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Contamination of organotin compounds and imposex in molluscs from Vancouver, Canada

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Abstract

Gastropods and bivalves were collected at 15 sites at Vancouver and Victoria, Canada between 24 May and 7 June, 1999, to establish tissue concentrations of butyltin and phenyltin compounds, to record imposex symptoms in gastropods, and to assess the present status of organotin contamination around Vancouver. No neogastropods (such as *Nucella lima*) were found around Vancouver. Neogastropod populations could have been extirpated by severe TBT contamination in Vancouver, as relatively high concentrations of TBT were detected in tissues of *Mytilus trossulus* from Vancouver, and the neogastropods distributed in Vancouver might be sensitive to TBT. Recovery from imposex, however, was observed in neogastropod populations from three sites at Victoria and Mission Point. TBT contamination has continued around Vancouver, arising from continuous use of TBT in antifouling paints for vessels larger than 25 m in length; however, TBT has decreased around Victoria and Mission Point. Different patterns of TBT accumulation in tissue were observed among the bivalve species from Vancouver. The highest TBT concentration detected in *Tresus capax* suggested some possible adverse effects. TBT was the most predominant butyltin component in almost all bivalve specimens surveyed, suggesting a low rate of TBT metabolism. Phenyltin compounds were not detected in any molluscan specimens in this study.

Keywords: Imposex; Recovery; Tributyltin (TBT); Neogastropods; Bivalves; Pollution monitoring; Vancouver harbour

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1. Introduction

Organotin compounds, such as tributyltin (TBT) and triphenyltin (TPhT), have been used worldwide in antifouling paints for ships and fishing nets since the mid-1960s, and have caused imposex in neogastropods and mesogastropods around the world (e.g., Bright & Ellis, 1990; Bryan, Gibbs, Hummerstone, & Burt, 1986; Ellis & Pattisina, 1990; Fioroni, Oehlmann, & Stroben, 1991; Gibbs, Bryan, Pascoe, & Burt, 1990; Gibbs, Spencer, & Pascoe, 1991; Goldberg, 1986; Horiguchi, Shiarishi, Shimizu, & Morita, 1994; Horiguchi, 2000; Stewart, deMora, Jones, & Miller, 1992). Imposex is defined as a superimposition of male sexual organs (penis and vas deferens) on female gastropods, and may bring about reproductive failure at severely affected stages, resulting in population decline (Bryan et al., 1986; Gibbs & Bryan, 1986; Gibbs, Bryan, Pascoe, & Burt, 1987; Gibbs, Pascoe, & Burt, 1988; Gibbs et al., 1990, 1991; Smith, 1971). Imposex is thought to arise from endocrine disruption induced by TBT and TPhT in gastropods and some hypotheses about possible modes of endocrine action of TBT in gastropods have been proposed (Bettin, Oehlmann, & Stroben, 1996; Bryan, Gibbs, & Burt, 1988; Bryan, Bright, Hummerstone, & Burt, 1993; Féral & Le Gall, 1983; Horiguchi, Shiarishi, Shimizu, & Morita, 1997a; Matthiessen & Gibbs, 1998; Oberdörster & McClellan-Green, 2000; Spooner, Gibbs, Bryan, & Goad, 1991; Ronis & Mason, 1996). Monitoring surveys of imposex and TBT contamination in gastropods have been carried out in many countries (for example, Horiguchi, Shiraishi, Shimizu, & Morita, 1997b; Horiguchi et al., 1998; Miller, Fernandes, & Read, 1999; Minchin, Stroben, Oehlmann, Bauer, Duggan, & Keatinge, 1996; Morgan, Murphy, & Lyons 1998; Nias, McKillup, & Edyvane, 1993; Prouse & Ellis, 1997; Saavedra Alvarez & Ellis, 1990; Tester, Ellis, & Thompson, 1996;).

The use of TBT has been banned in antifouling paints for ships smaller than 25 m in length in many developed countries, such as many European countries, Canada, the United States and Australia, since the 1980s (Stewart, 1996). The production, import and use of organotins (TBT and TPhT) have been restricted by law and administrative guidance since 1990 in Japan, resulting in no domestic production in 1997 (Horiguchi, 2000). TBT-based antifouling paints, however, are still used in developing countries, such as most Asian countries, and their use has also continued worldwide for most of the vessels larger than 25 m in length (Horiguchi, 2000; Stewart, 1996). Fortunately, however, a treaty for the worldwide ban of TBT was adopted at the International Maritime Organization (IMO) in October 2001, and it is expected to start from January 2003.

There has been considerable research on TBT and imposex in coastal waters of Canada, including British Columbia (e.g., Chau, Maguire, Brown, Yang, & Batchelor, 1997a, 1997b; Maguire, 2000; Maguire, Tkacz, Chau, Bengert, & Wong, 1986; Maguire, Chau, & Thompson, 1996; Saavedra Alvarez & Ellis, 1990; Stewart & Thompson, 1994, 1997; Tester & Ellis, 1995; Tester et al, 1996). Extensive butyltin contamination and widespread neogastropod imposex were observed in British Columbia around 1990 (Saavedra Alvarez & Ellis, 1990; Stewart & Thompson, 1994). Recovery of neogastropods from imposex began to be observed after the mid

1990s, due to the decrease of TBT concentration in the marine environment following the TBT control in 1989, except for contaminated sites near harbours, such as Vancouver Harbour and Victoria (Agriculture Canada, 1989; Tester & Ellis, 1995; Tester et al., 1996). Biological/environmental monitoring of neogastropod imposex and TBT contamination is still necessary because of the long persistence of TBT in sediment (Chau et al., 1997a; Maguire, 2000; Stewart & Thompson, 1997). During the PICES Practical Workshop in Vancouver, Canada from May 24 to June 7, 1999, we studied imposex symptoms and tissue concentrations of organotin compounds, such as TBT, in gastropod specimens, and organotin concentrations in tissues of bivalve specimens from Vancouver and Victoria as described below. The present status and temporal trends of imposex and organotin contamination in molluscs around Vancouver are also discussed, and it will be useful to gauge the success of Canadian regulation of TBT.

2. Materials and methods

2.1. Sampling for molluscs and imposex examination in neogastropods

Molluscs (gastropods and bivalves) were collected at 15 sites around Vancouver and Victoria during the Workshop (see Fig. 2 of Levings, Stein, Stehr, & Samis, 2003) After sampling, raw or frozen gastropod specimens were used for imposex identification. Sex determination was first performed based on accessory sex organs: females were recognized by the existence of albumen, sperm-ingesting and capsule glands, and imposex-exhibiting individuals were identified as females that had either penis or vas deferens with albumen, sperm-ingesting and capsule glands (Gibbs et al., 1987). The degree of imposex at each site was expressed as incidence (frequency) of imposex among female specimens (%), Relative Penis Length (RPL) Index (%), Relative Penis Size (RPS) Index (%) and Vas Deferens Sequence (VDS) Index through the measurement of penis length and observation of the development of vas deferens (Gibbs et al., 1987; Horiguchi et al., 1994). RPL and RPS Indices are defined as {(mean penis length in females)/(mean penis length in males)} $\times$ 100 and {(mean penis length in females)^3/(mean penis length in males)^3} $\times$ 100, respectively (Gibbs et al., 1987; Horiguchi et al., 1994; see Gibbs et al., 1987 for the detailed description for VDS Index). Both RPL and RPS Indices were measured in this study for comparison with other studies in which either the RPL Index or the RPS Index was reported (e.g., Gibbs et al., 1987; Horiguchi et al., 1994).

2.2. Chemical analysis of organotin concentrations in tissue of molluscan specimens

Chemical analyses of organotin (butyltin and phenyltin) compounds in tissues of both gastropod and bivalve specimens were conducted as described in Horiguchi et al. (1994). Briefly, composite tissues for each molluscan species were extracted with 0.1% tropolone/benzene and 1 N HBr/ethanol by ultrasonication, derivatized with propylmagnesium bromide, cleaned up by silica gel column chromatography and quantified by gas chromatography with a flame photometric detection (GC–FPD). The detection limit of the instrument was 50 pg, and certified reference material of Japanese sea bass, *Lateolabrax japonicus*, for TBT and TPhT analysis (prepared by National Institute for Environmental Studies; NIES CRM No. 11) was used for quality assurance and quality control. The analytical conditions are described in more detail in Horiguchi et al. (1994).

3. Results

3.1. Imposex symptoms in neogastropods

No neogastropod specimens (e.g. *Nucella lima*) were collected at sites in Vancouver (I-1, 2A, 3A, 3B, 3C, 4A, 4B, 5A, 5B, 6 and 7; Levings et al. 2003, Fig. 2) in this study. Huge colonies of barnacles, the prey of carnivorous neogastropods like *Nucella lima* and *N. lamellosa*, were common at the above sites.

Summaries of the imposex survey results are shown in Tables 1 and 2 [raw data on imposex surveys for the dogwinkles, *Nucella* sp. from Victoria (Ogden Point, Clover Point and Ten Mile Point) and at Mission Point in Wilson Creek are shown in Table 47 of the PICES data report (Stehr & Horiguchi, 2001)] The incidence of imposex among female dogwinkles was generally still high, i.e. over 71% for the file dogwinkle (*Nucella lima*), 100% for the emarginate dogwinkle (*N. emarginata*) and 100% for the frilled dogwinkle (*N. lamellosa*), except for *N. lima* and *N. lamellosa* from Ten Mile Point. The imposex symptoms, however, were not very serious. The values of RPL Index were below 19.0, 33.6 and 23.1 for *N. lima*, *N. emarginata* and *N. lamellosa*, respectively; those of RPS Index were below 0.7, 3.8 and 1.2 for *N. lima*, *N. emarginata* and *N. lamellosa*, respectively, and those of VDS Index were below 2.9, 2.1 and 2.5 for *N. lima*, *N. emarginata* and *N. lamellosa*, respectively (Tables 1 and 2). Some differences in the imposex survey results were observed between the investigators (e.g. incidence of imposex in females of *N. lima* and *N. lamellosa* from Ten Mile Point: Tables 1 and 2) but this may result from differences between the shell height of the dogwinkle specimens: larger gastropod specimens may be older than smaller ones, and therefore, the imposex symptoms of larger ones could reflect previous contamination levels of TBT which may have been more severe than recent contamination levels. No imposex was observed in the dire whelk (*Searlesia dira*) from Clover Point.

3.2. Butyltin concentrations in tissues of dogwinkles

Butyltin concentrations in tissues of both *N. lima* and *N. lamellosa* are shown in Fig. 1. Phenyltin compounds were not detected in either *N lima* or *N. lamellosa*. TBT concentrations of 2.4–14.4 and 6.5–22.0 ng g^{-1} wet wt. were detected in *N. lima* and *N. lamellosa*, respectively. Total butyltin concentrations in tissue [sum of TBT and its metabolites, monobutyltin (MBT) and dibutyltin (DBT)] of *N. lima* and *N. lamellosa* were 7.3–28.8 and 10.8–44.0 ng g^{-1} wet wt., respectively.

Table 1
Summary of results of imposex survey of gastropods collected by T. Horiguchi from the shores around Victoria and at Mission Point (*N*, Number of specimens; M, Male; F, Female; min, minimum value; max, maximum value; S.D., standard deviation; IOI, Incidence of Imposex; RPL, Relative Penis Length Index; RPS, Relative Penis Size Index; VDS, Vas Deferens Sequence Index)

Site	Date	Species	N (M:F)	Shell height in mm (mean ± S.D.; min-max)		IOI (%)	Penis length in mm (mean ± S.D.)[a]		RPL (%)	RPS (%)	VDS (%)
				Male	Female		Male	Female			
Ogden Point	05/31/99	*Nucella lima*	30 (12:18)	31.8 ± 2.6; 27.8–36.8	35.0 ± 3.2; 29.2–41.2	100	10.0 ± 2.0	1.9 ± 0.9	19.0	0.7	2.9
Clover Point	05/31/99	*N. lima*	20 (2:18)	28.3 ± 0.8; 27.7–28.8	30.2 ± 2.2; 25.5–33.9	72.2	9.3 ± 2.5	1.1 ± 0.8	11.8	0.2	2.1
Ten Mile Point	05/31/99	*N. lima*	10 (3:7)	29.4 ± 1.7; 28.0–31.3	27.7 ± 1.9; 26.0–30.5	71.4	9.0 ± 2.2	0.3 ± 0.4	3.3	0.004	1.1
Ten Mile Point	05/31/99	*N. lamellosa*	10 (4:6)	39.1 ± 4.0; 35.4–44.4	37.8 ± 2.7; 34.9–41.4	100	7.3 ± 0.5	0.6 ± 0.5	8.2	0.1	1.0
Mission Point	06/02/99	*N. lamellosa*	10 (7:3)	35.5 ± 2.2; 32.7–38.8	39.4 ± 1.6; 38.2–41.2	100	7.8 ± 1.2	1.8 ± 0.3	23.1	1.2	1.0

[a] Penis length was measured along its curve, as the penis form was curved.

Table 2
Summary of results of imposex survey of gastropods collected by Z. Li from the shores around Victoria and at Mission Point (*N*, Number of specimens; M, Male; F, Female; min, minimum value; max, maximum value; S.D., standard deviation; IOI, Incidence of Imposex; RPL, Relative Penis Length Index; RPS, Relative Penis Size Index; VDS, Vas Deferens Sequence Index)

Site	Date	Species	N(M:F)	Shell height in mm (mean±S.D.; min-max)		IOI (%)	Penis length in mm (mean±S.D.)		RPL (%)	RPS (%)	VDS (%)
				Male	Female		Male	Female			
Ogden Point	05/31/99	*Nucella lima*	30(11:19)	27.3±6.1; 16.5-33.5	30.0±4.1; 20.5-36.3	89	7.60±1.70	1.11±0.87	14.6	0.312	1.84
Clover Point	05/31/99	*N. lima*	30 (16:14)	29.4±2.4; 25.3–33.6	29.1±1.6; 26.0–32.0	78	6.26±1.52	0.50±0.45	7.98	0.051	1.50
Ten Mile Point	05/31/99	*N. lima*	30 (11:19)	24.8±1.8; 22.2–27.5	25.5±2.7; 20.0–30.9	21	7.42±1.17	0.04±0.13	0.568	1.83E-5	0.26
Ogden Point	05/31/99	*N. emarginata*	30 (5:25)	22.4±1.6; 20.3–23.7	23.0±1.8; 20.3–26.3	100	5.78±0.83	1.94±0.46	33.6	3.80	2.08
Ten Mile Point	05/31/99	*N. emarginata*	5 (1:4)	22.0	20.0±2.5; 16.4–22.0	100	6.00	0.80±0.32	13.3	0.237	2.00
Ten Mile Point	05/31/99	*N. lamellosa*	30 (14:16)	36.6±3.7; 31.9–44.0	39.7±2.9; 33.2–45.0	19	5.99±0.94	0.09±0.22	1.57	3.84E-4	0.38
Mission Point	06/02/99	*N. lamellosa*	24 (12:12)	36.6±2.5; 34.1–43.0	40.5±3.5; 35.0–47.2	100	6.41±0.89	1.27±0.47	19.8	0.776	2.50
Clover Point	05/31/99	*Searlesia dira*	10 (6:4)	33.7±4.6; 26.8–39.0	29.6±5.1; 23.9–36.0	0	8.90±2.46	0	0	0	0

3.3. Butyltin concentrations in tissues of bivalves

Biological monitoring of organotin contamination using the foolish mussel (*Mytilus trossulus*) was also carried out to establish the present status of contamination around Vancouver. Analytical results on organotin concentrations in tissues of the foolish mussel specimens are shown in Fig. 2 (see Table 44 in the PICES data report (Stehr & Horiguchi, 2001, for the raw data). Phenyltin compounds were not detected in *Mytilus trossulus* specimens. Butyltin compounds were detected in *Mytilus trossulus* specimens from all of sites surveyed, including a reference site (I-7), with a maximum concentration of TBT of 173.2 ng g^{-1} wet wt. of mussel tissues from I-4-A. TBT was the predominant butyltin detected in *Mytilus trossulus*, except at site I-3-A where DBT was predominant, possibly reflecting a local source of DBT, such as PVC stabilizer (Davies, 1997).

Remarkable differences of TBT accumulation in tissue were observed among the bivalve species (Fig. 3; see Table 46 in the PICES data report (Stehr & Horiguchi, 2001 for the raw data). The highest concentration of TBT (2229.9 ng g^{-1} wet wt.) was observed in the horse clam (*Tresus capax*) (Fig. 3). TBT was the most predominant in almost all bivalve specimens surveyed, suggesting a low rate of metabolism of TBT in these bivalve species. Phenyltin compounds were not detected in bivalves other than the foolish mussel (*Mytilus trossulus*)

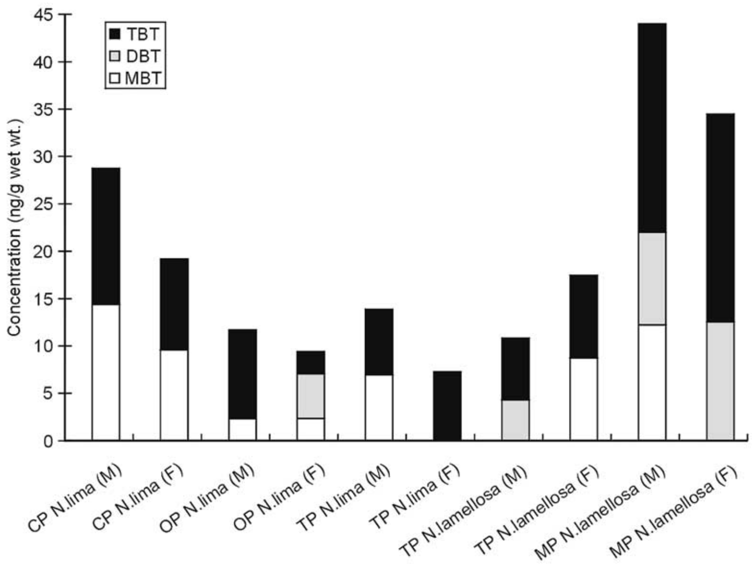

Fig. 1. Tissue concentrations of butyltin compounds in the dogwinkles, *Nucella lima* and *N. lamellosa* (May–June 1999). F: females including imposex-exhibited individuals; M: males. CP: Clover Point (Victoria); MP: Mission Point (Wilson Creek); OP: Ogden Point (Victoria); TP: Ten Mile Point (Victoria); TBT: tributyltin; DBT: dibutyltin; MBT: monobutyltin.

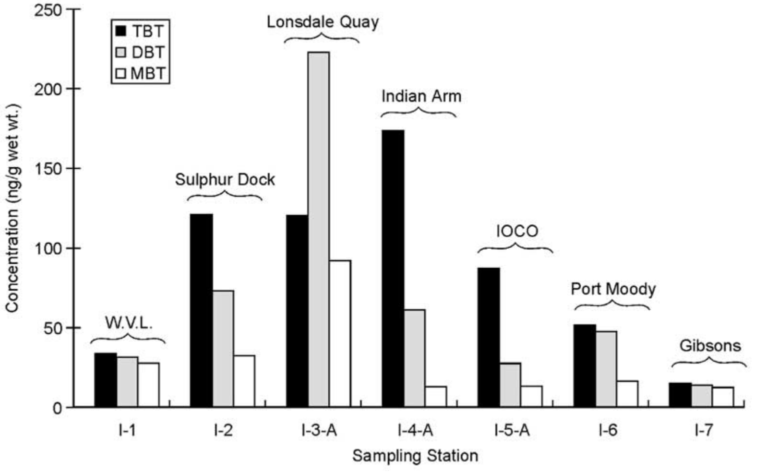

Fig. 2. Tissue concentrations of butyltin compounds in the foolish mussel, *Mytilus trossulus*, from Vancouver Harbour and the reference site, I-7 (May–June 1999) TBT: tributyltin; DBT: dibutyltin; MBT: monobutyltin. See Levings et al. (2003) Fig. 2, for a map of the stations.

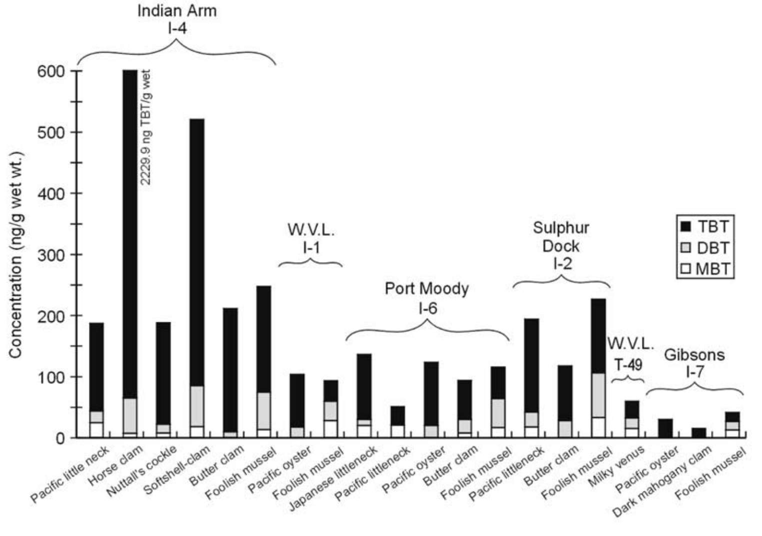

Fig. 3. Butyltin concentrations in tissues of bivalves from Vancouver Harbour and the reference site, I-7 (May–June 1999); TBT: tributyltin; DBT: dibutyltin; MBT: monobutyltin. See Levings et al. (2003) Fig. 2 for a map of the stations.

4. Discussion

4.1. Possible effects on Nucella populations, caused by reproductive failure resulting from imposex induced by TBT contamination in Vancouver

No neogastropod populations (e.g. *N. lima*) have been observed in the waters around Vancouver since at least 1994 (this study and Tester et al. 1996). As neogastropod populations, such as *N. lamellosa*, were observed in Vancouver Harbour in the mid-1980s (Barreca, 1984), it is possible that neogastropod populations have been extirpated by some biological and/or environmental factors around Vancouver since the late 1980s. The huge colonies of barnacles, prey of carnivorous neogastropods like *Nucella* spp., observed at the sites around Vancouver Harbour implies reduced or very low feeding pressure of barnacles by carnivorous animals including gastropods, which indirectly supports the possible extinction of neogastropod populations at the sites along Vancouver Harbour.

The analytical results for butyltin concentrations in tissues of *Mytilus trossulus* from the waters around Vancouver could also support the hypothesis of local extinction of neogastropod populations. This could have occurred through reproductive failure resulting from imposex induced by TBT contamination from antifouling paints at the sampling sites along Vancouver Harbour. TBT concentrations in tissues of *Mytilus trossulus* from Vancouver in this study were higher, compared with those in marine organisms, such as cornet fish (*Fistularia* sp.), croaker (*Nibea* sp.), scorpion fish (*Sebastiscus marmoratus*) and blue mussel (*Mytilus galloprovincialis*), reported in recent publications, although they were below the tolerable average residue level (={TDI (tolerable daily intake) * 60 kg b.w.}/average daily seafood consumption) of Canada (Belfroid, Purperhart, & Ariese, 2000; Environmental Agency of Japan, 1999; Takahashi, Tanabe, & Kubodera, 1997). TBT concentrations in sediment core samples collected from Vancouver Harbour (Burrard Inlet) showed no recent temporal declines, but still remained high (Stewart & Thompson, 1997; Thompson, 1997). This could result from either continuous release of TBT from the hulls of vessels larger than 25 m in length or the persistence of TBT in the bottom sediments around Vancouver Harbour after the controls on TBT introduced in 1989. TBT contamination, therefore, is concluded to continue in Vancouver Harbour.

Comparison of the analytical TBT and/or butyltin values in the dogwinkles from the sites of Victoria (Ogden Point, Clover Point and Ten Mile Point) and Mission Point (Wilson Creek) with those in marine fish, shellfish and other invertebrates reported in recent publications shows that TBT and/or butyltin concentrations in the dogwinkles from the sites of Victoria and Mission Point were generally lower (Takahashi et al., 1997; Tanabe, Prudente, Mizuno, Hasegawa, Iwata, & Miyazaki, 1998; Environmental Agency of Japan, 1999; Belfroid et al., 2000). As extensive imposex of dogwinkles seems to have been caused by relatively low contamination levels of TBT, it is suggested that dogwinkles distributed in Victoria and Mission Point may be especially sensitive to TBT. In case of *Nucella lapillus*, a similar species to the dogwinkles in Victoria and Mission Point, imposex was induced at concentrations of approximately 0.5 ng l^{-1} (as Sn) or less of TBT (Bryan, Gibbs, Burt, & Hummerstone, 1987; Gibbs et al., 1987). In laboratory flow-through exposure experiments with *N. lima*, imposex was induced at an average exposure concentration of TBT of 64 ng l^{-1} for 120 days, and the bioconcentration factor of TBT was estimated to be approximately 2200 (Stickle, Sharp-Dahlm, Rice, & Short, 1990).

From the results mentioned above, we strongly suggest that TBT from antifouling paints could have been one of the causes which led to extirpation of neogastropod populations, such as *Nucella* spp., in Vancouver, and we conclude that no, or only limited, recovery of neogastropod populations has taken place in Vancouver, as discussed in Tester et al. (1996).

4.2. Recovery from imposex in neogastropod populations around Victoria

In contrast to the above results for Vancouver, dogwinkles from the sites of Victoria (Ogden Point, Clover Point and Ten Mile Point) and Mission Point (Wilson Creek) seemed to be recovering from imposex, as indicated by the RPS Index, although not necessarily in terms of the incidence of imposex among females (Tables 1 and 2, Fig. 4). Imposex symptoms represented by RPS Index seemed to have decreased although female penis length did not clearly decrease, compared to the results from Tester et al. (1996), though this may reflect an increase of male penis length. Both the incidence of imposex and the RPS Index in the dire whelk (*Searlesia dira*) from Clover Point clearly decreased between 1994 and 1999 surveys (Table 2; Tester et al., 1996). Reitsema, Thompson, Scoltens, and Spickett (2002) conducted re-surveys for imposex in neogastropods like a few species of *Nucella* and *S. dira* at

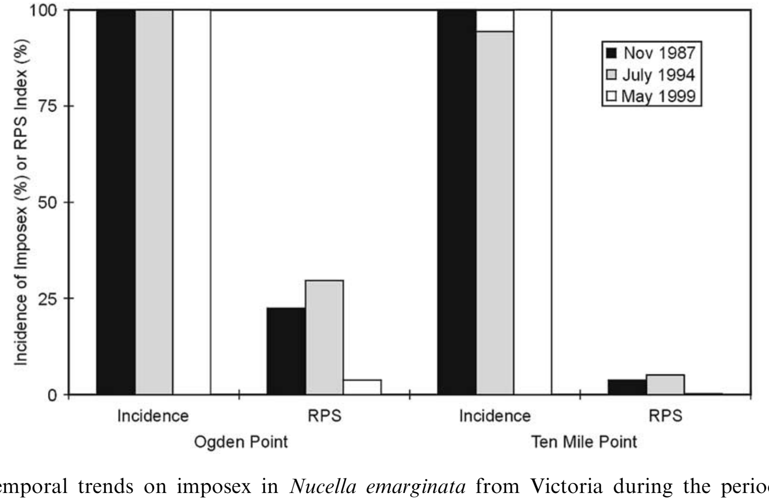

Fig. 4. Temporal trends on imposex in *Nucella emarginata* from Victoria during the period between November 1987 and May 1999. Data of imposex in *Nucella emarginata* in November 1987 and July 1994 were cited from Bright and Ellis (1990) and Tester et al. (1996), respectively. RPS Index = {(mean penis length in females)^3/(mean penis length in males)^3} × 100 (Gibbs et al., 1987).

the same stations as Tester et al. (1996), and observed a continuing decline of both the incidence of imposex and imposex symptoms (represented by RPS Index) of neogastropods collected at Victoria, the Strait of Georgia and West Vancouver Island, except for the incidence of imposex in neogastropods from Breakwater (Ogden Point), Victoria.

4.3. TBT accumulation and possible adverse effects in bivalves from Vancouver

The remarkably high concentration of TBT detected in tissue of *Tresus capax* may have caused some adverse effects (Fig. 3). This is possible because some chronic toxicities have been observed in other species of bivalves by exposure to low TBT concentrations: 0.24 μg l^{-1} of TBT inhibited sexual maturation of gonad and 0.1 μg l^{-1} or less of TBT inhibited the larval growth in oysters, while 0.08 μg l^{-1} of TBT was estimated as the no observable effective level (NOEL) for growth in mussels (Alzieu & Heral, 1984; Lawler & Aldrich, 1987; Salazar & Champ, 1988; Thain & Waldock, 1986). If the bioconcentration factor of TBT in marine bivalves is approximately 10000 (Waldock & Thain, 1983), the expected maximum tissue concentration of TBT would be 2.4 μg g^{-1} wet wt., which is almost the same level as the TBT concentration detected in tissue of *T. capax*. Further detailed studies on the adverse effects of TBT, the bioconcentration factor of TBT and/or bioavailability of TBT through the contaminated sediment will be necessary to assess the ecological risks of TBT to bivalves, such as *T. capax*, distributed in contaminated sites.

The highest TBT concentration in tissue was consistently observed in the Pacific oyster (*Crassostrea gigas*), among the marine invertebrates collected in every intertidal zone of 3 sites of Japan (Horiguchi et al., unpublished data). TBT predominated among butyltin species in tissue of *C. gigas*, implying low metabolic activity towards TBT in this species (Horiguchi et al., unpublished data). As TBT concentrations in tissues were also consistently higher in *C. gigas* than those in *M. trossulus* in this study (Fig. 3), *C. gigas* could be a useful species for biological monitoring of TBT contamination.

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