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Cytochrome P450 differences in normal and imposex-affected female whelk *Buccinum undatum* from the open North Sea

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Abstract

Normal and imposex-affected female *Buccinum undatum* were sampled from the open North Sea at three locations, one with low, and two with high shipping densities. Cytochrome P450 components and P450 aromatase activity were determined in the microsomal fractions isolated from pooled digestive gland/gonads. Cytochrome P450 aromatase activity was significantly higher ($P<0.05$) in normal females collected in the low shipping density area (1325 ± 295 fmol/h/mg protein) than levels from imposex animals from a high shipping density area (620 ± 287 fmol/h/mg protein). A negative correlation was found between aromatase activity and organotin body burden ($r=-0.99$). Levels of CYP450, cytochrome b_5 and NADPH cytochrome *c* reductase activity did not show differences among groups. This is the first field evidence of depressed aromatase activity in imposex affected females, although additional research under laboratory controlled conditions is required to fully understand the mechanisms underlying the development of imposex in this species.

Keywords: Organotin compounds; Aromatase activity; *Buccinum undatum*; Imposex; North Sea

The whelk *Buccinum undatum* is a benthic subtidal snail that occurs in continental shelf seas of the Northern Atlantic Ocean from France in the East, USA (Maine) in the West, and up to the Arctic in the North. Previous research has shown that whelk

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populations from the open North Sea show imposex, which is positively correlated with shipping density. However, vertical stratification of the water-column also decreases the exposure of benthic organisms to organotins leaching from ship hulls in the surface layer (ten Hallers-Tjabbes, Kemo, van Hattum, Wegener, & Boon, in press). A decline of the *Buccinum undatum* populations has occurred over the last decades in several areas in the North Sea, for which TBT contamination and benthic fisheries are the most likely causes (Mensink, 1999). In juvenile whelks, chronic exposure to TBT has been demonstrated to induce imposex at concentrations >7 ng Sn/l (Mensink, 1999). The mechanism leading to imposex in female prosobranchs is not well understood, but the inhibition of the P450-mediated aromatase (CYP19) responsible for the conversion of androgens to estrogens has been suggested as a possible mechanism for imposex, resulting in increased tissue androgen levels (Bettin, Oehlmann, & Stroben, 1996). However, direct measurement of aromatase activity in female gastropods affected by imposex has only been done for the neogastropod *Bolinus brandaris* (Morcillo & Porte, 1999). Therefore, this study aimed to investigate the activity of the P450-mediated aromatase (CYP19) and the response of other CYP450 system components in normal and imposex-affected female *B. undatum* from the North Sea.

Female *B. undatum* were collected by beam trawl in August/September 1999 in the North Sea, at three locations of different shipping density (high >10 ships per day within 15 nautical miles; low <5 ships per day). Whelks were sexed, and in females the degree of imposex was determined according to Mensink (1999). After imposex assessment, the length of the shell was measured and the shell then broken with a vice and the digestive gland/gonad complex immediately frozen in liquid nitrogen. Only snails larger than 7 cm in length were used for the biochemical determinations. The pooled digestive gland/gonad of two snails were used for each replicate. Microsomes were prepared as described by Livingstone (1988). The cytochrome P450 aromatase activity was determined by measuring the tritiated water release from 1β-^{3}H-androstenedione (Morcillo & Porte, 1999). Cytochrome P450 system components (levels of total cytochrome P450, cytochrome b_5 and NAD(P)H-cytochrome *c* reductases) were also measured in the microsomal fraction as described in Livingstone (1988). Organotin analysis was done by means of gas chromatography with an ion trap mass spectrometric detector according to the methodology describe in ten Hallers-Tjabbes et al. (in press). Statistical significance was assessed using one-way ANOVA with a multiple comparison test (Student–Newman–Keuls) using Statgraphics 7.0.; values of aromatase activity and NAD(P)H-cytochrome *c* reductases were log transformed to stabilise the variance.

The P450 aromatase activity ranged from 620 to 1325 fmol/h/mg protein, and was significantly higher ($P<0.05$) in normal females than in the ones showing imposex sampled at the station with the highest imposex rates (station 26: Fig. 1a; Table 1). A negative correlation was found between aromatase activity and organotin body burden ($r=-0.99$), the lower aromatase activity being found in the snails that showed the highest organotin content (Fig. 1b). Levels of total CYP450 and cytochrome b_5 did not show differences among groups (Table 1). In contrast, NADH cytochrome *c* reductase activity was significantly higher in imposex females (114

nmol/min/mg protein) than in normal ones (30 nmol/min/mg protein) collected in the low shipping density area. NADPH cytochrome *c* reductase activity which ranged from 2.8 to 8.6 nmol/min/mg protein, was slightly higher in normal females, but differences were not statistically significant.

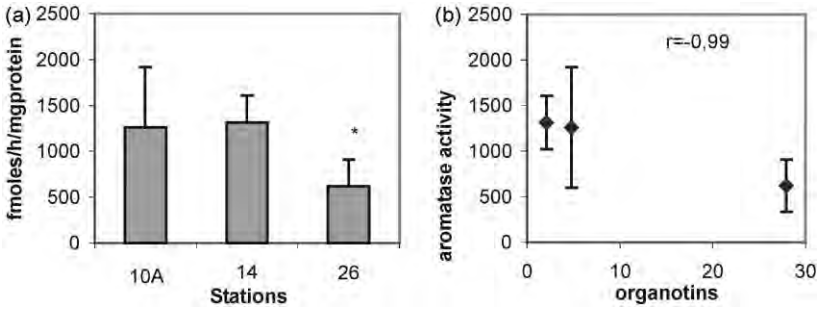

Fig. 1. (a) CYP450 aromatase activity; (b) Correlation between organotins body burden (ng Sn/g wet weight) and aromatase activity (fmol/h/mg protein). *Significantly different from station 14 ($P<0.05$).

Table 1
Sampling position, shipping density, summer water stratification (Y—yes, N—no), percentage of imposex female *Buccinum undatum*, biochemical determinations and organotin concentrations in snail tissue (ng Sn/g wet weight) for the three sampling sites

	Station 14	Station 10A	Station 26
Position	57.30N; 02.00E	54.55N; 01.00E	54.06N; 08.12E
Shipping density	low	high	high
Summer water stratification	Y	Y	N
% imposex females[a]	0	4.60	38.81
% imposex females[b]	0	0	75
Imposex index[c]	0.00	0.14	0.51
Cyt. 450 (pmol/mgprotein)	146.5±87.5	163.4±65.72	106.3±53.3
418 peak (a.u./mg protein)	123.3±56.4	120.9±87.24	156.1±97.4
Cyt. b_5 (pmol/mg protein)	30.3±8.3	31.4±21.7	37.3±20.7
NADPH cytochrome *c* reductase activity (nmol/min/mg protein)	5.3±2.5	8.6±7.9	2.8±1.2
NADH cytochrome *c* reductase activity (nmol/min/mg protein)	30.7±12.2	109.1±58.5*	114.0±39.7*
Tributyltin (TBT)	0.2	0.2	0.5
Dibutyltin (DBT)	0.8	0.9	6.3
Monobutyltin (MBT)	0.6	0.9	6.3
Triphenyltin (TPT)	0.5	1.3	12
Diphenyltin (DPT)	<0.3	1.3	<0.6
Monophenyltin (MPT)	<0.1	0.2	2.8

For biochemical determination values are mean±SEM ($n=4$–12); a.u.; arbitrary units

[a] %imposex females in the population.

[b] %imposex females used in the biochemical determinations.

[c] Imposex index in the population according to Mensink (1999).

* Significantly different from station 14 ($P<0.01$).

The results show that digestive gland/gonad microsomes of female *B. undatum* have the enzymatic system required to convert androgens (androstenedione and testosterone) to estrogens (estradiol and estrone), and they are the first field evidence that imposex snails show lower aromatase activity than normal ones.

This is in agreement with previous laboratory experiments conducted with the neogastropod *Nucella lapillus* (Bettin et al., 1996; Spooner, Gibbs, Bryan, & Good, 1991) and *Hinia reticulata* (Bettin et al., 1996), which showed that exposure to TBT promotes imposex, and resulted in increased tissue levels of androgen and increased testosterone/estradiol ratio. These results linked the induction of imposex in snails with a potential inhibition of P450 mediated aromatase. Further support to this hypothesis was the fact that a specific aromatase inhibitor can promote imposex (Bettin et al., 1996). In the clam *Ruditapes decussata*, laboratory exposure to TBT induced a decrease in the aromatization of testosterone to estrone and estradiol (Morcillo, Ronis, & Porte, 1998) and, in the field, clams transplanted to a organotin-polluted marina showed a decrease in estradiol titres, concomitant to increased concentration of organotins in their tissues, suggesting an interaction of TBT with aromatase activity (Morcillo and Porte, 2000). Additionally, exposure of *N. lapillus* and *H. reticulata* to an anti-androgen (cyproterone acetate) can suppress imposex development, suggesting the involvement of androgens receptor in imposex promotion in these species (Bettin et al., 1996).

Other hypotheses have been put forward to explain imposex induction. Hence, Féral and Le Gall (1983) showed that TBT affected cerebropleural ganglia in *Ocenebra erinacea* causing a release of neural factors inducing imposex. Recently, Oberdorster and McClellan-Green (2000) demonstrated that the amidated tetrapeptide Ala-Pro-Gly-Trp-NH_2 (APGWamide) induces imposex in *Ilyanassa obsoleta*, indicating that imposex may initially be induced by neuropeptides, which in their turn would affect enzymes controlling the production and metabolism of sex steroid hormones.

This study shows that imposex female *B. undatum* collected in open North Sea in August/September 1999 had lower P450 aromatase activity than normal ones. However, whether depressed aromatase activity is due to neuropeptide control or a direct inhibitory effect of organotins in this enzymatic activity is still unknown.

In field studies, factors other than organotins, e.g. differences in water temperature, salinity, or other chemicals, may have an effect on the physiology of *B. undatum*. Therefore, additional research under controlled laboratory conditions has to be done to fully understand the relevance of these findings and the mechanisms underling the development of imposex in female whelks.

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