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Imposex in *Stramonita haemastoma* (Gastropoda: Muricidae) along the Atlantic and Mediterranean coasts of Morocco

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Abstract. The objective of this study was to assess the incidence and severity of imposex in the Neogastropod *Stramonita haemastoma,* collected from 12 sites along the north Moroccan Atlantic and Mediterranean coasts during 2005 and 2006. The most contaminated sites were harbour areas, such as Bouznika and M'diq where highest imposex indices (I% = 100, RPLI = 73.39; VDSI = +4) were registered. Industrial and estuarine sites were only slightly contaminated compared to harbours.

Keywords: *Stramonita haemastoma,* Gastropoda, Muricidae, imposex, Morocco.

L'imposex chez *Stramonita haemastoma* (Gastéropode Muricidae) le long des côtes atlantiques et méditerranéennes du Maroc.

L'objectif de cette étude est d'évaluer l'incidence et la gravité de l'imposex sur le gastéropode *Stramonita haemastoma*, prélevé dans 12 sites le long des côtes atlantiques et méditerranéennes du nord du Maroc au cours de la période 2005-2006. Les sites les plus contaminés sont les zones portuaires de Bouznika et M'diq où les plus hautes valeurs de l'indice imposex ont été notées (I% = 100 ; RPLI = 73,39 ; VDSI = +4). Les sites industriels et les estuaires sont relativement moins contaminés.

Mots-clés : *Stramonita haemastoma*, Gastéropode, Muricidae, Imposex, Maroc.

INTRODUCTION

Tributyltin (TBT) is an organotin compound largely used in the composition of antifouling paints intended for the protection of the hulls of boats, stabilization of PVC (polyvinyl chloride) and prevention of clogging of cooling systems (Borghiand & Port 2002). Despite these benefits, TBT remains extremely dangerous for the environment and threatens several organisms, especially molluscs in both marine and freshwater ecosystems (Sayer *et al*. 2006). Indeed, many species are sensitive to very low levels of this biocide and a concentration of only 0.4 $ng.l^{-1}$ has been shown to disturb endocrine activity (Huet *et al.* 2004).

Correlations between dose or concentration and harmful biological effects of TBT have been demonstrated in the laboratory (Bryan *et al*. 1988). Transplantation experiments where gastropods were moved to a contaminated harbour area have clearly demonstrated initiation of imposex (Stewart & Thompson 1994). Furthermore, this stimulation of imposex is considered to be an irreversible process for gastropods (Gibbs *et al.* 1987).

Since the beginning of the 1980s, certain ecological disorders have been observed and His & Robert (1985) suggested that the weak recruitment of oyster spawn in the Arcachon Basin (France) could be a consequence of strong larval mortality caused by TBT. Alzieu *et al.* (1981) highlighted the role of TBT in disturbing shell formation in oysters *Ostrea edulis.* As a consequence of the TBT threat to shellfisheries, France was the first country to regulate the use of organotin-based antifouling paints (Alzieu 2000).

The muricid gastropod *Stramonita haemastoma* was selected as bioindicator species for assessing pollution by TBT along the Moroccan Atlantic and Mediterranean coasts. This species was chosen because it is common in this region and has a strong tendency to develop imposex (Spence *et al.* 1990, Terlizzi *et al*. 1997). Furthermore, the incidence of imposex in *S. haemastoma* is known to be closely linked with TBT pollution (Liu & Suen, 1996, Horiguchi *et al.* 1997, Rilov *et al.* 2000, Chiavarini *et al.* 2003).

Little is known about imposex and TBT contamination in gastropods collected along the Moroccan Atlantic and Mediterranean coasts. The exception is the study by Lemghich & Benajiba (2007) that reported imposex in *Hexaplex trunculus*, *Bolinus brandaris* and *S. haemastoma* along the Mediterranean coast of Morocco. As a consequence, the main objective of this study was to assess the incidence and severity of imposex in *S. haemastoma* collected at selected sites along the Atlantic and Mediterranean coasts of Morocco.

MATERIALS AND METHODS

Stramonita haemastoma was sampled during 2005 and 2006 at 12 sites along the Moroccan coasts (Fig. 1). In each

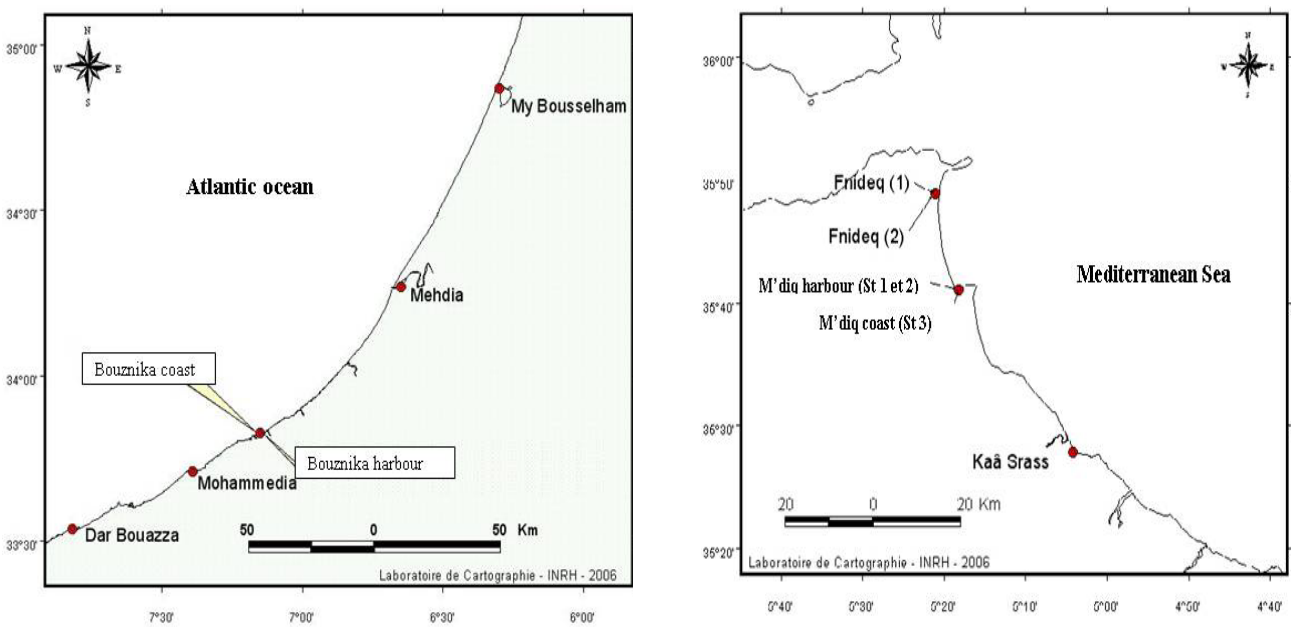

Figure 1. Location of the sampling stations. Illustration of the imposex phenomenon in *Stramonita haemastoma*

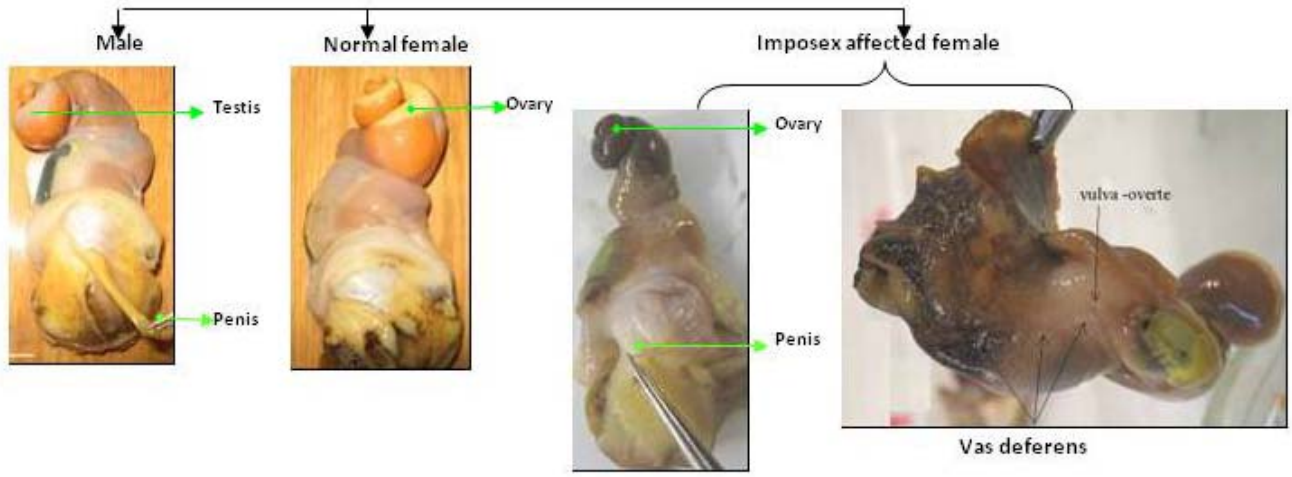

Figure 2. Illustration of the imposex phenomenon in *Stramonita haemastoma*

station, a number of adult *S. haemastoma* were collected manually or by means of a piston sampler during periods of low tide. In the laboratory, individuals were stored in the refrigerator at –20°C until analysis. Shell length was measured and sexes were identified: males by the presence of prostate, penis, seminal vesicle and the yellowish colour of the testis; females by the presence of vagina, capsule gland and albumin gland of whitish colour (Spence *et al.* 1990, Gibbs & Bryan 1994, Horiguchi *et al.* 1994, Fernandez *et al.* 2002) (Fig. 2).

The imposex incidence was estimated through the percentage of imposex-affected females in each site, using the following equation:

$$I\% = \frac{\text{number of females with imposex}}{\text{total number of females}} \times 100$$

Penis length was measured in both males and imposex-affected females using a graduated micrometer inserted in a binocular microscope. Subsequently, the average length of the penis in males (MPL) and in females (FPL) was estimated (Horiguchi *et al.* 1998).

Two indices of imposex severity based on penis measurements (relative penis length index - RPLI - and relative penis size index – RPSI -) were calculated through the following equations (Fernandez *et al.* 2002):

$$RPLI = \frac{FPL}{MPL} \times 100$$

$$RPSI = \frac{FPL^3}{MPL^3} \times 100$$

The different stages of development of the vas deferens and penis in imposex-affected females were classified using

Table I. Summary of imposex data in *Stramonita haemastoma* collected along the Atlantic and Mediterranean coasts of Morocco during 2005 and 2006. Data presented as mean ± s.d. SL: shell length; F: female; M: male; SR: Sex Ratio; I%: Imposex percentage; MPL: length of the penis in males; FPL: length of the penis in females; RPLI: relative penis length index; RPSI: relative penis size index; VDSI: vas deferens stages index.

Sites	Date	SL (mm)	F	M	SR (F:M)	I%	MPL	FPL	RPLI	RPSI	VDSI
Atlantic											
Dar Bouazza	2005	81.62±7.49	47	51	0.92	0	37.78±9.23	0	0	0	0
Dar Bouazza	2006	82.69±6.69	38	49	0.78	0	16.22±5.14	0	0	0	0
Mohammedia	2005	62.78±8.98	32	31	1.03	45.23	25.77±7.93	0.95±1.91	3.68	0.05	0.04
Mohammedia	2006	63.57±7.97	44	48	0.92	16.66	21.27±2.35	4.05 ± 1.21	19.05	0.69	1.72
Bouznika harbour	2005	82.44±6.69	6	3	2.00	100	27.25±7.03	20 ±7.02	73.39	39.54	4.62
Bouznika coast	2005	71.35±6.86	20	17	1.18	0	27.60±4.46	0	0	0	0
Bouznika coast	2006	60.87±6.77	19	30	0.63	0	24.92±5.66	0	0	0	0
Mehdia	2006	72.45±8.94	53	59	0.90	8.75	24.03±5.66	16.75 ±5.14	69.70	33.87	4.57
Moulay Bousselham	2005	67.37±5.31	6	1	6.00	33.33	35±7.02	2.66± 0.03	7.6	0.04	0.003
Moulay Bousselham	2006	40.06±6.21	16	43	0.37	19.5	27.62±7.02	6.14 ±3.26	22.23	1.09	2.25
Mediterranean											
Fnideq 1	2005	33.15±5.22	36	29	1.24	13.80	13.77±3.42	0.05±0.13	0.37	$5x10^{-6}$	$2x10^{-6}$
Fnideq 2	2005	35.74±5.09	53	47	1.13	2.13	12.09±2.32	0.10±0.72	0.87	$6x10^{-5}$	$2x10^{-4}$
M'diq harbour 1	2005	42.10±4.94	170	126	1.35	96.93	25.69±7.36	19.65±6.34	76.49	44.75	4.64
M'diq harbour 2	2005	59.04±4.71	63	57	1.11	100	24.66±4.02	20.28±3.58	82.24	55.41	4.67
M'diq coast	2005	44.30±6.16	27	24	1.13	92.80	16.33±7.93	2.67±1,60	16.35	0.43	1.40
Kaa Srass	2005	38.06±4.34	94	91	1.03	2	16.10±3.46	2.64±0.39	16.39	0.44	$15x10^{-5}$

a sequential scale of vas deferens stages (VDS), as defined by Gibbs *et al.* (1987) and Oehlmann *et al.* (1991):

Stage 0: normal female without any male characteristics.
Stage 1: beginning of penis formation behind the right ocular tentacle.
Stage 2: beginning of vas deferens formation at the level of the genital papilla.
Stage 3: penis with a closed penis duct continuing in an incomplete distal section of the vas deferens.
Stage 4: amalgamation of two portions of the vas deferens and lengthening of the penis.
Stage 5: vulva blocked by growth of vas deferens tissue.
Stage 6: dark mass of aborted capsules in the capsule gland.

The vas deferens sequence index (VDSI) was calculated as the average VDS stages observed in all females in a given sample (Bryan *et al.* 1988, Oehlmann *et al.* 1992).

RESULTS

Data on imposex incidence and severity in *S. haemastoma* from the Moroccan coasts are compiled in Table 1. Imposex indices were generally higher in the samples collected in harbour areas: M'diq harbour in the Mediterranean coast and Bouznika harbour in the Atlantic coast. In all the sampling sites located in harbour areas, 100% of the observed females showed some degrees of sexual alteration (M'diq and Bouznika harbours). The imposex phenomenon was widespread in the study area and females from 12 sites showed at least some signs of imposex. Only in the Bouznika coast and at Dar Bouazza harbour all *S. haemastoma* females were not affected by imposex (I% = 0).

The RPLI, RPSI and VDSI in *S. haemastoma* from the Atlantic coast varied between lowest values at Moulay Bousselham (RPLI = 22.23; RPSI = 1.09) and highest values in Bouznika harbour (RPLI = 73.39; RPSI = 39.54). Similarly, VDSI ranged from 0.005 at Moulay Bousselham up to ≈ 4 in Bouznika harbour and in Mehdia. On the Mediterranean coasts, RPLI and RPSI varied between lowest values in Kaa Srass (RPLI = 16.39; RPSI = 0.44) and highest values in M'diq harbour 1 (RPLI = 76.49; RPSI = 44.75) and M'diq harbour 2 (RPLI = 82.24; RPSI = 55.41), whereas the VDSI was comprised between $2x10^{-4}$ in Kaa Srass and ≈ 4 in M'diq harbour l and 2 (see Table I).

Along the Atlantic coast, imposex in *S. haemastoma* was also observed in areas with industrial activities, namely Mohammedia and Moulay Bousselham. Along the Mediterranean coast, the specimens collected in the M'diq coast showed the lowest impact and in general the imposex degree was relatively low. Contamination in these areas might be explained by the high activity of artisanal fishing boats. Individuals collected in Dar Bouazza and Bouznika were not affected by imposex.

The RPLI was strongly correlated with RPSI (R^2 = 0.928), VDSI (R^2 = 0.973) but not statistically significant ($P \geq 0.05$) with the percentage of imposex (R^2 = 0.516) (Table II).

DISCUSSION

Evidence to date suggests that imposex is generally caused by tributyltin (TBT) (Gibbs *et al.* 1987, Spooner *et al.* 1991, Sayer *et al.* 2006), a manufactured chemical massively used in self-polishing antifouling paints, which until recently was the most effective solution to prevent fouling on boat hulls (Terlizzi *et al.* 2004). Numerous investigations have linked imposex in Neogastropods with TBT contamination from antifouling paint in the marine environment (Bryan *et al.* 1987; Gibbs *et al.* 1988;

Table II. Matrix of the correlations established between the indices of imposex incidence (I%), imposex severity (FPL, RPLI, RPSI, VDSI) and TBT body burden in *Stramonita haemastoma*.

	FPL	**RPLI**	**RPSI**	**VDSI**
I%	$r^2 = 0.22$ $P = 0.054^{ns}$	$r^2 = 0.516$ $P = 0.042^{ns}$	$r^2 = 0.524$ $P= 0.041^{ns}$	$r^2 = 0.470$ $P = 0.055^{ns}$
	FPL	$r^2 = 0.608$ $P = 0.036^{*}$	$r^2 = 0.424$ $P = 0.059^{ns}$	$r^2 = 0.710$ $P = 0.038^{*}$
		RPLI	$r^2 = 0.928$ $P = 0.009^{**}$	$r^2 = 0.973$ $P = 0.009^{**}$
			RPSI	$r^2 = 0.829$ $P = 0.008^{**}$
				VDSI

Abbreviations: ns, not significant ($P \geq 0.05$); ^{ns}, significant ($P < 0.05$); *, highly significant ($P < 0.01$) **.

Oehlmann *et al.* 1996 a,b). Accordingly, we suggest that TBT from antifouling paints on large ships seems the most likely source of imposex in the populations of *S. haemastoma* from the Moroccan coast.

This phenomenon is known to be induced by TBT at very low ambient concentrations, just a few ng/l are sufficient and imposex development seems dose-dependent (Bryan *et al.* 1986). Furthermore, high correlations between likely sources of TBT and imposex induction have been confirmed in several studies by chemical analyses of water, sediments and gastropod tissues. For instance, significant correlations between TBT concentration in seawater and imposex indices have been reported for *Nucella lapillus* (Stroben *et al*. 1995, Birchenough *et al.* 2002), *Thaïs clavigera* (Horiguchi *et al.* 1994), *Hexaplex trunculus* (Axiak *et al.* 2003) or *S. haemastoma* (Fernandez *et al.* 2002). On the other hand, the fact that *S. haemastoma* exhibited imposex at very low levels at Mehdia, Moulay Bousselham and M'diq stations suggests that this species is a good bioindicator for detecting "hot spots" of TBT pollution.

In the Moroccan Mediterranean coasts, the first survey on imposex was conducted in 2000-2001 in three neogastropod species: *Hexaplex trunculus*, *Bolinus brandaris* and *S. haemastoma* (Lemghich & Benajiba 2007). These authors detected imposex in the five sites surveyed and confirmed that the rates of occurrence and degree of imposex were more important in harbour sites with heavy shipping activity (Tangier and Mdiq) than in the three seaside stations (Martil, Azla and Amsa). In the present study, were detected imposex frequencies of up to 100% in high shipping activity areas, such as Bouznika and M'diq harbours. Correspondingly, the VDSI varied between 4 and 5 in M'diq harbour, generating an incidence of female sterility of 8%, while the RPLI oscillated between 73.4 and 82.2. At the other stations, the imposex incidence varied from 2 to 45.2%, with VDSI varying between $2x10^{-5}$ to 1.73 and RPLI ranging from 0.36 to 68.86.

The relative abundance of imposex-affected females in certain sites is a tool for impact assessment when TBT concentrations in the water are relatively low and only a fraction of females is masculinised (Curtis 1994, Gibbs & Bryan 1994). At the harbour areas: M'diq and Bouznika, 100% of the observed females showed a certain degree of sexual alteration. Lower percentages of imposex were recorded in industrial and estuary sites, whereas only in the Bouznika and Dar Bouazza coastal sites imposex was not detected (I% = 0). Highest VDSI (4+) at M'diq harbour (sites 1 and 2) in the Mediterranean coasts and Bouznika harbour and Mehdia in the Atlantic coasts, might indicate that some females were sterile (VDSI ≈ 5), according to Stroben *et al.* (1992). In *S. haemastoma*, female sterilization is caused by the development of the vas deferens from the base of the penis and blockage of the vaginal opening, as reported for *Nucella lapillus* (Gibbs *et al.* 1987, Oehlmann *et al.* 1991). For the sites moderately affected, Mohammedia, Moulay Bousselham and M'diq coast, the VDSI varied between 1 and 2. For the sites of Dar Bouazza and Bouznika, VDSI = 0 and the negative effect of imposex were not recorded. In summary, imposex was recorded in *S. haemastoma* from six sites on the Mediterranean coasts and four sites on the Atlantic coasts. Imposex levels and organotin body burden can be assumed to be highest in populations nearest to major shipping activities and gradually lower with increasing distance from these sources (Leung *et al.* 2006, Lemghich & Benajiba 2007).

CONCLUSIONS

Imposex appears to be a widespread phenomenon affecting *S. haemastoma* along the Atlantic and Mediterranean coast of Morocco. Severe symptoms of this phenomenon were recorded near harbours in the Atlantic (Bouznika harbour) and Mediterranean (M'diq harbour) coasts of Morocco. This sexual aberration is mainly attributable to TBT in seawater (Fernandez *et al.* 2002; Trigui El-Menif *et al.* 2007). The present results corroborate a previous study and confirm the high contamination detected in some sites along the Moroccan coast (Lemghich & Benajiba 2007). The wide distribution of *S. haemastoma* along the Moroccan coasts makes the species a good bioindicator for monitoring "hot spots" of TBT pollution. Finally, it is necessary that the Moroccan legislation controls the use of TBT-based paintings and introduces alternative TBT-free antifouling products (e.g. Jelic-Mrcelic *et al.* 2006).

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