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Butyltins biomagnification from macroalgae to green sea urchin: a field assessment

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Biomagnification of butyltins (BTs) was examined in a simple food web including seawater, macroalgae (*Alaria esculenta, Laminaria longicruris, Ulvaria obscura*) and green urchin (*Strongylocentrotus droebachiensis*). The study was conducted in shallow waters of the St Lawrence Estuary (Canada) adjacent to two areas potentially contaminated by BTs. Levels of tri- (TBT), di- (DBT) and mono-BT (MBT) were determined in seawater, green urchin (including faecal matter after sampling) and macroalgae surrounding the urchins at each sampling site. The concentrations of TBT in seawater from all stations were relatively low (3–7 ngSn l^{-1}), and both the TBT and the total BTs ($\sum$ BT = MBT + DBT + TBT) concentrations decreased with increase in distance from the BT sources. The concentrations of TBT in algae were 0.35 ngSn g^{-1} dry weight (DW) in *A. esculenta*, 0.40 ngSn g^{-1} DW in *L. longicruris* and 3.58 ngSn g^{-1} DW in *U. obscura*. Following their location, green urchins feeding mainly on these algae accumulated BTs at levels ranging from 4 to 85 ngSn g^{-1} DW in gonads and from 35 to 334 ngSn g^{-1} DW in gut. The mean bioconcentration factor (BCF) calculated from seawater to algae ranged from 17 in *A. esculenta* to 151 in *U. obscura*, whereas the biomagnification factor (BMF) from algae to urchins ranged from 2 to 17 in gonads and from 10 to 67 in gut. The overall bioaccumulation factor of TBT between seawater and internal organs of urchins reached an average value of 1.2×10^3. These results are the first to illustrate high BT BCFs and BMFs in human-edible macroalgae and urchins sampled from northern coastal areas with a low TBT contamination level.

KEYWORDS: tributyltin; green urchin; *Strongylocentrotus droebachiensis*; macroalgae; benthic ecosystem; St Lawrence Estuary

INTRODUCTION

Although tributyltin (TBT) has been pointed out as a major pollutant in many coastal areas by numerous studies from academics and governmental researchers,[1,2] this biocide is still in use in antifouling paints for large commercial vessels and will be definitively banned from ship hulls only in 2008.[3] TBT is often present at very low concentrations (<10 ng l^{-1}) in coastal waters[4–6] and could be erroneously considered as harmless by the scientific community and regulators, as no obvious and alarming toxic effects can be observed or related to TBT and its degradation products. However, subtle effects on the immune and endocrine systems of invertebrates have been clearly identified for very low concentrations in seawater.[7–9] Furthermore, the lipophilic nature of TBT induces its rapid adsorption onto organic matter, and its penetration in biological membranes.[10] Plants and algae may take up TBT from seawater, followed by its stepwise transfer to primary consumers and upper predators, leading to its accumulation within the food web.

In shallow waters and hard-bottomed benthic ecosystems, macroalgae represent significant food sources for several primary consumers, including urchins, gastropods, small crustaceans and herbivorous fish. Little is known about the accumulation of butyltins (BTs) by macroalgae and their possible transfer to grazers. Available data from field monitoring are limited to the BT levels in bladder wrack,

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Fucus vesiculosis.[11,12] BTs accumulation in macroalgae seems to be species dependent, and levels in macroalgae are directly related to levels found in the surrounding seawater.[11] It could be hypothesized that the exposure of algivorous organisms to dietary BTs may lead to a biomagnification process that varies between sites and which is strongly dependent upon the bioconcentration factor (BCF) from seawater to macroalgae.

Green urchin, *Strongylocentrotus droebachiensis*, is an intensive grazer that is widespread in the shallow waters of North Atlantic coasts, including the estuary and the gulf of the St Lawrence, where macroalgae and green urchins are commercially harvested for food processing and potential nutraceutical applications. A number of field surveys in the last decade have shown that BT levels in sediment and benthic organisms of the St Lawrence system were relatively low and comparable to several other coastal sites in Canada.[13–15] BTs are expected to be taken up by the green urchin from seawater and macroalgae, as already observed for some other inorganic and organic chemicals,[16–18] but no field results have been available up to now.

Our objective was to characterize the bioconcentration/biomagnification process of BTs in a simple food web including seawater, macroalgae and green urchin using two field sites where TBT is chronically present at relatively low levels (<10 ng l^{-1}). The study was conducted in shallow waters along the south shore of the St Lawrence Estuary adjacent to Les Méchins shipyard and Rimouski Harbor. The sampling was conducted over a period corresponding to the time of final growth of green urchin gonads and the beginning of their commercial harvesting.

MATERIALS AND METHODS

Samples

In September 2002, seawater and biological samples were collected at low tide from three stations around Les Méchins shipyard (LM1, LM2, LM3) and two others near Rimouski Harbor (RK1, RK2). An additional station located near the shipyard (LM2a) was used for water sampling (Fig. 1). One seawater sample per station was collected at 0.5 m below the surface with 6 l polycarbonate bottles pre-rinsed with dilute nitric acid (HNO_3). Sea urchins were collected at the same sites, cleaned of all algal fragments and separated into three size classes: small (22.6 ± 2.2 mm in diameter, mean $\pm$ 1SD), medium (35.8 ± 3.0 mm) and large (50.4 ± 4.4 mm). Three macroalgae were collected at the same location as the seawater

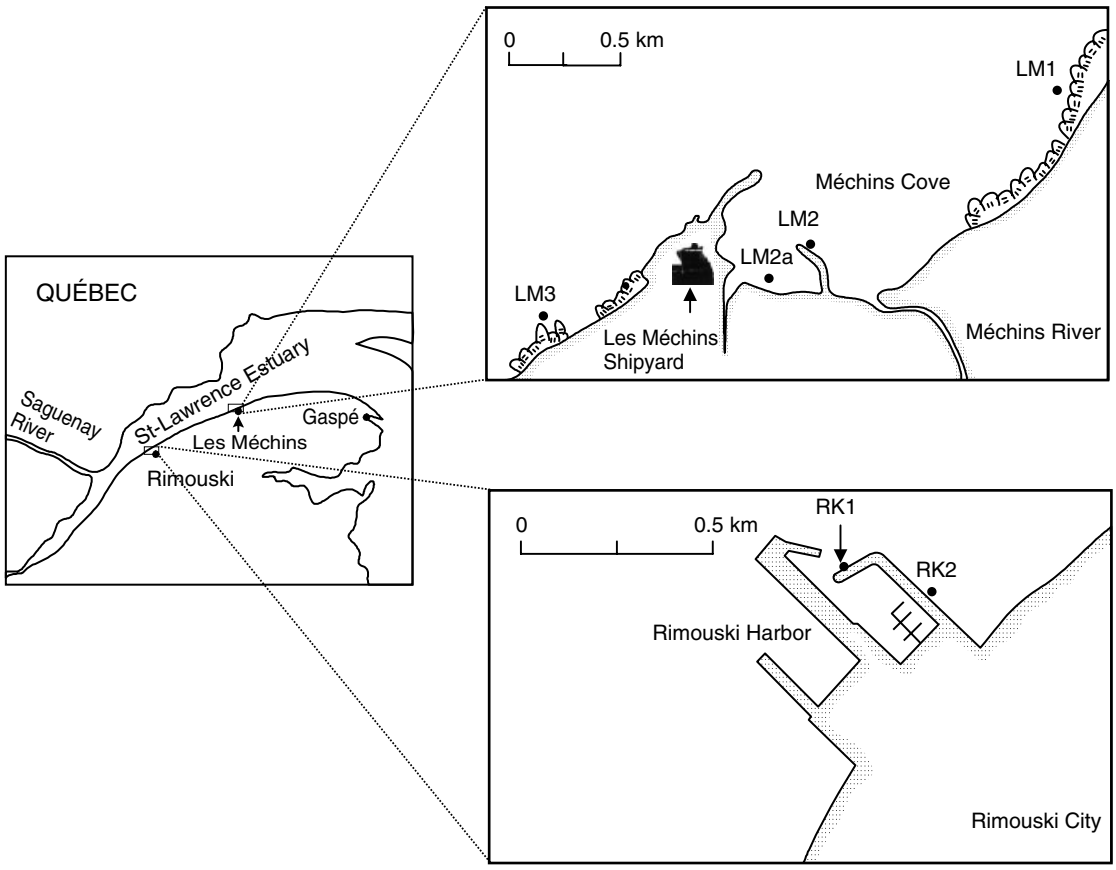

Figure 1. Sampling stations in southern St Lawrence Estuary. TBT sources are expected to be from the shipyard at Les Méchins and the inner harbour at Rimouski.

and urchins: *Alaria esculenta*, *Laminaria longicruris* and *Ulvaria obscura*. Collected fronds ($n = 3$) were cleaned of all epiphytes using seawater. All seawater and biological samples were transported to the nearby ISMER marine station (Pointe-au-Père, Qc, Canada), where the urchins were allowed to evacuate faecal matter over 3 h. Faecal matter was collected and separated from seawater by filtration. Urchins were dissected, and gonads and gut were taken for chemical analysis. Macroalgae, urchin organs and faecal matter were freeze-dried for 96 h. Dry samples were ground and kept frozen (−80 °C) until BTs analysis.

Analysis of BT species

The analytical procedures for BTs in seawater and biological samples were based on a previously described method.[19,20] Seawater was transferred immediately upon sampling in 3×2 l glass flasks and acidified with 3 M HNO_3 to reach pH 5.5. BTs were ethylated by vigorously shaking samples for 5 min with 0.5 ml of 4% sodium tetraethyl borate ($NaBEt_4$). After ethylation, BT were extracted with 10 ml of isooctane/pentane (1 : 4) by thoroughly shaking for 10 min. Tetrapentyltin (TePSn) was added as an internal standard. For analysis of BTs in biological samples, 0.1–0.5 g of pooled dry matter ($n = 3$–4) was digested for 60 min at 55 °C using 5 ml of tetramethylammonium hydroxide. The mixture was thereafter acidified with 25 ml of acetate buffer (pH 4.1). BTs were extracted with 2 ml of hexane. After adding internal standard (TePSn) the BTs were ethylated twice by shaking for 15 min with 0.6 ml of $NaBEt_4$. The organic layer was removed and 1 ml of this extract was dried with Na_2SO_4 and cleaned by passing through 5% silica gel (5 cm × 0.8 cm ID) and eluted with additional 5 ml of hexane. The cleaned mixture was evaporated under nitrogen flow to 0.2 ml.

Samples extracts were analysed for BTs using gas chromatography coupled to a mass spectrometer (MS: TraceGC 2000/PolarisQ, ThermoFinnigan, Mississauga ON, Canada). Chromatographic separation was performed on a 30 m × 0.25 mm ID capillary column with 0.25 µm film thickness coating (Rtx-5 MS, Restek Co., UK). The gas carrier was helium (1 ml min^{-1}). The injection temperature was increased from 100 to 250 °C at a rate of 10 °C s^{-1} and the oven was programmed from 80 to 250 °C at a rate of 12 °C min^{-1}. The identification of chromatographic peaks was performed in segmented scan mode with five typical ion groups of BT compounds. Standard curves for the quantification of mono-BT (MBT), di-BT (DBT), and TBT ethyl derivatives were prepared following the above the procedure by replacing biological samples with solutions of known concentrations of BTs in dry ethanol. The recovery of BT species from mussel standard CRM 477 ($n = 6$) was 92 ± 11% for MBT, 74 ± 11% for DBT and 79 ± 12% for TBT. The concentrations of BTs were not corrected for recovery. The correlation coefficients r^2 between MS responses and standard concentrations were >0.99 for MBT, DBT and TBT. The detection limit of MS for BT species was 1.5 pg of tin per injection (1 µl), giving a limit of quantification of 0.25 ngSn l^{-1} (for a 6 l water sample) and 0.075 ngSn g^{-1} (for 0.25 g of biological dry sample).

Data analysis

The BCF and the biomagnification factor (BMF) were calculated as follows:

$$\mathrm{BCF} = \frac{\text{BTs in tissue (ngSn g}^{-1}\text{ wet weight (WW))}}{\text{BTs in seawater (ngSn ml}^{-1}\text{)}} \quad (1)$$

$$\mathrm{BMF} = \frac{\text{BTs in tissue (ngSn g}^{-1}\text{ dry weight (DW))}}{\text{BTs in algal food (ngSn g}^{-1}\text{ DW)}} \quad (2)$$

To obtain BMFs realistically related to the field diet of sea urchins, the BTs in urchin algal food were calculated as a weighted mean of BTs using the BTs concentration in each alga and the relative proportion of each alga in the green urchin diet according to the food preference of *S. droebachiensis*. The preferred food composition (60% *L. longicruris*, 30% *A. esculenta*, 10% *U. obscura)* was determined from results previously obtained in our laboratory by feeding urchins of various sizes *ad libitum* with a mix of all three algae (Mamelona, unpublished data).

RESULTS

BTs in seawater

BT compounds were detected in all seawater samples collected from six stations (Table 1). Total BT concentrations ($\sum \mathrm{BT} = \mathrm{MBT} + \mathrm{DBT} + \mathrm{TBT}$) in water varied from 4.7 to 13.8 ngSn l^{-1}. Total BTs and individual BT species concentrations in seawater were about two times higher at Les Méchins (LM) than at Rimouski (RK) stations. The highest levels were found close to TBT potential sources, and both total BT and TBT concentrations in seawater decreased slightly with increasing distance from the sources. A comparable distribution of BT species was observed for both locations. TBT was generally the dominant species except for seawater collected from LM1, where the MBT level was up to two times higher than TBT. The TBT/DBT ratio was up to 7.4 for seawater sampled close to the shipyard (LM2a), and

Table 1. Distribution of BTs species (ngSn l^{-1}) in seawater collected from six stations in the southern St Lawrence Estuary in September 2002

Station	MBT	DBT	TBT	∑BTs	TBT/DBT
LM1	7.5	1.0	3.8	12.3	3.8
LM2	3.1	1.5	5.4	10.0	3.6
LM2a	5.4	1.0	7.4	13.8	7.4
LM3	3.2	1.4	5.1	9.7	3.6
RK1	1.6	0.7	3.3	5.6	4.6
RK2	1.3	0.6	2.8	4.7	4.7

Table 2. Concentration and distribution of BTs species in macroalgae collected from four stations in the southern St Lawrence Estuary in September 2002. Values are obtained from pooled samples ($n = 3$); ql: below quantification limit; nd: not determined because *Alaria* was absent in LM2

Station	Alga	BT concentration (ngSn g^{-1} DW)			
		MBT	DBT	TBT	∑BTs
LM1	*A. esculenta*	2.59	ql	0.49	3.08
	L. longicruris	1.56	ql	0.43	1.99
	U. obscura	0.94	0.48	0.87	2.29
LM2	*A. esculenta*	nd	nd	nd	nd
	L. longicruris	1.24	ql	0.62	1.86
	U. obscura	3.76	1.92	6.52	12.20
LM3	*A. esculenta*	ql	ql	0.18	0.18
	L. longicruris	2.25	ql	0.33	2.58
	U. obscura	1.53	0.78	2.35	4.66
RK2	*A. esculenta*	ql	ql	0.39	0.39
	L. longicruris	6.17	ql	0.23	6.40
	U. obscura	0.77	ql	4.59	5.36

this ratio decreased with increasing distance from the sources for Les Méchins stations (Table 1).

BTs in macroalgae and in faecal matter

BT compounds were detected in all three algal species collected from the four sites (Table 2). The concentrations of total BTs varied from 0.18 to 12.2 ngSn g^{-1} DW depending on the particular alga species. The distribution of BTs also varied with the alga. It decreased in the sequence TBT > MBT > DBT in *Ulvaria*. DBT was detected only at trace levels in *Ulvaria* collected from RK2. TBT was generally at a low level in *Alaria* and *Laminaria*. MBT was the dominant species of BT in *Laminaria*, with a mean concentration five times higher than TBT. This was the same for *Alaria* in LM1. DBT was not detected or was at trace levels in most samples. Although the highest concentrations of BT species and total BTs were found in the inner part of the shipyard (LM2 and LM2a), there is no clear relationship between the concentration of BTs in seawater and concentrations found in macroalgae. For example, total BTs and TBT concentrations were higher in seawater from LM3 than RK2, whereas the inverse relationship was found for *Ulvaria* from these two sites.

There was a significant bioconcentration (BCFs > 1) of BT compounds from surrounding seawater to macroalgae. BCFs ranged from 5 to 543 and 3 to 244 for individual BT species and total BTs respectively (Table 3). Variation of BCF between species was notably found for TBT. When considering all stations and sites, BCFs of TBT were strikingly higher in *Ulvaria* than in *Alaria* and *Laminaria* (ANOVA, $F_{1,2} = 5.419$, $p = 0.033$; SNK, $p < 0.05$). On average, there was over 150-fold more TBT in *Ulvaria* than in the surrounding water, whereas this factor averaged 10 in *Alaria* and *Laminaria*.

Table 3. BCFs of BTs species by macroalgae collected from four stations in the southern St Lawrence Estuary in September 2002; nd: not determined because BT concentration was below the quantification limit in macroalgae

Station	Alga	BCF [(ng g^{-1} WW)/(ng ml^{-1})]			
		MBT	DBT	TBT	∑BTs
LM1	*A. esculenta*	54.8	nd	20.9	40.3
	L. longicruris	23.5	nd	12.3	17.9
	U. obscura	25.7	93.1	45.5	37.6
LM2	*A. esculenta*	nd	nd	nd	nd
	L. longicruris	44.8	nd	12.3	21.3
	U. obscura	241.6	261.4	239.6	243.6
LM3	*A. esculenta*	nd	nd	11.3	6.4
	L. longicruris	78.4	nd	7.8	30.2
	U. obscura	93.1	112.9	91.1	95
RK2	*A. esculenta*	nd	nd	4.8	3.2
	L. longicruris	543.1	nd	9.0	154.5
	U. obscura	120.8	nd	324.8	227.7

BTs were found in faecal matter of urchins collected from the five stations (Fig. 2). BT concentration varied depending on both urchin size and sampling station. The highest total BT concentration in faecal matter was up to 5.5 ngSn g^{-1} and observed in large urchins collected close to the most contaminated station (LM2). However, the spatial variation of BTs in faecal matter did not follow the BTs' spatial variation in macroalgae. For example, the total BT concentration in faecal matter of medium urchins collected from LM3 was higher than from LM1, whereas the inverse relationship was found in *Laminaria* and *Ulvaria*. The composition of BTs in faecal matter also varied depending on urchin size and sampling station. Although, MBT and TBT were omnipresent, DBT was barely present or not detected, as already observed in macroalgae.

BTs accumulation in urchins

BTs were found in gonads and gut of all urchins collected from the five sites (Table 4). BT concentrations were significantly higher in gut than gonads for TBT alone ($t_{0.05,24} = 3.424$, $p = 0.002$), and also total BTs ($t_{0.05,24} = 2.688$, $p = 0.013$). TBT concentrations in urchins from Les Méchins were comparable to those from RK stations in both gonads ($t_{0.05,11} = 0.286$, $p = 0.780$) and gut ($t_{0.05,11} = 0.183$, $p = 0.858$). Similarly, total BT concentrations in urchin organs were not significantly different between sampling sites ($t_{0.05,11} = 0.285$, $p = 0.781$ for gonads and $t_{0.05,11} = 0.484$, $p = 0.638$ for gut).

Total BT concentration in gut decreased in the order LM2 > LM3 > LM1 and RK1 > RK2. The composition of BTs decreased in the sequence TBT > MBT > DBT except for LM1, where MBT in gut was higher than TBT (Table 4). At the three LM stations, the total BT concentration in gut was higher for large urchins than smaller ones. However, the total BT concentration in gut of individuals collected from the

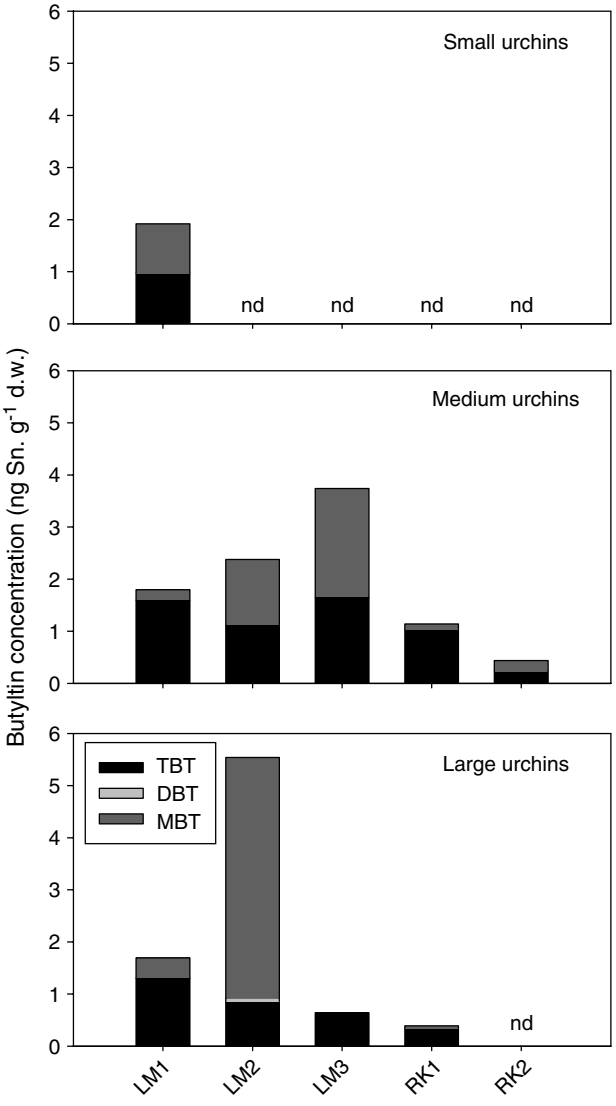

Figure 2. Concentration and distribution of BTs species in urchin faecal matter collected from five stations in the southern St Lawrence Estuary in September 2002; nd: not determined because faecal matter was not available.

two RK stations was in the order: small > large > medium urchins. The total BT concentration in gonads decreased in the order LM2 > LM1 > LM3 and RK1 > RK2. MBT was the dominant species of BT in gonads of urchins collected from LM1 and LM2, whereas TBT was first for those from LM3, RK1 and RK2, except for large urchins from RK1. The total BT concentration was usually higher in gonads of larger urchins.

BMFs in urchins

Following the method previously described, the concentrations of BT species in a typical urchin meal were calculated for four stations with available data and are summarized in Table 5. The mean concentrations are slightly above the individual *Alaria* and *Laminaria* values but are clearly below *Ulvaria*, which has a low contribution to the diet.

Significant BMFs (>1) of BTs in gonads and gut were found for all urchins and ranged from 2 to 22 in gonads and 10 to

Table 4. Concentration and distribution of BTs species in three size classes of urchin collected from five stations in the southern St Lawrence Estuary in September 2002. Values are means ($n = 2–4$) obtained from each urchin size and organ; ql: below quantification limit

			BT concentration (ngSn g^{-1} DW)			
Station	Size class	Organ	MBT	DBT	TBT	∑BTs
LM1	Large	Gonad	11.16	0.13	5.22	16.51
		Gut	35.41	0.80	17.03	53.24
	Medium	Gonad	42.94	0.38	7.99	51.31
		Gut	28.64	0.93	18.40	47.97
	Small	Gonad	13.35	0.27	4.12	17.74
		Gut	25.98	1.59	7.44	35.01
LM2	Large	Gonad	48.69	13.21	23.39	85.29
		Gut	174.48	35.39	123.94	333.81
	Medium	Gonad	31.33	2.17	26.75	60.25
		Gut	88.51	17.12	102.65	208.27
LM3	Large	Gonad	2.72	0.40	12.06	15.18
		Gut	26.07	6.65	48.79	81.51
	Medium	Gonad	0.57	0.20	11.65	12.42
		Gut	10.46	4.79	42.65	57.90
	Small	Gonad	0.38	ql	3.84	4.27
		Gut	15.02	4.61	27.08	46.71
RK1	Large	Gonad	51.74	6.81	13.68	72.23
		Gut	36.64	5.09	47.21	88.94
	Medium	Gonad	2.11	0.59	10.95	13.65
		Gut	24.13	5.44	36.58	66.15
	Small	Gonad	3.52	0.50	22.95	26.97
		Gut	29.49	9.88	117.97	157.34
RK2	Large	Gonad	3.32	0.11	8.91	12.34
		Gut	14.77	3.74	25.79	44.30
	Medium	Gonad	2.08	0.34	9.28	16.70
		Gut	19.49	4.58	36.17	60.23

Table 5. Mean concentration of BTs species in a typical green urchin algal meal based on the food preference of the urchins and used to calculate BMFs; nd: not determined because no DBT value available in the three macroalgae at that station

	BT concentration (ngSn g^{-1} DW)			
Station	MBT	DBT	TBT	∑BTs
LM1	1.81	0.05	0.49	2.35
LM2	1.99	0.19	2.39	4.96
LM3	2.04	0.08	0.55	2.13
RK2	3.78	nd	0.65	4.43

Table 6. BMFs of BTs species between algal food and urchin internal organs; nd: not determined because no DBT value available in algal food

Station	Size class	Organ	BMF [(ng g^{-1} DW)/(ng g^{-1} DW)] MBT	DBT	TBT	∑BTs
LM1	Large	Gonad	6.2	2.6	10.7	7.0
		Gut	19.6	16.0	34.8	22.7
	Medium	Gonad	23.7	7.6	16.3	21.8
		Gut	15.8	18.6	37.6	20.4
	Small	Gonad	7.4	5.4	8.4	7.6
		Gut	14.4	31.8	15.2	14.9
LM2	Large	Gonad	24.5	69.5	9.8	17.2
		Gut	87.7	186.3	51.9	67.3
	Medium	Gonad	15.7	11.4	11.2	12.1
		Gut	44.5	90.1	42.9	42.0
LM3	Large	Gonad	1.3	5.0	21.9	7.1
		Gut	12.8	83.1	88.7	38.3
	Medium	Gonad	0.3	2.5	21.2	5.8
		Gut	5.1	59.9	77.6	27.2
	Small	Gonad	0.2	nd	7.0	2.0
		Gut	7.4	58.1	49.2	21.9
RK2	Large	Gonad	0.9	nd	13.7	2.8
		Gut	3.9	nd	39.7	10.0
	Medium	Gonad	0.6	nd	14.3	3.8
		Gut	5.2	nd	55.6	13.6

67 in gut (Table 6). BMF of TBT alone varied from 7 to 21 in gonads and from 15 to 89 in gut. The BMFs of total BTs in gut were the highest at the most contaminated station (LM2), although the BMFs of TBT in gut were higher at LM3 than at LM2. In general, the BMFs of both total BTs and TBT in gut were higher in large and medium urchins than in small urchins. Both the total BTs and TBT BMFs in gonads did not show a clear correlation with either sampling station or urchin size (Table 6).

DISCUSSION

Once released in seawater, TBT is rapidly adsorbed onto suspended particulate matter, macroalgae, and organic-coated surfaces, and eventually degraded to DBT and MBT. The TBT half-life in seawater is generally estimated in the range of a few days to a few weeks.[2,21,22] The relatively high proportion of TBT among BT derivatives found in the present study suggests that its low but fresh input in the waters adjacent to our two sampling areas most probably comes from shipyard and boating activities. This study first reported TBT seawater level in the St Lawrence Estuary. TBT concentrations in all stations (3–7 ng l^{-1}) were far below those reported in some highly contaminated coastal shallow waters (up to 630 ng l^{-1}).[23–25] Though considered quite low, the observed TBT water concentrations could be deleterious to the health of marine ecosystems because they exceed the concentration (~1 ng l^{-1}) that causes chronic effects to the reproduction of several organisms[2,7,10] and impairs their immune system.[8] Furthermore, these seawater TBT levels were sufficiently high to induce a stepwise bioaccumulation within the food web.

The only previous TBT levels reported in macroalgae were for the bladder wrack, *F. vesiculosis*,[11,12] a species usually avoided by several macrobenthic grazers because of its high chemical defence against herbivory.[26] The TBT level was as high as 42–97 ngSn g^{-1} (WW) in algae from the German North Sea,[11] whereas it was 2–4 ngSn g^{-1} in the same species collected along Danish coasts.[12] The calculated BCF was up to 2×10^4 for *F. vesiculosis* collected from the North Sea. We first report the TBT level and BCFs in three macroalgae, which are not only dainty food sources for several herbivores living in benthic hard-bottomed ecosystems but are also used by man as seafood or in nutraceutical and pharmaceutical applications.[27] The observed BCFs were far lower than those reported for bladder wrack in the North Sea, although seawater TBT level (up to 4 ngSn l^{-1}) was in the same range as the one reported in our own stations. At our sampling sites, the BCFs of TBT ranged from a low 5 in *Alaria* to a high 325 in *Ulvaria* collected at the same station, indicating a huge range of values for the macroalga species and the location. As a major source of organic carbon used by nearshore benthic ecosystems, macroalgae may therefore represent significant initial sources of TBT for the rest of the food web.

Sea lettuce *U. obscura* accumulated TBT at a much higher level than the two brown algae. This significant difference in TBT accumulation and BCFs between macroalgae might be related to the difference in biochemical and physical nature of these algae. The thin sheet-like thallus of *Ulvaria* probably increases its relative adsorption surface[28] compared with the two brown algae. Moreover, this alga is formed by only two cell layers and contains much more fat (7% DW) than the two brown algae (4% DW), which probably favours rapid TBT incorporation and scavenging in algal components.

The presence of BT species in the daily diet of urchins is clear from the presence of BTs in their faecal matter at a level that is often higher than in some individual algae collected at the same location. This apparent discrepancy is linked to the specific diet of urchins in the hours before their sampling, as they grazed an unknown proportion of the three algae collected or even of algal species not sampled but which are also part of their diet.[29] The use of a weighted mean of BTs with a standardized meal based on food preference circumvents the problem of calculating the BMF with only one alga species.

A biomagnification of TBT more than 20 times higher than in algal food sources has been observed in gonads and gut of urchins. Although a direct bioaccumulation of BTs from seawater into sea urchin organs cannot be totally excluded, it is reasonable to assume that food is by far the main source of BTs for internal organs such as gonads and gut as they are energy storage tissues in urchins. Our BMF data are the first

to be reported for BTs in a macrophyte grazer. The observed values are much higher than the BMFs of TBT previously reported (0.04–7.5) in shellfish and fish from laboratory experiments,[30–34] and from field studies at the upper end of the food chain, such as in sea bird or mammals.[35–40] A comparison with an other echinoderm species (*Leptasterias polaris*) living in the same area as the green urchin showed a much lower BMF in gonads (BMF = 1.3, $n = 9$ sites) and its digestive tracts (BMF = 1.5) (recalculated from Pelletier and Normandeau[13]). It seems that sea star *L. polaris* has a much better capacity to biodegrade and eliminate BT species than sea urchin. Starting from a low 4.63 ngSn l^{-1} in seawater (mean for all stations), the TBT concentration reached an average of 5620 ngSn kg^{-1} WW (mean value calculated for all stations and urchin sizes) in urchin soft tissues (gonads and gut pooled together and using a conversion factor of 0.18 from DW to WW). The resulting effect corresponds to an overall bioaccumulation factor (BAF) of approximately 1214. The BAF reaches 1950 when only gut concentrations are used in the calculation.

We compared our data with data previously reported by Takahashi *et al*.[23] for the related species *Strongylocentrotus intermedius*. This shows that the total BTs and TBT mean concentrations in *S. droebachiensis* soft tissues from the St Lawrence Estuary are respectively four and six times lower than the levels in *S. intermedius* living in shallow waters, with total BTs (17 ng l^{-1}) and TBT (4 ng l^{-1}) comparable to those found in the present study (5–14 ng l^{-1} and 3–7 ng l^{-1}, respectively), thus confirming the high retention and apparently low excretion of BTs by the *Strongylocentrotus* species. Finally, it should be mentioned that we found more $\sum$BTs in gut than in gonads, regardless of sampling stations and urchin sizes. This is explained by the fact that gut was in direct contact with dietary BTs and its high contains in total lipids (23% DW) compared with gonads (16% DW).

CONCLUSION

This paper presents the first detailed field results on the transfer of dissolved BT species to macroalgae and to the grazer *S. droebachiensis*. Although these are low levels of TBT present in seawater for both sites sampled, the average BCF in macroalgae reached 71 and the BMF for green urchin was in the order of 31. Significant differences in the capacity of macroalgal species to adsorb BTs are observed, especially of *Ulvaria*, which seems particularly sensitive to bioaccumulation of BTs. TBT was found to be about 1200 times more concentrated in sea urchin soft tissues than in the surrounding seawater. These results point out the risk of BTs contamination of edible macroalgae and the highly appreciated green urchin in an estuarine environment with a quite low contamination level by BT species. These results also point out the need for the rapid enforcement of a world ban of TBT paints.

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