

## Imposex incidence in *Hexaplex trunculus* from Kaštela Bay, Adriatic Sea

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*Imposex, a pseudo-hermaphroditic condition characterized by the development of male sexual characteristics in female prosobranch gastropods, is used as a bioindicator of pollution by tributyltin (TBT)-based antifoulants. The incidence and the intensity of imposex, evaluated from the vas deferens sequence index (VDSI) and the female penis length (FPL), were investigated in Hexaplex trunculus populations from seven locations in Kaštela Bay (Adriatic Sea, Croatia). Analysis of 131 females revealed the absolute prevalence of imposex at all investigated locations. Furthermore, most of the females showed signs of advanced imposex development (VDSI=4.4±0.8, range=2-5). Female penis homologues were found to be well developed (max=20 mm) with mean values ranging from 5.2-12.6 mm. Significant differences were detected regarding both the VDSI (H=36.24, p<0.001) and the FPL (F=7.08, p<0.001) between the locations. The results presented in the study confirm the widespread incidence of imposex and can provide reference values for future monitoring programs.*

**Key words:** imposex, *Hexaplex trunculus*, Kaštela Bay, Adriatic Sea, Croatia

### INTRODUCTION

Many worldwide studies relate imposex development, a pseudo-hermaphroditic condition characterized by the development of male sexual characteristics in female prosobranch gastropods, to the use of organotin-based anti-fouling paints (AXIAK *et al.*, 1995; TERLIZZI *et al.*, 1998; TERLIZZI *et al.*, 1999; POWER & KEEGAN, 2001; AXIAK *et al.*, 2003; VASCONCELOS *et al.*, 2006). Imposex is considered to be mediated by organotin interference with steroid metabo-

lism (BETTIN *et al.*, 1996; OBERDÖRSTER *et al.*, 1998; OBERDÖRSTER & MCCLELLAN-GREEN, 2002; NISHIKAWA, 2005).

Since elevated organotin concentrations are most commonly measured near yacht marinas and shipyards, this study aimed to investigate the incidence and the intensity of imposex in *Hexaplex trunculus* populations from Kaštela Bay. The bay is a semi-enclosed, relatively small body of water, historically known as a "Black Spot" due to intensive anthropogenic pollution (BARIĆ, 1989) exacerbated by poor

flushing characteristics (ZORE-ARMANDA, 1980) and is currently characterized by intense shipping activity which encompasses a large shipyard and a growing number of nautical marinas.

The target species, *H. trunculus*, is one of the most common muricids in the Mediterranean (TERLIZZI *et al.*, 1998). It appears to be highly susceptible to the development of imposex and thus an important indicator species for organotin contamination (AXIAK *et al.*, 1995). Since *H. trunculus* is also a direct developer species (PELLIZZATO *et al.*, 2004) it could possibly be used to indicate point sources of organotin pollution.

## MATERIAL AND METHODS

Between 40 and 60 adult *Hexaplex trunculus* specimens ( $\geq 4$  cm in length) were collected by snorkelling and SCUBA diving at seven locations in Kaštela Bay: Bene, Marina Kaštela and Kaštel Stari in winter 2004, followed by Lora, Lučica, Resnik and Arbanija in summer 2006 (Fig. 1). Sampling locations were chosen to cover the entire bay area. Collected *H. trunculus* specimens were brought to the laboratory where the shell length (SL) was measured to the nearest 0.1 mm using vernier callipers, upon which they were cracked in a bench vice and then the soft body was removed and examined.

Males were recognized by the presence of only one cavity opening (anus), penis and vas deferens and by the absence of an egg capsule

gland. Females, both normal and affected, were recognized by the presence of a second opening (vagina) and the egg capsule gland (AXIAK *et al.*, 1995). The incidence and the intensity of imposex in females were evaluated from the mean female penis homologue length (FPL) and the vas deferens sequence index (VDSI). Using graduated graph paper, FPL was measured, to the nearest 0.1 mm, from the tip to the base without straightening the penis. VDSI was calculated as the mean of the imposex stages present in the sample. Different imposex stages were determined using AXIAK *et al.* (1995) and TERLIZZI *et al.* (1999) classifications as shown in Table 1.

One-way ANOVA was used to test for significant differences in the FPL between locations while a Kruskal-Wallis analysis determined the differences in the VDSI between locations. All of these analyses were carried out on the statistical package "Minitab 13".

## RESULTS AND DISCUSSION

A total of 131 females (length=66.3 $\pm$ 7.5 mm) were analyzed from seven locations in Kaštela Bay: Bene (N=20), Marina Kaštela (N=19), Kaštel Stari (N=18), Lora (N=14), Lučica (N=20), Resnik (N=20) and Arbanija (N=20). Imposex data is summarized in Fig. 2 and Table 2. All collected females showed signs of imposex development (VDSI=4.4 $\pm$ 0.8), with observed imposex stages ranging from 2

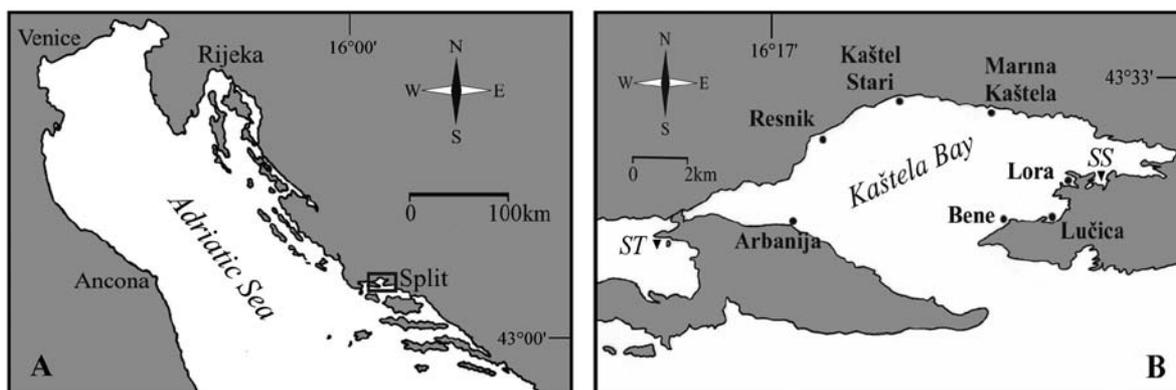


Fig. 1. (A) Position of Kaštela Bay relative to the Adriatic Sea, (B) Map of Kaštela Bay showing the positions of the sampling locations (●) and shipyards (▼); SS - Shipyard Split, ST - Shipyard Trogir

Table 1. Classification of *vas deferens* sequence index (VDSI) stages

VDSI stage	Description
Stage 0	female with no male sexual characteristics
Stage 1	presence of incipient penis behind the right ocular tentacle, vas deferens is still not developed
Stage 2	penis with initiation of vas deferens
Stage 3	more prominent penis and vas deferens still not reaching the vaginal opening
Stage 4	vas deferens reaches the vaginal opening
Stage 4.3	vas deferens passes the vaginal opening
Stage 4.7	vas deferens extends beyond the point in stage 4.3 and now runs along the capsule gland by approximately 30% of its length
Stage 5	vas deferens runs along the entire length of the capsule gland. No vulva is visible and the capsule gland is swollen, frequently split with its lumen open into the mantle cavity. Egg capsule release is prevented and aborted capsules are often present. These females can be regarded as sterile

to 5. The most advanced imposex stage 5 was observed at all sampling locations, showing a 20-90% frequency. Female penis homologues were found to be well developed (max=20 mm) with mean values ranging from 5.2 mm to 12.6 mm. Some females also exhibited excrescences on the penis.

Significant differences were detected regarding both the VDSI (Kruskal-Wallis,  $H=36.24$ ,  $p<0.001$ ) and the FPL (ANOVA,  $F=7.08$ ,  $p<0.001$ ) between the locations. The most severely affected of the analyzed locations, yacht marina Lučica and navy harbour Lora, are the closest to the Split shipyard and are also by themselves a source of contamination (Fig. 1). The results show that Lučica and Lora have the highest proportion of specimens exhibiting stage 5 development (Fig. 2) and in turn also the highest VDSI values of 4.9 and 4.7, respectively. Only in samples from Resnik and Arbanija was imposex not so pronounced, as indicated by the lowest proportion of advanced imposex stages with less than 30% of females in stages higher than 4.7, and minimal values of VDSI and FPL being 3.8 and 6.7 mm for Resnik and 3.9 and 5.2 mm for Arbanija. These two locations are

the furthest from the main point sources of organotin contamination in Kaštela Bay, the Split shipyard situated at the opposite end of the bay and numerous dispersed marinas. Even though Resnik and Arbanija exhibited the lowest values in this study, when compared to a few detailed studies carried out in the eastern Adriatic (TERLIZZI *et al.*, 2004; GARAVENTA *et al.*, 2006b; PRIME *et al.*, 2006), the results correspond to polluted locations. Since the impact of organotin toxicity may be seen over several kilometres from a known pollution source (AXIAK *et al.*, 2000), it indicates that the proximity of another smaller shipyard in the town of Trogir (Fig. 1) may also impact upon these locations.

Although the intensity of imposex is primarily related to organotin concentrations in the tissue, and in turn to the concentrations measured in the environment (AXIAK *et al.*, 2000; CHIAVARINI *et al.*, 2003; PELLIZZATO *et al.*, 2004; GARAVENTA *et al.*, 2006b), the precise biochemical mechanism of imposex induction still remains unresolved. Furthermore, the studies of AXIAK *et al.* (2003) and GARAVENTA *et al.* (2006a) have suggested that there is incidence of early imposex development as a result of various stresses.

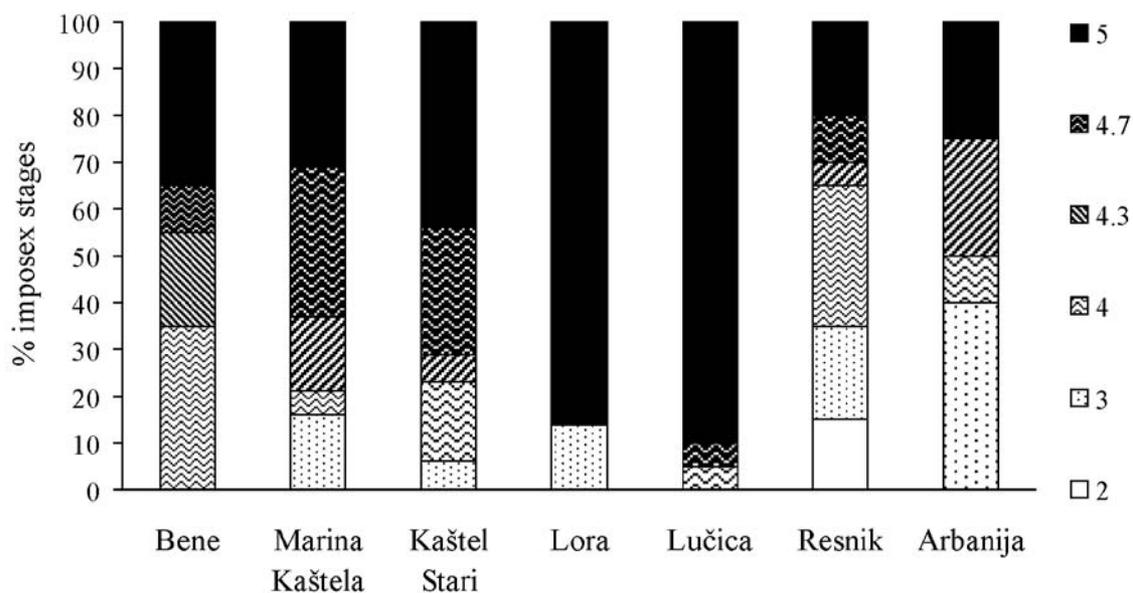


Fig. 2. Percentage of imposex stages for *Hexaplex trunculus* at each of the sampling locations in Kaštela Bay

All of the studies which determined the incidence of imposex along the eastern coast of the Adriatic Sea (TERLIZZI *et al.*, 2004; GARAVENTA *et al.*, 2006b; PRIME *et al.*, 2006), including this one, confirm its widespread incidence and can provide reference values for future monitoring programs. Especially now, with Croatia's recent adoption of the total ban on the use of organotin-based anti-foulants (NN, 17/2006) there is a need for further research aimed at understanding the imposex

induction mechanism and assessing the effectiveness of the regulations' implementation.

## CONCLUSIONS

All analyzed female *H. trunculus* (N=131) were affected by imposex. The most advanced imposex stage 5 was observed in 46% of females. The lowest imposex stage found, stage 2, accounted for 2% of the total number of analyzed females.

Table 2. Summary of mean  $\pm$  SD shell length (SL), female penis homologue length (FPL) and the vas deferens sequence index (VDSI) measurements of *Hexaplex trunculus* from seven locations in Kaštela Bay

Location	SL (mm)	FPL (mm)	VDSI
Bene	67.5 $\pm$ 4.6	8.1 $\pm$ 2.4	4.5 $\pm$ 0.4
Marina Kaštela	65.0 $\pm$ 4.0	12.6 $\pm$ 5.0	4.4 $\pm$ 0.7
Kaštel Stari	66.3 $\pm$ 9.2	6.8 $\pm$ 3.0	4.6 $\pm$ 0.5
Lora	72.0 $\pm$ 11.8	10.6 $\pm$ 4.5	4.7 $\pm$ 0.7
Lučica	70.7 $\pm$ 5.7	11.8 $\pm$ 3.3	4.9 $\pm$ 0.2
Resnik	64.8 $\pm$ 6.3	6.7 $\pm$ 2.6	3.8 $\pm$ 1.0
Arbanija	59.4 $\pm$ 2.9	5.2 $\pm$ 2.4	3.9 $\pm$ 0.8
Total	66.3 $\pm$ 7.5	8.7 $\pm$ 4.3	4.4 $\pm$ 0.8

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## Rasprostranjenost imposeksa kod vrste *Hexaplex trunculus* u Kaštelanskom zaljevu, Jadransko more

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### SAŽETAK

Imposeks, pseudohermafroditizam karakteriziran pojavom muških spolnih obilježja u ženki prednjoškržnih puževa, upotrebljava se kao bionidikator zagađenja tributil-kositrom (TBT), sastojkom protuobraštajnih boja. Za procjenu pojave i intenziteta imposeksa korištene su vrijednosti indeksa stupnja vas deferensa (VDSI) i dužine penisa ženki (FPL) dobivene analizom primjeraka vrste *Hexaplex trunculus* prikupljenih sa sedam postaja u Kaštelanskom zaljevu. Na istraživanim postajama analizom 131 ženke (visina kućice=66,3±7,5 mm) utvrđena je potpuna zahvaćenost imposeksom. Štoviše, većina zahvaćenih ženki bila je u uznapredovalom stadiju imposeksa (VDSI=4,4±0,8; raspon vrijednosti=2-5). Penisi su bili dobro razvijeni kod ženki (max=20 mm) sa srednjim vrijednostima od 5,2 do 12,6 mm. Utvrđene su statistički značajne razlike u VDSI (H=36,24, p<0,001) i FPL (F=7,08, p<0,001) između postaja. Rezultati istraživanja potvrđuju široku rasprostranjenost imposeksa i mogu se koristiti kao referentne vrijednosti za daljnje praćenje.

**Ključne riječi:** imposex, *Hexaplex trunculus*, Kaštelanski zaljev, Jadran, Hrvatska