A Survey of Imposex in the Dogwhelk (*Nucella lapillus*) in Eastern Canada (1995)

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Abstract

The occurrence of imposex in the dogwhelk (*Nucella lapillus*) was surveyed in eastern Canada for the first time in 1995. Dogwhelks were absent at 14 sites (all in harbours) with seemingly ideal habitat. Imposex was present at 13 of 34 sites (8 out of 17 harbours), with 100% imposex at two sites (two harbours). Seven sites showed no imposex. Relative Mean Female/Male Penis Size Index (RPSI) ranged 0.4-38.5%. These results provide a databank for future monitoring.

Introduction

Tributyltin (TBT) biocides are used in marine antifouling paints. TBT was first shown to cause detrimental effects in feral molluscs by Alzieu et al. (1986). Field surveys and bioassays have established that TBT can induce masculinization of female neogastropod molluscs (marine snails), known as "imposex", at levels as low as 0.064 ug $TBT \cdot L^{-1}$ (Stickle et al. 1990). Other causes of imposex, e.g. copper, environmental stress (Nias et al. 1993), have been suggested, but remain unproven.

TBT is partially controlled in Canada (CAPCO 1990) and elsewhere (e.g., France, U.K., U.S.A., New Zealand, Australia), but not globally (Khalimonov 1995). Even where controlled, it can still be used on aluminum boats and vessels over 25 m length. Thus, contamination may be continuing, especially within harbours. TBT release from sediments is known (Sarradin et al. 1994). The effectiveness of controls has been monitored (e.g. Page 1995; Tester et al. 1996), which showed that environmental concerns remain.

Neogastropod molluscs are generally carnivorous, often feeding on bivalve molluscs, and have separate sexes (Fretter and Graham 1994). Imposexed females develop a penis and vas deferens. Imposex intensity is measured by penile length and extent of vas deferens development. Surveys have been conducted in many areas using intertidal species (e.g., Ellis and Pattisina 1990). For example, high TBT loadings in Victoria and Vancouver Harbour sediments (Cullen et al. 1990; Stewart and Thompson 1994) are accompanied by 100% incidence of imposex in the nearest neogastropod stocks.

During 1995, sites in eastern Canada were surveyed for imposex using the common North Atlantic dogwhelk (*Nucella lapillus)*, which ranges from southern Labrador to New York and into the St. Lawrence estuary (Bousfield 1955, 1960). Egg capsules are laid on rocks during spring and summer. The young emerge from the capsules as miniature, crawling adults. Limited mobility facilitates use as a bioindicator of TBT.

Materials and Methods

Rocky, intertidal sites (Figure 1) with mussels and barnacles, food for dogwhelks, were surveyed during May-October, 1995. Sites were selected in harbours, generally one site a harbour, with extensive shipping and boat traffic, near marinas, or shipyards. Areas away from any marine activity were surveyed as controls. Most sites were visited once.

A more thorough survey was made of Halifax Harbour (Figure 2) which has the highest amount of ship traffic in eastern Canada (Statistics Canada 1993), the largest Canadian naval installation, numerous fishing vessels and recreational craft, and several dockyard facilities. The presence of juvenile and mature dogwhelks at each site was recorded. Up to 50 mature specimens (>20 mm shell length) were collected. Shell length and height were measured. The shell was cracked open, the body extracted, and sex determined (Gibbs et al. 1987; Fretter and Graham 1962; Hall and Feng 1976). The mantle cavity was opened with a longitudinal cut and examined with a dissecting microscope. Imposex frequency, the percent females affected at a site, was recorded (Ellis and Pattisina 1990; Saavedra Alvarez and Ellis 1990). Penile length was measured using an ocular micrometer. The mean female and male penile lengths (FPL & MPL) were determined and the Relative Mean Female/Male Penis Size Index (RPSI) ($FPL^3/MPL^3 \times 100$) calculated for each site (Gibbs et al. 1987). The development of the vas deferens (Gibbs et al. 1987) and blockage of the genital pore by vas deferens tissue (indicated by aborted egg capsules in the oviduct) were noted.

Results and Discussion

Imposex was present in 13 of 34 sites (Table 1, Figures 1 & 2). Imposex frequencies ranged from 29 up to 100% at sites in Sydney and Halifax Harbours. Both harbours rank high for ship traffic, fishing vessel tonnage, and industrial and commercial activity (Prouse 1994). Other sites were Les Méchins (95%), a small port with a dry dock and ship maintenance facility; Chester (88%), a marina; Lunenburg (81%), a fishing port with ship building and repair; Saint John (66%), a commercial and industrial harbour; Arnold's Cove (56%), a fishing port; and Come-by-Chance (29%), an oil terminal.

Halifax was the most seriously affected harbour (Figure 2). Six of the 11 sites had dogwhelks with imposex frequencies ranging 65-100%. Dogwhelks were not found at the remaining 5 sites, which appeared to have good habitat. Highest frequency (100%) was near the harbour mouth adjacent to the main ship channel (site A10), which annually carries over 2000 vessels exceeding 15 register tons (Statistics Canada 1993). Imposex close to areas used by large vessels has been observed elsewhere (Ten Hallers-Tjabbes et al. 1994; Davies and Bailey 1991). The extent of imposex outward from Halifax Harbour is unknown.

The 5 sites without dogwhelks were in the inner harbour near marinas, ship yards, and large docking facilities (sites A2, A4-7, Figure 2). The historical presence of dogwhelks at these sites is unknown. Unfavourable conditions, such as low salinity, may have prevented the establishment of viable dogwhelk populations or caused their disappearance. TBT concentrations in sediments sampled near these sites in 1994 were higher than in 1988, prior to TBT regulation (Ernst et al. 1995). Fresh TBT inputs from ship traffic and repair facilities were suggested. The absence of dogwhelks as a result of TBT contamination has been observed in the U.K. (e.g., Gibbs et al. 1991). Imposex in neogastropods is irreversible (Foale 1993), leading to reproductive failure and population decline (Gibbs and Bryan 1986; Bryan et al. 1986). Dogwhelks were found in the outer harbour (sites A1, A8-11) and in Bedford Basin (site A3) away from the area of concentrated vessel activity.

Dogwhelks were absent at 9 other sites, all within active harbours. Included are Hubbards, Mahone Bay, Sydney, Port Hawkesbury, Pictou, and Caribou in Nova Scotia, Summerside in Prince Edward Island, Rimouski in Quebec, and Holyrood in Newfoundland. TBT is present in sediments from Sydney, Port Hawkesbury, and Pictou (Maguire et al. 1986) and blue mussels (*Mytilus edulis*) from Pictou, Sydney, and Summerside Harbours in 1994 (Environment Canada, unpublished data).

Dogwhelks from 7 sites, including Cleveland Beach and Queensland Beach, Nova Scotia; Bellevue Cove, Prince Edward Island; Métis-sur-Mer, Quebec; and Rocky Harbour and 2 sites in Argentia, Newfoundland had no imposex. All sites except Argentia have little or no boating activity and are not near TBT sources. Argentia Harbour was a U.S. naval base until October 1994 and had been used by naval ships and small industrial support vessels. TBT was detected in sediments taken from this harbour in 1982 and 1985 (Maguire et al. 1986). Perhaps the absence of imposex resulted from the U.S. Navy's policy implemented in 1985 to manage use of TBT paints on its ships (Schatzberg 1987) and the base closure.

Juveniles and, usually, egg capsules were present at most sites where adults were found, confirming recruitment. However, dogwhelks collected at Les Méchins, in the St. Lawrence estuary, were severely imposexed (highest FPL of all sites) and no egg capsules were found. Les Méchins had an imposex frequency of 95% and the highest RPSI, at 38.5%. This was the only site where some females had aborted egg capsules, indicating reproductive failure.

Studies have reported recoveries from TBT contamination based on decreases in imposex measurements and reappearance of neogastropod populations following regulation of TBT use (Tester and Ellis 1995; Evans et al. 1995; Evans et al. 1994; Douglas et al. 1993). For example, a temporal decrease in RPSI values has been used in the U.K. to show the effectiveness of TBT controls (Evans et al. 1996; Evans et al. 1995). Imposex frequencies have declined in shoreline neogastropods on the west coast of Canada over time (Tester and Ellis 1995; Tester et al. 1996). There are no previous surveys of imposex in eastern Canada. The Canadian east coast does not have a readily available short-lived (1-2 years) species, which can be resampled more frequently to observe imposex changes (Tester et al. 1996; Tester and Ellis 1995). Monitoring recovery following controls is more difficult with a longer-lived species such as *N. lapillus* (6+ years). This species, the dominant intertidal neogastropod in eastern Canada, cannot be easily aged. This means that sites surveyed in 1995 yielded an unknown number of older individuals that were exposed to TBT prior to controls in 1989. Butyltin residues in sediments in 1994 were higher in large vessel harbours in Atlantic Canada than in pleasure craft areas and had increased since a previous survey in 1988 (Ernst et al. 1995).

Imposex affected all females at only 2 sites compared with numerous sites with frequencies at or close to 100% reported elsewhere, e.g. the Pacific coast of Canada (Tester et al. 1996; Tester and Ellis 1995; Saavedra Alvarez and Ellis 1990; Bright and Ellis 1990), the U.K. (Evans et al. 1994; Evans et al. 1991).

Despite restrictions, TBT contamination is a widespread and continuing concern (Stewart and Thompson 1994). Problem areas were found in eastern Canada in 1995. Sites with both high imposex frequencies and high RPSI values should be studied to determine if sediments are releasing TBT or if there is continuing release from aluminum boats and large vessels or if other sources are present.

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Table 1. Imposex data for dogwhelks (*Nucella lapillus*) at sites in eastern Canada in 1995

	Site	Date of Survey day/mo	No. of Specimens male	female	Shell Length (mm)	FPL (mm) ±SD	RPSI[a] (%)	Imposex Frequency (%)
<u>Nova Scotia</u>								
	Halifax Harbour							
A1.	Northwest Arm (mouth)	9/08	29	25	19.0-33.7	1.6 ±0.7	4.8	88
A2.	Northwest Arm (head)	17/08	0	0				
A3.	Bedford Basin	23/06	24	26	21.7-31.4	1.6 ±0.6	10.8	92
A4.	Dartmouth Shipyard	18/07	0	0				
A5.	Irving Oil Wharf	18/07	0	0				
A6.	Queens Wharf	20/06	0	0				
A7.	Eastern Passage	19/09	0	0				
A8.	Container Pier	3/08	19	31	19.0-27.1	1.4 ±0.4	6.9	65
A9.	Fergusons Cove	12/08	17	20	20.0-31.0	1.2 ±0.5	7.1	95
A10.	Portuguese Cove	6/09	24	16	20.0-27.0	1.5 ±0.5	11.3	100
A11.	Hartlen Point	19/09	24	16	18.4-22.6	0.7 ±0.2	1.7	81
	St. Margarets Bay							
B.	Cleveland Beach	15/05	29	21	19.0-26.8	0	0	0
C.	Queensland Beach	25/08	4	11	22.2-28.9	0	0	0
D.	Hubbards	13/07	0	0				
	Other Nova Scotia Harbours							
E.	Chester	0/06	12	16	20.3-30.9	1.0 ±0.4	3.4	88
F.	Lunenburg	11/07	14	36	19.8-30.3	1.5 ±0.5	11.3	81
G.[b]	Mahone Bay	11/07	0	0				
H1.	Sydney (north of South Bar)	9/08	21	15	20.3-31.0	1.7 ±0.4	16.5	100
H2.[b]	Sydney (South Arm, Pt. Edward, South Bar)	9/08	0	0				

Table 1 (cont.)

	Site	Date of Survey day/mo	No. of Specimens male	female	Shell Length (mm)	FPL (mm) ±SD	RPSI[a] (%)	Imposex Frequency (%)
I.[b]	Port Hawkesbury, Point Tupper	10/08	0	0				
J.[b]	Pictou	7/07	0	0				
K.	Caribou Ferry Terminal	10/08	0	0				
Prince Edward Island								
L.	Bellevue Cove	7/10	3	21	21.2-27.0	0	0	
M.[b]	Summerside, MacCallums Pt.	8/07	0	0				
New Brunswick								
N.	Saint John (at Red Head)	16/06	23	27	24.0-35.7	1.1 ±0.4	1.4	66
Quebec								
O.[b]	Rimouski, Pointe-au-Père	28/08	0	0				
P.	Métis-sur-Mer	28/08	14	18	23.3-31.4	0	0	0
Q.	Les Méchins	22/09	23	21	23.2-30.6	3.2 ±0.4	38.5	95
Newfoundland								
R.	Rocky Harbour	11/09	20	17	21.2-33.8	0	0	0
S.	Come-By-Chance	14/09	23	17	21.4-31.5	0.5 ±0.3	0.4	29
T.	Arnold's Cove	14/09	7	9	16.0-21.0	0.6 ±0.4	1.8	56
U1.	Argentia (inner harbour)	13/09	12	17	20.0-26.9	0	0	0
U2.	Argentia (outer harbour)	13/09	6	18	18.3-26.6	0	0	0
V.	Holyrood	13/09	0	0				

[a] Mean female penis length (FPL) and mean male penis length (MPL) were used to calculate relative penis size index (RPSI). RPSI = $(FPL)^3/(MPL)^3 \times 100\%$.

[b] More than one sample location was visited.

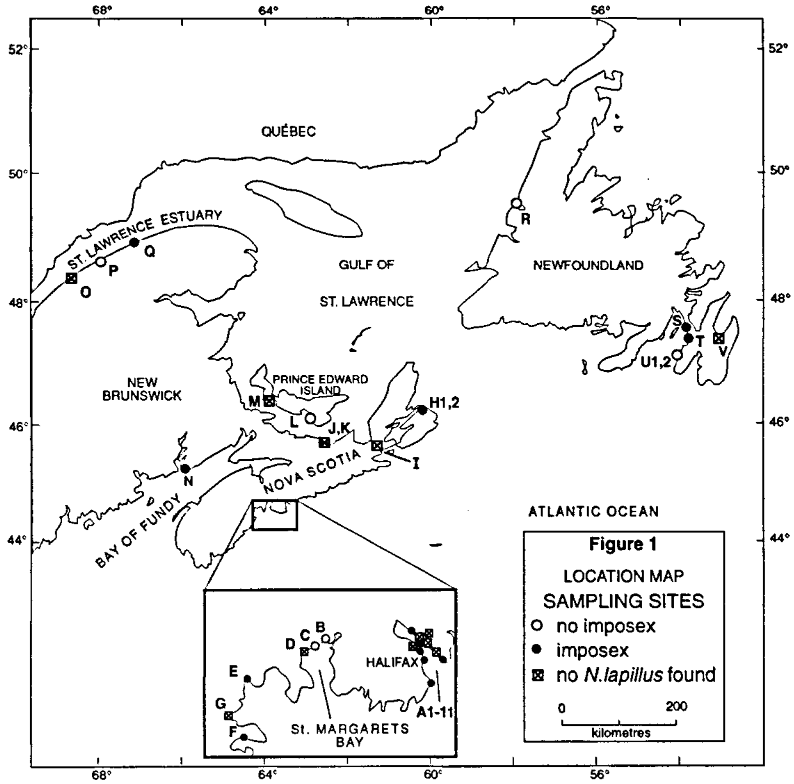

Figure 1
LOCATION MAP
SAMPLING SITES

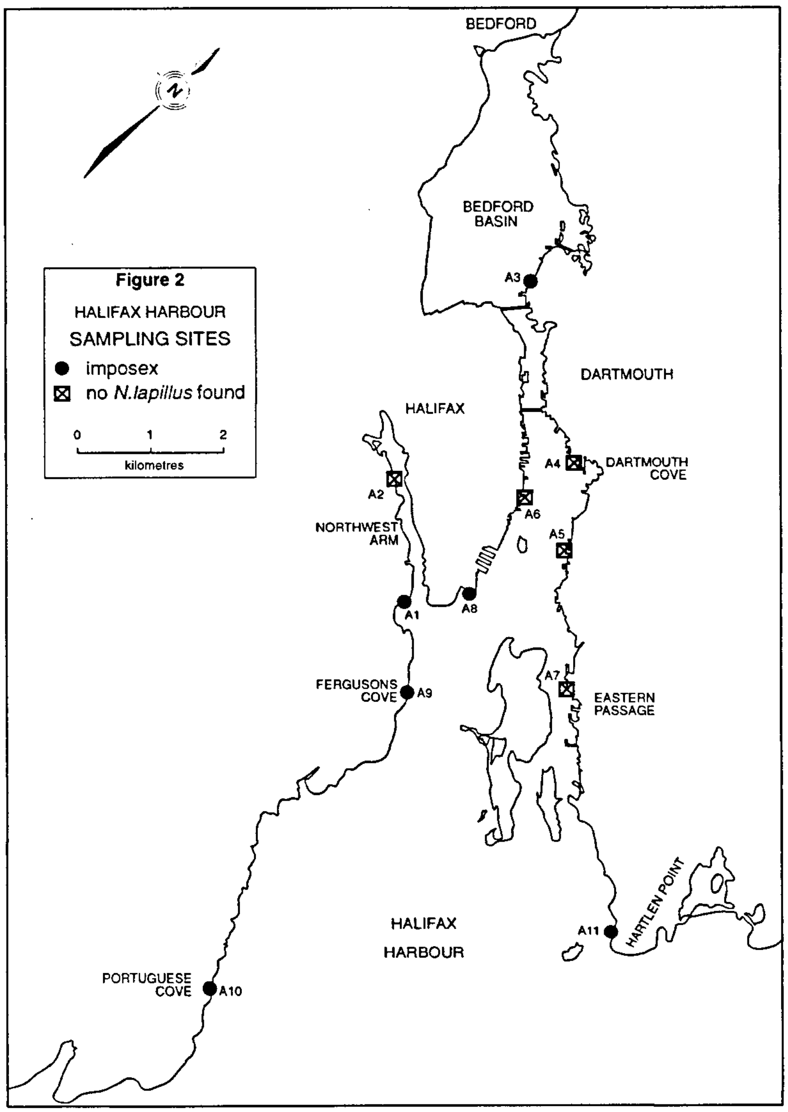

Figure 2
HALIFAX HARBOUR
SAMPLING SITES