

Inshore/offshore gradients of imposex in *Bolinus brandaris* (Gastropoda: Muricidae) from the Gulf of Gabès (southern Tunisia, Central Mediterranean Sea)

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The present study provides the first data available on imposex in a gastropod species caught offshore the Tunisian coast. Imposex was analysed in the purple dye murex (*Bolinus brandaris*) from ten collecting sites offshore the Gulf of Gabès (southern Tunisia, Central Mediterranean Sea). From a total of 584 individuals analysed, females outnumbered males, leading to a female-biased sex ratio (1 F:0.7 M). Among the 353 females analysed, 117 were affected by imposex. On the whole, these females presented low to moderate imposex incidence (I%=33.1) and severity (FPLI=0.3, RPLI=2.6 and VDSI=0.8). The study revealed that imposex is a widespread phenomenon in the Gulf of Gabès and identified a clear inshore/offshore gradient in the imposex indices of *B. brandaris*. Regression analysis detected highly significant negative correlations between imposex indices and the distance to the coastline and depth of the collecting sites. Principal components analysis highlighted the specific influence of the distance to the major harbours, either in number of calling vessels (commercial harbour of Sfax) or shipping tonnage (oil terminal of Skhira). This inshore/offshore gradient of imposex development in *B. brandaris* reflects marked spatial variation in pollution by organotin compounds associated to the shipping activity. Overall, the study provides valuable baseline data for assessing the future evolution of imposex in *B. brandaris*, and subsequently the spatial and temporal trends of pollution by organotin compounds offshore the Gulf of Gabès.

Key words: *Bolinus brandaris*, imposex, pollution biomonitoring, inshore/offshore gradient, Gulf of Gabès, southern Tunisia

INTRODUCTION

Imposex is a masculinisation phenomenon that consists of the development and superimposition of male secondary sexual characters (penis and/or vas deferens) onto females of prosobranch gastropods. The endocrine disruption

that induces imposex has been causally associated to pollution by organotin compounds (tributyltin - TBT and triphenyltin - TPT), which were used worldwide as biocides in antifouling paints applied on ships and boats hulls (TERLIZZI *et al.*, 2001). Because of this ubiquitous environmental contamination, imposex became

a widespread phenomenon, affecting an increasing number of both coastal and offshore gastropods (ELLIS & PATTISINA, 1990; HORIGUCHI *et al.*, 2000; STERNBERG *et al.*, 2010). Indeed, nowadays imposex is reported to affect more than 260 species worldwide, distributed among 33 families and largely dominated by species belonging to family Muricidae (100 species) (TITLEY-O'NEAL *et al.*, 2011).

The purple dye murex, *Bolinus brandaris* (Linnaeus, 1758), is a common muricid gastropod found along the entire Mediterranean Sea, while in the Atlantic Ocean its occurrence is mainly restricted to the Portuguese and Moroccan coasts (POPPE & GOTO, 1991; HOUART, 2001). Imposex in *B. brandaris* has been monitored in several locations throughout its distributional range, especially in the northern Mediterranean, firstly in Spain (MORCILLO & PORTE, 1998, 1999; SOLÉ *et al.*, 1998; RAMÓN & AMOR, 2001, 2002; TIRADO & SALAS, 2001) and then in Italy (CHIAVARINI *et al.*, 2003). Along the southern Mediterranean, imposex studies with *B. brandaris* are scarcer and limited to Moroccan (LEMGHICH & BENAJIBA, 2007) and Tunisian coasts, namely in the Gulf of Tunis and in the Bizerte lagoon (ABIDLI *et al.*, 2009a, 2011, 2012; LAHBIB *et al.*, 2011a). In the Atlantic Ocean, the only information available on imposex in *B. brandaris* was obtained off southern Spain (GÓMEZ-ARIZA *et al.*, 2006) and along southern Portugal (LANGSTON *et al.*, 1997; COELHO, 2005; VASCONCELOS *et al.*, 2010, 2011).

Tunisia has an impressive coastline of 1300 km bordered by the Central Mediterranean Sea. Since the last decade there has been a remarkable effort in monitoring imposex and its effects in some gastropod species distributed along the Tunisian coasts, with particular emphasis on sites with intense shipping activity and consequent organotin pollution. In Tunisian waters, imposex was firstly described in *Hexaplex trunculus* by LAHBIB *et al.* (2004) and since then this species has been subject of numerous studies in this country (TRIGUI EL MENIF *et al.*, 2006, 2007; ABIDLI *et al.*, 2007, 2009a,b, 2012; LAHBIB *et al.*, 2007, 2008a,b, 2009a,b, 2010, 2011a,b). As mentioned above, other gastropod species monitored for imposex include *B. brandaris* (ABIDLI *et al.*,

2009a, 2011, 2012; LAHBIB *et al.*, 2011a), and more recently *Conus mediterraneus*, *Cyclope neritea*, *Nassarius mutabilis*, *Nassarius nitidus* and *Stramonita haemastoma* have also been studied for imposex in Tunisia (LAHBIB *et al.*, 2010, 2011a).

Typically, imposex monitoring is performed in coastal areas, particularly in the vicinities of commercial harbours, fishing ports, shipyards and marinas, in order to detect point sources (hotspots) of contamination by organotin compounds. In contrast, studies in offshore areas are much scarcer, but allowed confirming the widespread character of the imposex phenomenon (ELLIS & PATTISINA, 1990), affecting gastropod species even in presumably more pristine regions (e.g. STRAND *et al.*, 2006, 2009). Similarly, in the Gulf of Gabès (southern Tunisia), imposex has been monitored in *H. trunculus* collected in coastal waters (TRIGUI EL MENIF *et al.*, 2007; LAHBIB *et al.*, 2007, 2008a, 2009a), but there are no studies on imposex in gastropod species caught in offshore areas.

The purple dye murex (*B. brandaris*) is among several gastropod species caught as by-catch by bottom trawling in the Gulf of Gabès. Recent surveys in this area showed that *B. brandaris* is distributed mainly in the 10–80 m depth range, with highest by-catches per unit effort (BCPUE = total biomass hour⁻¹) occurring between 40 and 60 m depth (K. ELHASNI, unpublished data). These catches provide an excellent opportunity for assessing the incidence and severity of imposex in *B. brandaris*, as well as their spatial and depth distribution in offshore areas of the Gulf of Gabès. To the author's best knowledge, this study reports the first data available on imposex in a gastropod species caught offshore the Tunisian coast.

MATERIAL AND METHODS

Study area and collecting sites

The Gulf of Gabès is situated in southeastern Tunisia (southern Ionian Sea, approximately between 33 and 35°N), spreading from Cap Kapoudia in the north to the Libyan border in the south (Fig. 1). The gulf opens eastwards to

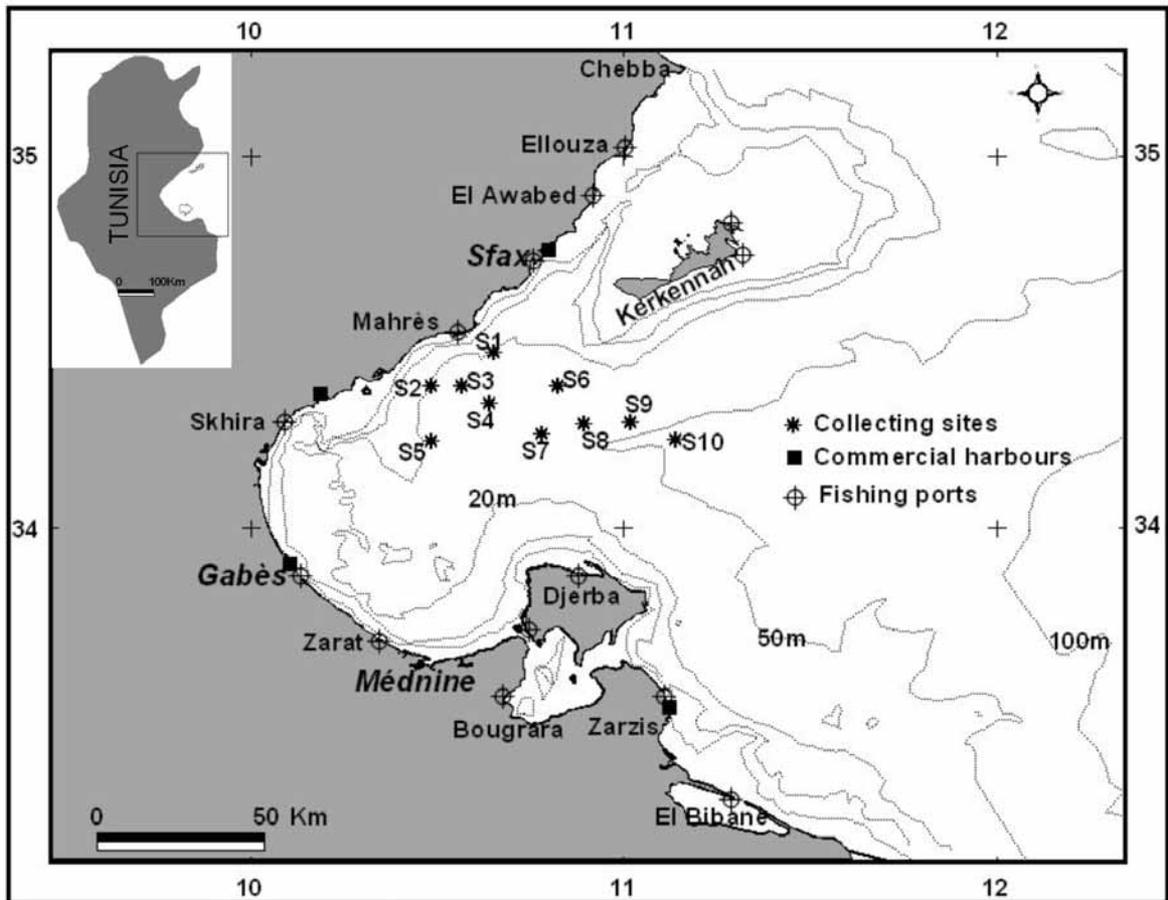


Fig. 1. Map showing the study area offshore the Gulf of Gabès (southern Tunisia, Central Mediterranean Sea) and the collecting sites surveyed for analysing imposex in *Bolinus brandaris*

the offshore and comprises some large islands (Kerkennah and Djerba) and coastal lagoons (Bougrara and El Bibane). This area corresponds roughly to 58% of the Tunisian coastline, comprising a wide continental shelf with soft slope, characterised by shallow bottoms and weak currents. The Gulf of Gabès has an intense tidal regime compared to other Mediterranean areas, with semidiurnal tides and a mean tidal range reaching 1.6 m (SERBAJI, 2000).

The study area is characterised by an intense shipping activity associated to the main commercial harbours in the Gulf of Gabès (from north to south: Sfax, Skhira, Gabès and Zarzis) (Fig. 1). According to official data (OMMP, 2007), in 2007 these harbours received 43.9% of the ships calling Tunisian harbours and represented 52.6% of the national cargo tonnage (Table 1). In terms of shipping traffic, the most important

harbour is Sfax (1608 ships), followed by Zarzis (1009 ships) and Gabès (720 ships). However, in terms of total cargo tonnage, the oil terminal of Skhira assumes prevalence (5402 thousand tonnes), followed by the commercial harbours of Sfax (5145 thousand tonnes) and Gabès (4261 thousand tonnes) (Table 1).

The Gulf of Gabès is the most important fishing area in Tunisia. In the last decade (2000-2009), this area contributed for 34 to 49% (mean \approx 43%) of the country annual fishery production, although with decreasing landings in recent years (DGPA, 2009). Therefore, the study area is also subjected to a significant boating activity connected to the main fishing ports (from north to south: Sfax, Mahrès, Skhira, Gabès and Zarzis) in the Gulf of Gabès (Fig. 1). The typology of the fishing fleet includes from small boats operating inshore to large

Table 1. Number of calling vessels and shipping tonnage in the main harbours of the Gulf of Gabès during 2007 (Data source: OMMP, 2007)

Harbour	Number	Calling vessels		Tonnes (1000)	Shipping tonnage	
		Gulf of Gabès (%)	Tunisia (%)		Gulf of Gabès (%)	Tunisia (%)
Sfax	1608	45.1	19.8	5145	33.2	17.4
Zarzis	1009	28.3	12.4	704	4.5	2.4
Gabès	720	20.2	8.9	4261	27.5	14.4
Skhira	226	6.3	2.8	5402	34.8	18.3
Total	3563	100.0	43.9	15512	100.0	52.6

bottom trawlers operating offshore. The most important fishing port is also Sfax, harbouring a high number of small boats and around 270 bottom trawlers (virtually all bottom trawling fleet operating in the Gulf of Gabès). The fishing effort exerted by bottom trawlers is considerable and concentrated mainly in the areas between 20 and 40 m depth (K. ELHASNI, personal observation).

Fishing surveys were carried out in 2007, on-board commercial bottom trawlers based in the port of Sfax and operating in the trawlable bottoms off the Gulf of Gabès. This fishing fleet targets commercially valuable demersal fishes, crustaceans (mainly shrimps) and cephalopods, while the purple dye murex is caught as by-catch. Individuals of *B. brandaris* were collected at ten sites, located between 5.9 and 37.4 Nm from the coastline and ranging from 20.0 to 60.0 m depth (Fig. 1). Approximately 50–70 individuals were collected from each of the ten sampling sites. Geographic coordinates and depth of the collecting sites were registered using the fishing boat echo sounder. The distances of each collecting site to the coastline, to the closest commercial harbour (Sfax or Skhira), to the most important harbour in shipping traffic (Sfax) and to the most important harbour in total cargo tonnage (Skhira), were calculated using GIS software (ArcView, version 3.2).

Biological sampling and imposex indices

In the laboratory, individuals were measured for shell length (SL) with a digital calliper (pre-

cision 0.05 mm). The shell of *B. brandaris* is quite fragile and frequently damaged by bottom trawling (particularly in the siphonal canal). Therefore, to minimise bias in the measurement of shell length, individuals with damaged shells were discarded. Because *B. brandaris* lacks external sexual dimorphism, individuals were subjected to gender identification after breaking and removing the shell: females by the presence of a vulva and capsule gland and males by the presence of a penis and the absence of a capsule gland. The penises of both males and imposex-affected females were measured (PL) under a binocular microscope with a graduated eyepiece. Females were also inspected for any signs of sterility (either by occlusion of the vulva or splitting of the capsule gland).

Imposex incidence (I%) was expressed as the percentage of affected females in each sample. Imposex severity was quantified through the three following indices: Female Penis Length Index (FPLI = mean penis length of all females in the sample), including the zero values of aphyllid females; Relative Penis Length Index (RPLI = female mean penis length / male mean penis length * 100), including the zero values of aphyllid females (GIBBS *et al.*, 1987); Vas Deferens Sequence Index (VDSI = mean of the VDS stages exhibited by all females in the sample). The original VDSI scheme with six sequential stages (VDS 0 to VDS 5) proposed by GIBBS *et al.* (1987) was classified following the scoring system modified by STROBEN *et al.* (1992a). The VDSI is particularly useful whenever females

present aphyllid development of imposex (type b VDS stages) (STROBEN *et al.*, 1992b), which influences the calculation of imposex indices based on penis measurements (e.g. FPLI and RPLI). Recently, and after the completion of the present study, ABIDLI *et al.* (2011) and LAHBIB *et al.* (2011a) partially modified the pathways of imposex development in *B. brandaris* from Tunisian waters (by proposing VDS stages of the types a, b, d, d' and e), which however does not influence the calculation of the VDSI.

Statistical analysis

The sex ratios (F:M) of *B. brandaris* from the ten collecting sites were compared with parity (1:1) using the chi-square test (χ^2 -test). Analysis of variance (ANOVA) was employed to compare specimen size (SL) between sexes. Whenever ANOVA assumptions (normality of data and homogeneity of variances) were not met, the non-parametric Kruskal-Wallis test was performed.

As a preliminary approach, the relationships between the distance to the coastline and depth of the collecting sites and the imposex indices in *B. brandaris* (I%, FPLI, RPLI and VDSI) were analysed through regression analysis. Depth is a consequence of the sloping ocean floor, i.e. depth increases with increasing distance from the shore. However, since some collecting sites located at similar distances from the coastline displayed different depths (Table 2), the influence of both variables on the imposex indices was investigated. The linear function ($Y=a + bX$) was fitted to raw data and the degree of association between variables was assessed by the correlation coefficient (r).

Subsequently, principal components analysis (PCA) was used to generate a two-dimensional overview of the relatedness of the collecting sites by physical (distances and depths) and biological (imposex indices) variables. In order to better discern the influence of the shipping activity in the Gulf of Gabès, PCA was performed with the following variables: distance to the coastline, depth, distance to the closest commercial harbour (Sfax or Skhira), distance

to the commercial harbour of Sfax, distance to the oil terminal of Skhira, imposex incidence (I%) and severity (FPLI, RPLI and VDSI) in *B. brandaris*.

Statistical analyses were carried out following ZAR (1996), with significance level set at $p < 0.05$. ANOVA was performed using the software package SigmaStat® (version 3.5). PCA was performed after normalisation of data and using the software package Canoco for Windows (version 4.5).

RESULTS

Descriptive statistics of the samples and data on the imposex incidence and severity in *B. brandaris* are compiled in Table 2. A total of 584 individuals were caught at the ten collecting sites in offshore areas of the Gulf of Gabès (minimum=47 in S1; maximum=71 in S6). In the overall samples, females ($n = 353$) clearly dominated over males ($n=231$), leading to a female-biased sex ratio (1 F:0.7 M) significantly different from parity (χ^2 -test=25.07, $p<0.01$). Nevertheless, and although females outnumbered males in all collecting sites, only in S5 the sex ratio (1 F:0.6 M) was statistically unbalanced (χ^2 -test=4.02, $p<0.05$). Both sexes presented individuals with a broad size range (females: 47.9 – 80.3 mm SL; males: 46.4 – 77.9 mm SL). On average, females (64.2 ± 5.6 mm SL) were significantly larger (K-W: $H=19.162$, $p<0.001$) than males (62.5 ± 5.4 mm SL) (Table 2).

On the whole, of the 353 females analysed 117 were affected by imposex (I%=33.1). Imposex incidence varied depending on the collecting site, ranging from 0% in S10 (the only site without imposex-affected females) to 53.6% in S1. Besides the existence of 317 aphyllid females (89.8% of the females analysed), average female PL (2.8 ± 0.9 mm) was noticeably smaller than average male PL (11.7 ± 4.0 mm). In addition, female PL ranged from aphyllid females in S8, S9 and S10 to 3.9 ± 0.5 mm in S3, whereas male PL was reasonably similar among collecting sites (between 10.3 ± 3.4 mm in S2 and 13.3 ± 4.3 mm in S5). In terms of imposex severity in the overall collecting sites, the indices based

Table 2. Descriptive statistics of the samples and summary of imposex data in *Bolinus brandaris* from offshore areas in the Gulf of Gabès

Site	Dist.	Dpt.	Sex	N	SR (F:M)	SL (mm)	PL (mm)	F(%)	I(%)	FPLI	RPLI	VDSI	VDSr	VDS stages (%)				
														0	1	2	3	4
S1	5.9	20.0	F	28	1:0.7 ^{ns}	64.7±4.4	3.7±0.6	59.6	53.6	0.8	7.1	1.6	0-4	46.4	7.1	7.1	17.9	21.4
			M	19		61.6±4.6	11.3±3.4											
S2	6.7	28.0	F	34	1:0.6 ^{ns}	63.2±5.3	2.3±0.5	61.8	52.9	0.6	5.8	1.7	0-4	47.1	5.9	2.9	17.6	26.5
			M	21		59.3±3.4	10.3±3.4											
S3	9.4	30.6	F	32	1:0.6 ^{ns}	62.6±4.5	3.9±0.5	64.0	46.9	0.5	4.6	1.2	0-4	53.1	15.6	6.3	12.5	12.5
			M	18		60.5±4.4	10.9±3.3											
S4	13.4	35.3	F	36	1:0.7 ^{ns}	59.7±3.7	3.0±0.7	59.0	41.7	0.3	2.7	1.1	0-4	58.3	8.3	5.6	16.7	11.1
			M	25		60.8±6.7	11.3±3.7											
S5	13.9	30.0	F	36	1:0.6*	65.2±6.3	2.8±0.8	64.3	44.4	0.7	5.3	1.5	0-4	55.6	2.8	5.6	11.1	25.0
			M	20		63.7±3.3	13.3±4.3											
S6	17.5	40.0	F	43	1:0.7 ^{ns}	61.4±6.5	1.5±0.1	60.6	32.6	0.1	0.9	0.6	0-4	67.4	18.6	9.3	0.0	4.7
			M	28		60.9±5.8	11.0±3.8											
S7	22.1	40.8	F	31	1:0.6 ^{ns}	67.5±4.3	1.2±0.2	60.8	35.5	0.1	0.9	0.5	0-4	64.5	29.0	0.0	0.0	6.5
			M	20		61.6±4.9	11.2±4.6											
S8	25.8	42.0	F	37	1:0.6 ^{ns}	65.1±3.3	-	62.7	21.6	0.0	0.0	0.2	0-2	78.4	18.9	2.7	0.0	0.0
			M	22		63.2±5.7	12.0±4.6											
S9	29.6	54.0	F	35	1:0.8 ^{ns}	64.7±4.7	-	54.7	14.3	0.0	0.0	0.2	0-3	85.7	11.4	0.0	2.9	0.0
			M	29		66.5±5.3	13.1±3.6											
S10	37.4	60.0	F	41	1:0.7 ^{ns}	68.2±5.6	-	58.6	0.0	0.0	0.0	0.0	0	100.0	0.0	0.0	0.0	0.0
			M	29		64.7±4.7	12.4±4.3											
Total			F	353	1:0.7**	64.2±5.6	2.8±0.9	60.4	33.1	0.3	2.6	0.8	0-4	66.9	11.6	4.0	7.4	10.2
			M	231		62.5±5.4	11.7±4.0											

Abbreviations: Dist., distance to the coastline (nautical miles); Dpt., depth (metres); SR, sex ratio; SL, shell length; PL, penis length; F(%), percentage of females; I(%), percentage of imposex-affected females; FPLI, female penis length index; RPLI, relative penis length index; VDSI, vas deferens sequence index; VDSr, vas deferens sequence range; ns, not significant ($p > 0.05$); *, significant ($p < 0.05$); **, highly significant ($p < 0.01$)

on penis length were $FPLI=0.3$ and $RPLI=2.6$, with both indices being lowest in S8, S9 and S10 ($FPLI=0$ and $RPLI=0$) and highest in S1 ($FPLI=0.8$ and $RPLI=7.1$). In all of the females analysed, imposex stages ranged between VDS 0 and VDS 4, corresponding to an average VDSI of 0.8 (ranging from VDSI = 0 in S10 to VDSI = 1.7 in S2). All females collected in S10 were imposex-free (VDS 0), the initial and intermediate imposex stages (VDS 1 and 2) were mainly found in farther and deeper sites (S6 to S9: 17.5 – 29.6 Nm and 40.0 – 54.0 m depth), whereas most advanced imposex stages (VDS 3 and 4) were mostly found in closer and shallower sites (S1 to S5: 5.9 – 13.9 Nm and 20.0 – 30.0 m depth). Sterile females (VDS stages 4+) were not detected in the study area (Table 2).

The linear regressions established between the distance to the coastline and depth of the collecting sites and the indices of imposex incidence (I%) and severity (FPLI, RPLI and VDSI) in *B. brandaris* are presented in Fig. 2. Both imposex incidence (Fig. 2A) and severity (Figs. 2 B-D) decreased markedly with increasing distance and depth of the collecting sites, as evidenced by the highly significant correlation coefficients ($r=0.86$ to 0.98 , $p<0.01$) obtained in all linear regressions. Higher correlations were registered for I% (distance: $r = 0.98$; depth: $r=0.97$; Fig. 2A) and VDSI (distance: $r=0.94$; depth: $r=0.92$; Fig. 2D), than for RPLI (distance: $r=0.88$; depth: $r=0.89$; Fig. 2C) and FPLI (distance: $r=0.86$; depth: $r=0.88$; Fig. 2B). In general, the highest imposex indices (I%, FPLI, RPLI and VDSI) were registered in closer and shallower collecting sites (S1 to S5: – 13.9 Nm and – 35.3 m depth), decreased steadily towards the transitional sites (S6 and S7: 17.5 – 22.1 Nm and 40.0 – 40.8 m depth), and reached lowest values in farther and deeper sites (S8 to S10: 25.8 Nm and – 42.0 m depth). Moreover, the only collecting sites with all aphyllic females ($FPLI=0$ and $RPLI=0$) were S8, S9 and S10 (Figs. 2B,C), and the only site with all imposex-free females ($I%=0$ and $VDSI=0$) was S10 (Figs. 2A,D).

The PCA confirmed the effects of distance to the coastline and depth of the collecting

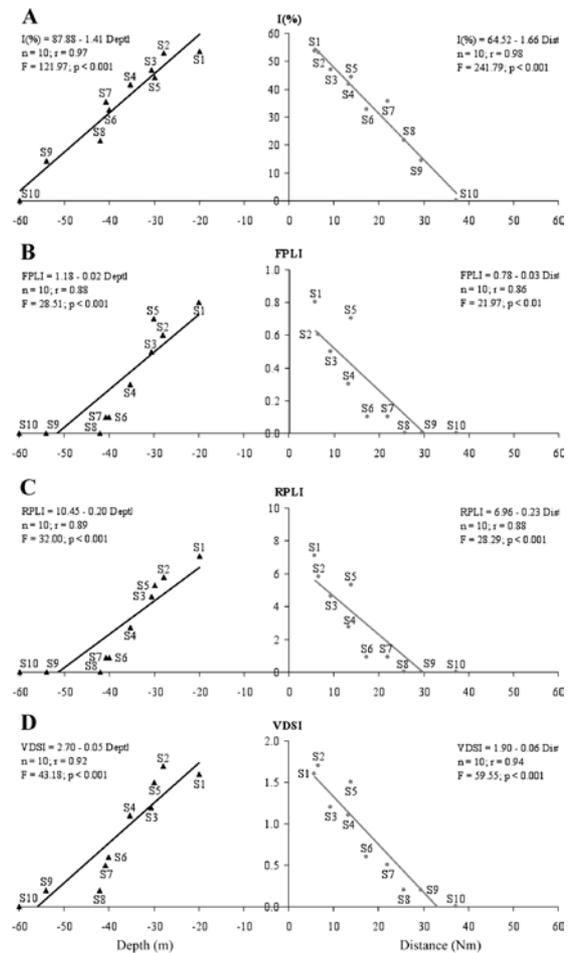


Fig. 2. Variation in the imposex indices of *Bolinus brandaris* according to the distance to the coastline and depth of the collecting sites in the Gulf of Gabès. A. Imposex incidence (I%). B. Female penis length index (FPLI). C. Relative penis length index (RPLI). D. Vas deferens sequence index (VDSI). For further details on the collecting sites see Fig. 1 and Table 2

sites, as well as provided helpful insights on the influence of the distance to the main harbours (commercial harbour of Sfax and oil terminal of Skhira) on the imposex indices of *B. brandaris* (Fig. 3). The PCA further evidenced that increasing distances (to the coastline and to the main harbours) and depths are closely related to decreasing incidences and severities of imposex. In addition, the projection of the sites S1 to S5 in separate axis relatively to sites S6 to S10 (once again with S6 and S7 assuming an intermediate position), highlights the distinctness of the imposex indices in *B. brandaris* from

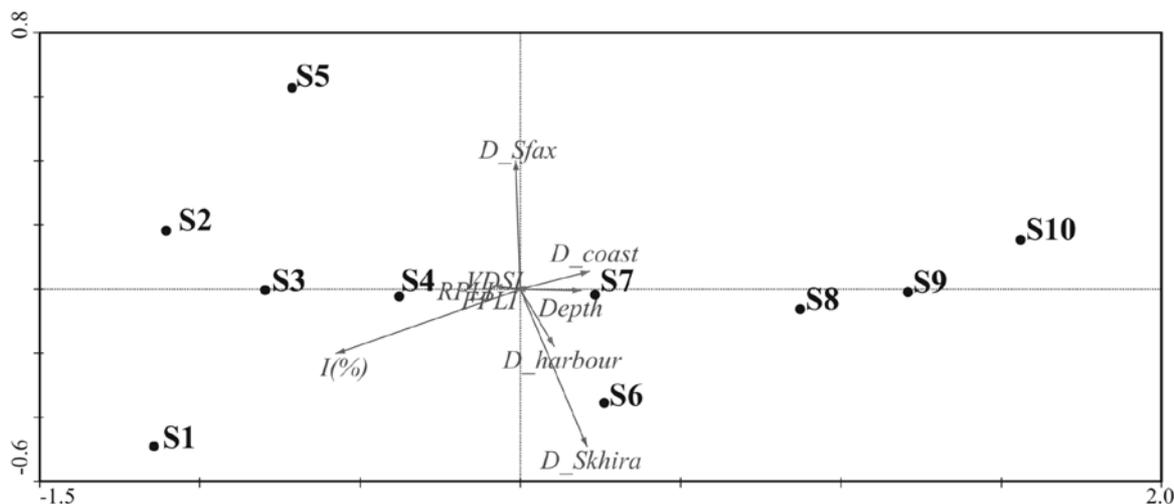


Fig. 3. PCA biplot showing the collecting sites of *Bolinus brandaris* in offshore areas in the Gulf of Gabès (based on normalised data, with 85.9% of the total variance explained by PC1 and 8.3% explained by PC2). Abbreviations: *D_coast*, distance to the coastline; *D_harbour*, distance to the closest commercial harbour (Sfax or Skhira); *D_Sfax*, distance to the commercial harbour of Sfax; *D_Skhira*, distance to the oil terminal of Skhira. For further details on the collecting sites see Fig. 1 and Table 2

those groups of sites. In particular, the smaller dispersion displayed by sites S8 to S10 denotes their lowest imposex indices, with the farthest position of S10 reflecting the absence of imposex in *B. brandaris* collected in this farther and deeper site. In every case, imposex incidence (I%) was the index that allowed for greater separation between collecting sites. Concerning the distances of the sites to the main harbours (Sfax and Skhira), the projection of their vectors in opposite quadrants of the ordination diagram reflects their distinct influence on the imposex indices registered at particular sites (Fig. 3). For instance, S5 is the closest site to the oil terminal of Skhira (17.5 Nm), which explains the relatively high imposex indices registered at this site (namely when considering only its distance to the coastline and depth). Similarly, S1 is the nearest site to the commercial harbour of Sfax (20.2 Nm) and where the highest values of I(%), FPLI and RPLI were recorded. Altogether, these findings support the importance of the near-shore shipping activity in the Gulf of Gabès, and particularly, the influence of the main commercial harbours (Sfax and Skhira, and respective navigation routes) on the imposex incidence and severity in *B. brandaris*.

DISCUSSION

Previous studies have analysed imposex in the purple dye murex from different coastal locations in Tunisia, particularly in the Gulf of Tunis and in the Bizerte lagoon (ABIDLI *et al.*, 2009a, 2011, 2012; LAHBIB *et al.*, 2011a). The present study reports upon the first data available on the imposex incidence and severity in *B. brandaris* from offshore areas in the Gulf of Gabès. Besides expanding the current knowledge on this topic, this study revealed that imposex in *B. brandaris* is a widespread phenomenon in this important fishing area in southern Tunisia.

On the whole, *B. brandaris* females from the Gulf of Gabès presented low to moderate imposex incidence (I%=33.1) and severity (FPLI=0.3, RPLI=2.6 and VDSI=0.8). It is well known that imposex might affect the reproductive potential and population dynamics of highly affected species, namely through male-biased sex ratios and female sterility (e.g. GIBBS & BRYAN, 1986; BAILEY *et al.*, 1995; GIBBS, 1996; SCHULTE-OEHLMANN *et al.*, 1997; SHI *et al.*, 2005). Fortunately, this is not the case of the studied populations of *B. brandaris* from the Gulf of Gabès. Firstly, the female-biased sex ratio registered in the whole collecting sites (1 F:0.7

Table 3. Comparison of imposex indices in populations of *Bolinus brandaris* from the southern Mediterranean Sea (Morocco and Tunisia)

Country	Location	Date	F(n)	SL (mm)	FPL (mm)	F(%)	I(%)	RPLI	VDSI	VDSr	Reference
Morocco	Tangier harbour	Mar-00 / Apr-01	7 ^a		5.6±1.3	53.8	100.0	54.6 ^a			LEMGHICH & BENAJIBA (2007)
	Martil	Mar-00 / Apr-01	36 ^a		0.4±0.9	34.0	33.3	4.6 ^a			LEMGHICH & BENAJIBA (2007)
	Azla	Mar-00 / Apr-01	26 ^a		0.5±0.9	32.9	30.8	4.2 ^a			LEMGHICH & BENAJIBA (2007)
	Amsa	Mar-00 / Apr-01	49 ^a		0.2±0.7	45.8	14.3	2.4 ^a			LEMGHICH & BENAJIBA (2007)
Tunisia	Bizerte lagoon	Feb-07 / Jan-08	558	55.8±2.2 ^b	1.6±0.8	48.5 ^a	93.9±3.8	18.0±9.4	3.3±0.5	0 – 4.3	ABIDLI <i>et al.</i> (2011)
	Bizerte lagoon (Menzel Abderrahmen)	Jun-10	17	59.1±4.5	1.9±1.4	56.7 ^a	100	17.6±13.2	3.4±0.9	2 – 4	LAHBIB <i>et al.</i> (2011a)
	Bizerte lagoon (Menzel Bourguiba)	Jun-10	12	58.1±4.1	2.6±1.3	54.5 ^a	100	22.9±11.3	3.9±0.4	3 – 4+	LAHBIB <i>et al.</i> (2011a)
	Bizerte lagoon (Lagoon Centre)	Jun-10	14	57.5±3.5	1.0±1.5	60.9 ^a	71.4	7.9±12.5	1.8±1.4	0 – 4	LAHBIB <i>et al.</i> (2011a)
	Bizerte lagoon (El Azib)	Jun-10	19	53.9±1.8	0	82.6 ^a	47.4	0	0.9±1.0	0 – 2	LAHBIB <i>et al.</i> (2011a)
	Bizerte lagoon (Menzel Jemil)	Jun-10	17	55.2±2.9	0	56.7 ^a	29.4	0	0.5±0.8	0 – 2	LAHBIB <i>et al.</i> (2011a)
	Small Gulf of Tunis	Feb-07 / Jan-08	519	56.7±2.2 ^b	1.1±0.2	49.4 ^a	66.2±11.4	11.5±3.9	2.4±0.4	0 – 4	ABIDLI <i>et al.</i> (2009a, 2011)
	Gulf of Gabès (offshore areas)	May-07 / Nov-07	353	64.2±5.6	2.8±0.9	60.4	33.1	2.6	0.8	0 – 4	Present study

Abbreviations: F(n), number of females; SL, shell length; FPL, female penis length; F(%), percentage of females; I(%), percentage of imposex-affected females; RPLI, relative penis length index; VDSI, vas deferens sequence index; VDSr, vas deferens sequence range; ^a, calculated from original data; ^b, sexes combined

M) indicates that sex-selective mortality due to female imposex is not expected to occur in these populations. In addition, like in most imposex studies with this species, female sterility was not detected ($VDSr=0-4$). The few reported cases of sterile females until now, either by occlusion of the vulva or by splitting of the capsule gland (VDS stage 5), are limited to *B. brandaris* collected in highly polluted sites along the Catalan coast (northwestern Mediterranean) (SOLÉ *et al.*, 1998; RAMÓN & AMOR, 2001).

Overall, the imposex indices registered in *B. brandaris* from the Gulf of Gabès are comparable and within some values reported for other populations of this species from the southern Mediterranean (Moroccan and Tunisian coasts) (Table 3). Taken as a whole, the imposex incidence ($I\% = 33.1$) was very similar to that observed in populations from the least affected location in the Bizerte lagoon (Tunisia) and from Martil and Azla (Morocco), being much lower than those reported for other locations along the Tunisian (remaining collecting sites in the Bizerte lagoon and Small Gulf of Tunis) and Moroccan (Tangier harbour) coasts, where the frequency of imposex-affected *B. brandaris* often reached 100% (i.e. with all females displaying signs of masculinisation) (Table 3). Concerning the imposex intensity in *B. brandaris*, the comparison of RPLI and VDSI values between locations reveals that the severity of the phenomenon is highly variable among collecting sites, reflecting different degrees of pollution by organotin compounds. Indeed, the imposex severity in *B. brandaris* from the Gulf of Gabès (RPLI = 2.6 and VDSI = 0.8) is within the range of values observed in the least affected locations monitored in the southern Mediterranean, namely along the Moroccan coast (Amsa, Azla and Martil for RPLI) and in the Bizerte lagoon (El Azib and Menzel Jemil for VDSI), but significantly lower than those reported for most other Tunisian and Moroccan populations of this species (Table 3).

Several previous studies detected gradients in the imposex indices of the biomonitoring species according to the distance to point sources of pollution by organotin compounds (mainly har-

bours) and/or to the intensity of shipping activity (e.g. BARROSO *et al.*, 2005; GÓMEZ-ARIZA *et al.*, 2006; RATO *et al.*, 2006, 2008; WIRZINGER *et al.*, 2007 and references therein). Similarly, the present study identified an evident inshore/offshore gradient in the imposex indices of *B. brandaris*, with a clear decreasing trend in both the imposex incidence ($I\%$) and severity (FPLI, RPLI and VDSI) with increasing distance of the collecting sites to the coastline and main harbours in the Gulf of Gabès. Firstly, the regression analysis detected highly significant negative correlations between the imposex indices and the distance to the coastline and depth of the collecting sites. Indeed, lower indices were observed in more distant and deeper sites, whereas higher indices were registered in shallower sites, closer to the coastline and harbours, reflecting the proximity to the point sources of organotin pollution (i.e. main harbours). Subsequently, the principal components analysis further confirmed this trend in the imposex indices of *B. brandaris* and highlighted the specific influence of the distance to the most important harbours in the Gulf of Gabès, either in number of calling vessels (commercial harbour of Sfax) or shipping tonnage (oil terminal of Skhira). In practice, this inshore/offshore gradient of imposex development in *B. brandaris* reflects marked spatial variation in the pollution level by organotin compounds, undoubtedly associated to the shipping traffic and shipping routes towards the major harbours in the Gulf of Gabès. Altogether, this phenomenon apparently results from the combined effects of the location of the point sources (main harbours) along the coastline and the higher shipping activity in inshore areas, and possibly also from stronger hydrodynamics in offshore areas that might promote a greater dispersion / dilution of the organotin compounds.

Among the great variety of gastropod species that occur in the Mediterranean Sea, the muricids *H. trunculus* and *B. brandaris* are certainly the most susceptible to develop imposex, even at very low ambient levels of organotin compounds. The high sensitivity of *B. brandaris* has been confirmed during laboratory experiments that successfully induced imposex development

after exposure to TBT (SANTOS *et al.*, 2006; ABIDLI *et al.*, 2012). Being unanimously considered a highly sensitive bioindicator of TBT pollution, *B. brandaris* has been employed in several biomonitoring studies throughout its distributional range (for a compilation of surveys involving this species, both in the Mediterranean Sea and in the Atlantic Ocean, see the Introduction section and VASCONCELOS *et al.*, 2010). Previous studies have demonstrated that spatial and temporal comparisons of imposex in *B. brandaris* should be cautiously interpreted, because of penis length variation throughout the reproductive cycle (RAMÓN & AMOR, 2002; VASCONCELOS *et al.*, 2011) and significant short-term variability in imposex indices (VASCONCELOS *et al.*, 2010). The overall results gathered in the present study, and especially the evident inshore/offshore gradient in the imposex indices in *B. brandaris* reflects a noticeable spatial variation (even at small scale) in the pollution level by organotin compounds in the Gulf of Gabès. In practice, this particularity recommends additional caution in the spatial and temporal comparisons of imposex incidence and severity in *B. brandaris*, which should carefully consider some factors that play a key role in the imposex indices, such as the distance (and subsequent depth) of the collecting sites to the coastline and to the point sources of organotin pollution (i.e. main harbours in the area).

CONCLUSIONS

The present study provides valuable baseline data for assessing the future evolution of imposex incidence and severity in *B. brandaris*, and subsequently the spatial and temporal trends of pollution by organotin compounds in the Gulf of Gabès. The IMO Antifouling System Convention, banning globally the use of organotin compounds (namely TBT) in antifouling paints on ships' hulls, entered into force in September 2008. Accordingly, declining TBT pollution and decreasing imposex indices in marine gastropods are expected to occur worldwide in the near future. In this context, the timing of sample collection in the present study (May–November 2007) is particularly useful for monitoring the local effectiveness of the IMO global ban, and hopefully, to confirm the short- to medium-term reduction of pollution by organotin compounds and imposex in *B. brandaris* from offshore areas in the Gulf of Gabès.

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Pojava imposeksa kod vrste *Bolinus brandaris* (Gastropoda: Muricidae) duž gradijenta priobalno/otvoreno more u zaljevu Gabes (južni Tunis, središnji dio Sredozemnog mora)

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SAŽETAK

U radu se iznose prvi podaci o pojavi imposeksa kod puževa uhvaćenih u otvorenom moru Tunisa. Imposeks je analiziran na jedinkama bodljikavog volka (*Bolinus brandaris*) prikupljenim na deset postaja u otvorenom moru zaljeva Gabes (južni Tunis, središnji dio Sredozemnog mora). Ukupno su analizirane 584 jedinke, a omjer spolova je bio u korist ženki (1 Ž: 0,7 M). Od 353 analizirane ženke, 117 ih je bilo pogođeno imposeksom.

Općenito, učestalost imposeksa kod ženki je bila niska do osrednja ($I\%=33.1$) i jačina ($FPLI=0.3$, $RPLI=2.6$ i $VDSI=0.8$). Studija je pokazala da je pojava imposeksa raširena u zaljevu Gabes te je utvrđen jasan gradijent pojavnosti imposeksa kod *B. brandaris* na relaciji priobalno/otvoreno more. Regresijskom analizom otkrivene su vrlo značajne negativne korelacije između pojavnosti imposeksa, udaljenosti od obalne linije te dubine na kojoj se uzorkovalo. Analiza glavnih komponenti (PCA) istaknula je određeni utjecaj udaljenosti od glavnih luka, i to ovisno o broju plovila (komercijalna luka Sfax) ili brodske tonaže (naftni terminal Skhira). Ovaj priobalno/otvoreno more gradijent razvoja imposeksa odražava značajne prostorne varijacije kod onečišćenja organskim spojevima kositra povezanih s pomorskom djelatnošću. Ova studija pruža vrijedne temeljne podatke za buduću procijenu razvoja imposeksa kod vrste *B. brandaris*, te prostorno i vremenskih trendova onečišćenja organskim spojevima kositra u otvorenom moru zaljeva Gabes.

Ključne riječi: *Bolinus brandaris*, imposeks, biomonitoring zagađenja, gradijent priobalno/otvoreno more, zaljev Gabes, južni Tunis

