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First record of butyltin body burden and imposex status in *Hexaplex trunculus* (L.) along the Tunisian coast

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Abstract:

We have assessed for the first time the current status of tributyltin (TBT) contamination in *Hexaplex trunculus* along Tunisian coastal waters. Two sampling campaigns have been performed in July 2004 and July 2007 at seven different sites. The snails were analyzed for imposex status, *i.e.* Imposex incidence, Relative Penis Length Index (RPLI) and Vas Deferens Sequence Index (VDSI), followed by the quantification of butyltins in their tissues. Imposex was detected in six populations from the 2004 samples and in five populations from the 2007 samples. The imposex incidence ranged from 0 (no imposex) to 100%, the RPLI from 0 to 56.7 and the VDSI from 0 to 4.4. TBT was also detected in six populations in 2004 and five populations in 2007. The concentrations ranged from 1.5 to 73.7 ng Sn g^{-1} dw in 2004 samples and from 1.2 to 23.9 ng Sn g^{-1} dw in 2007 samples. Temporal comparison of the data indicates some evidence of imposex recovery in most affected populations. Improvements in reduced body concentrations of TBT in both sexes were also seen in most stations. These TBT concentrations were only significantly correlated to imposex indices in samples from 2007 ($r > 0.8$, $n = 7$, $p < 0.05$). The present results will serve as a reference for long-term monitoring of butyltin contamination in the Tunisian coast, where TBT restrictions were not yet implemented.

Keywords: Tributyltin - TBT - Hexaplex trunculus - Imposex - Tunisia

1. Introduction

Imposex, the superimposition of male sexual features (i.e. penis and vas deferens) in female gastropods, has now been widely recognized. It was reported to occur in 195 gastropod species worldwide.[1] This deformity is specifically caused by tributyltin (TBT), a main ingredient of marine antifouling paints, at an ambient concentration of just a few nanograms per litre.[2,3]. Besides TBT, some other stressors such as copper [4], nonylphenol [5], polychlorinated biphenyls (PCBs) and aroclor [6] are also suspected to cause imposex. However, some authors reported that imposex may be a natural phenomenon[7,8]. The effects of imposex vary depending on the species. In some cases it does not impair reproduction, but in some others it can lead to population decline, as a consequence of sterility and reproduction failure.[9] Because imposex is a sensitive biomarker of TBT exposure and is easy to quantify, it has been widely adopted for monitoring TBT contamination and assessing the effectiveness of TBT control measures in many coastal environments.[10-13].

The Tunisian coast spreads over 1300 Km and include seven commercial ports. These ports shelter 6641 boats per year (passengers, car-ferries, roll-on-roll-off, bulk carriers, containers, oil tankers and gas tankers) with 5809 foreign boats coming from 84 locations notably from Italy (1827 boats), Malta (1034 boats) and Turkey (868 boats). This fleet insures a number of 13282 yearly commercial travels over the Tunisian coast. Besides this, we found 57 fishing harbours, namely 10 big harbours hosting trawlers, tuna boats, sardine boats and artisanal boats, and 47 small harbours hosting only artisanal boats. Although a large amount of TBT is anticipated to leach from hulls of these boats all over the Tunisian costal waters, especially in sites with high boating traffic, the assessment of butyltin contamination was very scare and to our best knowledge the only study on this subject has been undertaken by Mzoughi *et al*.[14], who studied organotin distribution in marine sediments and mussel tissues of the Bizerta Lagoon. Until now, *H. trunculus* from the Tunisian coastal waters has only been subjected to studies dealing with imposex analysis, [15-17] and the assessment of butyltin contamination has never been undertaken. Outside Tunisia, studies on imposex and butyltin contamination are also very scarce all over the Northern African Mediterranean coastal waters. The only study on this subject was reported by Lemghich *et al.*[18], who surveyed the occurrence of this deformity in three gastropod species (*H. trunculus, Stramonita haemastoma* and *Bolinus brandaris*), however without analysing butyltin contamination. On the contrary, studies on imposex and butyltins contamination in gastropods from the European Mediterranean countries, and particularly in *H. trunculus*, are well documented.[19-23] This reflects the general lack of baseline data on TBT contamination along the African Mediterranean countries, including Tunisia. Without such information, it is impossible to determine the effectiveness of any measures to be taken in the future to control the application of TBT-containing paints. For this reason, the present study aimed to provide the first data available on butyltin contamination in *H. trunculus*, together with imposex status in the Tunisian costal waters. In the future, this baseline information could be most useful for assessing the effectiveness of TBT restrictions in Tunisia.

2. Materials and methods

2.1. Sampling and biological analysis

Two sampling campaigns have been performed in July 2004 and July 2007 at seven stations along the Tunisian coast. These stations are: Cereal Office (Bizerta Channel), Menzel Jemil (Bizerta Lagoon), Small Channel (Tunis North Lake), Sfax fishing harbour, Gabès fishing farbour, Sea of Zarat and Gigthis- Djorf (Fig. 1). The number of sampled *H. trunculus* (adult specimens with 50-60 mm in shell length) per station ranged between 30 and 78. Data on boating traffic in 2004 was obtained from harbour authorities and in the annual report of the ministry of agriculture and aquatic resources (Table 1). Immediately after collection, individuals were taken to the laboratory and frozen. After thawing, specimens were measured for shell length (SL) using a vernier calliper to the nearest 0.1 mm and then crushed using a bench vice. The soft body was extracted from the shell and the sex was determined under a binocular microscope, based on the presence of a capsule gland and a vagina in females and the presence of a big penis in males. For each individual, the presence or absence of a penis was recorded. For those with a penis, the length of this organ was measured under a dissecting microscope using a calibrated eyepiece. Freezing and thawing increases penis measurements in dead specimens compared to live individuals (Y. Lahbib, personal observation). However, because this occurs in both sexes, it is supposed not to influence the calculation of imposex indices based on penis measurements. Relative Penis Length Index (RPLI), defined as the length of the female penis relative to that of the male,[24] was calculated using the following formula: [(penis length of each female/average male penis length) x 100]. The degree of imposex in each individual was also quantified using the Vas Deferens Sequence Index (VDSI).[16,19,25] The VDSI varies from 0 to 6, with stage 0 indicating no signs of imposex and stages 5 and 6 indicating female sterility. In stage 5, the genital papilla has been overgrown by tissues of vas deferens or by the splitting of the capsule gland. In stage 6, the capsule gland contains aborted egg capsules[25]. Between these extremes are imposex stages 1–4, whose diagnostic characteristics in *H. trunculus* have been listed and discussed in detail for their deviations from other species by Lahbib *et al.*[17]

2.2. Butyltins analysis

Five males and five females (50-60 mm in SL) were randomly selected from each station, the operculum was removed and the soft body was finely grinded in glass bottles using a T 18 basic Ultra-Turrax® disperser at 6000 rotations min^{-1}. Thereafter, tissues were freeze-dried, weighed and maintained at -20°C in the dark until analysis. Approximately 200 mg of freeze-dried ground tissue was used to quantify contents of tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT). The tissues digestion was investigated using a tetramethylammonium hydroxide solution (TMAH, 25%) in deionised water. Samples were simultaneously derivatised and extracted using sodium tetraethylborate ($NaBEt_4$) and n-hexane. The derivatisation reaction was controlled using a tripropyltin (TPrT) standard solution. The isolated hexane was concentrated by evaporation under a gentle stream of pure nitrogen. Samples were then cleaned up using solid phase extraction (SPE) Florisil cartridges and eluted with isooctane. A known volume of tetrabutyltin (TeBT) standard solution was added to verify the GC-FPD (gas chromatography flame photometric detection) performance throughout the analyses. The same procedure was applied for preparing external standards of TBT, DBT and MBT. The quantity and speciation of butyltins in each sample were determined using a Varian 3400 gas chromatograph. The system was equipped with a CP Sil 5 CB capillary column (inner diameter 320 µm, length 25 m, film thickness 0.25 µm) and a modified commercial flame photometric detector (by addition of a quartz burner to increase sensitivity). The sample was injected under splitless injection mode. The column temperature was maintained at 80°C for 2 min and then increased to 230°C at 8°C min^{-1}. Both injector and detector were maintained at 240°C. Hydrogen was used as the carrier gas at a flow rate of 12 ml min^{-1}. The flame photometer was equipped with a 610-nm filter selective for tin-containing compounds and operated using a hydrogen–air–nitrogen flame. The detection limits were 0.8 ng Sn g^{-1}dw for MBT, 0.7 ng Sn g^{-1}dw for DBT and 1.0 ng Sn g^{-1}dw for TBT. Analysis of a certified reference material (mussel tissue BCR 477, 6 replicates) using this procedure resulted in the following concentrations (as µg Sn g^{-1} dw): 1.03±0.04 for MBT, 0.75±0.03 for DBT and 0.86±0.04 for TBT. The certified values are 1.01±0.19, 0.79±0.06 and 0.90±0.08, for MBT, DBT and TBT, respectively. This procedure does not allow for the quantification of phenyltins that are not eluted at florisil purification stage.

2.3. Statistical analysis

Calculation of statistical tests was conducted using the software Sigmastat® 3.5 for Windows. The paired sample *t*-test was used to compare differences in RPLI, VDSI and tissue burden of TBT. Regression analysis was conducted to examine the relationship between TBT and the imposex indices (Imposex incidence, RPLI and VDSI) separately for 2004 and 2007 samples, using the curve estimation method with the software SPPS® 10.0 for Windows.

3. Results

3.1. Biological analysis

The results of imposex analysis are summarised in Table 2. In 2004, only one station out of seven was found free of imposex (Sea of Zarat). However, in the remaining stations, imposex was found at varied levels and degrees. The highest indices were recorded in Bizerta Channel, which is the only site where sterile females (14.3%) were found. These sterile females showed an occluded vulva and especially a split capsule gland. The stage 6 of imposex, characterized by the presence of aborted capsules, was not detected. High imposex incidence and degree were also recorded in the fishing harbour of Sfax, but without reaching sterility. In the four remaining stations (2, 3, 5 and 7), imposex indices were low to moderate. In general, the stations with imposex-affected *H. trunculus* showed an imposex incidence varying between 12.5% and 100%, a mean RPSI between 0.5 and 56.7, and a VDSI ranging between 0.3 and 4.4.

In 2007, only two stations showed significant variation in the incidence of imposex compared to 2004: the North Lake of Tunis, where a recovery from this deformity occurred, and the Gigthis Djorf, where imposex incidence increased markedly from 12.5% to 48.6% (*Chi*-square-test, $p < 0.05$). All the stations showed a reduction in the imposex degree, except at Gigthis-Djorf, where the phenomenon became more pronounced. The mean RPLI remained consistent in the fishing harbour of Sfax and Gightis-Djorf, but decreased significantly in the remaining stations (1, 2, 3 and 5) (paired *t*-test, $p < 0.05$). The Zarat Sea is still a sampling station free of imposex. The VDSI was the only index that showed significant variation in all the affected stations. With the exception of the increase in Gigthis-Djorf, this index decreased in all the remaining stations (paired *t*-test, $p < 0.05$). The maximum values were recorded in Bizerta Channel and Sfax fishing harbour, where VDSI reached stage 5 (sterility) at percentages of 4.7 and 1.6, respectively (Table 2).

3.2. Chemical analysis

Tributyltin concentrations ranged from 1.5 to 73.7 ng Sn g^{-1} dw in 2004 samples and from 1.2 to 23.9 ng Sn g^{-1} dw in 2007 samples (Table 3). In general, TBT concentration varied according to sampling site, sampling period, and sex. Snails showing higher TBT body burden were collected in Gabès fishing harbour and Bizerta Channel, both in 2004 and 2007, with the first site being the most polluted in 2004 and the second in 2007. In the Sea of Zarat (in both campaigns) and Tunis North Lake (only in 2007), tributyltin was below the detection limit (< 1 ng Sn g^{-1} dw). For most sites, TBT concentrations in the 2007 samples were lower than those from the 2004 samples (paired *t*-test, $p < 0.05$). The only station where TBT concentration increased markedly in 2007 was the fishing harbour of Sfax (Table 3). Females showed higher TBT concentration in both campaigns, except at Gabès fishing harbour in 2007, where males showed higher accumulation than females (paired *t*-test, $p < 0.05$).

Among the three species of butyltins, DBT was the compound most detected, while MBT was the least detected (Table 3). TBT accounted for 26–51% of the total butyltins and ranged from 1.2 to 73.7 ng Sn g^{-1}dw. In general, DBT was the predominant butlytin species in samples collected in both campaigns (41 to 62 % of total butyltins), ranging from 1.1 to 71.5 ng Sn g^{-1}dw. MBT accounted for 3–28 % of the total butyltins and varied between 1.2 and 37.9 ng Sn g^{-1}dw.

3.3. Correlation between imposex and tissue burden of TBT

Using the curve estimation method, the best fit was obtained with the logarithmic equation ($Y = a \ LnX + b$). In 2004, all imposex indices were not correlated with TBT concentrations ($p > 0.05$, $n = 7$). The r values were 0.6 ($p = 0.2$), 0.4 ($p = 0.3$) and 0.5 ($p = 0.2$), respectively for imposex incidence vs. TBT, RPLI vs. TBT and VDSI vs. TBT. In the opposite, in 2007 statistically significant ($p < 0.05$) positive correlations were obtained in all relationships. The r values were 0.9 ($p = 0.003$) for imposex incidence vs. TBT and 0.8 ($p = 0.03$) for both RPLI vs. TBT and VDSI vs. TBT.

4. Discussion

The temporal trend of imposex indices in *H. trunculus* collected along the Tunisian coast indicates some evidence of imposex recovery between 2004 and 2007. Based on the fact that TBT is the causal factor of imposex development in *H. trunculus*, this partial recovery probably results from the new generation of paints (without TBT) introduced into the Tunisian market, as well as in countries that adopted legal restrictions to the use of TBT-based in anti-fouling paints. Thus, the number of TBT-painted boats (local and foreign) certainly decreased between 2004 and 2007. In this study, only one station showed significant increase in the imposex incidence and degree (VDSI) between 2004 and 2007 (Gigthis-Djorf), probably because of the increasing number of boats near the harbours of Boughrara and Adjim. According to the annual report of the ministry of agriculture and aquatic resources, the number of fishing boats in these two harbours increased from 293 in 2004 to 474 boats in 2006. However, TBT body concentration recorded in females coming from this site was low and consistent between the two campaigns (1.5 and 1.8 ng Sn g^{-1} dw, respectively), suggesting that low TBT concentrations are able to induce imposex in *H. trunculus*. This observation is in agreement with data reported for this species by Axiak *et al.*[19], who detected the initiation of imposex at TBT concentrations lower than 1 ng Sn g^{-1} d.w. Concerning the RPSI, the highest degree of imposex found in the present study (RPSI = 18.2%) was lower than that reported for the same species along the Italian Coast by Terlizzi *et al.*[25] and Pellizzato *et al.*[22] (36.2% and 77.2%, respectively) and along the Maltese coast by Axiak *et al.*[19] (107.4%). In addition, high sterility was reported by these authors, reaching values of 76.9% in the Lagoon of Venice [22] and 100% in SM di Pagan [25], against only 14.3% of sterile females in the present study. This comparison suggests that imposex in Tunisia is less pronounced compared to these neighbouring locations in the Mediterranean Sea.

In males, it seems that TBT pollution may also impact secondary sexual organs. In fact, the penis length increased according to the degree of imposex (VDSI) in both campaigns. However, significant correlations were only obtained for 2007 samples ($r = 0.6$, $p = 0.2$ in 2004 and $r = 0.8$, $p = 0.04$ in 2007). Similarly, the male penis length also increased in function of the TBT concentrations, but only in 2007 samples ($r = 0.02$, $p = 0.8$ in 2004 against $r = 0.9$, $p = 0.01$ in 2007). In *Nucella lapillus*, Castro *et al.* [26] also reported significant increase of penis length in males exposed to TBTCl.

In females, the relationship between TBT concentration and the incidence/degree of imposex was opposite in the stations of Sfax and Gabès fishing harbours, namely in 2004 samples. In the first site, the concentration of TBT was quite low, but imposex indices were high. On the contrary, TBT concentrations in the second site were high, but imposex indices were low. This observation suggests that factors other than TBT could be implicated on the development of imposex and thus agrees with recent findings reported by Garaventa *et al.*[6]. In fact, these authors have experimentally developed imposex in *H. trunculus* with stressors other than TBT, such as polychlorinated biphenyls (PCBs) and aroclor. Besides this, Garaventa *et al.*[8] have found an initial development of imposex in *H. trunculus* specimens from museum collections collected in the period between 1845 and 1930, i.e. 30–115 years before the use of TBT. Altogether, these observations might infer new implications on the use of imposex in *H. trunculus* as a specific biomarker for TBT contamination. However, these findings should be supported by additional experimental investigations, namely the induction of imposex in healthy females (imposex-free) with suspected stressors (separately and together with TBT). In this research, particular care should be taken to avoid the risk of contamination by seawater and from the food provided to snails. This could eventually allow to confirm the ability of these stressors to initialise imposex in *H. trunculus* and consequently to validate the use of imposex in this species as a specific indicator of TBT contamination. In fact, Garaventa *et al*.[6] have studied a population already showing low levels of imposex, and consequently we ignore if the contaminants used are able to induce imposex. Significant increase of VDSI compared to the control (non treated), in snails narcotised with $MgCl_2$ and in narcotised snails injected with 100% ethanol have also been reported by these authors, being explained by the use of contaminated *Mytilus galloprovincialis* as food for reared snails.

By comparing the higher concentrations of TBT in female *H. trunculus* recorded in this work with similar studies that involved this species, we verify that Tunisian snails accumulated less TBT than those from the Sicilian coast (91 ng Sn g^{-1} dw),[21] the Lagoon of Venice (117 ng Sn g^{-1} dw), [22] and the Israeli coast (447.7 ng Sn g^{-1}dw).[20] The concentration of MBT recorded in this study was lower than that of DBT and TBT, probably reflecting a recent TBT contamination in the collecting sites. This predominance of DBT was also reported by other authors.[21,22] According to Chiavarini *et al*.[21], significant TBT concentrations and the accumulation of DBT and not MBT in *H. trunculus* from the Sicilian coast are likely to indicate that the studied area is submitted to a continue input of TBT. However, these hypotheses must be supported by the study of metabolic ability of *H. trunculus* to eliminate butyltin compounds. Tacking the sex of the specimens into consideration, it appears that females accumulate more TBT than males. The same finding was also reported in *H. trunculus* from the Lagoon of Venice.[22]

5. Conclusions

Imposex analysis of *H. trunculus* sampled along the Tunisian coast showed the occurrence of imposex in six stations out of seven, with an incidence reaching 100% in Bizerta Channel and Sfax fishing harbour. In 2007, decreasing degrees of imposex were observed in most stations, but the phenomenon is still pronounced in the more contaminated sites.

Chemical analyses showed high contamination in snails of Bizerta Channel, Gabès fishing harbour, and more recently in the Sfax fishing harbour, suggesting the existence of a continuous input of TBT in the marine environment. Nevertheless, the contradiction detected in the relationship between TBT concentration and the incidence/degree of imposex in Sfax and Gabès fishing harbours might provide new insights on the use of *H. trunculus* as an indicator species of TBT pollution in the marine environment.

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Table 1
Number and type of boats (in 2004) in the studied sites along the Tunisian coast

No.	Sampling Sites	Boats	Type
1	Bizerta Channel	1062	505 fishing boats (trawlers, sardine boats, crawfish boats, small artisanal boats), 557 commercial boats (oil tankers, gas tankers, passengers, containers) and navy boats
2	Menzel Jemil	60	Small artisanal fishing boats
3	Tunis North Lake	78	Small artisanal fishing boats
4	Sfax fishing harbour	736	Fishing boats (trawlers, sardine boats, tuna boats, small artisanal boats)
5	Gabès fishing harbour	108	Fishing boats (trawlers, tuna boats, small artisanal boats)
6	Zarat Sea	81	Small artisanal fishing boats
7	Gigthis-Djorf	20	Small artisanal fishing boats

Table 2

Summary of imposex analyses in *Hexaplex trunculus*. (*) denotes statistically significant differences (paired *t*-test or *Chi*-square test, $p < 0.05$) between the samples collected in 2004 and 2007 campaigns

No.	Site	Sex	N	Shell length (mm)	Penis length (mm)	RPLI	VDSI	% Imposex	% Sterile females
First campaign (July 2004)									
1	Bizerta Channel	M	17	52.2 ± 2.1	17.2 ± 5.7				
		F	21	52.7 ± 2.9	9.8 ± 3.9	56.7	4.4	100	14.3
2	Menzel Jemil	M	16	51.1 ± 1.1	15.0 ± 5.7				
		F	30	52.1 ± 2.1	0.2 ± 0.4	1.8	1.3	60.0	0
3	Tunis North Lake	M	13	52.8 ± 2.9	21.0 ± 2.3				
		F	17	54.4 ± 3.4	0.1 ± 0.3	0.5	0.3	17.6	0
4	Sfax fishing harbour	M	14	51.6 ± 1.4	24.7 ± 3.6				
		F	22	52.6 ± 2.1	8.2 ± 3.2	33.3	4.2	100	0
5	Gabès fishing harbour	M	38	53.3 ± 3.8	14.8 ± 2.8				
		F	25	55.5 ± 5.0	0.8 ± 1.8	5.6	1.6	48.0	0
6	Zarat Sea	M	31	53.3 ± 3.3	14.2 ± 4.5				
		F	29	54.4 ± 3.8	0	0	0	0	0
7	Gigthis Djorf	M	17	51.1 ± 1.8	10.1 ± 1.9				
		F	40	52.2 ± 3.1	0.0 ± 0.1	0.3	0.3	12.5	0
Second campaign (July 2007)									
1	Bizerta Channel	M	15	57.4 ± 2.1	19.8 ± 4.2				
		F	43	56.8 ± 2.8	8.2 ± 4.2	41.5*	4.2*	100	4.7
2	Menzel Jemil	M	13	51.9 ± 2.4	13.6 ± 1.9				
		F	38	51.5 ± 1.9	0.1 ± 0.2*	0.5*	0.7*	44.7	0
3	Tunis North Lake	M	11	52.4 ± 2.7	14.6 ± 3.1*				
		F	39	51.2 ± 1.8	0*	0*	0*	0*	0
4	Sfax fishing harbour	M	14	54.3 ± 3.3	22.4 ± 2.1				
		F	61	54.4 ± 3.3	7.4 ± 3.7	33.1	4.0*	100	1.6
5	Gabès fishing harbour	M	36	55.4 ± 3.3	20.4 ± 3.0*				
		F	42	56.6 ± 3.0	0.0 ± 0.2*	0.2*	1.0*	59.5	0
6	Zarat Sea	M	35	53.9 ± 3.9	15.6 ± 4.7				
		F	32	55.6 ± 4.2	0	0	0	0	0
7	Gigthis Djorf	M	12	51.5 ± 1.8	16.4 ± 3.2*				
		F	37	50.9 ± 1.5	0.0 ± 0.0	0.0	1.0*	48.6*	0

Table 3
Mean and standard deviation (n = 2) of butyltin concentrations (ng Sn g^{-1} dw) in male (M) and female (F) tissues of *Hexaplex trunculus*. (+) denotes statistically significant differences (paired *t*-test, $p < 0.05$) between sexes, (*) denotes statistically significant differences (paired *t*-test, $p<0.05$) between the samples collected in 2004 and 2007 campaigns, (dl): detection limit.

No.	Site	Sex	MBT		DBT		TBT		Total butyltins	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
First campaign (July 2004)										
1	Bizerta Channel	M	20.3	1.7	38.9	2.8	23.0	1.3	88.2	5.8
		F	37.9^{+}	1.1	59.3^{+}	0.4	35.5^{+}	0.4	132.7^{+}	0.1
2	Menzel Jemil	M	3.4	0.6	8.8	1.3	4.9	0.4	17.1	2.3
		F	<dl	-	8.2	1.0	6.4^{+}	0.2	14.5^{+}	1.3
3	Tunis North Lake	M	<dl	-	3.4	0.5	2.4	0.2	5.9	0.7
		F	1.2	0.3	5.2^{+}	0.3	5.1^{+}	0.6	11.5^{+}	1.3
4	Sfax fishing harbour	M	2.5	0.5	6.3	1.1	4.0	0.3	12.8	1.9
		F	2.0	0.7	7.1	1.8	4.2	0.8	13.1	1.8
5	Gabès fishing harbour	M	12.9	2.1	71.5	0.1	58.7	1.3	143.1	3.3
		F	8.2^{+}	0.2	61.1^{+}	4.5	73.7^{+}	4.0	143.0	8.6
6	Zarat Sea	M	<dl	-	1.4	0.2	<dl	-	-	-
		F	<dl	-	1.4	0.6	<dl	-	-	-
7	Gigthis Djorf	M	<dl	-	1.6	0.3	1.6	0.2	3.2	0.5
		F	<dl	-	1.1	0.3	1.5	0.1	2.6	0.4
Second campaign (July 2007)										
1	Bizerta Channel	M	2.1*	0.0	15.5*	1.5	18.7*	1.4	36.3*	2.9
		F	3.5*	0.5	20.0^{+}*	0.6	23.9^{+}*	0.6	47.4^{+}*	1.7
2	Menzel Jemil	M	<dl	-	2.1*	0.5	1.2*	0.0	3.2*	0.5
		F	<dl	-	2.3*	0.4	1.8*	0.0	4.1*	0.4
3	Tunis North Lake	M	<dl	-	1.5*	0.4	<dl	-	-	-
		F	<dl	-	1.2*	0.3	<dl	-	-	-
4	Sfax fishing harbour	M	3.0*	0.5	20.3*	2.0	14.6*	1.2	37.9*	3.7
		F	3.1*	0.5	24.3^{+}*	2.5	13.8*	1.4	41.2^{+}*	4.3
5	Gabès fishing harbour	M	1.3*	0.2	19.6*	1.9	18.2*	1.2	39.0*	3.3
		F	1.5*	0.3	11.7^{+}*	2.1	11.4^{+}*	1.5	24.6^{+}*	3.9
6	Zarat Sea	M	<dl	-	<dl	-	<dl	-	-	-
		F	<dl	-	<dl	-	<dl	.	-	-
7	Gigthis Djorf	M	<dl	-	3.0*	0.2	2.0*	0.1	5.1*	0.3
		F	<dl	-	2.9*	0.2	1.8	0.2	4.7*	0.3

Fig. 1. Sampling sites of *Hexaplex trunculus* along the Tunisian coast. 1: Bizerta Channel, 2: Menzel Jemil, 3: Tunis North Lake, 4: Fishing harbour of Sfax, 5: Fishing harbour of Gabès, 6: Sea of Zarat, 7: Ghigthis- Djorf.