Comparison of isotopic niches of four sea cucumbers species (Holothuroidea: Echinodermata) inhabiting two seagrass meadows in the southwestern Mediterranean Sea (Mostaganem, Algeria)

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Abstract. Among the fauna inhabiting the *Posidonia oceanica* seagrass meadow, holothurians are particularly abundant and provide essential ecological roles, including organic matter recycling within seagrass sediments. This study aimed to investigate the trophic niche of four holothurians of the order Holothuriida [*Holothuria poli* (Delle Chiaje, 1824), *Holothuria tubulosa* (Gmelin, 1791), *Holothuria sanctori* (Delle Chiaje, 1823) and *Holothuria forskali* (Delle Chiaje, 1823)] inhabiting *P. oceanica* meadows, through the measurement of nitrogen and carbon stable isotope ratios. Two shallow and contrasting sites of the littoral region of Mostaganem (North West Algeria) were chosen. The first site, located in Stidia, is weakly impacted by human activities. The second site, located in Salamandre, is highly impacted by human activities (industries, harbor facilities). High values of $\delta^{15}N$ in holothurians and their food sources were observed at both sites. The $\delta^{13}C$ values showed a lower contribution from detritic *Posidonia* than in other areas. This could be a consequence of *P. oceanica* bed degradation in the studied area. The stable isotope approach did not reveal dietary differences between species, and the four holothurians species exhibited significant isotopic niche overlap. However, niche sizes differed between species showing more variable individual trophic diversity in some species (*H. tubulosa* and *H. sanctori* in Salamandre; *H. forskali* in Stidia). If niche segregation does occur, it is not in terms of general resource use. More likely, it would be the abundance of food sources, the different life habits and their micro-habitats that may explain their co-existence in the *P. oceanica* seagrass meadow.

Keywords. Echinodermata, holothuroids, stable isotopes, Mediterranean.

Introduction

The *Posidonia oceanica* (L.) Delile 1813 seagrass meadow is considered as a pivotal ecosystem of Mediterranean coastal waters (Boudouresque *et al.* 2006; Gobert *et al.* 2006) and as a powerful integrator of the overall marine water quality (Richir *et al.* 2015). The *P. oceanica* ecosystem has

significant primary production that is only partially consumed by herbivorous organisms (VIZZINI 2009). The remaining primary production (seagrass litter, composed mainly of *P. oceanica* dead leaves), represents an important source of organic matter for benthic detritivore communities (WALKER *et al.* 2001; REMY *et al.* 2018). Thus, dead leaves are part of the diet of a great number of species, such as echinoderms (echinoids and holothuroids), copepods, isopods, amphipods, decapods and polychaetes (VIZZINI 2009; MASCART *et al.* 2018; REMY *et al.* 2018). This detrital biomass is also exported from the *P. oceanica* meadow to adjacent areas where it contributes to local food webs (VIZZINI 2009; REMY *et al.* 2018).

Holothuroid echinoderms, commonly known as sea cucumbers, especially of the order Holothuriida (formerly Aspidochirotida partim) are major representatives of the benthic compartment of *P. oceanica* ecosystem (MEZALI 2008). They are detritus-feeders, participating in the transfer of organic matter produced by the seagrass, contributing to the recycling of organic matter and its re-mineralization and generating an important "turn over", thus playing a key role in the oxygenation of the soft substrate (AMARO *et al.* 2010; MACTAVISH *et al.* 2012; COSTA *et al.* 2014). Several holothurian species are present in the *P. oceanica* meadows. The most common are *Holothuria poli* (Delle Chiaje, 1824), *Holothuria tubulosa* (Gmelin, 1791), *Holothuria sanctori* (Delle Chiaje, 1823) and *Holothuria forskali* (Delle Chiaje, 1823). *Holothuria tubulosa* and *H. poli* are found at the "intermatte" (spaces without *P. oceanica* leaves within the meadow), whereas, *H. sanctori* and *H. forskali* frequent much more commonly the eroded vertical edge (thickness of the "matte" that is observed at the level of inter-mattes) (MEZALI 2004). Other studies indicate that *H. poli* frequents sparse meadows, while *H. tubulosa* frequents dense ones (FRANCOUR 1990).

Sea cucumbers are an economically important fisheries resource and constitute an interesting food and medicinal market in Asian and some European countries (SICURO *et al.* 2012; PURCELL *et al.* 2013). Despite their ecological importance, data on the diet of aspidochirotid holothurians remain scarce (MEZALI *et al.* 2003). Studies on feeding behavior were conducted by several authors worldwide (SONNENHOLZNER 2003; AMARO *et al.* 2010) and in the Mediterranean (COULON & JANGOUX 1993; İŞGÖREN-EMIROĞLU & GÜNAY 2007). According to BELBACHIR *et al.* (2014), *H. poli* tends to select the finest sedimentary particles; while other species show selectivity for organic matter (MEZALI *et al.* 2003; MEZALI & SOUALILI 2013).

Several food components of various origins (plant and animal) have been found in the digestive tract of holothurians inhabiting *P. oceanica* meadows (BELBACHIR & MEZALI 2018).

Food-niche studies aim to identify the alimentary resources exploited by individuals or populations. Several techniques are used to study the selection of food resources and trophic relationships between species. Traditional techniques rely on direct observations of feeding behavior (CARAVEO-PATINO & SOTO 2005), or on indirect methods such as the analysis of stomach or fecal contents (SMITH & WHITEHEAD 2000; JARMAN *et al.* 2002). However, these methods are difficult to apply to deposit-feeding organisms such as holothurians. Moreover, these approaches do not inform us about the nature of the assimilated material. A biochemical marker, such as carbon and nitrogen stable isotope ratios, provides an indirect method for identifying animal dietary components. The advantage of these biochemical markers is the integration of animal diet information over a longer time (DALERUM & ANGERBJORN 2005). Moreover, isotopic compositions of various species can be compared to establish the relative extent of the trophic niches (JACKSON *et al.* 2011) or the extent to which they overlap. Isotopic variability may also reveal fluctuations of the inter-individual diet of animals (BEARHOP *et al.* 2004). This approach has recently been applied in invertebrate communities colonizing the *P. oceanica* litter exported outside of the seagrass meadows (MASCART *et al.* 2018; REMY *et al.* 2018).

Marine coastal ecosystems are sensitive to disturbances associated with human activities. Wastewater from urban areas and organic and inorganic fertilizers from agricultural areas may have significant side

effects on benthic invertebrates, inducing changes in food resource availability, leading to a change in food web functioning (COOPER *et al.* 2007).

The aims of our study were to characterize the isotopic niches of holothurian species and to test for niche overlaps between four sympatric species inhabiting *P. oceanica* meadows. We tested these study aims at two sites Stidia and Salamandre, which differ in their levels of anthropogenic disturbance in term of farmland fertilizers and urban wastewater, respectively. These different levels of anthropogenic disturbance could potentially influence the feeding behavior of holothurians and affect their trophic niches.

Material and methods

Sampling

The sampling was carried out during autumn 2016 in the littoral region of Mostaganem (North West Algeria). We chose this period of the year because *P. oceanica* leaf detritus resulting from natural leaf fall accumulates as *litter* after autumn storms (GOBERT *et al.* 2006). Two shallow water stations were selected (3 m average water depth and 15 km distant from one another) (Fig. 1). The first site is located at Stidia (35°49′ N; 0°01′ W) in a poorly urbanized and industrialized area (Fig. 1). However, it is very close to farmland, leaving it exposed to some sources of organic pollution (fertilizers rich in phosphorus and nitrogen) (BELBACHIR 2012). At this site, *P. oceanica* seagrass develops on rocky substratum from 1.5 m depth. This meadow is of type II according to the classification of GIRAUD (1977) and is in front of small patches of *Cymodocea nodosa* meadows (BELBACHIR 2012). The second site located at Salamandre (35°54′ N; 0°03′ E) (Fig. 1), is adjacent to the fishing harbor of Mostaganem. This site is characterized by a large residential area and the presence of several factories [milk production (Orolait), pasta products (Safina) and cosmetic products]. The infralittoral area of Salamandre shows signs of degradation, namely a reduced biodiversity (BENZAIT 2015) and *P. oceanica* meadow in very bad condition (i.e., reduced shoot density, increased patchiness).

This meadow is sparse and develops on a rocky substratum (BELBACHIR 2012). This situation is likely a direct consequence of the construction of the fishing harbor over the last 10 years altering the water flow and directly affecting the health of the *P. oceanica* meadow. In these two sites, we find, in order of abundance, four aspidochirotid holothurians species: *Holothuria poli,* which frequents the detrital bottoms and *P. oceanica* meadows (MEZALI 2004); *Holothuria tubulosa* found much closer to the rocky
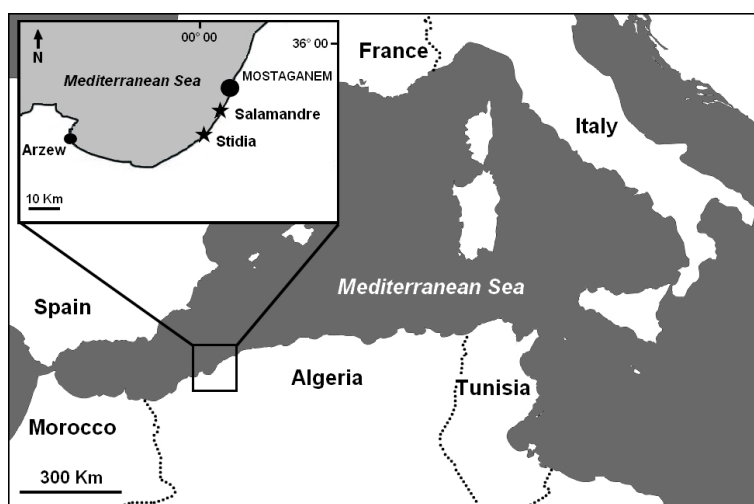

Fig. 1 – Geographical location of the two sampling sites (stars): Stidia and Salamandre (from SOUALILI *et al.* 2008, modified).

substratum and within *P. oceanica* meadows (FRANCOUR 1990); and two other species (*H. sanctori* and *H. forskali*), which are cryptic species found on the hard substratum, under rocks and between rhizomes of *P. oceanica* (MEZALI 2004).

Ten individuals of each holothurian species (20 cm mean size) were collected from each study site (n = 80) at 3 m mean depth. Their potential food sources were also sampled from the biotope where they lived. The three considered food sources were: *P. oceanica* dead leaves (PM) (sampled from the litter); meiofauna (Me) (i.e.,: fauna passing through a 1mm mesh sieve and retained on 38μm mesh sieve) and the epiphytes of the *P. oceanica* leaves (Ep). The latter were obtained after carefully scraping the *P. oceanica* leaves using a razor blade (DAUBY & POULICEK 1995). The epiphytes contribute to the enrichment of the organic matter contained in the sediment and are likely to be assimilated by holothurians.

Stable isotope analysis

The retractor muscles of each individual were carefully removed and then dried separately for 48h at 50°C. Afterwards, they were ground, with mortar and pestle to a very fine powder. Each of the three food sources was ground separately in a ball mill (Retsch Mixer Mill MM301), to obtain a very fine and homogeneous powder. In order to eliminate the carbonates (HCO_3^{2-}) potentially contained in the retractor muscles, the latter were exposed to 37% hydrochloric acid (HCl) fumes for 24h. The *P. oceanica* epiphyte (Ep) samples were also decalcified directly in 1N HCl for 24h. The isotopic ratios of C and N were measured on a mass spectrometer (EA-IRMS) (Isoprime 100, Isoprime, UK) coupled to a N-C-S elemental analyzer (Vario Microcube, Elementar, Germany at the Laboratory of Oceanology, University of Liège, Belgium). Stable isotope ratios were expressed using the notation δ (‰) (COPLEN 2011). Certified materials were IAEA-N2 (ammonium sulphate) ($\delta^{15}N$ = +20.30 ± 0.20 ‰); IAEA C6 (sucrose) ($\delta^{13}C$ = -10.80 ± 0.47 ‰) and glycine ($\delta^{15}N$ = 2.25 ± 0.3 ‰, $\delta^{13}C$ = -47.5 ± 0.3 ‰). Standard deviations on the composition measurements replicated on a sample were 0.3 ‰ for $\delta^{13}C$ and 0.4 ‰ for $\delta^{15}N$, respectively.

Statistical analysis

A non-parametric Kruskal-Wallis test was performed to test for a possible difference in C and N isotopic ratios between the studied holothurian species, as well as between the three food sources. The differences in C and N isotopic ratio for the holothurians and food sources between the two sites were also examined with a non-parametric Kruskal-Wallis test. In case of significant difference, the Kruskal-Wallis test was followed by the Dunn's posterior test (with Bonferonni's adjustment method for the P value). The test results were considered significant, when P < 0.05. The statistical analyses were performed using the R v3.4.1 software (R CORE TEAM 2017). The different parameters of the isotopic niche (trophic niche proxy established by stable isotopes) were calculated using the SIBER package (Stable Isotope Bayesian Ellipses in R, Version 4.2, JACKSON *et al.* 2011). The SIBER package was used to generate the bivariate standard ellipses that represent the holothurians' isotopic niches. Calculation of niche area and overlap between the ellipses was performed using Bayesian SEAc inference (JACKSON *et al.* 2011). Comparison between areas of the ellipses associated to each holothuroid was also performed using Bayesian modeling (SEA_b).

Results

Isotopic composition

The $\delta^{15}N$ value of potential food sources used by the holothurians of the Stidia site showed a significant difference (Kruskal-Wallis test, *P* = 0.006). The $\delta^{15}N$ of the *P. oceanica* epiphytes differed from that of the *P. oceanica* dead leaves (Dunn's test, *P* = 0.002); whereas the $\delta^{15}N$ of *P. oceanica* dead leaves and

meiofauna showed no difference (Dunn's test, $P = 0.2$) (Fig. 2). The $\delta^{13}C$ value of food sources showed a significant difference (Kruskal-Wallis test, $P = 0.005$). The *P. oceanica* epiphytes showed a $\delta^{13}C$ value significantly different from that of the dead *P. oceanica* leaves (Dunn's test, $P = 0.002$) (Fig. 2). The nitrogen isotopic composition varied between the holothurian species in the Stidia site (Fig. 2) with values ranging between 13.2 ‰ and 16.8 ‰ (15.1 ± 1.1 ‰); 12.9 ‰ and 15.2 ‰ (14.3 ± 0.9 ‰); 12 ‰ and 15.6 ‰ (14 ± 1.1 ‰) and between 12.5 ‰ and 15 ‰ (13.7 ± 0.7 ‰) respectively in *H. sanctori*, *H. poli*, *H. forskali* and *H. tubulosa*. The values obtained for *H. sanctori* were significantly different from those of *H. tubulosa* (Kruskal-Wallis test, $P = 0.05$; Dunn test, $P = 0.02$). The values of $\delta^{13}C$ varied between -17 ‰ and -15.7 ‰ (-16.3 ± 0.3 ‰); -17.2 ‰ and -15 ‰ (-16.5 ± 0.6 ‰); -17.1 ‰ and -16.1 ‰ (-16.8 ± 0.3 ‰), and between -17.3 ‰ and -16.2 ‰ (-16.9 ± 0.3 ‰) respectively for *H. tubulosa*, *H. forskali*, *H. sanctori* and *H. poli*. There were significant differences between *H. tubulosa* and *H. poli* (Kruskal-Wallis test, $P = 0.04$; Dunn's test, $P = 0.01$).

The $\delta^{15}N$ values of potential food sources consumed by holothurians of the Salamandre site showed a significant difference (Kruskal-Wallis test, $P = 0.008$). The $\delta^{15}N$ value of *P. oceanica* epiphytes differed from that of the dead *P. oceanica* leaves (Dunn's test, $P = 0.007$) and differed from that of the meiofauna (Dunn's test, $P = 0.02$) (Fig. 2). The $\delta^{13}C$ values of the three food sources showed a significant difference (Kruskal-Wallis test, $P = 0.001$). *Posidonia oceanica* epiphytes had a $\delta^{13}C$ value that was significantly different from those of dead *P. oceanica* leaves (Dunn's test, $P = 0.0006$) (Fig. 2). Low variation of nitrogen isotopic composition was observed between holothurians species of the Salamandre site (Fig. 2), the values ranging between 11.4 ‰ and 13.8 ‰ (mean ± standard deviation, 12.5 ± 0.6 ‰); 12 ‰ and 15 ‰ (13.6 ± 0.9 ‰); 10.7 ‰ and 13.6 ‰ (12.1 ± 0.9 ‰) and 12 ‰ and 13.7 ‰ (12.9 ± 0.6 ‰) respectively for *H. poli*, *H. forskali*, *H. sanctori* and *H. tubulosa*. There was a significant difference between the $\delta^{15}N$ values of holothurians. *Holothuria forskali* was significantly different from *H. sanctori* (Kruskal-Wallis test, $P = 0.01$; Dunn's test, $P = 0.003$). The $\delta^{13}C$ values obtained for holothurians ranged between -18.1 ‰ and -16.1 ‰ (-17.2 ± 0.5 ‰); -18.3 ‰ and -16.8 ‰ (-17.5 ± 0.5 ‰); -18.3 ‰ and -16.3 ‰ (-17.7 ± 0.7 ‰), and between -19.2 ‰ and -15.7 ‰ (-17.7 ± 0.9 ‰) respectively for *H. poli*,
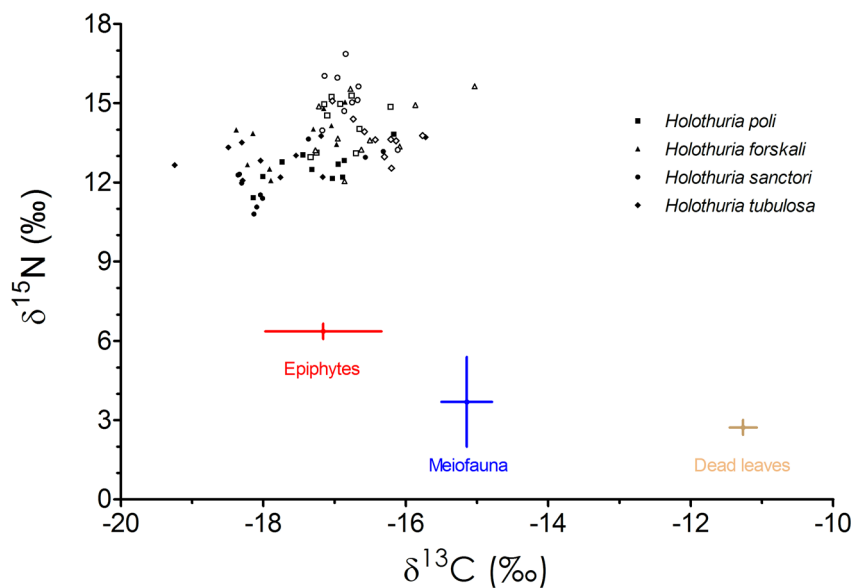

Fig. 2 – Carbon isotope ratio ($\delta^{13}C$ in ‰) and nitrogen isotope ratio ($\delta^{15}N$ in ‰) of the four studied holothuroids (individual measurements) and their food sources (mean values ± standard deviation) at Stidia (open symbols) and Salamandre (black symbols) sites, respectively.

H. forskali, *H. sanctori* and *H. tubulosa*. Carbon isotopic composition showed no difference between the studied holothurian species (Kruskal-Wallis test, $P = 0.1$) (Fig. 2). The δ^{15}N and δ^{13}C values of the food sources showed no difference between the two studied sites (Kruskal-Wallis test, $P = 0.1$). However, the δ^{15}N and δ^{13}C values of holothurians showed a difference between the two studied sites (Kruskal-Wallis test, $P = 0.05$), the holothurians of the Stidia site being more enriched in ^{15}N and ^{13}C.

Isotopic niche characteristics

At the site of Stidia, *H. forskali* occupied the largest isotopic niche (SEA$_C$: 2.56 ‰²), followed by *H. sanctori*, *H. poli* and *H. tubulosa* (with SEA$_C$: 1.11 ‰², 1.05 ‰² and 0.73 ‰² respectively) (Fig. 3A). A strong overlap between isotopic niches was observed between all holothurian species (Fig. 3A). The most important overlap was that between *H. poli* and *H. forskali* (6.18 ‰², i.e., 6.34% of *H. poli* SEA$_C$ and 15.33% of *H. forskali* SEA$_C$); while the least important overlap was observed between *H. poli* and *H. tubulosa* (2.59 ‰², i.e., 6.34 % of *H. poli* SEA$_C$ and 4.40 % of *H. tubulosa* SEA$_C$) (Fig. 3A).

Through Pairwise comparisons of model estimated ellipse areas (SEA$_b$), we found that the *H. poli* isotopic niche width was smaller than that of *H. forskali* in 98% of the model estimates (Fig. 3B). The standard ellipse area (SEA$_b$) of the other holothurian species taken in pairs was not significantly different as the probability differences were less than 95%.

In Salamandre, *H. tubulosa* occupied the largest isotopic niche (SEA$_C$: 2.03 ‰²), followed by *H. sanctori*, *H. forskali* and *H. poli* (with SEA$_C$: 1.86 ‰², 1.66 ‰² and 0.98 ‰² respectively) (Fig. 4A). The largest isotopic niche overlap was between *H. sanctori* and *H. tubulosa* (7.57 ‰², i.e., 11.15 % of *H. sanctori* SEA$_C$ and 12.15 % of *H. tubulosa* SEA$_C$); while the least significant overlap, was observed between *H. poli* and *H. forskali* (3.81 ‰², i.e., 5.92 % of *H. poli* SEAc and 9.95 % of *H. forskali* SEAc) (Fig. 4A). Through the pairwise comparison of model estimated ellipse areas (SEA$_b$), we found that the standard ellipses area (SEA$_b$) of holothurians species taken in pairs did not show any significant difference (Fig. 4B).
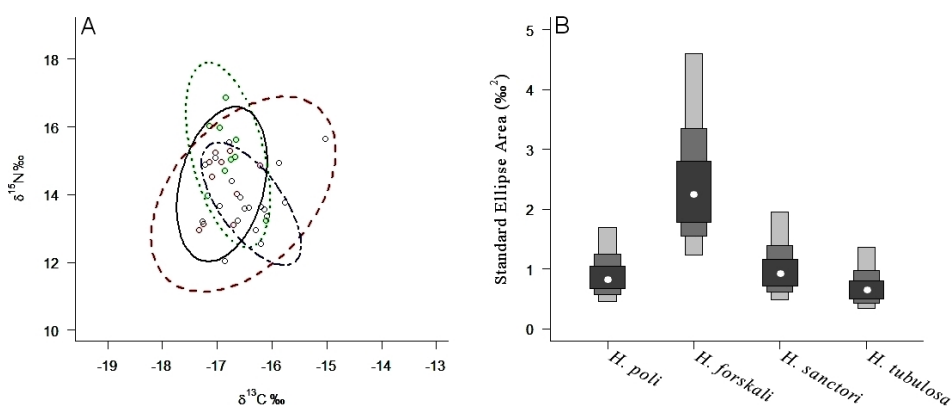

Fig. 3 – Isotopic niches of the four holothuroids of the Stidia site. The lines represent the bivariate standard ellipses associated with each sea cucumber species (*H. tubulosa*, *H. poli*, *H. forskali*, *H. sanctori* in blue, black, red and green line respectively). Boxplots of the bivariate ellipses standard estimation model (SEA$_b$); dark gray, medium and light boxes are respectively 50 %, 75 % and 95 % credibility intervals of the probability of density function distributions of the model solutions, and white dots are the modes of these distributions at Stidia.
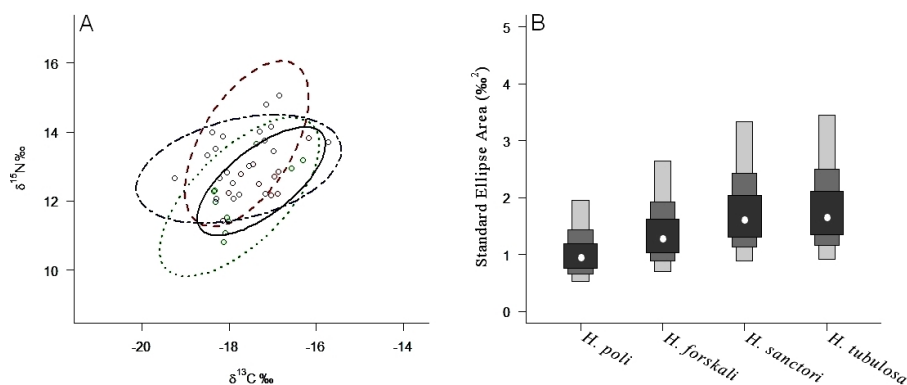
Fig. 4 – Isotopic niches of the four holothuroids of the Salamander site. The lines represent the bivariate standard ellipses associated with each sea cucumber species (*H. tubulosa*, *H. poli*, *H. forskali*, *H. sanctori* in blue, black, red and green line respectively). Boxplots of the bivariate ellipses standard estimation model (SEA$_b$); dark gray, medium and light boxes are respectively 50 %, 75 % and 95 % credibility intervals of the probability of density function distributions of the model solutions, and white dots are the modes of these distributions at Salamander.

Discussion

The potential trophic sources used by the holothurians of the two studied sites were more enriched in $\delta^{15}N$ compared to other Mediterranean sites (LEPOINT *et al.* 2000). The site of Stidia is edging an agricultural zone; nitrification and denitrification following the application of fertilizers on these farmlands could increase the $\delta^{15}N$, which could be transported to the marine environment (FINLAY & KENDALL 2007) and influence the $\delta^{15}N$ of primary producers (COLE *et al.* 2005). The classification of PERGENT *et al.* (1995), which incorporates the depth factor in the estimation of the state of the *P. oceanica* meadows, indicates that the vitality of the *P. oceanica* meadow of Stidia site is abnormal (BELBACHIR 2012). The wastewaters and sewage spills at the Salamandre site may also have an impact on the $\delta^{15}N$ of the primary producers (VERMEULEN *et al.* 2011) including *P. oceanica* (LASSAUQUE *et al.* 2010). Therefore, our results indicate that both sites are influenced by human activities, even though the Stidia site remains relatively better preserved than the Salamandre site.

The studied holothurians species showed higher $\delta^{15}N$ and $\delta^{13}C$ values than those obtained by LEPOINT *et al.* (2000), in *H. tubulosa* from Calvi Bay (Corsica) [(5.5 ± 1.0 ‰) for $\delta^{15}N$; (-13.2 ± 1.7 ‰) for $\delta^{13}C$]. The $\delta^{15}N$ values obtained for the studied holothurians in the present work are also higher than those obtained by RICART *et al.* (2015) for *H. tubulosa* from Catalan coasts (Spain); whereas the $\delta^{13}C$ values obtained by these authors [(9.17 ± 0.43 ‰) for $\delta^{15}N$; (-16.41 ± 0.32 ‰) for $\delta^{13}C$] are very close to our results.

The $\delta^{15}N$ values in holothurians of Stidia site were higher than those of the Salamandre site; this may suggest that the sources used at Stidia belong to higher trophic levels. However, in our case, it is estimated that anthropogenic inputs would have influenced the isotopic baseline. Indeed, the nitrogen isotope composition of primary producers reflects those of external sources (FRY 2006), including anthropogenic inputs (LASSAUQUE *et al.* 2010; VERMEULEN *et al.* 2011).

Dead leaves are an important source of organic matter for seagrass detritivore communities (WALKER *et al.* 2001), including holothurian species in *P. oceanica* beds (LEPOINT *et al.* 2000, COSTA *et al.* 2014),

but paradoxically, our δ¹³C data could indicate that this source is less consumed in our area than in the Calvi bay, for example (Lepoint *et al.* 2000). This result could be explained in two ways: 1) the sampled meadows in our study are unique since they develop on rocky substratum, whereas, for example, the meadows sampled by Lepoint *et al.* (2000) develop on sandy substrate. This is likely to affect the amount of sediment and the *P. oceanica* organic matter available for holothurians. Alternatively, at least one of the meadows (especially that of Salamandre) is much degraded and the *P. oceanica* contribution to the sedimentary material is probably small; 2) the tissues used in our study (i.e., retractor muscles) are different from the tissues commonly used (i.e., integument), however our choice seems appropriate insofar as muscle tissues do not contain carbonate unlike the integument, which is generally difficult to decalcify and grind. This difference in tissues implies a difference in biochemical composition but also in renewal time (Tieszen *et al.* 1983; Ogden *et al.* 2004). Our results show that in the few months preceding the sampling, the contribution of *P. oceanica* to the holothurians' diet seemed limited.

Sampled Holothurians seemed to rely mostly on food sources with lower δ¹³C, namely of algal origin (i.e., epiphytes, phytoplankton).

At the two sites of Mostaganem region, the studied holothurian species had a high isotopic niche overlap, which suggests that these benthic invertebrates used the same food sources and therefore exhibited similar trophic niches. The similarity of trophic niches between species raises two very important issues: the sharing of the resource and competitive interactions (Dromard 2013). Analysis of the gut contents of the same four studied holothurian species confirmed that the ingested diet of these animals is based on the same trophic resources (Belbachir & Mezali 2018). This finding leads us to say that the coexistence of these holothurians cannot be explained by food resources partition, but rather by a better partition of their habitat (spatial and behavioral segregation). Indeed, *H. poli* and *H. tubulosa* mainly prefer detrital and rocky beds, near *P. oceanica* meadows, while *H. forskali* and *H. sanctori* are located under the boulders and can even be found on rocky bottoms rich in plant species, close to *P. oceanica* meadows (Francour 1990; Mezali 2004). The micro-distribution of the species studied during the present work was different from that observed by Mezali (2004) on the same holothurian species in Sidi Fredj site (central region of Algeria). This author found that *H. tubulosa* and *H. poli* preferred *P. oceanica* "intermattes", whereas *H. forskali* and *H. sanctori* were situated at the level of the eroded vertical edge and between *P. oceanica* rhizomes. It is possible that this difference in the micro-distributions of holothurians is due to the nature of *P. oceanica* meadows. Indeed, the meadows of Stidia and Salamandre are installed on rocky substratum, while the meadow of Sidi Fredj is installed on *Posidonia* "matte". This indicates that the micro-niche of these species could be, to some extent, variable and plastic according to local environmental conditions.

The high overlap could also be explained by the abundance of the food resources, which excludes competition between holothurian species and thus allows for coexistence. The difference in the relative contribution of each food resource to the diet of the studied species, or the timing difference in their supply, could also explain their coexistence. *Holothuria forskali* of the Stidia site occupied the largest isotopic niche, which is probably due to its use of a wide range of trophic sources compared to other species. The difference between the habitats frequented by these holothurian species could also be at the origin of the difference between the dimensions of their isotopic niches (especially between *H. poli* and *H. sanctori*). Holothurian species of Stidia displayed higher δ¹⁵N variability than those of Salamandre, suggesting that they may eat across a broader range of trophic level, mainly from animal sources. However, holothurians in Salamandre were more variable according to their δ¹³C (especially *H. tubulosa*), which could indicate that holothurians rely on multiple and diverse basal food sources, thus it leads us to suppose that they have a higher trophic plasticity and a generalist character. It is important to note that Salamandre is a highly impacted site with a much-degraded and very sparse *Posidonia* meadow. The organic matter content of basal resources decreases in sparse Posidonia meadows and species trophic generalism increases (Calizza *et al.* 2013). Therefore, we suppose that the trophic

plasticity of the holothurians at Salamandre is due to the poor state of their *Posidonia* meadows. Indeed, according to the optimal foraging theory, the trophic niche broadening is a consequence of disturbance, decrease in the quality of available resources, and reduced food availability, where the consumers relying on a scarcity of preferred food items are required to add less profitable resources to their diet (PYKE *et al.* 1977; ROSSI *et al.* 2015). This situation constrained the consumers to forage at greater distances, which may enable them to target various resources with diverse $\delta^{13}C$ values.

Conclusion

We did not run a mixing model to determine the respective contributions of food sources to holothurian diet. Sea cucumber isotopic values did not fit the mixing polygon determined by isotopic composition of food sources, which is a basic assumption for applying mixing models (e.g., FRY 2006). To apply a mixing model to our data, it would be necessary to experimentally determine fractionation factors (i.e., isotopic differences between diet and sea cucumber tissues). We predict that these factors would be more elevated than generally assumed for detrivorous animal, as that is the only way to explain such discrepancy between consumer $\delta^{15}N$ values and those of their potential food sources.

From the $\delta^{13}C$ data, we observed that, in this disturbed area, holothurians could not exploit the same food sources as in a pristine seagrass meadow (i.e., Calvi Bay, Corsica), with, probably, a lower contribution of seagrass material to the holothurians' diet than would occur in a pristine area. The holothurian species studied here were able to respond to *P. oceanica* local conditions by adapting resources utilization. We also showed that the four species shared a large part of their trophic niches and that, if niche segregation occurs, it is not in terms of general food sources use but, more likely, in term of habitat size, micro-habitat use and behavioral differences.

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