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Imposex incidence in *Stramonita haemastoma* (Gastropoda: Muricidae) from the Mediterranean and Atlantic coast after Tributyltin global ban

Tahani El Ayari[a,b,c,*], Nicolas Bierne[b,c], Najoua Trigui El Menif[a]

[a] University of Carthage, Faculty of Sciences of Bizerta, Laboratory of Environment Bio-monitoring, 7021 Zarzouna, Bizerta, Tunisia
[b] Université de Montpellier, Place Eugène Bataillon, 34095 Montpellier Cedex 5, France
[c] ISEM - CNRS, UMR 5554, Station Marine OREME, 2 rue des Chantiers, 34200 Sète, France

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ABSTRACT

The development of male genital tract by female gastropods, or imposex, can be caused by the tributyltin used in antifouling paints. A spatial survey of imposex in the gastropod *Stramonita haemastoma* was conducted across five Western Mediterranean and eleven North-Eastern Atlantic sites, in order to monitor the effectiveness of the tributyltin regulation imposed in the International Maritime Organisation. Imposex still occurs in eight out of eleven Mediterranean sites and in three out of five Atlantic sites. Extreme values of imposex incidence (I%) and degree (VDSI) were recorded in Tunisia, mainly in Bizerta channel (I% = 96.2%, VDSI = 0.96). However, the Relative Penis Length index (RPLI) was higher in Western Mediterranean sites where values varied between 0.56 in Algiers (Algeria) and 11.80 in Bouznika (Morocco). In the European sites, moderate to low imposex level and degree were recorded. All the affected sites were below the Ecotoxicological Assessment Criteria (EAC) derived for TBT.

1. Introduction

Decades of research on genital deformities in gonochoristic gastropods have revealed a strong cause-effect relationship between the occurrence of imposex and the presence of organotin compounds such as tributyltin (TBT) and, in some cases, triphenyltin (TPT) in the aquatic system (Smith, 1981; Gibbs et al., 1987; Stroben et al., 1992; Smith, 1996). More than 260 gastropod species are affected by imposex (Sternberg et al., 2010; Titley-O'Nea et al., 2011), among which 76 species belong to the family of Muricidae (Shi et al., 2005), including the geographically widespread *Stramonita haemastoma* (Limaverde et al., 2007; Claremont et al., 2011; Toste et al., 2013; El Ayari et al., 2015). Since 2005 the known number of affected species has increased considerably to include *Thais deltoidea* (Costa et al., 2008), *Pugilina morio* (de Azevedo et al., 2012), *Heleobia australis* (Neves et al., 2013), *Nassarius mutabilis* (Lahbib et al., 2013), *Xanthochorus buxea* (Guabloche et al., 2013), *Gemophos viverratus* (Lopes-dos-Santos et al., 2014), *Babylonia spirata* (Afsar et al., 2015), *Plicopurpura pansa* (Domínguez-Ojeda et al., 2015), *Olivella minuta*, *Hastula cinerea* (Petracco et al., 2015) and *Oliva peruviana* (Batista et al., 2016).

Despite the abundance of *S. haemastoma* in the Mediterranean and Atlantic Ocean (Harding and Harasewych, 2007; Claremont et al., 2011), information about the status of pollution by organotin compounds is still limited geographically. Few studies have been conducted in the Eastern Mediterranean basin (Rilov et al., 2000; Rilov et al., 2001), or the Atlantic Coast (Spence et al., 1990; El Mortaji et al., 2011), in contrast, studies in Western Mediterranean basin have been much more abundant (Chiavarini et al., 2003; Lemghich and Benajiba, 2007; Lahbib et al., 2010; El Mortaji et al., 2011; Boulajfene et al., 2015; El Ayari et al., 2015). Gastropods therefore provide ideal as biological indicators of organotin pollution as chemical analysis are very expensive (Minchin et al., 1995; Camillo et al., 2004) and imposex surveys have become part of national and international monitoring programmes (OSPAR, 1997). The snail *S. haemastoma* has proven an effective bioindicator of TBT pollution (Limaverde et al., 2007; Toste et al., 2013; Boulajfene et al., 2015; El Ayari et al., 2015). Imposex is induced in 90% of the females *S. haemastoma* after only 15 days of transplantation to a polluted site (Rossato et al., 2014), and, coupled with its abundance and ease of collection, make it an ideal bioindicator species. Several authors have discussed the importance of using gastropod as biological indicators of organotin pollution as chemical analysis are very expensive (Minchin et al., 1995; Camillo et al., 2004). Undeniably, imposex surveys are becoming part of national and international monitoring programmes (OSPAR, 1997).

Among the gastropod species used for imposex monitoring, only *S. haemastoma* has the advantage of wide geographic distributional range.

* Corresponding author at: Faculty of Sciences of Bizerta, Laboratory of Environment Bio-monitoring, 7021 Zarzouna, Bizerta, Tunisia.
E-mail address: tahaniayari@yahoo.fr (T. El Ayari).

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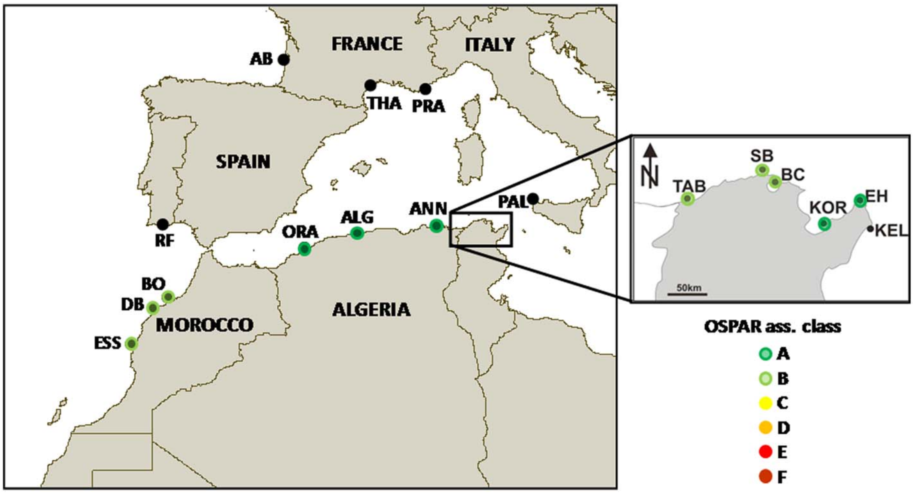

Fig. 1. Sampling locations of *Stramonita haemastoma* in the North-Eastern Atlantic Ocean: Arcachon Bay (AB), Faro (FAR), Bouznika (BO), Dar Bouaaza (DB) and Essaouira (ESS) and in the Western Mediterranean basin: Etang de Thau (THA), Tarragona (TAR), Oran (ORA), Algiers (ALG), Annaba (ANN), Tabarca (TAB), Sidi Bechir (SB), Bizerta Channel (BC), Korbous (KOR), EL Houaria (EH), Kelibia (KEL) and Palermo (PAL). The green colors in A and B mean that the Ecological Quality Objective is met (OSPAR, 2005).

While the muricids *Hexaplex trunculus* and *Bolinus Brandaris* are limited to the Mediterranean Sea and *N. lapillus* to the Atlantic Ocean. Hence, using *S. haemastoma* has the advantage to make reliable assessment of both Mediterranean and Atlantic coast while avoiding species extrapolation in monitoring programs. In the present study we used *S. haemastoma* to assess (1) the status of TBT pollution in eleven Mediterranean and five Atlantic sites using imposex. (2) We also provide a baseline information that could be useful, in the future, for assessing the effectiveness of TBT banning at wide range scale.

2. Materials and methods

2.1. Imposex analysis

Individuals of *S. haemastoma* were collected by hand using either snorkel or SCUBA from eleven sites in the Western Mediterranean and 5 in the North Eastern Atlantic Ocean (see Figure 1 and Table 1). At each site between 14 and 50 individuals with a shell length from 31 to 85 mm were collected and frozen for laboratory analysis. All samples were collected at the same period 2013 (Table 1). Information on the boating traffic in 2013 was obtained from harbour authorities (Table 2).

In the laboratory, shell length (SL) was measured using a vernier calliper to the nearest 0.1 mm and then crushed using a bench vice. The soft body was carefully extracted and the sex was determined under a binocular microscope. Sex was identified based on the presence of a capsule gland and a vagina in females and a large penis with a vas deferens and a prostate gland in males. Female penis length (FPL) and male penis length (MPL) were measured under a dissecting microscope using a calibrated eyepiece.

The assessment of the imposex development was conducted using the indices reported in El Ayari et al. (2015):

(1) Imposex incidence (I %) = percentage of imposex-affected females compared to the total number of females in the sample;
(2) Vas Deferens Sequence Index (VDSI) = sum of imposex stages of all females/total number of females. VDS stages were determined according to the general scheme proposed by Stroben et al. (1992), as partially modified for *H. trunculus* (Lahbib et al. 2007) and recently for *S. haemastoma* by Toste et al. (2013). The VDS varies from 0 to 4, with stage 0 corresponding to healthy females. In stage 1, two types were recorded: the first sign of imposex development could be the appearance of an incipient penis (VDS1a) or a vas deferens sequence appeared halfway between the penis site and the vagina (VDS1d). In stage 2, two types were also observed: a small penis with a penis duct (VDS2a) or a longer vas deferens sequence (VDS2d). Two types were again observed in stage 3, a penis with a penis duct and a vas deferens sequence (VDS3a). Finally, stage 4 was observed the simultaneous presence of a penis and a vas

Table 1
Stramonita haemastoma sampling sites in the North-Eastern Atlantic and Western Mediterranean basin.

	Sample name	Sample code	Date of sampling	GPS coordinates	
				Latitude	Longitude
North-Eastern Atlantic	Arcachon Bay	AB	December 2013	44°35′33.36″N	1°12′52.56″W
	Faro	FAR	April 2013	36°59′19.38″N	7°59′1.30″W
	Bouznika	BO	April 2013	33°48′48.18″N	7°10′37″78W
	Dar Bouaaza	DB	April 2013	33°32′12.53″N	7°49′26″95W
	Essaouira	ESS	April 2013	31°41′32.87″N	9°48′56.38″W
Western Mediterranean basin	Etang de Thau	THA	June 2013	43°23′36.03″N	3°42′10.74″E
	Oran	ORA	April 2013	35°54′5.07″N	0°19′54.95″W
	Algiers	ALG	April 2013	36°45′4.11″N	2°50′44″77E
	Annaba	ANN	April 2013	36°53′53.66″N	7°46′17.15″E
	Tabarca	TAB	May 2013	37°30′51.27″N	9°52′9.94″E
	Sidi Bechir	SB	July 2013	37°13′57.05″N	9°19′4″00E
	Bizerta channel	BC	May 2013	36°50′9.70″N	11°6′4.81″E
	Korbous	KOR	May 2013	36°4′0.65″N	10°34′1.02″E
	El Houaria	EH	May 2013	37.1′59.90″N	11°3′33.73″E
	Kelibia	KEL	May 2013	38°7′34.60″N	12°47′37.40″E
	Palermo	PAL	September 2013	43°19′57.87″N	3°36′44.69″E