

IMPOSEX IN THE MARINE NEOGASTROPOD *HEXAPLEX TRUNCULUS* FROM TUNISIAN COASTS: GEOGRAPHICAL DISTRIBUTION AND DEVELOPMENT INTENSITY

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HEXAPLEX TRUNCULUS
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ABSTRACT. – The superimposition of male sexual characteristics in neogastropod females, or imposex, was used as a reliable bioindicator for the evaluation of sea water tributyltin (TBT) contamination. We recorded in Tunisian coasts the first observations of this phenomenon in *Hexaplex trunculus* in 16 stations, among 19 investigated sites, from Bizerta to Djerba. Imposex indices revealed a frequency of 100% in high shipping activity areas such as the Bizerta channel, Menzel Bourguiba, Menzel Abderrahmen, NPK Sfax, Sfax Harbour and the Adjim channel, with a VDS index value varying between 4 and 5 in the Cement factory station, generating a sterility of 8%, while the RPL index values oscillated between 29.96 and 69.13. In the other stations, the imposex rate varied from 0 to 93% with a variable VDS index of 0 to 3.32 and an RPL index ranged from 0 to 8%. A classification (tree clustering), of all the stations where this anomaly was found, taking into account the female length of the penis and VDS index, enabled us to highlight three groups of sampling intensity of imposex.

INTRODUCTION

Gastropods are invertebrates most affected by organotin compounds such as tributyltin (TBT). According to Shulte-Ohlman *et al.* (2000) more than 150 species of proso-branches are sensitive to these compounds. Generally used as a biocide in antifouling paints, TBT induces abnormalities in the gastropod female sexual apparatus at a very low concentration of 1 to 2 ng/l of water (Bryan & Gibbs 1991). This phenomenon, or imposex (Smith 1971), or pseudo-hermaphroditism (Jenner 1979, Fioroni *et al.* 1991) is especially a genital disorder due to a hormonal imbalance whereby the females of gonochorist gastropods develop male sex organs, particularly a penis and/or a *vas deferens* (Spooner *et al.* 1991). Observations of imposex in *Hexaplex trunculus* were first made outside Tunisia, by Martoja & Bouquegneau (1988) and later by Axiak *et al.* (1995), El Hamdani (1998), Terlizzi *et al.* (1998), Rilov *et al.* (2000), Pellizzato *et al.* (2004) on the coasts of Malte, Spain, Italia, and Israel. The morphological aspects of this phenomenon in *H. trunculus* were described by Axiak *et al.* (1995) and Terlizzi *et al.* (1999). However, these authors clarified the last imposex stages of the classification as proposed by Stroben *et al.* (1992) for *Hinia reticulata*.

In Tunisia, imposex was detected by Lahbib *et al.* (2004a) for the first time in 2002 in the gastropod *Hexaplex trunculus*. The phenomenon was observed in all individuals in the Bizerta Channel with a frequency progressively decreasing when moving away from this site towards Bizerta Lagoon.

The aims of this study were to give the geographical distribution of imposex in *H. trunculus* from Tunisian waters and to assess the development intensity of a penis and / or a *vas deferens* in each station. Data were gathered through a statistical study, to highlight the stations along the Tunisian littoral most affected by imposex and thus most polluted by stannic compounds (TBT).

MATERIAL AND METHODS

Specimens of *H. trunculus* with a shell length from 40 to 60 mm were collected between March and July 2004 in 19 stations along the Tunisian coast from Bizerta to Djerba (Fig. 1). In the laboratory, the animals were killed by freezing and preserved until analysis. Shell lengths were measured. After thawing, the shell was broken and the soft parts of the gastropod were carefully removed for observation. The mantle was cut longitudinally through the hypobranchial gland to expose the pallial oviduct. The animals were observed under a low-power stereomicroscope. Sex was determined by presence or absence of the capsule gland and the vagina. For each station, imposex intensity was assessed by the following indices:

Imposex frequency:

$$IF = \frac{\text{Abnormal female number}}{\text{Total number of females}} \times 100$$

Relative penis length index (Minchin *et al.* 1996):

$$RPL \text{ index} = \frac{\text{Average length of female penis}}{\text{Average length of male penis}} \times 100$$

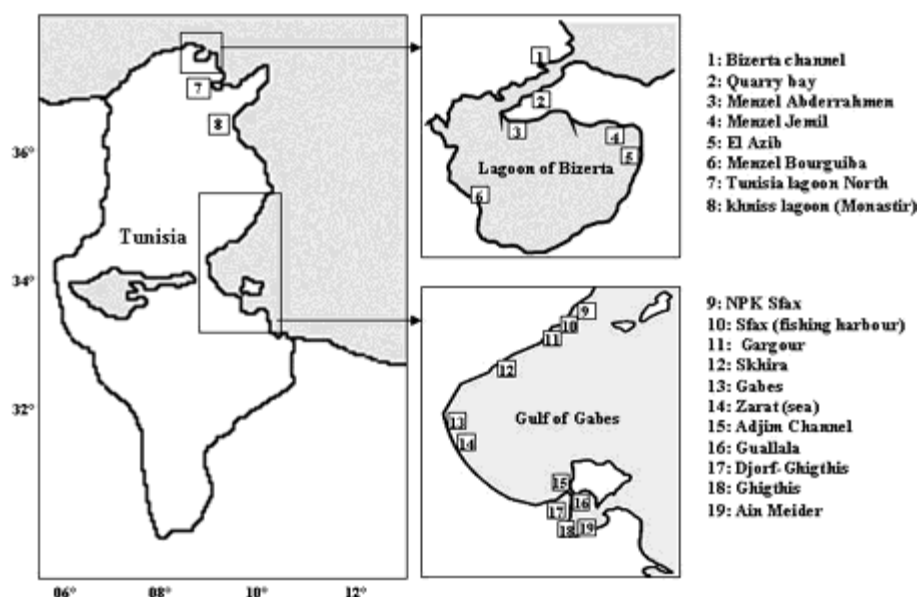


Fig. 1. – Sampling sites of *Hexaplex trunculus* along Tunisian Mediterranean waters.

Relative penis size index (Gibbs *et al.* 1987):

$$\text{RPS index} = \frac{(\text{Average length of female penis})^3}{(\text{Average length of male penis})^3} \times 100$$

Vas deferens sequence index (Axiak *et al.* 1995):

$$\text{VDS index} = \frac{\text{Sum of imposex stage values of all females sampled}}{\text{Total number of females}} \times 100$$

The RPS Index was determined by measuring the length of the male and female penis in order to carry out a comparison with previous studies. VDS index was calculated from results obtained from thorough observations of the genital tract development state using a low-power stereomicroscope. The different stages of imposex were determined according to the general scheme proposed by Stroben *et al.* (1992) in *Hinia reticulata*, as partially modified by Axiak *et al.* (1995) in their study on imposex in *H. trunculus*. At least, the classification proposed by these previous authors was applied.

Zarat, 170 km further south, the rates decreased from 100% to 93% at Gargour, 84.37%, at Skhira, 51.51% in the Gabes Gulf and to 0% in the Zarat station. With special regard to the stations situated in the semi-closed sea of Boughrara (Ain Meider, Guallala, Gighis-Djorf and Gighis), the values were relatively low (Fig. 2). As for Bizerta Lagoon stations, the recorded rates decreased when moving away from the communication channel: 66.6% at Menzel Jemil and 62% at El Azib. In the Tunis northern Lagoons and Khniss, the rates of imposex were 34.37% and 0%, respectively.

The RPL index values (Fig. 2) recorded in the most affected areas (seven in total), varied between 29.96% in Menzel Bourguiba and 69.13% in the cement factory station. In specimens collected from Sfax fishing harbour, a relatively low value of the RPL index was calculated, 33.08%, compared to that calculated for specimens collected in Bizerta Channel (69.13%). This phenomenon could be explained by the mean size of male penis that

RESULTS

Of the 19 stations studied, 16 exhibited the imposex phenomenon in *H. trunculus* females, the intensity of the phenomenon varied from site to site. The more affected areas with a frequency of 100% were located from north to south: Bizerta Channel (cement factory and Quarries Bay), Menzel Abderrahmen, Menzel Bourguiba, NPK Sfax factory, Sfax fishing harbour and Adjim Channel. The imposex rate was lower in the other stations. Leaving the fishing harbour of Sfax towards the station of

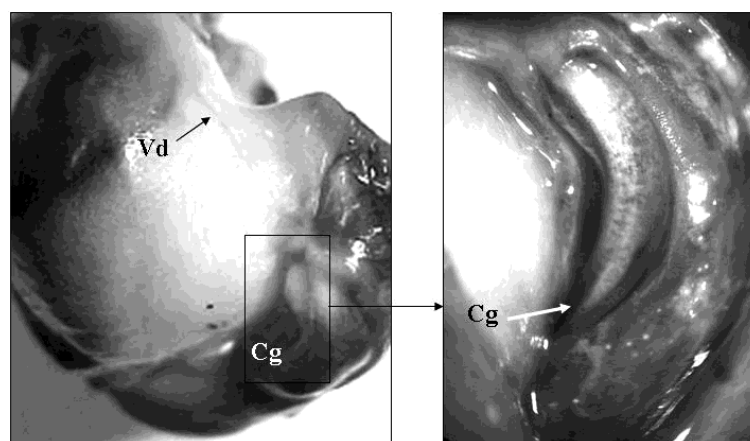


Fig. 2. – Sterility of *Hexaplex trunculus* due to the fissure in the gland of the capsule. Vd: vas deferens, cg: capsule of gland.

Table I. – Summary of imposex results of *Hexaplex trunculus* from Tunisian waters. F.N, females number. M.N, males number. T, total number. SR, sex ratio. IF, imposex frequency. RPSI, relative penis size index. RPLI, relative penis length index. VDSI, *vas deferens* sequence index. VDSS, *vas deferens* sequence stages. FPL, female penis length. MPL, male penis length. ST, sterility

Stations	F.N	M.N	SR(f/m)	IF %	RPSI	RPLI	VDSI	VDS	FPL	MPL	ST %
Ain Meider (AM)	28	37	0.76	3.57	0.000	0.00	0.04	0 - 1	0.00	9.05 ± 1.53	0
Guallala (GAL)	27	26	1.04	3.70	0.000	0.05	0.04	0 - 1	0.0065 ± 0.03	14.28 ± 4.37	0
Ghigthis (GH)	81	61	1.32	0.00	0.000	0.29	0.60	0 - 4	0.071 ± 0.19	13.14 ± 3.94	0
Ghigthis-Djorf (GD)	81	62	1.33	6.17	0.000	0.09	0.12	0 - 3	0.016 ± 0.07	9.16 ± 1.97	0
Adjim channel (AC)	37	18	2.00	100.00	3.236	31.87	4.09	3 - 4.7	5.8 ± 3.26	18.2 ± 5.39	0
Zarat (Z)	30	33	0.91	0.00	0.000	0.00	0.00	0	0.00	13.86 ± 4.6	0
Gabes (G)	33	46	0.72	51.51	0.016	5.48	1.82	0 - 4	0.81 ± 0.25	14.78 ± 3.25	0
Skhira (SK)	32	38	0.84	84.37	0.033	6.96	2.50	0 - 4	1.06 ± 1.58	15.37 ± 5.46	0
Gargour (GA)	72	65	1.11	93.00	0.050	8.00	3.32	0 - 4	0.83 ± 0.56	10.29 ± 3.31	0
Sfax fishing harbour (SH)	32	14	2.28	100.00	3.620	33.08	4.21	4 - 4.7	8.16 ± 2.91	24.72 ± 3.62	0
NPK Sfax (NP)	30	51	0.59	100.00	17.000	55.38	4.00	4 - 4.3	5.63 ± 2.38	10.31 ± 3.73	0
Khmiss lagoon (KL)	32	10	3.2	0.00	0.000	0.00	0.00	0	0.00	17.49 ± 1.53	0
Tunisia Lagoon north (LN)	32	15	2.13	34.37	0.000	0.47	0.21	0 - 2	0.08 ± 0.35	17.14 ± 2.16	0
El Azib (EA)	21	29	0.72	62.00	0.002	2.52	0.38	0 - 3	0.37 ± 0.38	14.33 ± 5	0
Menzel Jemil (MJ)	66	75	0.88	66.60	0.001	2.05	0.31	0 - 4.3	0.28 ± 0.44	13.56 ± 5.85	0
Menzel Bourguiba (MB)	27	29	0.93	100.00	2.690	29.96	3.97	3 - 4.7	3.54 ± 2.82	12.25 ± 3.17	0
Menzel Abderrahmen (MA)	29	33	0.88	100.00	5.130	37.16	3.73	3 - 4.7	5.18 ± 2.31	14.64 ± 4.6	0
Quarry Bay (QB)	37	26	1.42	100.00	28.470	65.79	4.09	4 - 4.3	7.77 ± 3.02	11.81 ± 4.36	0
Bizerta channel (BC)	37	63	0.59	100.00	33.030	69.13	4.24	4-5	8.23 ± 3.53	12.05 ± 5.44	8

was larger for specimens collected in the first station than those collected in the cement factory site.

Determination of *vas deferens* stages (Table I) showed that, with the exception of the stations of Zarat and Khmiss, where imposex was absent, the other sites can be divided into three classes. The first, from 0 to 1 (presence of a penis bud or the most generally case, a *vas deferens* portion), indicated that individuals were hardly affected by imposex. The second class, from 0 to 4, showed an acceleration of the phenomenon, and especially, the third class, from 4 to 5, the most affected by the abnormality, was in the stations where the imposex rate was 100 %. The VDS index 5 was the highest imposex stage recorded in this study. It was found in the cement factory station with a frequency of 8% causing sterility in females as indicated by the split capsule gland (Fig. 2).

The VDS index and the female length of the penis were considered as the most appropriate variables for the evaluation of the imposex intensity. The two variables were studied separately to obtain the best representation of the cluster analysis. The tree clustering stemming from the VDS index shows 2 groups of stations:

* The first group comprises similar stations from the point of view of the degree of development of the *vas deferens*. These stations are populated by females with completely developed *vas deferens*, and located in northern, central and southern Tunisia: the Bizerta Channel, Bay of Quarries, Menzel Abderrahmen, Menzel Bourguiba, NPK of Sfax, the fishing port of Sfax and the Adjim Channel. The second subgroup comprises the stations located in the gulf of Gabes, Gargour, Skhira and Gabes. Although the sampled stations were separated between them, by a

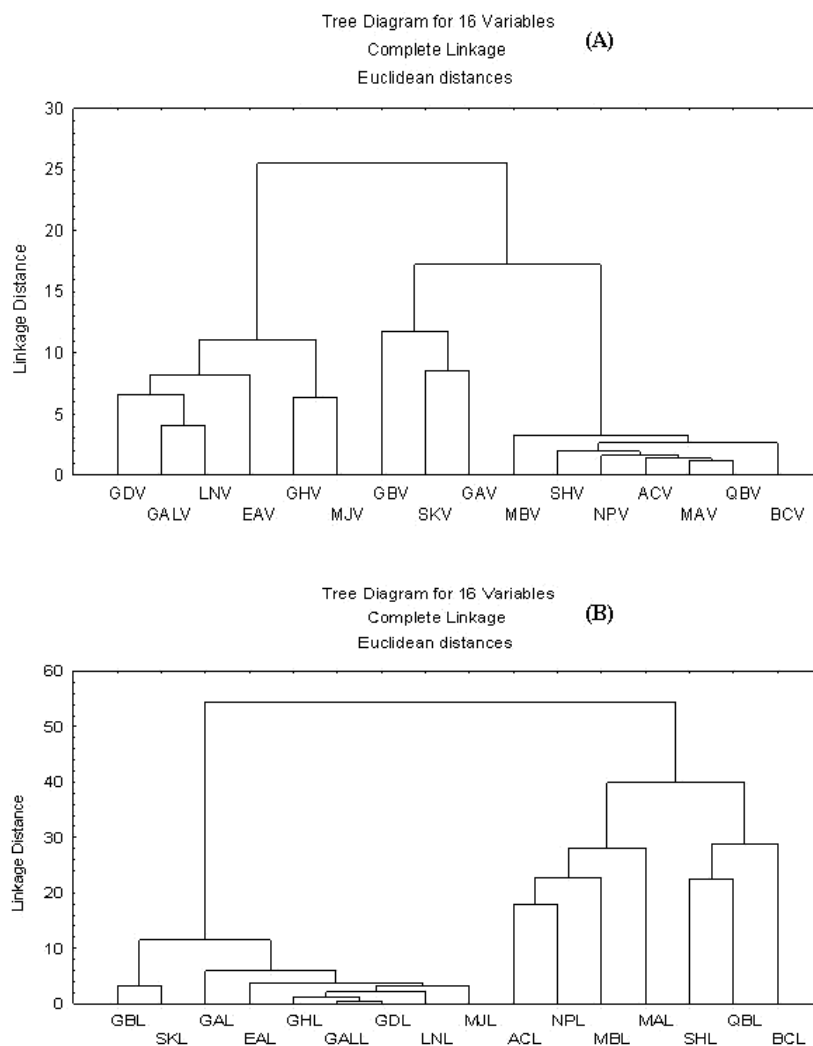


Fig. 3. – Distribution of all stations with the intensity of development for the *vas deferens* index VDSI (A) and the length of the female penis (B).

distance of 70 km approximately, the *H. trunculus* individuals collected in each station exhibited similar development of *vas deferens*.

* The second group comprises 6 stations where development of *vas deferens* in *Hexaplex trunculus* is less conspicuous than that observed in abnormal specimens from other stations. A first subgroup comprised Menzel Jemil and Ghigtis and a second El Azib, the northern lake of Tunis, Gallala and Ghigtis Djorf.

Cluster analysis was based on the penis length data (Fig. 3) and showed a first grouping comprising the same stations as those observed in the subgroup (Fig. 3). Indeed, these 7 stations can be divided into two groups. The first group includes Bizerta Channel, Bay of Quarries and the fishing harbour of Sfax. The second subgroup comprises Menzel Abderrahmen, Menzel Bourguiba, NPK of Sfax and the Adjim Channel.

The group comprising Menzel-jemil, the northern lake of Tunis, Ghigtis, Ghigtis Djorf, Gallala, El Azib and Gargour, forms a homogeneous population of *Hexaplex* compared to those noted in Skhira and Gabes stations.

DISCUSSION

Imposex intensity varied from site to site in relation to the maritime activity and the size of boats. The maximal value of the RPS index recorded in this survey (33.03%) was lower than the values reported for the same species in Italy by Pellizzatto *et al.* (2004) in the Venice lagoon (RPSI = 36.2%), Terlizzi *et al.* (2004) in the Italian marine reserve of Capo Rizzuto (RPSI = 50.1%), Terlizzi *et al.* (1998) along the Italian coasts (RPSI = 77.2%) and in Malta, in the Ta' Xbiex locality (Eastern Maltese coasts) by Axiak *et al.* (2003) (RPSI = 98.1%). These results could indicate that TBT concentrations in Tunisian waters, including those characterized by an intense maritime traffic, with boats over 25 m long as in the Bizerta Channel, are lower than those of Italian and Maltese waters.

According to Ramon *et al.* (2001), the RPL and RPS indices cannot be applied to measure imposex intensity in the Muricid *Bolinus brandaris* due to the seasonal changes in the size of the male penis depending on the reproductive cycle. *Hexaplex trunculus* was found to be

sexually active almost all the year round (Lahbib *et al.* 2004b) and, in order to avoid any possible variation in penis size related to the reproductive cycle, the animals were only collected at the end of June and in July, a period in which the sexual activity was generally lower (Lahbib *et al.* 2004b).

The use of the VDS index alone did not permit a better evaluation of imposex intensities in highly polluted sites like the Bizerte cement factory, the Quarries Bay, Menzel Abderrahmen, Menzel Bourguiba, NPK Sfax factory, the Sfax fishing harbour and the Adjim Channel. In these stations, the VDS index values were similar (4.24; 4.09; 3.73; 3.97; 4; 4.21 and 4.09 respectively) whereas the average lengths of the female penis were 5.8, 8.18, 5.71, 3.67, 5.44, 7.77 and 8.33 mm. According to Sole *et al.* (1998), this difference can be related to the variable concentration of the pollutant in the marine environment. So it is necessary to assess the imposex intensity by taking the VDS index and the average length of the penis in females into account.

For instance, Strand & Jacobsen (2002), who had studied imposex of *Buccinum undatum* and *Neptunea antiqua*, pointed out that the highest values of VDSI are observed in the stations with a high frequency of imposex. These authors have measured the stannic compound content in the body tissue of *Buccinum undatum* and found that the highest concentrations were measured within the specimens collected in the stations with a high rate of imposex (33 to 89%). According to the above authors, the tin concentrations recorded in the tissue of *Buccinum undatum* (9.4 to 24.9 ng Sn/g wet wt) were the highest in specimens collected in areas with the highest imposex rate.

The cluster analysis based on the female length of the penis and the VDS index data made possible to highlight 7 stations greatly affected by imposex, namely the Bizerte Channel where the sterility rate is 8%. This group indicates that the six stations where at the moment there are no cases of sterility (bay of the Quarries, fishing harbour of Sfax, Menzel Abderrahmen, Menzel Bourguiba, the NPK of Sfax and the Adjim Channel) are part of the same group as the station of the Bizerte Channel and can thus be considered as endangered.

Amongst the stations analysed, we found three stations with an intermediate stage of contamination: Gabes, Skhira and Gargour. The latter station of Gargour is intermediate between the lightly contaminated sites (Menzel jemil, El Azib, the northern Lake of Tunis, Gigthis Djorf, Gigthis, Guallala) and the two moderately contaminated sites (Gabes and Skhira).

Regular monitoring of the imposex rate and the intensity of the development of the male genital tract in females of *H. trunculus*, as well as the determination of TBT, DBT and MBT concentrations would make possible to assess the impact, over a period of time, of antifouling paints on these marine organisms along Tunisian coasts and to establish a relationship between the stannic com-

pound content in the water, in the tissues; and the intensity of imposex.

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