Limited effectiveness of marine protected areas: imposex in *Hexaplex trunculus* (Gastropoda, Muricidae) populations from Italian marine reserves

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Tributyltin (TBT) and its derivatives have been defined as the most toxic compounds deliberately released into marine environments by man (Fent, 1996). The presence of these chemicals in marine environments is mainly due to a massive use of antifouling (AF) paints incorporating TBT as a biocide. TBT-based AF paints are recognised as being the most effective coatings in protecting boat hulls against the settling of fouling organisms (Bosselmann, 1996), and not surprisingly, since their introduction on the market, in the late 60s, they have gained popularity, especially for application on recreational boats.

The negative effects that organotin compounds cause to non-target marine organisms (Terlizzi et al., 2001) have forced many governments to contain their use. To date, in Italy, as well as in all EU countries, USA, Australia, and New Zealand, the use of TBT is severely restricted, although not completely banned. Since the introduction of these regulations, efforts have been made to adequately assess their effectiveness in reducing TBT contamination (Evans, 1999).

The build-up of organotin compounds, even in low environmental concentrations, may to some extent affect marine organisms, which, therefore, become effective biomarkers for these toxicants. TBT, in particular, at an ambient concentration of just a few nanograms per litre, has the potential to cause a genital disorder in females of different species of marine snails. Such a disorder consists in the superimposition of male sex organs onto females. This phenomenon is widely known as *imposex* (Smith, 1971) or *pseudohermaphroditism* (Jenner, 1979) and is known to occur in 63 genera and 118 species (Fioroni et al., 1991). The effects of imposex can vary, depending on the species. In some cases, this phenomenon does not impair reproduction. In some others, it can lead to population decline as a consequence of reproduction failure. Imposex is a graded response and it has been used as a highly sensitive bioindicator of tributyltin pollution worldwide (Ellis and Pattisina, 1990), since no other toxicant is known to induce it (Bryan et al., 1986; but see also Nias et al., 1993).

Imposex studies in Mediterranean prosobranch species are fairly scant and mainly limited to the population of the following species: *Stramonita haemastoma* (Spence et al., 1990; Terlizzi, 2000), *Bolinus brandaris* (Solé et al., 1998; Ramòn and Amor, 2001), and *Hexaplex trunculus* (Axiak et al., 1995; Terlizzi et al., 1999; Rilov et al., 2000). Along the coasts of Italy, Terlizzi et al. (1998) found this phenomenon to be widespread and related to boat density in the investigated sites.

Over the last few decades, the recognition of the profound influence of humans on marine biota has led to stronger conservation efforts in marine systems. Marine protected areas (MPAs), in particular, have become a widely advocated form of marine conservation and their number is constantly increasing worldwide (Hixon et al., 2001; Fraschetti et al., 2002). Although our knowledge of the effects of protection measures on marine assemblages is still far from exhaustive (Planes et al., 2000), it is generally recognised that MPAs are essential for conservation as they can provide unique protection for critical areas and spatial escape for overexploited species. Further, MPAs can act as buffers against management miscalculation (Allison et al., 1998). MPAs, however, only safeguard populations or assemblages within their boundaries and fail to offer any protection from some major threats to marine environments. These include coastal modifications and subsequent changes in local hydrodynamic and sedimentary regimes, the spreading of exotic species, disease epidemics and, above all, contamination by chemicals (Allison et al., 1998).

This study aims to provide further information about the current extent of imposex in *H. trunculus* along the coasts of Italy and to evaluate the current situation of organotin impact inside Italian MPAs.

Approximately 40 adult specimens of *H. trunculus* (>4 cm) were collected by snorkeling and SCUBA diving from no-take and no-access zones (integral protection or A Zone) of 13 Italian MPAs (Fig. 1). All samples were taken at a depth ranging from 2 to 10 m.

The animals were sent to the laboratory, placed in plastic tanks with recirculating seawater, and examined

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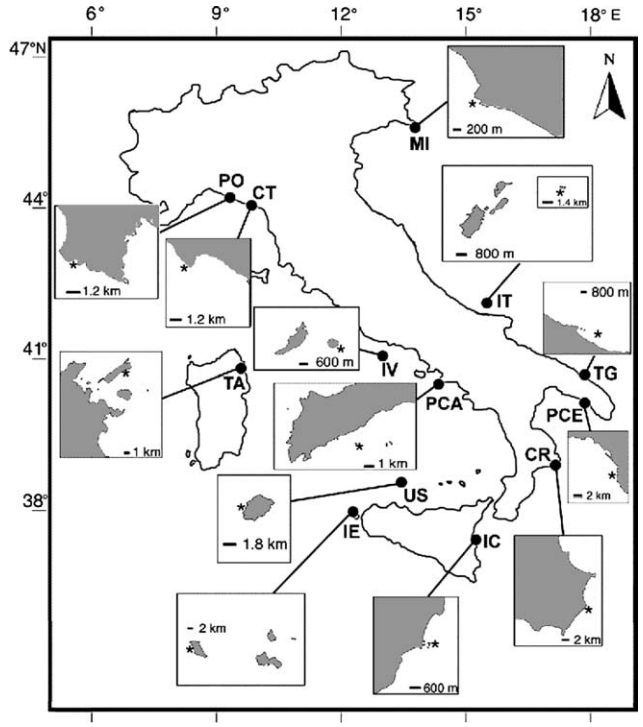

Fig. 1. Map of *H. trunculus* sampling locations. All locations were marine protected areas and are coded as in Table 1. Samples were taken inside no-take and no-access zones (A Zone, indicated by ∗).

within a maximum of 5 days. The shell length (apex to end of columella) was recorded to the nearest 0.1 mm using a vernier calliper. The animals were then narcotised in 7% $MgCl_2$ in seawater, the shell cracked in a bench vice and the soft body gently removed. The mantle cavity was opened under a stereomicroscope by a longitudinal cut through the hypobranchial gland to observe any abnormalities in sexual apparatus. Due to the widespread appearance of male characters in females, the presence/absence of a penis cannot be used to separate genders. Males were recognised by both the presence of a penis and a prostate and by the lack of a capsule gland. Females were recognised by certain characters present both in normal and imposexed females, namely the presence of the vagina and capsule gland. For each population two indices were calculated: (1) the cubed RPSI (relative penis size index, Gibbs et al., 1987), defined by the formula (mean female penis length)^3/(mean male penis length)$^3 \times 100$; and (2) the vas deferens sequence index (VDSI), i.e. the mean score of the various stages of imposex development in females (Fioroni et al., 1991). The determination of the different stages of imposex in females followed the general scheme proposed by Stroben et al. (1992) and partially modified by Axiak et al. (1995) and Terlizzi et al. (1999) for *H. trunculus*.

Stage 0 is assigned to females with no sexual abnormalities. Stage 1 is the initial sign of imposex and is identifiable by the occurrence of an incipient penis at the back of the right ocular tentacle. At stage 2, a small penis with a developed penis duct is observed. At stage 3, the penis duct extends proximally in an initial tract of vas deferens. At stage 4, the vas deferens reaches the vaginal opening, crosses it (stage 4.3) and runs towards the ventral portion of the capsule gland (stage 4.7). At stages 5 and 6, the vulva is absent. The capsule gland is frequently split with its lumen open into the mantle cavity. Aborted capsules are often present also in the form of an amorphous dark mass filling the lumen of the capsule gland (Terlizzi et al., 1999).

In certain cases, female genital system abnormalities were studied using a SEM-technique. Specimens were fixed in buffered 4% formalin, preserved in 70% ethanol, and dehydrated via a graded ethanol series, critical point dried, coated with gold and examined with a Stereoscan 250 MK3 Cambridge Instruments at a 500× magnification.

Table 1 summarises biological data for the various populations of *H. trunculus* as well as imposex intensities. Signs of imposex were recorded in all the investigated MPAs. The stages recorded ranged from 0 (absence of any sexual abnormalities) to 4.3. No sterile females (stages 5 and 6) were observed. The lowest RPS index values were recorded at Isole Tremiti (0.00), Ustica (0.03), Isole Egadi (0.10), Porto Cesareo (0.10), and Torre Guaceto (0.66); the highest at Isole Ciclopi (50.1), Miramare (37.20) and Tavolara (26.04) (Table 1). Similar information concerning the incidence of imposex was obtained by VDS index, with the lowest values at Porto Cesareo (0.11), Isole Tremiti (0.16), Isole Egadi (0.62), Torre Guaceto (0.66), and Ustica (0.77); and the highest at Miramare (4.13), Isole Ciclopi (4.05) and Tavolara (4.04) (Table 1). Stages 1, 3 and 4.3 are documented in Fig. 2.

This study has demonstrated the spread of imposex in *H. truculus* affecting populations from all the considered locations. Imposex was recorded in all the populations and, in eight out of 13 MPAs investigated, all females were recorded to exhibit imposex (though at different levels). In some cases, females were apparently free of sexual apparatus abnormalities and the recognition of early imposex stages (particularly stage 1) was possible only with the analysis of SEM photos. This finding was recently addressed by Axiak et al. (2003) in *H. trunculus* populations from Malta (Mediterranean Sea), showing that much care is needed in order to recognise females with earlier stages of imposex.

Imposex, thus, is still evident along the coast of Italy, more than 10 years after the enforcement of restrictions on trybutyltin use. Based on current data, it is clearly impossible to gain information about a likely decline in the severity of imposex because data on its incidence along the coasts of Italy are very few and sparse (Terlizzi et al., 1998; Chiavarini et al., 2003). There are, however,

Table 1
Summary of imposex results of *H. trunculus* populations from A Zone of 13 MPAs in Italy

Marine protected area	Date	Sex	No. of individuals	Shell length (mm)	% Imposex	Average penis length	% Sterile females	RPSI	VDSI	VDSI range
CAPO RIZZUTO = CR	14-Nov-02	M	20	34.4		4.32				
		F	7	40.7	100	3.42	0	50.1	3.94	3–4.3
CINQUE TERRE = CT	16-Oct-02	M	8	49.7		10.45				
		F	12	58.06	100	3.57	0	3.98	3.82	1–4.3
ISOLE CICLOPI = IC	12-Jul-02	M	18	46.3		6.1				
		F	26	47.7	100	3.5	0	18.90	4.05	3–4.3
ISOLE DI VENTOTENE E S. STEFANO = IV	20-Jun-02	M	11	46.7		9.4				
		F	21	55	100	1.5	0	0.40	2.68	1–4.3
ISOLE PELAGIE = IP	11-Jun-02	M	20	51.4		8.8				
		F	13	57.2	30.8	0.4	0	0.10	0.62	0–2
ISOLE TREMITI = IT	24-Jun-02	M	13	53.7		5.2				
		F	19	58.2	10.5	0.01	0	0.00	0.16	0–2
MIRAMARE = MI	19-Jun-02	M	25	63.9		8.9				
		F	7	67.9	100	6.4	0	37.20	4.13	4–4.3
PORTO CESAREO = PCE	24-Apr-02	M	16	44.5		6.9				
		F	24	48.4	54.2	0.7	0	0.10	0.11	0–3
PORTOFINO = PO	29-Jun-02	M	20	55		6.9				
		F	21	59.6	100	3.9	0	18.10	3.86	2–4.3
PUNTA CAMPANELLA = PCA	11-Jul-02	M	12	62						
		F	14	65.8	100	2.3	0	5.12	3.33	1–4.3
TAVOLARA = TA	29-Aug-02	M	23	45.6		8.3				
		F	14	51.5	100	5.3	0	26.04	4.04	3–4.3
TORRE GUACETO = TG	17-Apr-02	M	12	44.2		6.2				
		F	24	49.2	25	0.2	0	0.00	0.66	0–4
USTICA = US	01-Jun-02	M	27	45.7		7.6				
		F	18	52.5	38.9	0.5	0	0.03	0.77	0–2

RPSI = relative penis size index; VDSI = vas deferens sequence index.

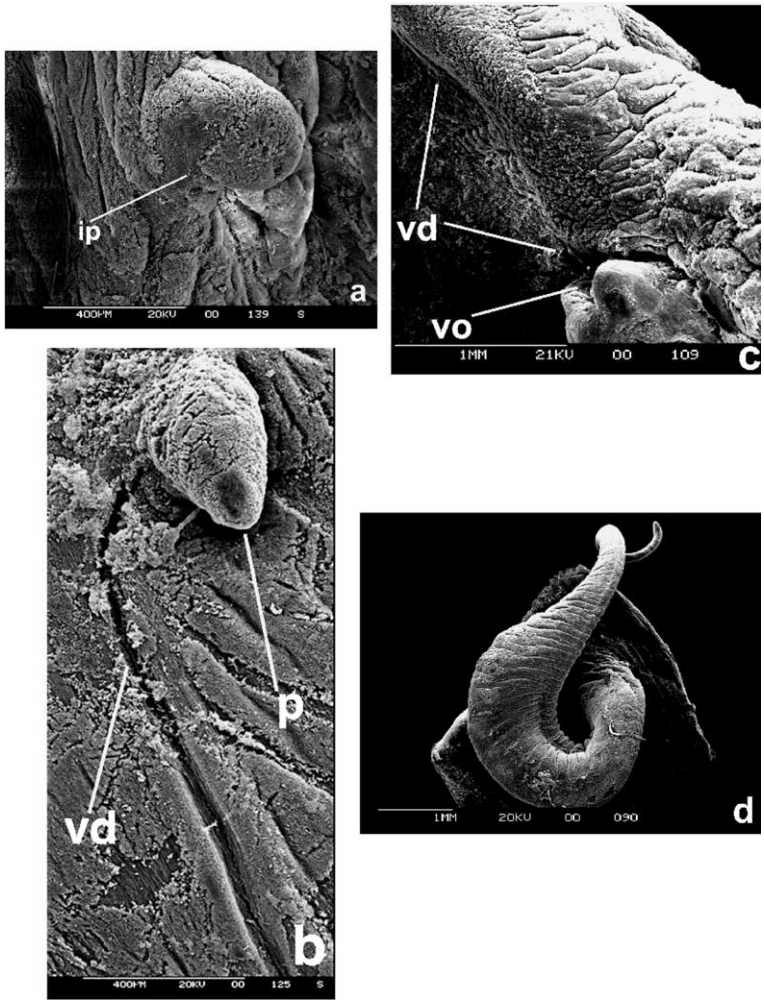

Fig. 2. *H. trunculus*. SEM photographs of (a) stage 1, presence of an incipient penis (ip); (b) stage 3, presence of a penis (p) with a well-developed vas deferens (vd); (c) stage 4, vas deferens reaching vaginal opening (vo); (d) penis in a female (shell length 61.3 mm) at a 4 stage of imposex.

some clues: for instance, the population from the AMP in Portofino recorded a VDSI of 3.9, with VDS stages ranging from 2 to 4.3. In a population from the same area (namely S.M. di Pagana), which was sampled in May 1995, Terlizzi et al. (1998) measured a VDSI of 5 with all females sterilized. The population from the AMP of Ustica recorded a VDSI of 0.8, with VDS stages ranging from 0 to 2. In a population from the same area sampled in October 1999 and May 2000 Chiavarini et al. (2003) measured an average VDSI of 3.0.

The vast majority of studies on imposex have been conducted near marinas, harbours or likely sources of TBT contamination. In this study, we found imposex in *H. trunculus* populations from relatively pristine areas. This finding confirms that there are certain threats to marine systems (the dispersion of pollutants, in our case) against which MPAs fail to provide any direct protection (James, 2002).

The most important reason for the limited biological effectiveness of MPAs is that the scale of processes in marine systems is often much larger than scales the reserve can encompass (Allison et al., 1998). Thus, MPAs, though representing a critical component of any conservation strategy, must be coupled with other, complementary measures. The effectiveness of reserves, for instance, may greatly benefit from a better understanding of the sources, fate and impact of chemicals in the sea. Critical to this is the ability to predict the likelihood and magnitude of contamination inside the reserves originating from external sources. These forecasts should be based on accurate estimates on the origin, dispersal, and longevity of contaminants, as well as on the response of reserve inhabitants to potential levels of contamination.

In conclusion, this study generated data that could be used as a baseline for future monitoring programmes related to TBT pollution along the continental and insular coasts of Italy. It provides information about the time-related trend of imposex incidence on a wide scale. In such cases, MPAs will be essential not only for

protecting populations within their boundaries, but also outside, by providing guidelines for more comprehensive strategies of marine environment protection.

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