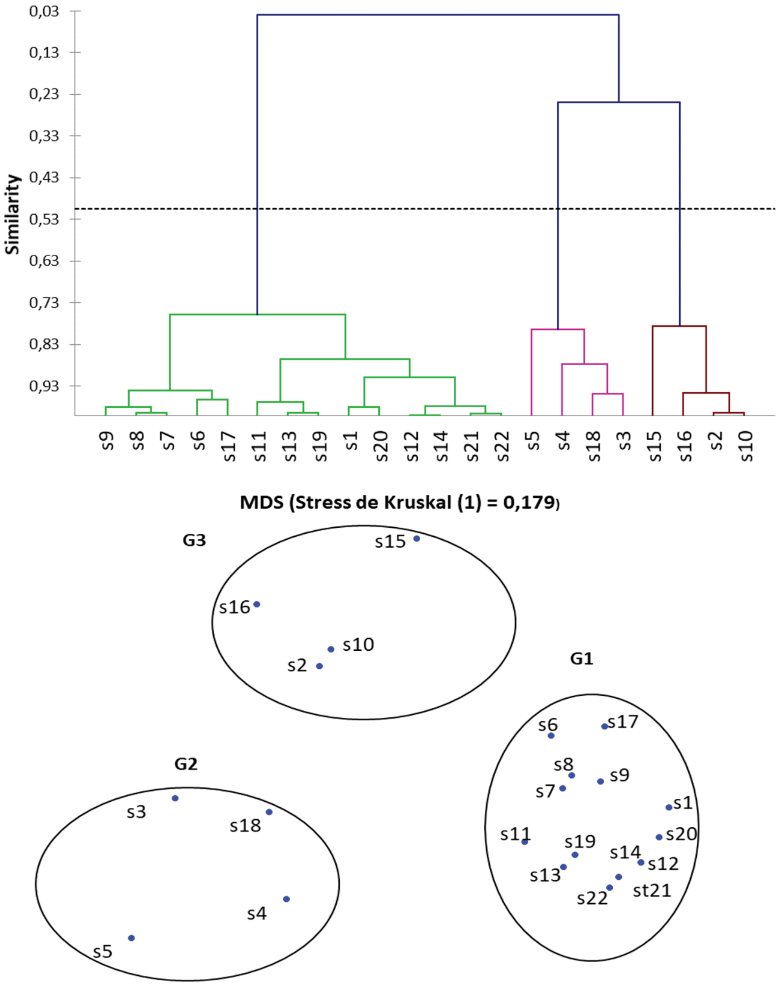

Fig. 6: Dendrogram and MDS ordination of Bray-Curtis similarities from abundance data (square root transformation) for 22 sampling stations in the Bizerte lagoon.

grasses, and mean dry biomass of the different functional groups of macroalgae). Results showed that average density of the isopod assemblage was mainly explained by the coarsely branched seaweeds (i.e. *Gracilaria* sp.) and the jointed calcareous seaweeds (i.e. *E.elongata*) (Spearman ρ = 0.504). Followed by the combination of coarsely branched seaweeds, jointed calcareous seaweeds and thick leathery seaweeds (i.e. *C. compressa*) (Spearman ρ = 0.491) (Table 2).

Trophic structure of the isopod assemblages

The analysis of trophic groups showed a clear dominance of herbivore deposit feeders (HeDF) over all seasons. This trophic group, composed of 9 species, accounted for 78.3% of the total abundance and displayed their maximum values in summer and spring. Deposit-suspension feeders (DSF), was the second most dominant feeding group accounting for 13.6% (4 species) of the total abundance with maximum values occurring in autumn and a minimum values in summer and winter. Finally, carnivores (C), accounted for 8.01% (4 species), displayed also maximum values in spring and minimum values in winter and autumn (Table 1, Fig.7).

Discussion

The present study, which followed the same sampling strategy used by Zaouali (1980), investigated a large number (22) of stations in Bizerte Lagoon. It allowed the recording of 7 and 17 isopod families and species, respectively, which represents approximately 17% of the total isopod species recorded by Castellò *et al.*, 2020 in the subregions Algeria, Tunisia, Libya and Malta. The species richness found in the present study, considerably exceeds those previously reported from the Bizerte lagoon

Table 2. Results of BIOENV analyses.

Number of variable	Correlation	Selections
2	0.504	Coarsely branched seaweeds, jointed calcareous seaweeds.
3	0.491	Coarsely branched seaweeds, jointed calcareous seaweeds and thick leathery seaweeds.
3	0.472	Coarsely branched seaweeds, jointed calcareous seaweeds and seagrasses.
2	0.470	Jointed calcareous seaweeds and thick leathery seaweeds.
3	0.466	Coarsely branched seaweeds, filamentous seaweeds and jointed calcareous seaweeds
4	0.451	Coarsely branched seaweeds, jointed calcareous seaweeds seagrasses and thick leathery seaweeds.
3	0.422	Jointed calcareous seaweeds, seagrasses and thick leathery seaweeds.
4	0.394	Coarsely branched seaweeds, filamentous seaweeds, jointed calcareous seaweeds and thick leathery seaweeds.
5	0.388	Coarsely branched seaweeds, filamentous seaweeds jointed calcareous seaweeds, seagrasses and thick leathery seaweeds.

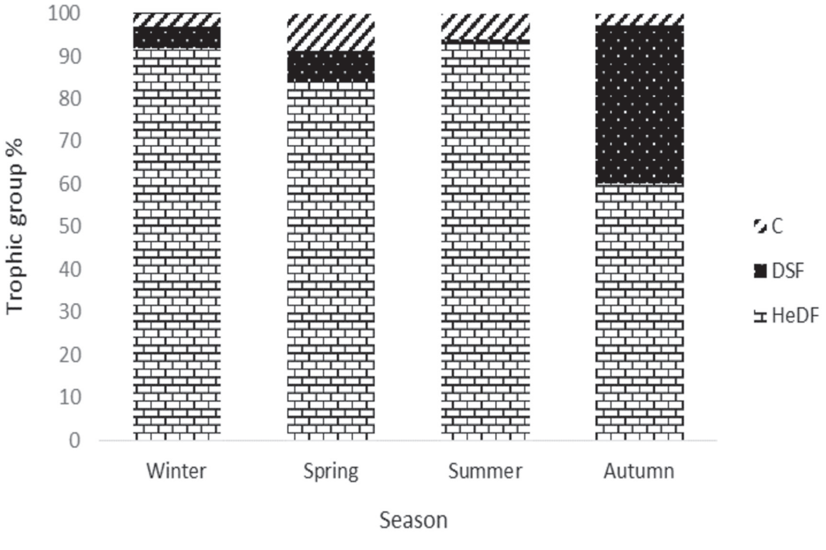

Fig. 7: Seasonal variation in the abundance of trophic groups of isopod communities in the Bizerte lagoon (C: carnivores, DsF: deposit-suspension feeders, HeDF: herbivores-deposit feeders).

(Zaouali, 1980; Afli *et al*., 2009; Khemaissia *et al.,* 2017; Fezzani *et al*., 2001; Zaabar *et al*., 2017). It also exceeds the number of species recorded by studies of neighbouring areas realized by Dridi & Prunus, 1980 (Northern coastal lagoon:10 species); Casagranda *et al*., 2006 (Ichkeul lake: 2 species); Ounifi-Ben Amor *et al*., 2017 (Tunis Southern Lagoon: 11 species) and to those from other Mediterranean lagoons containing similar wetlands (e.g., Evagelopoulos *et al.,* 2008 (Island: 3 species); Chaouti & Bayed, 2011 (Maroc: 4 species).

Among the 17 species recorded in the present study, 9 species were reported for the first time in the Bizerte lagoon (*A. mediterranea, A. gorgonophila, A. monodi, A. axeli, M. romulea, A. corsica, P. japonica, P. dianae* and *C. prismatica*). However, except for *A. mediterranea, A. gorgonophila, A. monodi, A. axeli, M.*cf. *romulea* and *A. corsica*, all other species have already been recorded in other Tunisian waters (e.g., Diawara *et al*., 2008; Ounifi-Ben Amor *et al*., 2016; Ounifi-Ben Amor *et al*., 2017; Zaouali & Ben Souissi, 2013).

The relatively high number of isopods recorded in the Bizerte lagoon may be due to a lack of sampling as well as due to anthropogenic factors such as the development of the navigable water network, the commercial transport of aquaculture species and the shipping traffic in the Bizerte lagoon (see Khammassi *et al*., 2019). It may be also due to the current environmental conditions such as the increased temperature resulting from global warming, which can affect the benthic community composition. Several studies have shown that increasing rates of invasion, predation and/or competition, caused by climate change, can affect the distribution and composition of certain faunal groups including isopods (Ingels *et al*., 2012). In fact, even though isopods are not very mobile, they may respond to climate change with migration (Barnes *et al*., 2009).

Recent studies have demonstrated that species introduction events increased to more than double the total number of NIS between 1970 and 2015 in the Mediterranean Sea with intensification of commercial shipping and aquaculture being the main reasons (Galil *et al*., 2016; Galil *et al*., 2017). Large harbours can be sink and source sites for marine NIS, while, the local traffic of smaller vessels may facilitate their spread towards other port localities, creating a hub-and-spoke network for further range expansion of NIS (Azmi *et al*., 2014; Marchini *et al*., 2015). Despite the fact that live fish market, aquarium trade and aquaculture have received less attention as a causing factor for the introduction of new species, studies elsewhere have indicated that these are the main vectors and pathway of introduction of NIS in lagoonal ecosystems (Kolar & Lodge, 2002; Rixon *et al*., 2004).

The current list of isopod species shows four NIS species (*M*. cf. *romulea, P. japonica, P. sculpta,* and *P. dianae*), contributing with 20% to the total abundance. These species are widely distributed throughout the Mediterranean Sea (Castelló *et al.*, 2020), and have been introduced either via maritime traffic or aquaculture, as shown by Marchini *et al*. (2014) and Ulman *et al*. (2019). The only NIS recorded previously in the Bizerte Lagoon by Zaabar *et al*. (2017) is the sphaeromatid *P. sculpta*, native to the northeast Pacific, whose human-borne spread on a global scale is well known (Hewitt & Campbell, 2001) and whose first record from Tunisian coasts dates back to Rezig (1978).

In a recent study on the biogeography of Mediterranean isopods, Castelló *et al.* (2020) updated the list of non-indigenus species to 23, suggesting that the isopod fauna of the Mediterranean is much more diverse than previously thought. Studies elsewhere have demonstrated that isopods are great invaders around the world (Galil *et al*., 2011) and that their expansion can follow many different pathways, such as vessels, live seafood, aquaculture, contaminated gear and footwear, floating plastic debris and marsh restoration (Carlton, 2011). An earlier study showed that the main introduction pathway of isopod species into the Tunisian lagoons are fouling and ballast waters (Ounifi-Ben Amor *et al*., 2017). In addition, Zaouali & Ben Souissi (2013) have shown that Tunisian fish farms may also be responsible for the settlement of NIS.

The present study shows that the isopod fauna of the Bizerte lagoon is very diverse and that the assemblages are directly linked to the vegetation pattern of the lagoon. In fact, the MDS analysis showed that the sampling sites were grouped mainly according to the nature and structure of the macrophyte, with coherent clusters associated with red and calcareous algae. This is of no surprise since the presence of vegetated habitats increases the habitat heterogeneity and complexity available for the epifauna (Vázquez-Luis *et al*., 2008), thus increasing also the abundance and diversity of the isopod fauna.

The analysis of trophic structure showed that herbivore deposit feeders accounted for over 78% of total abundance, a dominance which could be linked to the extensive presence of seagrass and macroalgae. Furthermore, the highest species richness and abundance were found in the biotope with photophilic algae, for which it is known that the chemical, nutritional and structural compositions provide a heterogeneous and very diverse food environment for herbivores (Rivera & Hay, 2001).

There are a large number of studies that show that the seasonal variations in the composition and structure of the isopod fauna inhabiting macroalgae are influenced by differences in the shapes of macroalgae (such as the degree of substrate complexity) and by seasonal changes in the population of macroalgae density (Nicotri, 1980; Gestoso *et al*., 2012; Veiga *et al*., 2014). Our results suggests that habitat morphology played an important role in shaping the structure of isopod assemblages. Isopods were attracted to large, tough and branched algae to which isopod morphology is well adapted. Schmidt & Scheibling (2006) and Cacabelos *et al*. (2010) proved that morphology and complexity of macroalgae might also be important factors in shaping the structure of these assemblages and determining habitat choice.

During the study period, the isopod community composition was subject to predictable seasonal changes: the isopod fauna density and the species richness showed seasonal variations in such a way that the maximum was recorded in spring and the minimum was recorded during winter. The seasonal variations in mean density and diversity are mainly caused by recruitment in spring (Reiss & Kroncke, 2005). Recruitment is reflected by a general increase in the number of species and their abundance during spring and a decline during winter. Recruitment is known to be highly variable in space and time and is mainly affected by diverse factors during the seasonal cycle, such as food availability, predation, water temperature and the hydrodynamic regime (Reiss & Kroncke, 2005).

However, an increase was determined in the abundance of the Arcturidae family during autumn and the majority of the individuals collected were adults; since the microscopic inspection of random specimens indicates the presence of eggs, this period (autumn) would be particularly favourable for the proliferation of *Astacilla* species. The highest abundances were found at three stations (S3, S4 and S17). These localities were characterized by the lowest average salinity (36 psu) and low hydrodynamism.

The three dominant species encountered in the Bizerte Lagoon were *I. balthica*, *C. truncata* and *I. chelipes*. Despite its marine preference, *I. balthica* appears to be the most abundant species compared to the lagoonal species *I. chelipes*. Zaabar *et al*. (2017) suggested that the decrease in *I. chelipes* in Bizerte Lagoon could be explained by the increase in salinity due to the construction of dams upstream of Ichkeul Lake. Similarly, Vlasblom *et al*. (1977) suggested, after experimental studies on osmoregulatory capacity, that the interaction of temperature and salinity may limit the distribution patterns of *I. chelipes*. On the other hand, Łapucki & Normant (2008) described *I. chelipes* as a brackish water species tolerating 3–30 psu. This tolerating range should be larger since our study reported the presence of the species (88 individuals) during spring at 40 psu (S12). Likewise, Leidenberger *et al*. (2012) reported that *I. chelipes* may have the highest capacity to adapt to significant environmental changes caused by climate change and other anthropogenic activities. Finally, the lowest abundances of *I. chelipes* with respect to *I. balthica* could be explained by their interspecific competition pressure as reported Korheina (1981).

There was a positive relationship between the maximum algal and seagrass development, during the warm season, and the highest abundance and species richness for the majority of isopod families reported in our study. Likewise, the distribution of isopods in the Bizerte lagoon appeared mainly linked to seasonal changes of water temperatures, salinity, and biomass of macroalgae and functional groups of macrophytes. Similarly, Zaabar *et al.* (2017) reported that the composition and structure of the isopod fauna inhabiting macroalgae and seagrass were influenced by same environmental factors such as the temperature and salinity. Thus, it is well known that temperature is the most significant factor that may affect algae and seagrass, which are habitats of the isopod assemblages (Aikins & Kikuchi, 2001).

Conclusions

A total of 6808 isopod individuals belonging to 17 species and 7 families were collected in the Bizerte Lagoon. Among them, 9 species were recorded for the first time in the Bizerte Lagoon. Sphaeromatidae was the family richest in species, while *I. balthica* was the most abundant species. Salinity, temperature, nature and structure of macroalgae were important in the composition and distribution of isopods in this area. In conclusion, the development of the navigable water network and the commercial transport of aquaculture species have opened the lagoon to international trade and to the introduction of NIS. This is particularly relevant for the Bizerte Lagoon, where the introduction of NIS has involved a variety of vectors and pathways that have changed over time. This aspect deserves increased attention for the conservation of lagoonal ecosystems.

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