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ORIGINAL ARTICLE

# Distribution and bioaccumulation of butyltins in the edible gastropod *Odontocymbiola magellanica*

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**ABSTRACT**
Butyltins (BTs) were found in sediments and body tissues of the edible gastropod *Odontocymbiola magellanica*, in which imposex has been recorded since 2000. BTs in sediments ranged from < MDL to 174.8 ng (Sn) $g^{-1}$ for TBT, < MDL to 19.2 ng (Sn) $g^{-1}$ for DBT, and < MDL to 71.8 ng (Sn) $g^{-1}$ for MBT. In body tissues BTs varied from < MDL to 147.1, < MDL to 77.0 and < MDL to 345.3 ng (Sn) $g^{-1}$ for TBT, DBT and MBT, respectively. BT concentrations were higher in gonads and digestive glands than in the albumen gland and foot (edible). The highest concentrations of BTs in both sediments and gastropods were found in the harbour area, decreasing with distance to the harbour and areas with less maritime traffic. The Biota-Sediment Accumulation Factor (BSAF) in the different organs was between 0.02–0.42, 0.09–0.35 and 0.08–5.25 for TBT, DBT and MBT, respectively. There were positive correlations between concentrations of BTs in sediments and gastropod body tissues, suggesting that xenobiotic accumulation in *O. magellanica* occurs mainly through contaminated sediments, rather than water or the food chain. Considering current sediment quality guidelines, our results indicate that acute toxic effects would be expected from TBT exposure, which represents a serious environmental threat for the benthic community. Although the levels of BTs found in the foot of this edible gastropod did not exceed the recommended Tolerable Daily Intake in polluted areas, they should be monitored to ensure the safety of seafood consumers. The alternative antifouling biocides Irgarol and Diuron were not detected in sediments.

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## Introduction

Tributyltin (TBT) is a very toxic synthetic organic compound that has been released into the environment through anthropogenic activities (Bryan et al. 1988). It was introduced into the international market of antifouling paints during the 1960s and its use expanded rapidly due to its great efficiency and reasonable cost (Almeida et al. 2007). TBT has often been detected in coastal areas associated with maritime activities worldwide (Evans 1999), and its effects on marine organisms (i.e. imposex in marine gastropods, malformation in oysters and gastropods, hormonal imbalance in dolphins and metabolic disruption in fishes) have been widely documented (Smith 1981; Harino et al. 1998; Alzieu 2000; Horiguchi 2009; Lahbib et al. 2010; Marquez et al. 2011; Meador et al. 2011). The moderately high partition coefficients of TBT favour its accumulation in organic matter (particulate phase) and in organisms (Stewart & de Mora 1990), while the water-sediment interface is considered to be the main route of bioaccumulation (Meador 2000). Molluscs, particularly gastropods, are the organisms most affected by TBT, due to a slow metabolism of this toxic compound (Lee 1991). In fact, the imposex incidence in molluscs is the most commonly used tool for monitoring organotin pollution (Titley-O'Neal et al. 2011). The imposex phenomenon is a dose-dependent response (the more contaminated by TBT the higher the imposex parameters); in the same way the length of the penis and vas deferens developed in females depends on the environmental concentrations of TBT (Sternberg et al. 2010). This phenomenon has been reported worldwide (Stewart et al. 1992; Nias et al. 1993; Vishwa-Kiran & Anil 1999; Gooding et al. 1999; Bech 1999a, 1999b; Evans & Nicholson 2000; Castro

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et al. 2012a, 2012b, 2012c; Miloslavich et al. 2007; Bigatti et al. 2009, among others), including the Argentinean coast (Penchaszadeh et al. 2001; Willers 2004; Bigatti & Penchaszadeh 2005; Bigatti et al. 2009). Although the use of TBT-based antifouling paints on vessels was banned around the world in 2008 by the Antifouling Systems Convention (AFS) of the International Maritime Organization (IMO), high imposex and TBT levels are still being detected in South American coastal areas (Castro & Fillmann 2012; Castro et al. 2012a).

In the NE Atlantic the imposex vigilance programme, mandatory within OSPAR (the Convention for the Protection of the Marine Environment of the NE Atlantic), has recently recommended the quantification of TBT and derivatives in tissues (Ruiz et al. 2015). Although there are no recommendations in this regard for the SW Atlantic, the documentation of occurrence and effects of BTs in the region is important to assess the global situation of TBT pollution and to eventually create the missing guidelines.

While environmental contamination by BTs is attributed to anthropogenic factors such as release from ship hulls via either legal or illegal paints (chronic pollution), other mechanisms may also be responsible for TBT pollution. In particular, desorption from sediments is a natural phenomenon that is expected to occur in disparate geographic settings. Detailed BT monitoring is expected to increase the field database and thus enhance our understanding of the long-term fates and effects of this paradigmatic pollutant (Ruiz et al. 2015). Because of its dual hydrophobic (Log Kow 4.1) and ionic characteristics, TBT tends to accumulate in the lipid tissues of many aquatic species (Davies & McKie 1987; Wade et al. 1988; Kannan et al. 1995). TBT also binds to macromolecules such as the protein glutathione in organs such as kidney and liver (Kannan & Falandyszt 1997). Due to the unique physicochemical properties of butyltin compounds, it has been suggested that significant amounts of TBT may be associated with particulate material and suspended sediments in coastal plain estuaries, and that TBT contaminated sediments may act as sources for dissolved TBT (Unger et al. 1987; Pereira et al. 1999). Benthic organisms appear to be good bioindicators because of their intimate contact with sediments, relative abundance, and ubiquitous and sedentary nature, particularly to determine the long-term environmental health of sediments contaminated by hydrophobic organic macro-pollutants (Martínez Lladó et al. 2007).

The gastropod *Odontocymbiola magellanica* (Gmelin, 1791) is an edible species that is collected commercially and consumed in northern Patagonia (Bigatti & Ciocco 2008). It is a fragile species due to its late sexual maturity, low population densities and low fecundity (lays few egg capsules with no more than 12 embryos) (Bigatti et al. 2008, 2014). In Golfo Nuevo, Patagonia, Argentina, where this study was carried out, imposex in *O. magellanica* has been recorded since 2000 in several studies (Bigatti & Penchaszadeh 2005; Bigatti et al. 2009, among others). However, there is no knowledge about the distribution of butyltin derivatives in the body tissue or the source of contamination. Consequently, the aim of this study was to assess the distribution of tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT) in several organs of the edible snail *O. magellanica*, a species well studied regarding imposex incidence in northern Patagonia. In addition, we evaluate the Biota Sediment Accumulation Factors (BSAFs), the sediments' health and the risk to human consumption. Also, BTs as well as Irgarol and Diuron (third-generation antifouling biocides used after TBT world banning) were determined in sediments. This work provides knowledge about TBT and derivatives in organisms from the SW Atlantic, following recent recommendations by OSPAR for the NE Atlantic.

## Materials and methods

### *Study area and sampling*

The study was conducted during December 2009 at four sites inside the Golfo Nuevo (GN) (42°45′S, 65° 02′W), Patagonia, Argentina (Figure 1). Three sites were positioned in the Bahía Nueva (BN), located in western GN, which has an area of 58 km$^2$ and a mean depth of 30 m. The city of Puerto Madryn (~90,000 inhabitants), situated in BN, has two ports with high maritime traffic. Since BT contamination is strongly associated with maritime traffic, three areas inside the BN were selected based on previous studies on TBT and imposex (Bigatti & Penchaszadeh 2005; Bigatti et al. 2009, among others): Luis Piedrabuena harbour (LPH), high maritime traffic; Parque Piedras (PP), medium maritime traffic; and Punta Este (PE), low maritime traffic. In addition, Cerro Avanzado (CA), located 17 km away from Puerto Madryn (outside the BN), was selected as a control site with little maritime traffic (Figure 1) and an absence of imposex incidence in gastropods (Bigatti & Penchaszadeh 2005; Bigatti et al. 2009). Samples of sediments and gastropods were taken by scuba diving, in depths between 5 and 20 m, at low tide. Since *Odontocymbiola magellanica* is a long-lived vulnerable species (Bigatti et al. 2008), and imposex incidence has been extensively studied

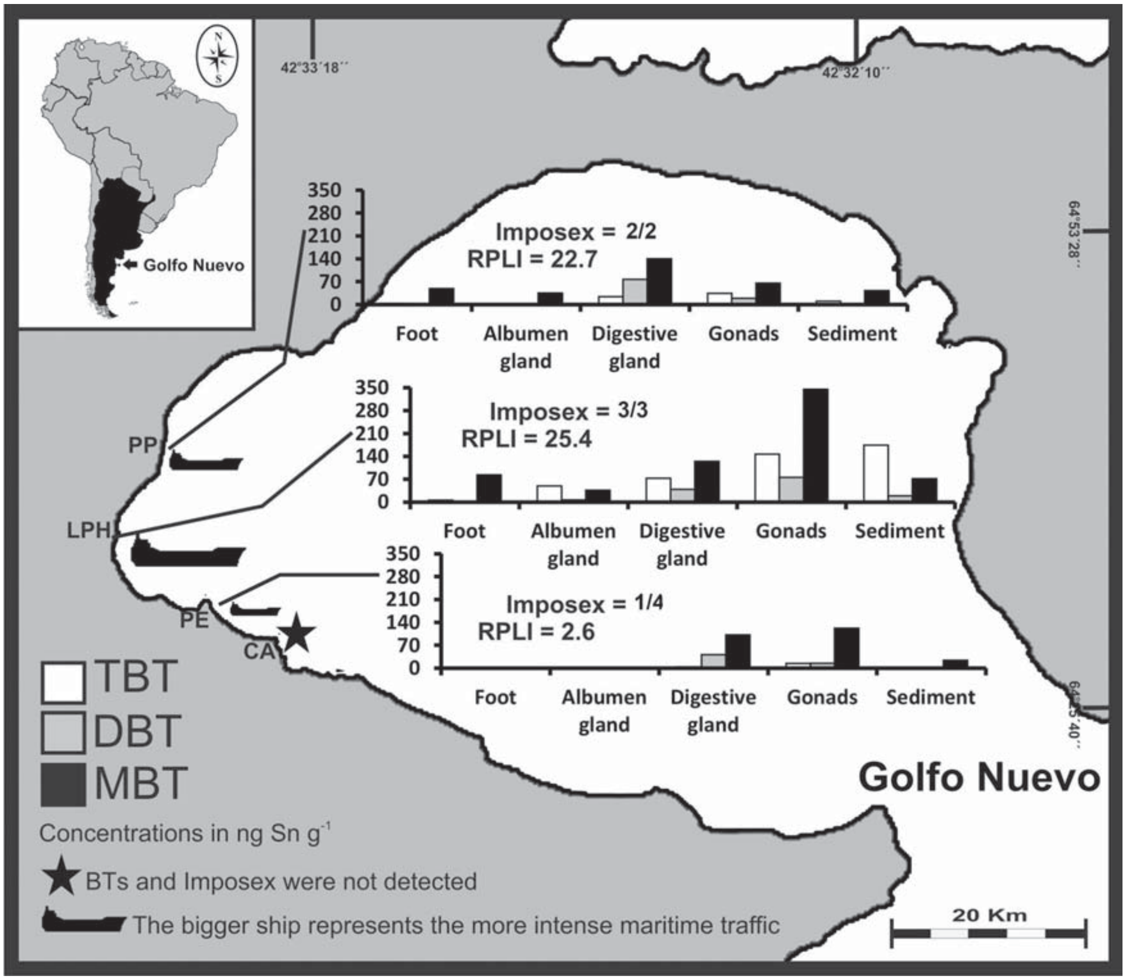


**Figure 1.** Marine traffic, imposex (as *n* females imposex/*n* total females), RPLI and concentrations of BTs in sediments of Golfo Nuevo and body tissue distribution in the gastropod *Odontocymbiola magellanica*. Imposex in CA was null but only one female was found ($n = 5$ for BT determination).



in the zone in recent years (Bigatti & Penchaszadeh 2005; Bigatti & Carranza 2007; Bigatti et al. 2009; Márquez et al. 2011), only five specimens were collected per site to analyse BT concentrations in the different organs, except for PP, where only four specimens could be collected, due to the low densities. *Odontocymbiola magellanica* is a relatively large gastropod (up to 18 cm length) allowing for reasonable quantities of tissue to be obtained from each organ for chemical analysis. The individuals were captured alive, immediately transported and dissected in the laboratory. Organ tissues from all specimens ($n = 4$–5) from each site were pooled, producing a composite sample. Gonads (G), digestive gland (DG), foot (edible portion) (F) and albumen gland (AG) (only in females), were homogenized and kept at −20°C before laboratory analysis. Twelve superficial sediment fractions (0–3 cm) from each location were collected using acrylic cores (4.5 cm diameter). The pooled fractions of sediments were homogenized into a composite sample, placed into a pre-cleaned glass flask and kept at −20°C until analysis could be carried out.

### *Characterization of sediments*

Humidity and granulometry were determined as described by Commendatore et al. (2000), while total organic carbon (TOC) was analysed in a Perkin Elmer 2400 Series II CHNS Elementary Analyzer after decarbonation in a desiccator containing HCl (37%) (Kristensen & Andersen 1987).

### *Characterization of gastropods*

#### *Lipid and water content*

A sub-sample of each tissue homogenate (between 0.2 and 3 g) was mixed with dry anhydrous sodium sulphate and Soxhlet extracted with 200 ml of

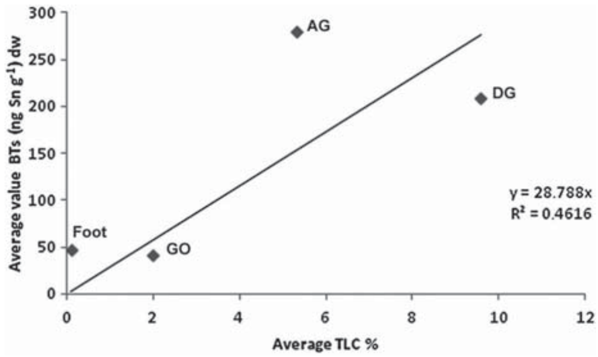


**Figure 2.** Correlation between average values of BTs (ng Sn $g^{-1}$) dw in each organ and TLC (%). Abbreviations: GO: gonads; AG: albumen gland; DG: digestive gland.

dichloromethane for 8 h. The extract was evaporated to dryness with a gentle flow of $N_2$ and weighed in a microbalance to determine the Total Lipid Content (TLC %) (Massara Paletto et al. 2008). The water content was analysed in each organ (gonad, digestive gland, foot and albumen gland) by drying in an oven at 60°C until constant weight was achieved.

### *Assessment of sex and imposex*

Gastropod shells were mechanically broken and body tissues were extracted. Sex was determined by observation of the albumen gland, capsule gland and vagina (only in females), as well as penis, vas deferens and gonad colour (brown in females and orange in males). Penis length was measured with a caliper for males and imposexed females. Information about imposex incidence in gastropods of the studied zone was available through previous studies that established associations to BT contamination in sediments (Bigatti et al. 2009); hence, only a proportion of imposex affected females (imposexed females/*n* females) and the Relative Penis Length Index (RPLI) were reported as a confirmation of sexual malformations due to TBT contamination (Gibbs & Bryan 1994).

### *Analysis of butyltin compounds (BTs) in sediments and organisms*

Butyltins (TBT, DBT and MBT) were analysed according to Castro & Fillmann (2012). Briefly, 5 g of dry sediments or 1 g of dry body tissue were placed into 40 ml vials and spiked with 100 ng of tripropyltin as a surrogate standard. Afterwards, organotins were extracted with 15 ml of 0.05% tropolone solution (w/v) in methanol and 1 ml of concentrated HCl in an ultrasonic bath. The extract volume was reduced to ~2 ml and derivatized with pentylmagnesium bromide in diethylether solution. The excess of Grignard reagent was destroyed by adding ultrapure water and HCl, both previously refrigerated to 4°C. After derivatization, the pentylated butyltins were recovered by a liquid-liquid extraction with hexane. The extracts were evaporated, transferred to a chromatographic column filled with activated silica and eluted with hexane/toluene (1:1). Finally, the solution was concentrated down to 0.9 ml under a gentle nitrogen flow, and 100 µl of tetrabutyltin solution (1000 ng Sn $ml^{-1}$) was added as an internal standard. Extracts were analysed in a Perkin Elmer Clarus 500MS gas chromatograph equipped with a mass spectrometer detector and an Elite-5MS capillary column ($30 \times 0.25 \times 0.25$). The quality assurance and quality control were based on analyses of blanks, spiked matrices and certified reference material (PACS-2/ National Research Council of Canada, Ottawa, Canada). Sample recoveries were between 78% and 107% and RSD (relative standard deviation) below 20% (IUPAC 2002). The method detection (MDL) and quantification limits (MQL) for sediment were 0.2 and 0.5 for TBT, and 0.3 and 0.8 ng (Sn) $g^{-1}$ for DBT and MBT, respectively. For gastropod tissues, limits were 0.5 and 1.5 for TBT, and 0.7 and 2.0 ng (Sn) $g^{-1}$ for DBT and MBT, respectively. In order to avoid matrix effects during BT analyses, the analytical curves were prepared using standard addition.

The BT level for a whole organism was estimated based on the sum of the relative concentration measured in each organ tissue. The amount of BTs (as Sn) in the foot (edible portion) was calculated based on BT concentration found at each site multiplied by the average weight of foot ($18.7 \pm 2.9$ g dw) in an average weight organism ($42.9 \pm 7.8$ g dw) and considering conversion factors of 2.74 for TBT, 2.56 for DBT and 2.38 for MBT. All concentrations are reported as ng Sn $g^{-1}$ (dry weight).

### *Biota-sediment accumulation factor (BSAF)*

According to Deng et al. (2015), organic pollutants can be directly ingested from contaminated sediments by sediment-dwelling organisms. The biota-sediment accumulation factor (BSAF) for butyltins is described by the equation: $BSAF_{BTs} = (C_{BTs}{-}bio/f_{lip})/(C_{BTs}{-}sed/f_{TOC})$, where $C_{BTs}$–bio and $C_{BTs}$–sed are concentrations in dry weight, $f_{lip}$ is the dry-weight fraction of lipid in tissue of the gastropod (g $g^{-1}$) and $f_{TOC}$ is the dry-weight fraction of organic carbon in sediment (g $g^{-1}$) (Deng et al. 2015). Normalization of the sediment contaminant concentration to the organic carbon content reduces variability in sediment characteristics, and normalization of the tissue contaminant concentration to the lipid content reduces variability in tissue concentrations among individuals of the same species as well as between different species (Pereira et al. 1999). In the current work BSAFs of TBT ($BSAF_{TBT}$), DBT ($BSAF_{DBT}$), MBT ($BSAF_{MBT}$) and ∑BTs ($BSAF_{BTs}$) were calculated for each analysed tissue (F, AG, DG and G).

### *Analysis of Irgarol and Diuron in sediments*

Irgarol and Diuron were analysed according to Biselli et al. (2000) and Gatidou et al. (2004). Briefly, 10 g of dry sediment was placed in an Erlenmeyer flask with 25 ml of acetonitrile, mechanically shaken and the extracts collected. This step was repeated twice and the extracts were combined and centrifuged at 4000 rpm. Afterwards, the supernatants were transferred to glass flasks and the volume reduced to 5 ml on a rotary evaporator. Then, 45 ml of ultrapure water was added and the extract was passed through a Strata C18-E cartridge (Phenomenex, Torrance, CA) previously conditioned with methanol followed by ultrapure water. The compounds were eluted with methanol and the final volume was reduced down to 1 ml under a gentle nitrogen flow. Analyses were carried out on a Waters Alliance 2695 liquid chromatograph equipped with a Micromass Quattro Micro API detector and a ESI Waters interface (LC-ESI-MS/MS system) (Milford, MA). The separation was performed using an XTerra analytical column (50 × 3×3.5) (Waters, Milford, MA). The mobile phase was acetonitrile:water (52:48 v/v) with 0.1% of formic acid at a flow rate of 0.4 mL $min^{-1}$. The quality assurance and quality control was based on analyses of blanks and spiked matrices. The results were in good agreement with SANCO (2009) with recoveries between 84–86% for Irgarol and 86–112% for Diuron. RSD values < 14% were obtained and the MDL was 0.03 ng $g^{-1}$ for both compounds.

## Results

### *Sediments*

Sediments at all sites were characterized by sandy particles, predominating over coarse and fine material. CA (control location) and PE sediments showed a similar distribution of particle grain sizes. At PP the sand fraction was higher (85%) than at all other sites, while fine material was relatively high (35%) in LPH (Table I). TOC had a similar order of magnitude at the four locations ranging between 0.19 and 0.39% (mean = 0.27 ± 0.09%, $n$ = 4) (Table I).

∑BTs in surface sediments ranged from < MDL to 174.8 ng (Sn) $g^{-1}$ for TBT, < MDL to 19.2 ng (Sn) $g^{-1}$ for DBT and < MDL to 71.8 ng (Sn) $g^{-1}$ for MBT (Figure 1 and Table II). BT concentrations at sites, imposex incidence and RPLI parameters increased with increasing levels of maritime traffic (CA (< MDL) < PE < PP < LPH). Levels of Irgarol and Diuron were below the method's detection limit (<MDL) in all analysed sediments.

### *Organisms*

All sampled gastropods were sexually mature (larger than 90 mm) (Table III). The TLC was higher in the digestive gland (7.4–11.5%) followed by gonads (3.2–7.3%), albumen gland (0.43–3.5%) and foot (0.03–0.47%) (Table IV).

In *Odontocymbiola magellanica* body tissues, butyltin derivatives varied from < MDL to 147.09 ng (Sn) $g^{-1}$ for TBT, <MDL to 77.0 ng (Sn) $g^{-1}$ for DBT and < MDL to 345.27 ng (Sn) $g^{-1}$ for MBT. MBT was the most abundant derivative in the organs (Figure 1, Table II). ∑BTs in body tissues were < MDL in CA and increased towards the areas with increasing maritime activities, showing a similar trend to that of the sediments ($R^2 = 0.855$; $P < 0.05$; $n = 4$). Butyltin concentrations were higher in gastropods from LPH, which had the highest levels in gonads and digestive gland, followed by albumen gland (females) and foot (edible portion). TBT, DBT and MBT were detected in almost all tissues of LPH and PP, except for DBT in foot (LPH and PP), and TBT and DBT in albumen gland (PP). In PE, where TBT and DBT concentrations were < MDL in sediment (MBT only was found), BTs (TBT, DBT and MBT) were detected in digestive gland and gonad. In CA (Control site) BT levels were < MDL for all gastropod tissues (Figure 1 and Table II). The average value of TLC in gastropods was positively correlated with the average of BT concentrations for each organ ($r^2 = 0.462$; $P < 0.05$; $n = 4$), considering all sampled individuals ($n = 19$) collected at all the sites ($n = 4$) (Figure 2).

### *BTs in foot (edible part)*

Considering average weights (foot and the whole organism) and conversion factors provided in the methods section, a whole gastropod collected from LPH would have around 70.2 ng Sn $g^{-1}$ corresponding to TBT (192.3 ng $g^{-1}$ of TBT), 34.3 ng Sn $g^{-1}$ corresponding to DBT (87.7 ng $g^{-1}$ of DBT), and 184.5 ng Sn $g^{-1}$ corresponding to MBT (439.0 ng $g^{-1}$ of MBT). The foot would have around 6.0 ng Sn $g^{-1}$ (16.3 ng

**Table I.** Granulometry and total organic carbon (TOC) in sediments and geographic position of sampling sites.

| Site | Latitude (S) | Longitude (W) | % gravel (>2 mm) | % sand (>63 µm, < 2 mm) | % fine (<63 µm) | % TOC |
|---|---|---|---|---|---|---|
| PP | 42°45′648 | 65°02′968 | 13 | 85 | 2 | 0.19 |
| LPH | 42°45′741 | 65°01′597 | 1 | 64 | 35 | 0.39 |
| PE | 42°46′776 | 64°57′444 | 41 | 58 | 1 | 0.24 |
| CA | 42°49′044 | 64°52′155 | 43 | 48 | 9 | 0.24 |

PP: Parque Piedras; LPH: Luis Piedrabuena harbour; PE: Punta Este; CA: Cerro Avanzado.

$g^{-1}$ of TBT), 3.5 ng Sn $g^{-1}$ (9.7 ng $g^{-1}$ of TBT) and < MDL in the organisms collected from LPH, PP, and PE and CA, respectively.

High bioaccumulation of BTs were registered in the foot for both LPH and PP gastropods, where the highest BSAF was found for MBT ($BSAF_{MBT} = 5.25$ in LPH and $BSAF_{MBT} = 1.4$ in PP). For other tissues the order was: AG > G > DG (Table IV).

The higher proportion of imposex affected females (imposexed females/*n* females) was registered in gastropods from the harbour zone (Figure 1). The females affected by imposex presented different penis development according to the site. Values for RPLI were 25.4 in LPH and 22.7 in PP, sites with high and medium maritime traffic, respectively, decreasing markedly at PE (RPLI= 2.6), a site with low maritime traffic, while no imposex was registered at CA (scarce marine traffic). In CA only one female was found, but previous reports from this site (see Discussion) determined this species as imposex-free (Figure 1).

**Table II.** TBT, DBT and MBT concentrations (ng (Sn) $g^{-1}$ dw) in sediments and in organs of *Odontocymbiola magellanica*.

| Site | Organ | TBT | DBT | MBT | ∑BTs |
|---|---|---|---|---|---|
| PP | Foot | 3.54 | <MQL | 48.49 | 52.03 |
| (2/2) | AG | <MQL | <MQL | 34.89 | 34.89 |
| | DG | 23.92 | 77.04 | 139.7 | 240.60 |
| | GO | 34.11 | 19.09 | 65.63 | 118.80 |
| | Sediment | 10.27 | < MQL | 42.34 | 52.61 |
| LPH | Foot | 5.95 | <MQL | 83.55 | 89.50 |
| (3/3) | AG | 49.27 | 6.45 | 36.21 | 91.92 |
| | DG | 72.91 | 39.41 | 124.8 | 237.10 |
| | GO | 147.09 | 75.46 | 345.27 | 567.80 |
| | Sediment | 174.81 | 19.19 | 71.79 | 265.80 |
| PE | Foot | <MQL | <MQL | <MQL | <MQL |
| (1/4) | AG | <MQL | <MQL | <MQL | <MQL |
| | DG | 4.20 | 41.39 | 102.30 | 147.80 |
| | GO | 14.51 | 14.83 | 121.82 | 151.20 |
| | Sediment | <MQL | <MQL | 24.05 | 24.05 |
| CA | Foot | <MDL | <MDL | <MDL | <MDL |
| (0/1) | AG* | na | na | na | na |
| | DG | <MDL | <MDL | <MDL | <MDL |
| | GO | <MDL | <MDL | <MDL | <MDL |
| | Sediment | <MDL | <MDL | <MDL | <MDL |

Note: Proportions in parentheses below each site correspond to number of affected females/*n* total.
PP: Parque Piedras; LPH: Luis Piedrabuena harbour; PE: Punta Este; CA: Cerro Avanzado; GO: gonads; AG: albumen gland; DG: digestive gland.
*na: not analysed (there was not enough tissue for the analysis).

## Discussion

### *BTs in sediment*

Although the use of TBT was banned worldwide in 2008 (IMO 2001), important levels of TBT were still detected in sediments and body tissues of *Odontocymbiola magellanica* from Bahía Nueva (Golfo Nuevo) in December 2009. Despite being an IMO member state, the IMO Convention has not yet been ratified by Argentina, but Prefectura Naval Argentina (the maritime authority) banned the use of antifouling paints in leisure and small boats from shipyards, recreational ports or marinas by Ordenanza 4/98 (DPMA). The fact that Irgarol and Diuron were not detected in sediments but BTs were present, particularly in the harbour zone, suggests that despite the prohibition and the availability of new biocides on the market, TBT-based antifouling paints were still being used by, at least, part of the maritime fleet in Golfo Nuevo up to the date of our study. However, these findings may also be a consequence of the persistence of TBT in sediments, given that the contaminant has a half-life from 1.85 to more than 10 years (Takeuchi et al. 2004). Concentrations of TBT and its degradation products in the environment are still a cause for concern for the aquatic environment (Martínez-Lladó et al. 2007). Taking into account that BT concentration has a tendency to increase with maritime traffic and harbour proximity (Maguire et al. 1986; Foale 1993; Gibbs & Bryan 1994; Bigatti et al. 2009), the higher levels of butyltins found at LPH could be directly associated with the relatively high maritime traffic in the area; the Golfo Nuevo receives an average of 654 ships per year (APPM 2012). In addition, the harbour area presented the greatest percentage of fine particles and TOC in bottom sediments, which favours the adsorption of these contaminants (Stewart & de Mora 1990). At the same time the lower levels of BT found at PP (500 nm north of LPH) relative to LPH could be associated with the dominance of coarse sediments and relatively low TOC. According to Pereira et al. (1999), concentrations of butyltins in benthic sediment appear to be controlled primarily by a partitioning process into the sediment organic carbon, with ionic interactions probably playing a minor role in the adsorption processes. The even lower BT concentrations in PE can be explained by coarse sediments and lower TOC, but also by the lower influence of maritime traffic. In CA, where sediments showed similar

**Table III.** General parameters and lipid content in *Odontocymbiola magellanica.*

| | | Parameters | | | | % of lipids in each tissue* | | | |
|---|---|---|---|---|---|---|---|---|---|
| Site | *n* | TW ± SD (g) | SW ± SD (g) | ShW ± SD (g) | ML ± SD (cm) | F | GO | AG | DG |
| PP | 4 | 157.1 ± 21.2 | 95.0 ± 24.5 | 62.1 ± 4.0 | 125.9 ± 7.7 | 0.12 | 3.20 | 3.50 | 8.80 |
| LPH | 5 | 240.0 ± 95.2 | 159.7 ± 52.2 | 80.5 ± 49.2 | 137.9 ± 11.0 | 0.10 | 6.80 | 0.43 | 9.90 |
| PE | 5 | 158.9 ± 37.4 | 102.2 ± 28.0 | 56.7 ± 12.7 | 120.5 ± 20.1 | 0.03 | 5.00 | 0.90 | 7.40 |
| CA | 5 | 221.4 ± 85.4 | 154.0 ± 60.5 | 67.4 ± 59.5 | 137.4 ± 21.7 | 0.47 | 7.30 | 1.50 | 11.50 |

PP: Parque Piedras; LPH: Luis Piedrabuena harbour; PE: Punta Este; CA: Cerro Avanzado; TW: total weight; SW: soft weight; ShW: shell weight; ML: maximum length; F: foot; GO: gonads; AG: albumen gland; DG: digestive gland.
*% lipid is expressed in tissue dry weight.

characteristics to PE, BTs were < MDL, associated with low maritime traffic. The process of BT adsorption onto sediments is fast and involves primarily particulate organic matter, constituents that act as sorbents (Berg et al. 2001). However, for this study, no correlation was found between BT concentrations and the relative TOC and grain size in sediments ($P > 0.05$). Rossi de Oliveira et al. (2010) did not find a correlation between BT concentrations and the relative organic carbon contents in sediments of Santa Catarina (Brazil). This fact can be explained by a patchy behaviour of BTs related to source proximity (maritime traffic) and/or a low and almost unaltered TOC content in the studied sites. This patchy behaviour made it difficult to correlate sedimentary TBT with grain size and TOC, which have both been reported as possible important factors in sediment BT distribution (Martínez-Lladó et al. 2007). In the same way, other authors did not find correlations between BT levels and those parameters (Dong et al. 2015). BT levels in sediments and in gastropods were positively correlated ($r^2 = 0.855$; $P < 0.05$; $n = 4$), suggesting that xenobiotic accumulation in *O. magellanica* occurs mainly through contaminated sediments, rather than water or the food chain. This gastropod lives most of the time buried in the substrate and moves to capture live prey on mixed or hard bottoms (Bigatti et al. 2010). According to Berto et al. (2007), desorbed TBT is not the only bioavailable form, and it is important to stress that the absence of TBT in sediments does not necessarily imply safe removal from the system. TBT can be directly taken up from contaminated sediments by scavenger organisms such as gastropods. The relationship between TBT and its derivatives (DBT and MBT) as TBT/(DBT + MBT) showed a predominance of TBT over DBT + MBT (value = 1.9) in LPH, denoting a recent input of TBT in the environment (Kuballa et al. 1995) or sediment desorption of aged but intact TBT. In contrast, in PP MBT was predominant while the TBT level was relatively low, probably due to smaller inputs of TBT at this site (lower maritime traffic) combined with the lower sorption capability of its coarse sediment. In PE only MBT was detected, suggesting aged TBT input. In summary, recent and aged contributions of TBT in this coastal zone suggest chronic pollution. However, due to the persistence of TBT in sediments and its bioavailability depending on various environmental factors, there is a need to monitor these compounds over time. The significant relationship ($r^2 = 0.8334$, $P < 0.05$) found between the TBT and [DBT + MBT] concentrations in *O. magellanica* from LPH indicated that most of the [DBT + MBT] residues in the tissues might be originated from TBT used in ship antifouling paints. Although BT derivatives can have multiple sources (Dong et al. 2015), in the Golfo Nuevo there is no evidence of sources other than antifouling paints.

In LPH and PE, TBT levels found in organ tissues were significantly correlated with the sum of [DBT + MBT] ($r^2 = 0.833$, $p < 0.05$, $n = 4$ and $r^2 = 0.444$, $p < 0.05$, $n = 4$). This positive correlation was not found in PP, where [DBT + MBT] had the highest values in the digestive gland, indicating a direct incorporation rather than metabolization of these degraded derivatives. At this site, the lower value of TOC (0.19%) could facilitate the bioavailability of these compounds passing to the water column. In fact, functional groups in organic matter allow adsorption of different pollutants. Thus, the content of organic carbon in soils and sediments should strongly influence their capability to retain TBT (Hoch & Schwesig 2004). According to Quevauviller et al. (1994), any resuspension of contaminated bottom sediments by the tide, storms or dredging activities can act as a new contamination source and may lead to enhanced BT concentrations in the overlying water column. As a consequence, TBT degradation in the bottom sediments and resuspension may affect the ratios of TBT versus DBT and MBT (Diez et al. 2005).

Waite et al. (1991) assessed TBT in sediments from UK estuaries and established four categories of sites, according to TBT concentration: 4–18, 22–73, 109–365 and >365 ng (Sn) $g^{-1}$ as corresponding to low, moderate, high and sediment containing paint particles, respectively. Following this categorization, sediments from LPH can be considered as high, PP

**Table IV.** Biota sediment accumulation factors and TLC %.

| | TBT | DBT | MBT | ∑BTs | TLC % |
|---|---|---|---|---|---|
| *LPH (0.39% TOC)* | | | | | |
| F | 0.15 | – | 5.25 | 1.52 | 0.09 |
| AG | 0.30 | 0.35 | 0.53 | 0.36 | 0.37 |
| DG | 0.02 | 0.09 | 0.08 | 0.04 | 8.55 |
| G | 0.06 | 0.26 | 0.32 | 0.14 | 5.87 |
| *PP (0.19% TOC)* | | | | | |
| F | 0.42 | – | 1.39 | 1.20 | 0.16 |
| AG | – | – | 0.03 | 0.03 | 4.57 |
| DG | 0.04 | – | 0.05 | 0.08 | 11.48 |
| G | 0.15 | – | 0.07 | 0.10 | 4.17 |
| *PE (0.24% TOC)* | | | | | |
| F | – | – | – | – | 0.04 |
| AG | – | – | – | – | 1.06 |
| DG | – | – | 0.12 | 0.17 | 8.71 |
| G | – | – | 0.21 | 0.26 | 5.89 |

and PE as moderate, and CA (control) as uncontaminated. In comparison to other studies done in South American coastal zones, TBT levels from LPH were lower than values found in hotspots, such as Bahía Blanca – Argentina (3228 ng Sn $g^{-1}$, Delucchi et al. 2006) and Paranaguá Bay – Brazil (2796 ng Sn $g^{-1}$, Santos et al. 2009), and in the same order of magnitude of Guayaquil Gulf – Ecuador (100 ng Sn $g^{-1}$, Castro et al. 2012a) and Babitonga Bay – Brazil (125 ng Sn $g^{-1}$, Rossi de Oliveira et al. 2010).

### *Imposex*

The species *Odontocymbiola magellanica* has been monitored for imposex related to marine traffic since the year 2000 in Golfo Nuevo (Bigatti & Penchaszadeh 2005), including the same areas studied in the present work. Similarly, Bigatti et al. (2009) confirmed TBT contamination in sediments of the studied zone, but not in body tissues and reported imposex in *O. magellanica* and other species inhabiting the same area. In the present study we confirmed for the first time a relationship between imposex incidence in *O. magellanica* and the presence of BTs in its organ tissues. Although TBT determinations are essential to determine pollution, our results corroborate the fact that imposex incidence in *O. magellanica* is a good and rapid tool to assess the history of TBT contamination at a site.

### *BTs in organisms*

*Odontocymbiola magellanica*, a long-lived organism (reaching up to 20 years), exhibits late maturity (Bigatti et al. 2007) and lays egg-capsules with few embryos; these characteristics and its low densities in Golfo Nuevo (Bigatti 2005) make this species very vulnerable to environmental impacts. *Odontocymbiola magellanica* is captured for local consumption in the studied zone (Bigatti & Ciocco 2008), and hence it is important to monitor levels of contamination in this gastropod for alimentary safety. Despite local consumption of *O. magellanica*, BT levels have not been analysed before. This study reports contamination levels in different organ tissues for the first time in Argentinean marine gastropods, and relates imposex with TBT pollution in sediments. Although the number of individuals collected for this study was small for statistical comparisons, our data confirms that imposex is a dose-dependent phenomenon in *O. magellanica* as in other species (Maguire et al. 1986; King et al. 1989; Krone et al. 1989; Shim et al. 2002). Previous results, in the same area of study, reported malformations in *O. magellanica* shells and losses of up to 10% in weight of females with imposex, attributing the phenomenon to TBT contamination (Bigatti & Carranza 2007; Marquez et al. 2011), which can be confirmed in the present study.

BT concentrations varied according to the organ analysed and, except for CA (< MDL for all tissues), gonads and digestive gland always presented higher BT levels than albumen gland and foot. Horiguchi et al. (2012) observed that one-third or more of total TBT in the gastropod *Reishia clavigera* (Küster, 1860) was accumulated in the digestive gland, followed by the gonad, whilst in *O. magellanica* the higher concentrations of TBT were detected in the gonad followed by the digestive gland. In addition, gonads had high TLC, probably due to the presence of gonads full of gametes at the time of collection (Bigatti et al. 2008). Taking this into account, the gonad seems to be a good matrix to detect and monitor BT contamination in future studies on *O. magellanica* from commercial fisheries. Both gonad and digestive gland presented higher TLC than the albumen gland and foot, similar to what has been observed for bivalves and cephalopods (Arai et al. 2009). Since BTs are lipophilic compounds (Hall & Pinkney 1984), higher lipid contents are related to highly contaminated tissues (Hoch 2001). In fact, the average value of TLC in gastropods was positively correlated with the average of BT concentrations of each organ ($r^2 = 0.462$, $P < 0.05$, $n = 4$).

The bioaccumulation of MBT was higher in the foot of gastropods from LPH and PP, which could be associated with mechanisms of metabolization of TBT. Both locations showed the highest TBT levels in sediments, which are reservoirs for this pollutant. Consequently, the incorporation of TBT in organisms and its ensuing metabolization and/or a direct assimilation of DBT and MBT from aged TBT would be expected.

Despite the low lipid amounts in the foot, relatively high MBT levels were observed at PP and LPH. This edible tissue, which should be monitored for public health purposes, could accumulate BTs as a final receptor of metabolic derivatives by direct contact with the contaminant in the sediment and/or by transference through the mucus secreted by the organisms (Pope 1998). In this study we hypothesize that BTs could also be transported by the haemolymph that irrigates the foot with numerous vascular connections. Contaminants could reach the haemolymph through food uptake, direct contact and/or respiration by ctenidia. In addition, the presence of BTs in the albumen gland suggests that these compounds could be transferred to egg capsule intracapsular fluid with consequent embryo contamination. Luzzatto (2006) registered malformation of embryos in *Adelomelon brasiliana* (Lamarck, 1811), probably related to TBT exposure (Goldberg et al. 2004); in this context, reproductive outputs could also be affected by TBT (Lahbib et al. 2009). Strand et al. (2009) reported TBT, DBT and MBT levels in gastropods from the Caribbean Virgin Islands in the following ranges: 3.8–13; 6.1–29; <1–20 ng (Sn) $g^{-1}$ dw in *Plicopurpura patula* (Linnaeus, 1758); 3.5–14; 3.2–17; 2.8–13 ng (Sn) $g^{-1}$ dw in *Stramonita rustica*; 8.1–101; 9.6–78; 12–62 ng (Sn) $g^{-1}$ dw in *Vassula deltoidea* (Lamarck, 1822); 17–119; 36–226; 12–111 ng (Sn) $g^{-1}$ dw in *Cittarium pica* (Linnaeus, 1758). In the current work, BT levels in *O. magellanica* varied from < MDL to 147.1 for TBT, < MDL to 77.0 for DBT, and < MDL to 345.3 ng (Sn) $g^{-1}$ dw for MBT, being relatively higher than those reported for the Caribbean Virgin Islands. Considering that the foot of *O. magellanica* is consumed regionally as seafood, the risk related to its consumption is relatively low. Even taking into account the most contaminated site of GN (LPH – 16.29 ng $g^{-1}$ of TBT), an average person of 70 kg of weight would need to consume around 3.2 kg of fresh foot (meat) per day to exceed the European Food Safety Authority (EFSA) recommended Tolerable Daily Intake (TDI) of 250 ng $kg^{-1}$ body weight of organotin compounds in food (EFSA 2004). However, this analysis did not take into consideration any other source of contamination, very common in harbours with high marine traffic, which could influence the sediment toxicity through synergistic effects.

TBT is included in the group of organic tin compounds on the OSPAR List of Chemicals for Priority Action (OSPAR 2009). Due to the persistence of TBT in sediments, with a half-life from 1.85 to more than 10 years (Takeuchi et al. 2004), TBT and its degradation products are still a cause of concern for the aquatic environment. As in Argentina, there are no Sediment Quality Guidelines (SQG) for TBT, so we used the Australian SQG and the Dutch RIKZ (National Institute for Coastal and Marine Management) to assess the sediment risk to benthic organisms. The Australian SQG for TBT established low and high trigger values of 5 and 70 ng (Sn) $g^{-1}$ respectively (ANZECC/ARMCANZ 2000). This criterion places LPH sediments (174.8 ng (Sn) $g^{-1}$) exceeding the safety range and therefore represents a risk to benthic organisms; PP sediments where TBT level was 10.3 ng (Sn) $g^{-1}$ is higher than the low trigger value; PE and CA do not represent a threat to organisms' health. In addition, the SQGs proposed by the Dutch RIKZ established the maximum permissible concentrations and the negligible concentrations as 0.7 and 0.007 ng (Sn) $g^{-1}$ dw respectively, based on a standard sediment having 10% organic matter, or an equivalent of 5% organic carbon. When normalized to total organic carbon, this corresponds to 0.014 µg (Sn) $g^{-1}$ TOC and 0.00014 µg (Sn) $g^{-1}$ TOC, respectively. Comparison of the OC-normalized TBT contained in sediments in LPH (44.8 µg (Sn) $g^{-1}$ TOC) and PP (5.4 µg (Sn) $g^{-1}$ TOC) against the Dutch criteria showed that TBT exceeded the Maximum Permissible Concentration (MPC). These values indicated that sediments in these areas were seriously polluted by TBT. TBT in sediments from LPH and PP was found to be high (174.8 and 10.3 ng (Sn) $g^{-1}$ dw, respectively). The comparison against the Dutch SQGs indicated that concentrations largely exceeded the MPC, posing a serious environmental threat.

## Conclusions

The butyltin assessment of tissue organs of the edible gastropod *Odontocymbiola magellanica* and the sediments that it inhabits in Golfo Nuevo confirmed the relationship between the contaminant and imposex incidence and highlighted recent and aged inputs of TBT in those areas more influenced by maritime activities. Bioaccumulation processes were evident in all body tissues of *O. magellanica*, particularly at LPH and PP, where BT concentrations were high. Even after the TBT ban at a global level, new antifouling agents were not detected in sediments, while populations of *O. magellanica* are still affected by imposex incidence and TBT contamination. Even so, TBT levels found in the foot of this edible gastropod do not prohibit its human consumption. However, considering that the species could be exploited as a fishery resource, studies on contaminants commonly found in marine traffic areas must be periodically carried out to ensure the safety for seafood consumers. The state of sediments from LPH and PP is of concern, as they exceeded quality values established by international directives. Our results add to the effort of mapping the occurrence and effects of BTs globally as recommended by OSPAR for the NE Atlantic.

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