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Butyltin contamination in Northern Chilean coast: Is there a potential risk for consumers?

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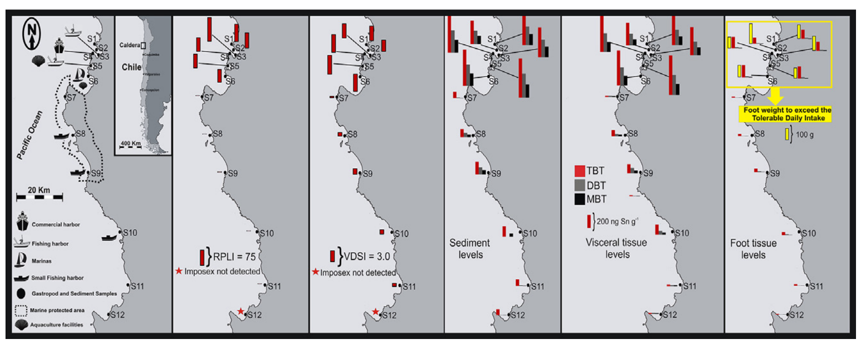
HIGHLIGHTS

- Imposex and TBT levels were analyzed in sediments and edible gastropod tissues.
- High contamination levels and evidences of fresh inputs of TBT were detected.
- TBT contaminated sites were located within "Isla Grande Atacama" marine reserve.
- The ingestion of *Thaisella chocolata* foots from the most contaminated sites is not safe.
- Regulatory actions to protect environment and food safety should be implemented.

GRAPHICAL ABSTRACT



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ABSTRACT

Imposex is the superimposition of non-functional male sex organs in gastropod females. This syndrome is a hormonal imbalance induced by tributyltin (TBT) which have been used in antifouling paints formulation. The present study aimed to perform an integrated environmental assessment of imposex and butyltin (BT) contamination using surface sediments and tissues of *Thaisella chocolata* (an edible gastropod) from northern Chile. The results showed imposex incidence in 11 out of 12 sites. In the most contaminated sites, which are areas under the influence of maritime activities, and also used for fishing and aquaculture, RPLI were over 60 and VDSI over 4 (high incidence of sterile females). Exceptionally high contamination levels and evidences of fresh inputs of tributyltin (TBT) were detected along the studied area. TBT levels above 300 and 90 ng Sn g^{-1}, respectively, were recorded in sediments and edible gastropod tissues of 6 sites. Thus, a daily ingestion of 90 to 173 g of *T. chocolata* foot (4 to 8 organisms) from the most contaminated sites will certainly lead to the consumption of BT exceeding the tolerable daily intake recommended by European Food Safety Authority. It is reasonable to consider that human risk is even higher if daily consumption of additional seafood is considered. Moreover, some contaminated sites were located within the marine reserve "Isla Grande Atacama", indicating that even marine protected areas are under the influence of TBT contamination. These findings suggest that current levels of TBT in the studied area

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are sufficient to induce harmful effects on the environment and constitutes a potential threat to seafood consumers. Thus, national regulatory actions toward environmental protection and food safety of local populations are still mandatory, even after 8 years of the TBT global ban by IMO.

1. Introduction

Tributyltin-based antifouling paints are used since 1960's as protective coatings on hulls of ships and boats (Almeida et al., 2007; Castro et al., 2011). Although they are very effective to prevent and minimize establishment of fouling, these products are highly toxic for non-target species (Laranjeiro et al., 2015; Lopes-dos-Santos et al., 2014). Several studies reported deleterious effects of tributyltin (TBT) on the wildlife, including imposex (imposition of male sexual organs on females) in gastropod species (Blaber, 1970; Castro et al., 2008), immunosuppression in cetaceans (Tanabe, 1999; Choi et al., 2011), obesogenic syndrome in fish (Meador et al., 2011) and shell malformations in bivalves (Alzieu et al., 1986; Alzieu, 2000). In addition, possible human exposure to tributyltin via seafood intake (Cardwell et al., 1999) associated to health risks (as immunosuppression, endocrine disruption, neurotoxic damages, cancer, among others) were also reported (Guerin et al., 2007). Hence, the use of TBT-based antifouling paints was initially regulated by local legislations in Europe (Gipperth, 2009) and later globally banned in 2008, through the convention on the control of harmful antifouling systems on ships (AFS Convention) (IMO, 2016). Furthermore, due to inherent human health risks, the U.S. Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) has set threshold limits (not exceeding 0.0015% in food composition and 100 ng Sn kg^{-1}, respectively) for the amounts of tin compounds in food (ATSDR, 2005; EFSA-Q-2003-110, 2004).

After TBT restrictions entered into force, its environmental levels as well as imposex incidence (a known biomarker of TBT contamination) begun to decline in many areas worldwide (Castro et al., 2012a, 2012b, 2012c, 2012d; Galante-Oliveira et al., 2011; Guomundsdóttir et al., 2011). However, recent studies have pointed out that present usage of tributyltin is still evident in many South American countries, including Argentina (Del Brio et al., 2016; Laitano et al., 2015; Quintas et al., 2016), Brazil (Artifon et al., 2016; Borges et al., 2013; Petracco et al., 2015; Santos et al., 2016), Central Chile (Batista et al., 2016), Ecuador (Grimón et al., 2016), Peru (Castro and Fillmann, 2012) and Venezuela (Paz-Villarraga et al., 2015). This scenario is, at least partially, caused by the absence of local regulations on the use of TBT-based antifouling paints (Batista et al., 2016). In addition, the gaps of knowledge on TBT contamination and impacts has also helped to hampered the implementation of actions to protect environmental and human health in most of these countries (Castro et al., 2012a, 2012b, 2012c, 2012d).

Chile is particularly susceptible to the environmental impacts produced by the use of antifouling biocides due to the several maritime and harbor activities developed along its 6435 km of coastline (Bravo, 2003). Imposex in marine gastropods (Gooding et al., 1999; Osorio and Huaquin, 2003) and TBT residues (Bravo et al., 2004; Pinochet et al., 2009) in surface sediment samples were previously detected in coastal areas under the influence of harbors and marinas in the central region of Chile. Recently, Batista et al. (2016) have also detected butyltin levels (TBT, dibutyltin (DBT) and monobutyltin (MBT)) in surface sediments and biota tissues and imposex in gastropods (*Acanthina monodon*, *Oliva peruviana* and *Xanthochorus cassidiformis*) from three out of ten regions of the Chilean coast under significant influence of ship and/or boat traffic.

In addition, the Chilean benthic invertebrate fishery (comprising over 60 species of mollusks, crustacean and echinoderms) represents an important food resource, which are consumed by the local population and traded on domestic and international markets (Leiva and Castilla, 2002). In this concern, *Thaisella chocolata* ("Locate") is a gastropod species exploited since 1978 for human consumption, being an important benthic resource caught by artisanal fisheries in northern Chile. Its extraction reached 8244 ton in 1986, but severely declined in subsequent years due to overexploitation (Avendaño et al., 1996). Currently, the "Locate" fisheries are regulated by the Chilean government, which established closed seasons and minimum size of capture (Avendaño et al., 1998). In 2015, the National Service of Fisheries and Aquaculture of Chile reported landings of 492 ton of *T. chocolata* (SERNAPESCA, 2015).

Thaisella chocolata is a good indicator of TBT contamination for the Pacific coast of South America by bioaccumulating butyltin residues and developing imposex (Castro and Fillmann, 2012). However, no studies were performed so far evaluating butyltin (BT) contamination and its potential implications to human health in Chile. Thus, the present study aimed to assess the environmental impacts and potential risk for consumers associated to intake of sea food from exploitation areas of Caldera, Northern Chile. For this, the spatial distribution of imposex in gastropods and butyltins (BT) levels in environmental samples (surface sediments and gastropod tissues) were appraised along a fishing and aquaculture area under different types and intensities of maritime traffic incidence. This assessment shall support the implementation of regulatory actions toward environmental protection and food safety of local populations.

2. Material and methods

2.1. Study area and sampling

The present study was carried out at the region of Caldera city (27°S), Atacama region, Northern Chile (Fig. 1). This region has been promoted as an area of high economic interest, where artisanal fisheries and aquaculture are performed supplying domestic and some international markets (Castillo and Valdés, 2011). Twelve sites (S1 to S12) representing different degrees of human intervention by maritime and/or harbor activities, which are known TBT sources (Mattos and Romero, 2016), were selected for study along 80 km of coast (Table 1).

All selected sites were sampled using a vessel at approximately 500 m from the coast. In each site, a single sample of surface sediments (upper 2 cm) was collected during April 2015 using a stainless steel "Van-veen" dredge. The sediment samples were packed in precleaned aluminum boxes, kept under refrigeration and taken to the laboratory. Simultaneously, at least 30 adult specimens of *T. chocolata* were also caught by SCUBA diving in the subtidal zones from the same 12 sites, totalizing 485 adult specimens along the 12 sites. The organisms were taken to the laboratory for imposex analysis. In the lab, sediment samples were frozen, freeze dried and stored at −20 °C until subsequent analysis.

2.2. Imposex assessment

The gastropods were narcotized with a 3.5% $MgCl_2$ solution (1:1 seawater/distilled water) for 2 h. Shells length (SL) were measured using a digital caliper (0.05 mm) from the apex to the top of the siphonal canal. Soft bodies were removed from the shell using a bench vice and sex determination was based on the presence or absence of sexual accessory glands (capsule, ingesting and albumen). The penis length (PL) were measured with a digital caliper and the presence of vas deferens in females and males were also registered. Imposex parameters were assessed using the following indices: % of imposex in females (I%),

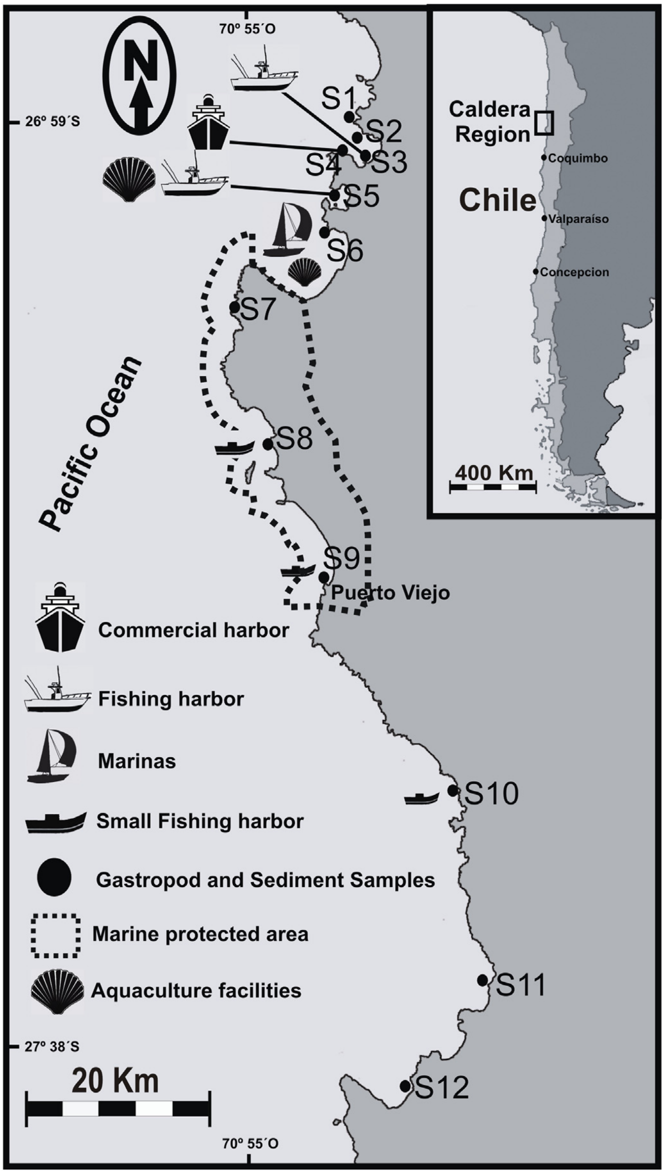

Fig. 1. Location of each sampling site (surface sediments and gastropods) and corresponding maritime activity along Northern Chile.

Female Penis Length Index (FPLI = mean penis length of all females sampled, including the zero values), Relative Penis Length Index (RPLI = [mean penis length in females / mean penis length in males] × 100) (Stroben et al., 1992). The vas deferens sequence index (VDSI), based on the development of male sexual characters (particularly the vas deferens) by females, was evaluated according to Gibbs et al. (1987). In addition, the % of sterile females presenting blocked vulva (VDS ≥ 5) was also calculated. After imposex determinations, the gastropod tissues (visceral coil and foot tissues) were dissected, and individually frozen (−20 °C), freeze dried and stored at −20 °C until subsequent analyses. For confirmation of sexual maturation stage, five females were randomly selected and their gonads were histologically analyzed according to Howard and Smith (1983).

2.3. Butyltin analyses

Chemical analyses were performed in duplicate for all sediment and tissue samples. Whole sediments (not sieved) were used for analysis, whereas each tissue (whole visceral coil or foot) was pooled from 3 females collected in each site. Butyltins (TBT, DBT and MBT) were determined according to Bravo et al. (2004). In brief, 1.0 g of freeze-dried sample were spiked with tripropyltin (TPrT) as surrogate standard and extracted with 20 mL of glacial acetic acid in an ultrasonic bath. This mixture was shaken for 24 h at 400 rpm, and centrifuged for 15 min at 4000 rpm. Then, 2 mL of centrifuged extract, 20 mL of acid acetic–acetate buffer (pH 4.8) and 1 mL of isooctane were directly introduced into the derivatization reactor. Ethylation was carried out using 500 μL of sodium tetraethylborate ($NaBEt_4$; 2% m/v). The mixture was immediately shaken for 1 h at 200 rpm. Subsequently, 1 or 2 μL of the organic phase were directly injected into the gas chromatographer coupled to a pulsed flame photometric detector (GC-PFPD). Butyltins were quantified using the standard addition method, using tetrabutyltin as internal standard. The analytical method was validated by analyzing the certified reference material PACS-2 (marine sediment) (N = 3). The results obtained for the PACS-2 (TBT - 809 ± 10 ng Sn g^{-1}; DBT - 1002 ± 9 ng Sn g^{-1} and MBT - 580 ± 11 ng Sn g^{-1}) were in good agreement with the certified (TBT - 890 ± 105 ng Sn g^{-1} and DBT - 1047 ± 64 ng Sn g^{-1}) and reported (MBT - 600 ng Sn g^{-1}) values. The recoveries ranged from 79% to 110% for sediment and 81 to 112% for tissue samples. The relative standard deviations among analyzed samples were below 20%. The limits of detection (LD) for sediments and tissues analyses were 0.1, 0.2 and 0.2 ng Sn g^{-1} for TBT, DBT and MBT, respectively. All results were reported as average concentrations (±SD; n = 2) as ng Sn g^{-1} (dry weight).

2.4. Sediment characterization and butyltin degradation index (BDI)

The total organic carbon content (TOC %) was analyzed in all sediment samples as described by Sadzawka et al. (2005). Granulometry was determined according to Gray (1981) by sieving dried sediments and expressing the results as % of fine fraction (<63 μm).

In order to assess the degree of TBT degradation and to predict whether the sediment contamination is recent or old, the butyltin degradation index (BDI) was calculated as: butyltin degradation index (BDI) = [MBT] + [DBT] / [TBT] (Díez et al., 2002). Typically, values of BDI > 1 are normally associated to old inputs of TBT (Díez et al., 2006).

Table 1
Location (latitude and longitude), depth (m), distance from the coast (m) and intensity of naval traffic of the sites sampled in Northern Chile.

Site code	Site name	Latitude	Longitude	Depth (m)	Distance from the coast (m)	Naval traffic
S1	Huasco River	27°01′56.34″S	70°49′12.05″O	24	500	High
S2	Black Rocks Maritime Terminal	27°02′52.81″S	70°48′52.77″O	26	500	High
S3	Caldera harbor	27°03′52.71″S	70°49′40.35″O	23	380	High
S4	Punta Padrones harbor	27°03′04.17″S	70°50′13.52″O	28	500	High
S5	Calderilla bay	27°04′42.07″S	70°50′55.95″O	25	500	High
S6	Inglesa bay	27°06′22.01″S	70°51′28.06″O	22	500	High
S7	Morro of Caldera	27°09′51.19″S	70°58′13.13″O	24	500	Moderate
S8	Cisne bay	27°04′08.23″S	70°57′12.34″O	15	560	Moderate
S9	Puerto Viejo	27°19′51.41″S	70°56′24.98″O	14	560	Moderate
S10	Barranquilla	27°30′39.68″S	70°53′34.29″O	16	560	Moderate
S11	Salada bay	27°37′28.25″S	70°54′18.85″O	16	560	Low
S12	Chasco bay	27°40′41.58″S	70°59′14.98″O	18	560	Low

2.5. Total lipids and water content in biota tissues

Two aliquots of each pooled tissue sample (obtained from 3 individuals) were weighed (~15 to 20 g), dried with dry anhydrous sodium sulfate and Soxhlet extracted for 8 h using 200 mL of dichloromethane. The extracts were evaporated to dryness and weighed in a microbalance to determine the Total Lipid Content (TLC %). The water content was analyzed in triplicate by oven drying (60 °C) visceral and foot tissues until constant weight. Results were expressed as average ± SD of water content (N = 3).

2.6. Amount of foot tissue to reach the tolerable daily intake (TDI)

The amount of foot tissue to reach the tolerable daily intake (TDI) for each site (S1–S12) was calculated as recommended by the European Food Safety Authority (EFSA-Q-2003-110, 2004). Wet weight BT concentrations, including TBT and DBT concentrations measured in the edible part (foot tissues), and an average adult weighting 60 kg were used to calculate the amount of foot tissue to be ingested to reach the TDI of 100 ng Sn g^{-1}/kg/day.

2.7. Statistical analysis

The normality and homogeneity of data (biometric parameters, imposex indices, BTs concentrations, TOC % and TLC %) were verified using Shapiro–Wilk and Levene tests, respectively. The relationship between shell and penis lengths of *T. chocolata* in males and females was evaluated by a linear regression due to its predictive power. After, the shell lengths were compared among all sampled sites using Kruskal–Wallis test. Non-parametric Spearman correlation was used to investigate the relationship between TBT and its degradation products (DBT and MBT) in sediments, visceral coil tissues and foot tissues since this analysis reduces the influence of extreme values. Similarly, the relations between imposex indices (FPLI, $RPLI_{stand}$, VDSI and Sterility %) and TBT levels in different matrices were also investigated by Spearman correlation. The differences between BT levels and total lipid content in visceral coil and foot tissues from different sites were evaluated by Mann-Whitney *U* Test. Spearman correlation was used to investigate the relationship between BT levels and total lipid content in biota tissues. All statistical analyses were performed using Statistica® (version 12.0 (Statsoft)) with a significance level of 0.05.

3. Results and discussion

3.1. Environmental implications of TBT contamination

3.1.1. Biometrics and Imposex levels

Average male shell lengths (MSL) ranged from 73.3 ± 7.1 mm (S8) to 94.9 ± 22.6 mm (S4) (N = 252), while average females shell lengths (FSL) varied between 77.5 ± 10.7 mm (S9) and 95.3 ± 23.9 mm (S4) (N = 233) (Table 2). Shell lengths (SL), considering all sampling sites, were significantly different between males and females (Kruskal-Wallis, $p < 0.0001$). As already reported for *T. chocolata* from Peru (Castro and Fillmann, 2012), male penis lengths (MPL) were statistically correlated to MSL (linear regression, $p < 0.0001$, $r^2 = 0.89$). Since FPL can only be measured in imposexed females, which is more TBT dependent than SL dependent (Matthiessen and Gibbs, 1998), a low correlation (linear regression, $p < 0.0001$, $r^2 = 0.28$) was seen between FPL and FSL. Similar findings were previously observed in other gastropod species used as indicators of TBT pollution in coastal areas around the world (Petracco et al., 2014; Vasconcelos et al., 2011). In any cases, bias may arise from differences among organisms' size (Paz-Villarraga et al., 2015). Thus, imposex indices based on biometric parameters (such as RPLI) were normalized by the shell length ($RPLI_{stand}$), as recommended by Castro and Fillmann (2012).

Imposex incidence in *T. chocolata* was detected in eleven out of twelve studied sites (Table 2). Although this species has already been reported as a good sentinel for TBT pollution along coastal areas of Peru (Castro and Fillmann, 2012), this is the first record of imposex in *T. chocolata* from Chile. The highest imposex levels were observed in the stations S1 (I% = 100, $RPLI_{stand}$ = 77.3 and VDSI = 4.0), S2 (I% = 100, $RPLI_{stand}$ = 72.2 and VDSI = 3.1), S3 (I% = 100, $RPLI_{stand}$ = 118.3 and VDSI = 3.9), S4 (I% = 100, $RPLI_{stand}$ = 67.1 and VDSI = 4.8), S5 (I% = 100, $RPLI_{stand}$ = 66.4 and VDSI = 5.2) and S6 (I% = 100, $RPLI_{stand}$ = 65.5 and VDSI = 4.7), which were located nearby potential TBT sources, such as fishing harbors (S1, S2, S3 and S5), commercial harbor (S4), and marinas (S6) (Fig. 1). The cause-effect relationship between imposex and TBT is well established in the literature (Titley-O'Neal et al., 2011). Therefore, the high and widespread levels of imposex over the study area are probably result of the known sensitivity of muricid species to TBT exposure (see below), as have been reported by Castro et al. (2012d).

Based on the histological investigation (Fig. 2A) and on the yellow-cream color of gonads, all the analyzed females (five organisms per site) were considered as sexually mature (Mattos and Romero, 2016). However, reproductive alterations including sterile females (VDSI ≥ 5) presenting developed penis (Fig. 2B) with blockage of the vaginal opening caused by the vas deferens tissue proliferation (Fig. 2C and D) were seen in S1 (3.2%), S2 (7.7%), S3 (14.8%), S4 (67.9%), S5 (76.9%) and S6 (66.7%). Moreover, females presenting aborted egg mass within the capsule gland (VDSI = 6) (Fig. 2E) were found in S1 (3.2%), S2 (4.0%), S3 (7.7%), S4 (14.8%) and S5 (41.7%). These VDS stages indicate high TBT impacts on the local muricid populations. Records of TBT-mediated female sterility are rare for South American muricid gastropods (Castro and Fillmann, 2012; Fernandez et al., 2002). In species with fully benthic development, this alteration may lead to unbalanced sex distribution resulting from selective mortality of females due to the

Table 2
Biometric and imposex parameters for *T. chocolata* collected in Northern Chile. MSL - Male Shell Length, MPL - Male Penis Length, FSL - Female Shell Length, FPL - Female Penis Length, RPLI - Relative Penis Length Index, VDSI - Vas Deferens Sequence Index, $RPLI_{stand}$ - size standardized Relative Penis Length Index, I% - percentage of Imposex, Sterility % - percentage of sterile females and SD - standard deviation.

Site	N♂	MSL ± sd (mm)	MPL ± sd (mm)	N♀	FSL ± sd (mm)	FPL ± sd (mm)	RPLI	VDSI ± sd	$RPLI_{Stand}$	I%	Sterility (%)
S1	33	80.3 ± 13.1	14.3 ± 4.8	31	88.4 ± 8.4	12.2 ± 0.9	85.1	4.0 ± 0.7	77.3	100	3.2
S2	34	85.7 ± 10.5	16.5 ± 4.3	26	89.8 ± 9.3	12.5 ± 0.8	75.6	3.1 ± 0.8	72.2	100	7.7
S3	34	83.8 ± 10.6	9.9 ± 4.1	27	82.8 ± 10.1	11.6 ± 0.1.5	116.9	3.9 ± 1.2	118.3	100	14.8
S4	32	94.9 ± 22.6	19.2 ± 6.2	28	95.3 ± 23.9	12.9 ± 2.5	67.3	4.8 ± 0.7	67.1	100	67.9
S5	17	91.3 ± 9.9	18.7 ± 4.2	13	93.9 ± 7.8	12.8 ± 0.7	68.3	5.2 ± 0.8	66.4	100	76.9
S6	15	91.5 ± 11.5	18.9 ± 4.7	15	88.3 ± 8.4	12.0 ± 1.8	63.2	4.7 ± 0.5	65.5	100	66.7
S7	15	79.7 ± 7.8	12.9 ± 1.7	15	80.6 ± 8.1	0.1 ± 0.1	0.5	0.4 ± 0.5	0.5	40	0.0
S8	16	73.3 ± 7.1	11.2 ± 0.9	14	82.1 ± 9.2	<0.1 ± 0.0	0.1	1.0 ± 0.0	0.1	100	0.0
S9	12	81.5 ± 7.4	13.1 ± 1.7	18	77.5 ± 10.7	0.1 ± 0.2	0.9	1.5 ± 0.5	1.0	100	0.0
S10	13	79.6 ± 9.2	11.5 ± 2.2	17	77.7 ± 7.5	<0.1 ± 0.0	0.2	1.2 ± 0.4	0.2	100	0.0
S11	15	79.2 ± 5.3	12.6 ± 0.8	15	79.1 ± 6.9	0.1 ± 0.1	0.5	0.9 ± 0.4	0.5	87	0.0
S12	16	79.1 ± 3.9	12.5 ± 0.5	14	77.6 ± 9.2	0.0 ± 0.0	0.0	0.0 ± 0.0	0.00	0	0.0

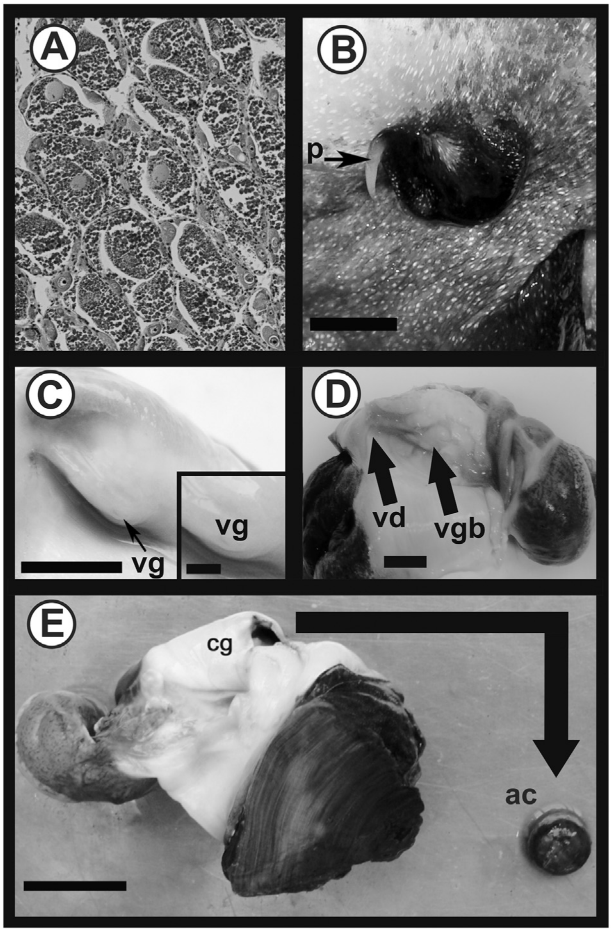

Fig. 2. Histological and morphological alterations in imposexed *T. chocolata* from the most contaminated sites of Northern Chile. (A) transverse histological section of ovary (400 x), (B) female penis detail, (C) external view of vagina opening (VDS ≤ 4), (D) external view of vaginal blockage by the vas deferens tissue proliferation (VDS ≥ 5) and (E) external view of the capsule gland dissected to extract aborted capsules (VDS = 6). p: penis, vg: vagina, vgb: vagina blocked, vd: vas deferens, cg: capsule gland, ac: aborted capsules. Scale bars = 10 mm.

blockage of vaginal opening. This phenomenon was described by Gibbs and Bryan (1986), Nicholson et al. (1998) and Blackmore (2000) for other regions of the world. However, considering that *T. chocolata* presents a planktotrophic teleplanic larvae, which after approximately one month in capsules is released to a planktonic life, the most affected populations may recruit larvae from unpolluted sites (Cantillanez and Avendaño, 2013) as already reported for *Stramonita haemastoma* populations from Azores, Portugal (Spence et al., 1990). Anyhow, the infertility rates observed in gastropods from S4 (Punta Padrones), S5 (Calderilla) and S6 (Inglesa bay) were exceptionally high, even in comparison with relatively high levels recently detected in other South American countries or any recent worldwide data available.

Imposex incidence was also seen at sites S7, S8, S9, S10 and S11 (40–100%). Since these areas are located on exposed beaches, RPLI (0.2–1.0) and VDSI (0.4–1.5) levels were relatively low due, probably, to lower TBT inputs from the local fishing harbors in combination to higher local hydrodynamics. Further south, with no evident maritime activities, no imposex were observed at site S12. Thus, the spatial distribution of imposex in Caldera region showed a direct relation with maritime activities since the high levels were observed in the stations S1 to S6 and lower imposex indices were detected in sites under less (S7, S8, S9 and S10) or no (S11 and S12) influence of naval traffic. However, no clear imposex gradient was observed due probably to the presence of multiple TBT sources associated to the Peru-Chile Counter current, which flows in Caldera region predominantly from north to south throughout the year (Thiel et al., 2007).

Thus, the contamination pattern detected in the present study confirmed ship/boat traffic zones as hotspots of imposex incidence probably caused by TBT contamination (Titley-O'Neal et al., 2011). Thus, differing from global trends, South American coastal shores still present relevant impacts related to the current use of TBT-based antifouling paints (Castro et al., 2012a, 2012b, 2012c, 2012d). Moreover, some of the less impacted sites S7 (I% = 40, $RPLI_{stand}$ = 0.5 and VDSI = 0.4), S8 (I% = 100, $RPLI_{stand}$ = 0.1 and VDSI = 1.0) and S9 (I% = 100, $RPLI_{stand}$ = 1.0 and VDSI = 1.5) are located inside the marine protected area “Isla Grande Atacama”. This conservation unit was created in 2004, including water column, sea bed and adjacent coastal zones, to protect the biodiversity of a portion of the Humboldt current ecosystem (Gaymer et al., 2008). Therefore, the occurrence of imposex affecting species within this protected coastal system points out to the need of more restrictive and specific management regulations. Similar situation was recently seen for Galapagos, where imposex incidence was detected in four muricid species (Grimón et al., 2016).

3.1.2. Butyltin levels in surface sediments

Butyltin residues were detected in all sediment samples collected along Caldera coastal zones (Table 3). TBT concentrations ranged from 90.4 ± 18.6 to 193.9 ± 32.1 ng Sn g^{-1} for sites S7 to S12, and from 363.8 ± 19.2 to 602.3 ± 14.5 ng Sn g^{-1} for sites S1 to S6. In fact, sites S4 (574 ng Sn g^{-1}), S5 (602 ng Sn g^{-1}) and S6 (554 ng Sn g^{-1}) presented one of the highest levels recently detected worldwide for sediments (Castro et al., 2012b; Titley-O'Neal et al., 2011). The sediment fine fraction (<63 μm) has presented a large variation among the studied sites (23.5 to 88.1% of fines) showing non-significant Spearman correlations with % TOC and butyltin levels ($p > 0.05$). On the other hand, the total organic carbon contents (TOC %) varied from 0.2 to 1.83% in sediments and were well correlated to TBT ($r = 0.97$), DBT ($r = 0.91$) and MBT ($r = 0.97$) levels. Moderate to strong relationships between these parameters have often been observed and are probable linked to inherent physicochemical properties of the BT compounds (Batista et al., 2016; Pinochet et al., 2009). However, some studies reported that different composition of organic matter, relative adsorbability onto inorganic particles, and the existence of biological activity in the sediment layers can bias those relationships (Artifon et al., 2016). In addition, the magnitude of local TBT sources also influence the correlations with TOC and sediment granulometry.

According to Waite et al. (1991), levels between 4 and 18, 22–73, and 109–365 ng Sn g^{-1} indicate sites under low, moderate, and high contamination of TBT, respectively. In addition, sites presenting TBT levels higher than 365 ng Sn g^{-1} are, in general, contaminated by antifouling paint particles. Thus, the sites S1, S3, S4, S5 and S6 may be

Table 3
Total organic carbon (TOC), granulometry (<63 μm - fine %) average (±sd) levels of tributyltin (TBT), dibutyltin (DBT) and monobutyltin (MBT), sum of butyltins (ΣBTs) and butyltin degradation indices (BDI) in sediment samples from Northern Chile. sd - standard deviation. N = 2.

Site	TOC %	Fine %	TBT ± sd	DBT ± sd (ng Sn g^{-1})	MBT ± sd	ΣBTs	BDI
S1	0.58	23.5	479.5 ± 23.6	285.5 ± 12.4	171.0 ± 4.3	936.1	1.0
S2	0.30	57.2	363.8 ± 19.2	208.8 ± 22.5	131.2 ± 15.0	703.7	0.9
S3	0.33	49.8	458.0 ± 19.3	272.1 ± 4.5	164.7 ± 6.2	894.8	1.0
S4	0.68	64.3	574.0 ± 18.0	343.1 ± 12.1	198.6 ± 6.1	1115.6	0.9
S5	1.83	32.0	602.3 ± 14.5	368.7 ± 5.5	203.7 ± 4.0	1174.6	1.0
S6	0.64	43.4	554.8 ± 10.2	328.2 ± 11.3	191.3 ± 7.5	1074.3	0.9
S7	0.22	77.7	100.5 ± 22.1	11.3 ± 20.1	5.5 ± 10.5	117.4	0.2
S8	0.23	84.4	124.7 ± 17.1	64.8 ± 13.3	39.7 ± 22.4	229.2	0.8
S9	0.24	77.1	193.9 ± 32.1	108.0 ± 7.2	68.4 ± 3.8	370.3	0.9
S10	0.24	74.4	157.8 ± 7.4	<0.2	49.1 ± 24.8	206.9	0.3
S11	0.24	88.1	105.5 ± 22.1	14.4 ± 21.0	8.8 ± 18.2	128.7	0.2
S12	0.20	66.8	90.4 ± 18.6	4.9 ± 13.7	1.3 ± 10.4	96.6	0.1

classified as more than highly contaminated, while S2, S8, S9 and S10 as highly contaminated and S7, S11 and S12 as moderately to highly contaminated by TBT. Regarding DBT and MBT, the concentrations varied between <0.2 and 368.7 ng Sn g^{-1} and 1.3 to 203.7 ng Sn g^{-1}, respectively (Table 3). Significant spearman non-parametric correlations were detected between TBT and DBT ($p < 0.05$ and $r = 0.93$) and MBT ($p < 0.05$ and $r = 0.99$), indicating that those dealkylated products are originated from the same sources. In fact, DBT and MBT inputs not related to antifouling paints are so far unknown in South American coastal shore (Castro et al., 2012a, 2012b, 2012c, 2012d).

The high sediment contamination detected in "Isla Grande Atacama" (sites S7, S8 and S9) indicates, as pointed out by the imposex indexes, a limited effectiveness of this conservation unit, which was designed to protect representative ecosystems, as well as geopolitical and social interests (Gaymer et al., 2008). These findings denote a partial effectiveness of the AFS convention and/or an important lack of local restrictions against TBT-based antifouling paints. TBT environmental levels and impacts (imposex) have been recently reported for several marine protected areas from South America, such as Paraty Island (Brazil) (Borges et al., 2013), Mochima National Park (Venezuela) (Paz-Villarraga et al., 2015) and Argentine Patagonian coast (Commendatore et al., 2015). Thus, due to the above-mentioned impacts, regulatory actions toward environmental protection of local populations should be pursued. Similar actions included by European Union in the Water Framework Directives have helped to run environmental monitoring programs (Laranjeiro et al., 2015).

TBT levels in sediments presented strong and significant Spearman correlations ($p < 0.05$) with imposex incidence, including FPLI ($r = 0.88$), $RPLI_{stand}$ ($r = 0.73$), VDSI ($r = 0.99$) and sterility % ($r = 0.91$), corroborating to the hypothesis that imposex incidence is directly related to the TBT environmental levels. Similar results have been detected by several studies pointing out the cause-effect relationship between TBT environmental levels and imposex (Cacciatore et al., 2016; Castro et al., 2012a; Castro et al., 2012b; Galante-Oliveira et al., 2010). Moreover, the relatively high levels of TBT in the sites S1 to S6 in combination to the low BDI values (0.9 to 1.0) (Table 3), suggest the occurrence of fresh local inputs (Díez et al., 2006). However, the even lower BDI levels (0.1 to 0.9) associated to relatively lower levels of BTs in the sites S7 to S12, indicate that TBT residues were probably brought over by coastal currents from more contaminated sites (S1 to S6). Therefore, considering that the naval traffic in the studied area is predominantly performed by small boats, these recent inputs of TBT and the high imposex incidence are in accordance to recent studies which have pointed out marinas and fishing harbors as sources of fresh TBT in South American coastal zones (Batista et al., 2016; Borges et al., 2013; Grimón et al., 2016; Paz-Villarraga et al., 2015). Thus, despite the AFS convention, the BT sediment levels confirmed that contamination is still a serious environmental issue along the Chilean coastal shore.

3.1.3. Butyltin body burdens

Tributyltin concentrations ranged from 16.1 ± 0.3 (S12) to 603.6 ± 10.9 (S5)·ng·Sn·g^{-1} in visceral coil tissues and from 14.7 ± 4.4 (S12) to 190.5 ± 52.7 (S5)·ng·Sn·g^{-1} in foot tissues of *T. chocolata*. DBT and MBT were also detected in all biological samples, although normally at lower levels. Levels ranged from 14.9 ± 0.0 (S12) to 305.9 ± 8.4 (S5)·ng·Sn·g^{-1} of DBT and from 12.4 ± 0.4 (S12) to 171.7 ± 8.1 (S5)·ng·Sn·g^{-1} of MBT in visceral coil tissues, being even lower in foot tissue (from 4.4 ± 3.8 (S11) to 22.4 ± 9.2 (S5)·ng·Sn·g^{-1} of DBT and from 4.0 ± 2.0 (S12) to 12.1 ± 12.0 (S5)·ng·Sn·g^{-1} of MBT) (Table 4). As seen for sediments, BTs also seem to have the same sources since TBT, DBT and MBT correlate to each other in both visceral (Spearman, $p < 0.05$ and $r = 0.99$) and foot (Spearman, $p < 0.05$ and $r = 0.97$) tissues. Likewise, imposex parameters were positively correlated with TBT concentrations measured in visceral coil tissues [Spearman, FPLI ($r = 0.86$), $RPLI_{stand}$ ($r = 0.71$), VDSI ($r = 0.99$) and Sterility % ($r = 0.91$)] and foot tissues [Spearman, FPLI ($r = 0.88$), $RPLI_{stand}$ ($r = 0.73$), VDSI ($r = 0.99$) and Sterility % ($r = 0.91$)] (Fig. 3). The same pattern has been often reported by several studies evaluating cause-effect relationship between levels of TBT and imposex incidence (Castro et al., 2012c; Lahbib et al., 2011; Paz-Villarraga et al., 2015). *T. chocolata* is a subtidal carnivorous muricid that lives on hard substrates feeding mainly on mussels and barnacles, which probably accumulate BTs from the water column. Its high capacity to bioaccumulate BTs may reflect contamination predominantly from the environment (Bryan et al., 1993; Castro and Fillmann, 2012).

Total lipid content (TLC %) varied from 8.1 ± 0.1 (S12) to 17.3 ± 0.3% (S5) in visceral coils and 1.3 ± 0.3 (S11) and 5.4 ± 0.5% (S5) in foot tissue (Table 4). Levels of TLC ($p < 0.001$) and, consequently, TBT ($p < 0.05$), DBT ($p < 0.001$) and MBT ($p < 0.001$) were significantly higher (Mann-Whitney *U* Tests) in visceral coils than in foot tissues. In fact, gastropods accumulate BT preferentially in tissues with higher lipid contents, as pointed out by studies using muricids (Horiguchi et al., 2012) and volutids (Del Brio et al., 2016) as sentinels of TBT pollution. The lipophilicity of butyltin compounds explains the preferential partitioning (Hoch, 2001). Taking this into account, visceral coils seem to be the best matrix to assess and monitor BT contamination in future environmental studies using *T. chocolata*. Similar pattern has also been observed for other gastropod species worldwide (Titley-O'Neal et al., 2011).

The results for body burden followed the same pattern seen for sediments and imposex incidence, where stations S1 to S6 presented the highest levels, followed by sites S8, S9 and S10, while S7, S11 and S12 showed the lowest levels of contamination. These sites S1 to S6 are

Table 4
Average (±sd) levels of butyltin and total lipid content (TLC) in visceral coil and foot tissues of *T. chocolata* from Northern Chile. Amount of foot tissue to reach the TDI Tolerable daily intake (TDI) by sample for foot tissues. TBT - Tributyltin, DBT - Dibutyltin, MBT - Monobutyltin, sd - standard deviation. N = 2.

Site	Visceral coil tissue				Foot tissue				Amount of foot tissue to reach TDI (g)
	TBT ± sd	DBT ± sd (ng Sn g^{-1})	MBT ± sd	TLC (%)	TBT ± sd	DBT ± sd (ng Sn g^{-1})	MBT ± sd	TLC (%)	
S1	472.3 ± 1.7	238.9 ± 6.9	131.9 ± 4.3	12.2 ± 0.4	144.4 ± 8.7	17.7 ± 5.9	9.1 ± 8.8	3.5 ± 0.4	118
S2	345.6 ± 1.8	179.8 ± 0.8	95.0 ± 0.5	11.6 ± 0.4	97.2 ± 19.4	13.5 ± 3.1	7.1 ± 10.1	3.0 ± 0.1	173
S3	428.2 ± 4.7	209.7 ± 2.8	124.3 ± 0.9	10.1 ± 0.2	138.4 ± 71.4	16.4 ± 2.6	8.5 ± 2.6	4.2 ± 0.1	123
S4	552.7 ± 14.4	288.6 ± 5.4	157.5 ± 1.7	15.4 ± 0.5	178.1 ± 11.0	21.0 ± 10.1	11.3 ± 27.5	5.2 ± 0.1	96
S5	603.6 ± 10.9	305.9 ± 8.4	171.7 ± 8.1	17.3 ± 0.3	190.5 ± 52.7	22.4 ± 9.2	12.1 ± 12.0	5.4 ± 0.5	90
S6	535.7 ± 8.1	274.7 ± 11.3	150.5 ± 0.4	13.4 ± 0.2	168.7 ± 10.8	19.5 ± 8.4	10.8 ± 13.4	4.4 ± 0.4	102
S7	19.0 ± 6.6	17.4 ± 11.7	16.7 ± 5.8	8.4 ± 0.2	18.0 ± 7.3	4.9 ± 0.5	4.4 ± 0.2	1.3 ± 0.3	836
S8	98.5 ± 5.4	38.9 ± 6.9	27.6 ± 7.7	9.3 ± 0.4	35.3 ± 11.9	5.2 ± 4.1	4.7 ± 14.3	2.5 ± 0.4	473
S9	144.5 ± 12.9	85.9 ± 3.1	44.6 ± 14.7	9.0 ± 0.2	55.6 ± 1.3	11.6 ± 6.0	5.4 ± 5.1	2.7 ± 0.1	285
S10	154.7 ± 12.9	58.0 ± 8.2	33.1 ± 0.9	9.9 ± 0.6	44.3 ± 7.5	10.3 ± 1.3	4.9 ± 8.4	2.5 ± 0.1	351
S11	29.0 ± 2.7	23.5 ± 2.9	21.8 ± 23.6	8.5 ± 0.8	23.7 ± 4.4	4.4 ± 3.8	4.6 ± 11.6	1.3 ± 0.3	683
S12	16.1 ± 0.3	14.9 ± 0.0	12.0 ± 0.4	8.1 ± 0.1	14.7 ± 4.4	5.1 ± 0.6	4.0 ± 2.0	1.5 ± 0.3	968

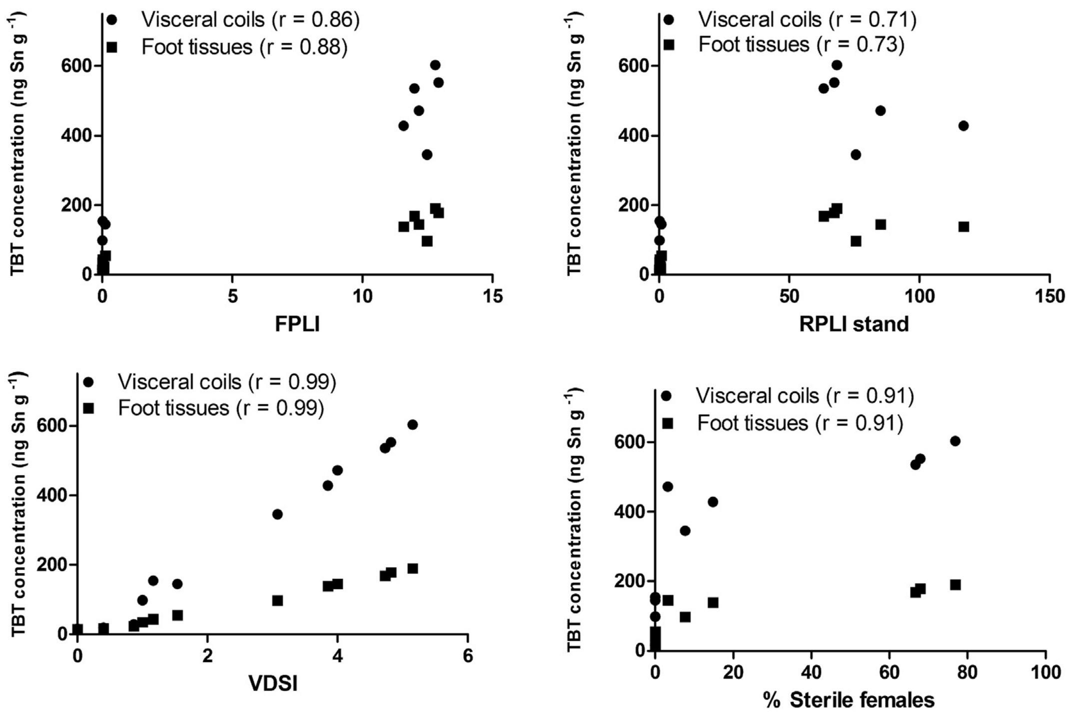

Fig. 3. Spearman correlations between TBT concentrations (in visceral coils and foot tissues) and imposex parameters (FPLI, $RPLI_{Stand}$, VDSI and % of sterile females).

located in a system of bays where small urban concentrations, fishing harbors and touristic activities represent the main anthropogenic impacts (Valdés and Castillo, 2014). In this concern, the high contamination levels detected in these bays (especially in Calderilla-S5) may be influenced by limited hydrodynamics due to their relative confinement. The long water residence time within the bays hinders the dispersion and/or dilution of the residues released. In addition, sheltered coastal areas are also often more used for nautical activities (Kim et al., 2015), making them more vulnerable to contamination by antifouling biocides (Castro et al., 2012b).

The lower levels of BT in environmental matrices sampled far from the main potential sources (S8, S9, S10, S11 and S12) may be related to other less significant maritime sources and to the transport of suspended matter from contaminated areas by the coastal currents associated to dilution produced by persistent upwelling system in the area (Thiel et al., 2007; Troncoso et al., 2003). These water fluxes may contribute to lower the contamination levels, but maintaining them more homogeneously distributed on these sites, as seen for imposex occurrence. The transport of TBT by suspended particles has recently been reported for other South American regions (Santos et al., 2016). On the other hand, no signs of imposex were observed in the organisms from Chasco bay (S12), despite the detected TBT levels in visceral (16.1 ± 0.3 ng Sn g^{-1}) and foot tissues (14.7 ± 4.4 ng Sn g^{-1}), and also sediment (90.4 ± 18.6 ng Sn g− 1). This may suggest that TBT is not fully bioavailable or the environmental levels in site S12 were not be enough to elicit imposex in *T. chocolata*.

3.2. The potential risk for consumers of TBT and DBT levels in T. chocolata

Due to the differential toxicity of the most frequently organotin compounds detected in seafood, the European Food Safety Authority (EFSA) Scientific Panel on Contaminants has established a tolerable daily intake (TDI) of 100 ng kg^{-1} b.w. (when expressed as Sn) which included TBT, DBT, TPT (triphenyltin) and DOT (dioctyltin) (EFSA-Q-2003-110, 2004). Despite TPT and DOT were not analyzed in the present study, a risk related to the consumption of *T. chocolata* from the most contaminated stations (S1, S3, S4, S5 and S6) was detected. A daily ingestion of as low as 90–120 g of foot tissue from S1, S3, S4, S5 and S6, assuming an average adult weighting 60 kg and taking into consideration only the contamination levels of TBT and DBT, may exceed the EFSA recommended TDI (Table 4). Those concentrations can be easily ingested in a single meal, considering that one foot of "locate" weighs approximately 20 g and, in northern Chile, a popular dish (butter fried) has seven or more feet. Assuming that there is no significant degradation of TBT during common household cooking procedures (microwave, frying pan, steamed and boiled) (Willemsen et al., 2004), BT levels detected in foot tissues may be completely available for seafood consumers.

Other three studies carried out in South America have also indicated (low to moderate) risks related to TBT intake through seafood (Artifon et al., 2016; Del Brio et al., 2016; Fernandez et al., 2005). In addition, TBT levels as high as 503 ng Sn g^{-1} were recently detected in fish muscle from the Polish coast of the Baltic Sea, exceeding the levels recommended by the Baltic Marine Environment Protection Commission, for witch, the good environmental status boundary for TBT in seafood is 31.2 ng Sn g^{-1}(Filipkowska et al., 2016). Thus, *T. chocolata* foots from S1, S2, S3, S4, S5, S6, S8, S9 and S10 exceeded also the limits imposed by the Baltic legislation According to Yi et al. (2012), dietary consumption of contaminated seafood is the main pathway of butyltin intake for humans. Thus, it worst highlighting that organotin contamination still poses a risk to some populations that regularly feed on seafood (Rosenberg, 2013).

Chile, together with China and Norway, is one of the largest global seafood net exporters (Smith et al., 2010). *T. chocolata* was exported by Chile until 1998 when the wild-stocks were exhausted (Leiva and Castilla, 2002). Currently, the "Locate" is collected by artisanal fisheries to supply the local market only, providing livelihood for fishing communities which regularly consume this resource (Avendaño et al., 1998). However, these sites in the Caldera region detected as highly contaminated by TBT are also fishing spots where several other edible species (gastropods, mussels, oysters, squids, crabs, sea urchins, ascidians and fishes) are caught to supply domestic and international markets (SERNAPESCA, 2015). The filter-feeder *Argopecten purpuratus* (scallop), for instance, is extensively farmed in the Calderilla (S5) and Inglesa (S6) bays. Although the present study has not assessed BT levels in another seafood species, it is reasonable to assume that local biota is similarly contaminated. Thus, due to the additional consumption of fish, mussels and other marine organisms from these highly-contaminated areas of Caldera, the human daily exposure may certainly be even higher than calculated based only on the consumption of *T. chocolata*. An analogous situation was seen for Lagoon of Venice (Italy) where high levels of TBT were detected in the edible tissues of six coexisting species (clams, mussels, littoral crabs, small fishes and European eels), causing the extrapolation of the tolerable daily intake for local population (Bortoli et al., 2003). Considering that several harmful health effects might be associated to BT exposure, such as a wide range of immunosuppressive, endocrine, neurotoxic, dermal, cardiovascular, respiratory, gastrointestinal, carcinogenic and reproductive effects (Antizar-Ladislao, 2008; Hiromori et al., 2016; Nakanishi, 2007; Nakanishi et al., 2005), the consumption of *T. chocolata* (together with other seafood) from the most contaminated sites (S1, S3, S4, S5 and S6) can be considered as a real health risk to humans.

4. Conclusion

Exceptionally high TBT impacts caused by fresh inputs were observed along the Caldera coastal zones, mainly on areas under the influence of small to medium boats (marinas and fishing harbors). The BT levels detected in surface sediments classifies nine out of twelve studied sites as highly (109–365 ng Sn g^{-1}) or more than highly (>365 ng Sn g^{-1}) contaminated. In addition, widespread imposex occurrence (11 out of 12 sites) associated to high incidence of sterility (notably in S4, S5 and S6) indicates that environmental levels were high enough to cause deleterious effects on the local biota. In this concern is reasonable to assume that other marine species may be affected by the local TBT contamination. Moreover, imposex and BT concentrations found in "Isla Grande Atacama" (sites S7, S8 and S9) indicates a limited effectiveness of this marine protected area designed to protect to protect the biodiversity of a portion of the Humboldt current ecosystem. Therefore, considering the clear cause-effect relationship found between TBT (accumulated in foot tissues) and imposex levels, *T. chocolata* may be used as an easy-application sentinel tool for TBT contaminated areas.

A daily ingestion of only about 100 g of "locate" foot tissue (4 to 8 organisms) caught from the most BT contaminated sites (S1, S3, S4, S5 and S6) may exceed the TDI recommended by the European Food Safety Authority. Moreover, the same areas identified as heavily contaminated are also used for fishing (artisanal and industrial) and farming of other marine resources, which certainly also contribute to increase the daily intake of BT. Thus, the health of local population regularly consuming seafood from Caldera can be considered as under a real risk. The environmental and human health implications detected in Caldera confirm TBT contamination as an environmental issue far from being solved within South American coastal areas. Thus, considering the high environment impacts and the potential human health risk associated to seafood consumption, regulatory actions toward environmental protection and food safety of local populations should be urgently implemented.

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