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Organotin compounds in *Brachidontes rodriguezii* mussels from the Bahía Blanca Estuary, Argentina

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ABSTRACT

Levels of tributyltin and its breakdown compounds, including the first record of monobutyltin (MBT) in history for Latin America, were determined in native mussels (*Brachidontes rodriguezii*) by means of CG-MS, after extraction/derivatization assisted by ultrasound. The samples were collected in 2013 in Bahía Blanca Estuary (Argentina) at 6 sites, which reflect different levels of maritime activities.

Total butyltins (TBts = TBT+ DBT+ MBT) levels ranged from 19.64 to 180.57 ng Sn g^{-1} dry weight. According to the Oslo-Paris commission, the results indicated that 73.9% of mussels could be under biological effects risks associated with TBT pollution. In accordance with the calculated bioaccumulation factors, approximately 56% of samples appeared to have accumulated TBT through the sediments.

All sampling sites were shown to be impacted by organotin compounds (OTCs) showing variable levels through seasons, which could be related with the variation of the water temperature.

Degradation index analyses suggested aged inputs of TBT possibly under a general degradation process at the area of study. In addition, the occurrence of DBT and MBT could not be uniquely attributed to the degradation pathway of the TBT; in fact, results outlined the possible contribution of some punctual and diffuse sources at the area such as proximity to plastic industries, industrial effluents, sewage outlets and domestic wastewaters.

1. Introduction

Organotin compounds (OTCs) have been used worldwide due to their large number of industrial applications (Hoch, 2001). In particular, dibutyltin (DBT) and monobutyltin (MBT) have been extensively used since 1940 mainly as stabilizers in the synthesis of polyvinyl chloride (PVC). Moreover, from the mid-70s, tributyltin (TBT) has been widely used in antifouling paints due to its action as a biocide. These coatings were applied as a protection system to combat the formation and establishment of biological material, such as certain bacteria, fungi, crustaceans and algae on any surface exposed to water.

The major pathway of entry of TBT into the aquatic environment is through direct leaching from antifouling paints into water, particularly in areas with frequent boat activities (Wang et al., 2008). The presence of TBT has generated a serious problem for marine ecosystems because of its persistence, ecotoxic action and its ability to be transferred along the trophic chains. Once in the environment, successive dealkylation reactions produce DBT and MBT, via ultraviolet irradiation (photolysis), chemical cleavage or biodegradation produced by certain microorganisms (bacteria, algae and fungi) (Ayanda et al., 2012; Hoch, 2001).

OTCs, especially TBT, are highly toxic towards marine organisms. Even at very low concentrations –in the order of 1 ng L^{-1}–they can cause changes in the endocrine system of marine organisms (Rainbow, 1995; Zhou et al., 2003). These changes include the development of male sexual characteristics in female gastropods, known as *imposex* (Fernandez et al., 2005), reduction of growth (Salazar and Salazar, 1991), larval mortality (Tanabe et al., 2000; Zhou et al., 2003), shell thickening (Alzieu et al., 1986), low progesterone levels and delay in sexual maturation (Siah et al., 2003). As a consequence, since the mid-80s many countries worldwide have banned the application of TBT-based paints to small vessels (< 25 m length). More recently, the European Commission Parliament included OTCs as priority hazardous substances subjected to cessation of emissions, discharges and losses into water (Amending Water Policy Directive 2000/60/EC) (Antizar-Ladislao, 2008; Castro and Fillmann, 2012). In agreement, the

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International Maritime Organization (IMO,1999) called for a global prohibition of the application of antifouling systems containing OTCs on ships by January 2003 and a complete prohibition by January 2008 (Rudel, 2003). In Argentina, the use of TBT-based antifouling paints in all types of vessels was banned in 1998 (Ohji et al., 2007). Despite this fact, even after the implementation of national and international regulatory actions, TBT levels are still present in the marine environment. This is particularly relevant in estuaries with restricted circulation, which are under the influence of harbors, marinas, and shipyards (Castro and Fillmann, 2012; Sousa et al., 2009b; Yamamoto et al., 2009). This is the case of the Bahia Blanca Estuary, a highly anthropized area at the South Atlantic that have two commercial harbors, cities (more than 350,000 inhabitants) and one of the top industrial parks in South America. Moreover, several studies have already reported OTCs contamination for South America's coasts, including data from sediments (Delucchi et al., 2007; Laitano et al., 2015; Mattos et al., 2017) and marine mammals (Artifon et al., 2016; Batista et al., 2016; Del Brio et al., 2016; Sant'Anna et al., 2014), but there is a scarcity of information regarding marine bivalves. Bivalves in general –and mussels in particular–are considered as a reliable environmental indicator in the marine environment pollution, due to their low enzymatic capacity, which turns them able to concentrate and accumulate pollutants from the environment without a significant metabolization (Goldberg, 1975; Laughlin et al., 1986; Lee, 1991). In addition, they are sessile, easy to collect, fairly tolerant to chemical contamination and many of them can be found in areas where more sensitive species may be absent (O'Connor, 2002). For these reasons mussels worldwide have been used as sentinel organisms for monitoring marine pollutants. For Argentina, the mytilid *Brachidontes rodriguezii* (d'Orbigny, 1846) is the dominant organism on intertidal rocky substrata in warm-temperate shores (Adami et al., 2004). It distributes from Uruguay to north Patagonia along the Argentinean coast (Adami et al., 2008; Torroglosa and Giménez, 2015) and then, it was the selected biomonitor for OTCs in this study.

Considering all the above-mentioned, the main objectives of this study were: (i) to assess, for the first time, the levels and distribution of TBT and its degradation products -DBT and MBT- in native mussels collected from the Bahia Blanca Estuary (South Atlantic, Argentina), an intensive anthropized area; (ii) evaluate their seasonal and spatial distribution; (iii) contrast the obtained information against worldwide previously reported values.

2. Materials and methods

2.1. Study area

The Bahía Blanca estuary is located in the south-west coast of Buenos Aires Province within Argentina (38°45′ − 39°40′ S and 61°45′ − 62°20′ W) (Fig. 1). The estuary could be described as a series of major NW-SE tidal channels which separate extensive coastal wetlands and islands (Piccolo and Perillo, 1990). The total surface is close to 2300 km^2 including ~410 km^2 of islands, ~1150 km^2 of intertidal sector and ~740 km^2 of subtidal zone (Piccolo et al., 2008). It is classified as mesotidal (Hayes, 1979); semidiurnal tides wave predominate and the mean tidal amplitude varies between 3.5 and 2.2 m at the head and mouth of the estuary, respectively. The water surface is ~400 km^2 during the low tide, while this area rises to nearly 1300 km^2 in the high tide (Perillo and Piccolo, 1991). The inner zone of the Bahía Blanca Estuary has been regarded as turbid with fine suspended sediments (i.e., silt and clay) (Perillo et al., 2007) and eutrophic environment (Guinder et al., 2009; Marcovecchio et al., 2008). The phytoplankton has shown a marked recurrent pattern with a single winter-early spring diatom bloom (Gayoso, 1998, 1999; Guinder et al., 2015; Popovich et al., 2008; Popovich and Marcovecchio, 2008).

At the northern boundaries of the estuary various ports (Cuatreros, Ingeniero White, Galván, Rosales and Belgrano port), cities (Bahía Blanca, Punta Alta and General Cerri) and industries (oil, chemical, and plastic factories) are located. This includes mostly refineries, petrochemical industries, plastic manufacturing plants and manufactures of fertilizer. Also textile plants, tanneries, silos and cereal mills, cold storage facilities and related industries are located within this area. They discharge their processing residues into the streams or directly into the estuary for which has been estimated to enter about 100 m^3/day of effluents without any treatment (Perillo et al., 2001; Piccolo and Perillo, 1990). In the same way, the domestic discharges of the cities are discharged into the estuary. In addition, the estuary is extensively used by fishing boats, oil tankers and cargo vessels, and therefore requires regular dredging in the Main Navigation Channel. This undoubtedly affects the resuspension, transport and redistribution of sediment, which has a significant impact in the pollutant transport.

2.2. Sample collection

The present study was carried out within the Bahía Blanca estuary, along the Main Navigation Channel. Six sampling stations were selected from Rosales Harbor (S1) to Villarino Viejo (S6), which were affected by different intensities of maritime traffic activities, fishing areas, industrial discharges and other activities. The distribution of sampling stations is shown in Fig. 1.

The selected sampling sites, from the outer to the inner zone within the estuary, were:

- Rosales Harbor (S1): This includes oil tankers and port service vessels. This site is in the proximity of the Belgrano Harbor, the most important military harbor of Argentina, where the hulls of many commercial and military ships are continuously repaired and painted.
- Ingeniero White Port (S2): one of the major commercial and general freight ports of Argentina with a fleet of fishing and recreational boats, too. This site is located in the proximity of the "Luis Piedra Buena" thermoelectric facilities, which supplies electric power to the area.
- Galvan Harbor (S3 and S4): heavily industrialized core of the estuary. While S3 is situated in an abandoned dock, S4 is located in the proximity of a petroleum and oil derivatives loading buoy.
- Cuatreros Harbor (S5): fishing and recreational port.
- Villarino Viejo (S6): located in the vicinity of rural lands with relatively low anthropogenic impact.

Sampling was performed during 2013, at low tide and every three months. Native mussels (*Brachidontes rodriguezii*) were collected on board of the *IADO IV* research vessel from natural banks, dock columns, platforms, surface sediments or in surface water (0–1 m), according to their availability. An average of 60 mussels from each site was used to make a composite sample. In each individual site, an attempt to collect samples with similar sizes was made. In general, the mussel lengths varied between 28 and 44 mm. Mussels from each sampling station were immediately ice-cooled and transferred to the laboratory. In the laboratory, mussels were extensively washed with distillated water and finally stored at − 20 °C until extraction. These storage conditions assured a high stability of OTCs, up to 44 months (Gomez-Ariza et al., 2000). To complete the pre-treatment of the samples, soft tissues were homogenized and lyophilized during 48 h and then smashed in a mortar.

2.3. Reagents and standards

MBT as Butyltintrichloride (97%), DBT as Dibutyltin dichloride (97%), TBT as Tributyltin chloride (96%) and TeBT as Tetrabutyltin (93%) used as internal standard, were purchased at Sigma-Aldrich; TPrT as Tripropyltin chloride (> 99%) used as recovery control standard was obtained from LGC Promochem. Standard stock solutions of

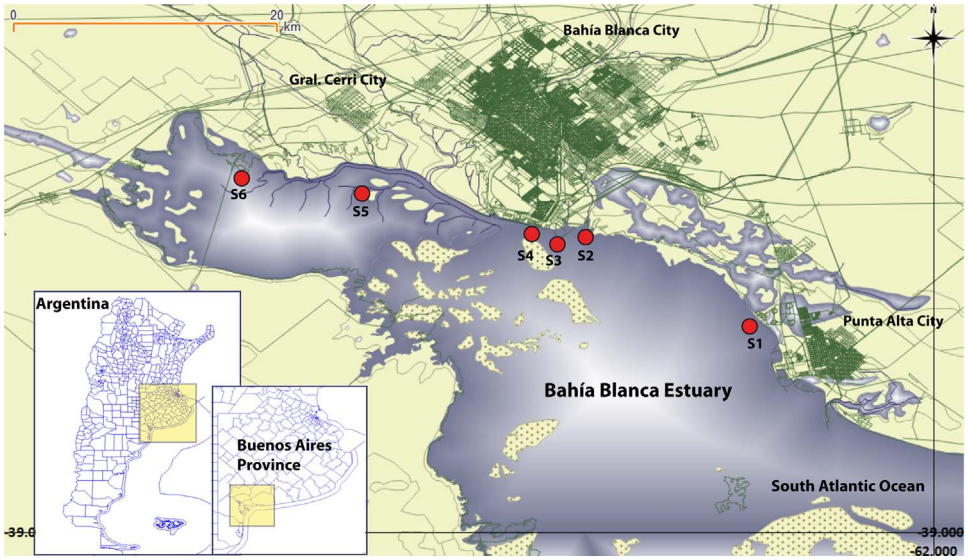

Fig. 1. Sampling sites at the Bahía Blanca Estuary, Buenos Aires, Argentina.

MBT, DBT, TBT, TPrT and TeBT -containing 1 mg mL^{-1} of Sn for each compound- were prepared in chromatographic grade methanol (U.V.E.) (Merck). Stock solutions were freshly prepared by appropriate dilutions in methanol. All standard solutions were stored in amber flasks and vials in the darkness at 0 °C (UNEP/IOC/IAEA, 1994). Sodium borohydride ($NaBH_4$ > 96%) was purchased at FlukaChemika. Sodium hydroxide methanolic solution was prepared by dissolving 1.0 g of sodium hydroxide (98%) in 1 L of chromatographic-grade methanol (U.V.E.) to obtain a 0.1% solution (UNEP/IOC/IAEA, 1994). Chromatographic-grade hexane (U.V.E) (Merck) was used. Throughout all the study, ultrapure deionized water (18.3 Ω cm^{-1}, Barnstead, Dubuque, USA) was used.

2.4. Organotin determination

The procedure used is similar to that previously applied to sediment samples described in Quintas et al. (2016) using 10 mL of 0.1% sodium hydroxide in methanol instead of the original volume.

2.5. GC-MS operational conditions

OTCs separation and quantitation were carried out by capillary gas chromatography (Agilent 7890 B) coupled with a mass spectrometer (Agilent 5977 A). An HP-5MS fused silica column (30 m; 0.25 mm i.d.; 0.25 μm film thickness) was used. One microliter of sample –or standard mixture, or blank– was injected on split-less mode at 220 °C. The carrier gas was Helium at a constant flow of 1 mL min^{-1}. The mass spectrometer was operated in the electron impact mode (70 eV). The temperature program was 55 °C for 1 min, then 20 °C/min to 200 °C, which was held for 5 min. The organotin hydrides were determined in the selected ion monitoring (SIM) mode; the selected ions are presented in Table 1. Each individual organotin compounds was confirmed by the retention time and the abundance of quantification/confirmation ions with respect to certified OTCs standards.

Table 1
Characteristic ions (*m/z*) and retention times of the compounds of interest.

Analite	Ions *m/z*	Retention time (seg)
$MBTH_3$	119–121-177–179	2.1
$DBTH_2$	121–177-179	5.2
TPrTH	119–121-163–165-205–207	5.6
TBTH	119–121-177–179-233–235	7.5
TeBT	119–121-177–179-233–235-289–291	9.2

3. Additional analysis and measurements

3.1. Physico-chemical parameters

Temperature, pH and conductivity were measured at each sampling point. Salinity was calculated by conductivity and expressed as practical salinity units. The measurements were recorded with a multisensory HORIBA U-10 (Japan).

3.2. Condition index

Condition index (CI) was suggested to be a good indicator of health condition in marine gastropods (Lau and Leung, 2004). The condition index (CI) was determined according to the following formula (Crosby and Gale, 1990; Freeman, 1974; Orban et al., 2002):

$$CI = \frac{\text{dry tissue weight(g)}}{\text{dry shell weight(g)}} \times 100$$

This formula is recommended by most authors because it involves stable and easily measurable variables (Davenport and Chen, 1987; Lucas and Beninger, 1985).

3.3. Validation requirements of the method

Accuracy of the method was assessed in terms of precision and trueness (Table 2). The study of trueness was carried out on the samples, i.e. by spiking tissues previously lyophilized with known amounts of the three OTCs analyzed. Three concentration levels for each

Table 2
Recovery percentages (%) obtained in mussels with known amounts of the TBT, DBT and MBT by applying the proposed method.

Analyte	Added concentration (ng Sn g^{-1} d.w.)	Obtained concentration (ng Sn g^{-1} d.w.)	%Recovery
TBT	10	10.3 (6.3)	96
	100	95.3 (0.9)	108
	1000	1013 (2.3)	92
DBT	10	10.9 (2.0)	87
	100	100.7 (4.0)	105
	1000	996 (1.0)	98
MBT	10	9.83 (3.1)	103
	100	110.5 (4.9)	96
	1000	912 (7.1)	85

RSD % is included in parentheses; d.w.: dry weight; n = 6.

organotin compound were added to the samples. In addition, six samples were analyzed for each concentration level of OTCs. Precision was evaluated under repeatability conditions and was estimated as percent relative standard deviation, RSD (%), n = 6. The recoveries varied between 91% and 110% and the associated RSD (%) were < 9%. The results were in accordance with analytical validation recommendations (EURACHEM, 1998; IUPAC, 2002), i.e., recovery of 70 − 120% and RSD (%) below 20%.

Detection limits (LOD) were calculated as three times the baseline noise of chromatograms taking into account the recoveries of the analytes. LOD obtained were 0.8, 1.1 y 3.5 ng Sn g^{-1} dry wt. for TBT, DBT and MBT, respectively.

In order to evaluate the extraction efficiency, TeBT was added in known concentrations and they were subjected to the analytical procedure using TPrT as internal standard. Thus, only the extraction step is evaluated because the TeBT does not suffer derivatization process. The recoveries obtained were 100%.

3.4. Statistical analysis

The results were statistically analyzed using INFOSTAT software version 2008. The normal distribution of the characteristics in each group was tested with the Shapiro-Wilk test ($p > 0.05$), the correlation analysis ($p < 0.05$ was regarded as significant) and Kruskal-Wallis test was used for the comparisons among different samples, sites and seasons data. Furthermore, the principal component analysis (PCA) was applied, using R2012b (The MathWorks), in order to evaluate the relationships among the concentration of the different organotin compounds, the sampling sites and other parameters.

4. Results and discussion

4.1. Physicochemical variables

Table 3 shows the pH, temperature and salinity values measured in the seawater column along the sampling period. These three variables did not show statistically significant differences (Kruskal Wallis, $p > 0.05$) among the studied sites. On the one side, the pH ranged between 7.6 (S4, autumn) and 9.6 (S6, summer). From a seasonal point of view, statistically significant differences between pH values obtained in autumn with respect to those achieved in winter and summer were observed. In turn, there was statistically differences between the pH values obtained in summer with respect to the ones obtained in winter, as well as between the values found in spring respect to the ones measured for autumn and winter ($\alpha = 0.05$). On the other side, the general behavior of temperature was similar as for pH. Thus, temperature fluctuations in the environment within each season were low, with high fluctuations between seasons. All sites registered the greatest temperatures in summer and the lowest in winter; these values ranged from 8.0 °C (S5) to 22.9 °C (S6). Seasonally, the temperature recorded for summer differed significantly from the one for autumn and winter; however, it was not possible to set differences with the spring season (Kruskal Wallis test, $p < 0.05$). In turn, the temperature found in winter differed significantly with that obtained in spring, but no significant differences were found with respect to autumn; simultaneously, spring and autumn did not show significant differences between them.

Salinity ranged from 27.8 (S6, spring) to 38.5 (S5, summer; Table 3). No statistically significant differences were found among autumn, winter and spring; whereas statistically significant differences were found against summer. During summertime, the salinity values increase due to the uniform evaporation throughout the estuary. In general, with the exception of summer, salinity increased from the head (S6) to the middle of the estuary (S1), probably as a result of the dilution effect caused by the brackish tributaries of the drainage basin.

In spite of the importance of these variables, no statistically significant correlations (Spearman coefficient, $p > 0.05$) were found between them and OTCs levels in mussels tissues. This fact points out the interaction of other factors affecting the distribution of OTCs in the area of the study, such as, the presence of microorganisms responsible for biodegradation, dredging works, characteristic tidal currents, among others.

4.2. Condition index

Results listed in Table 3 showed that the CI ranged between 4.2 and 20.2 for the analyzed mussels. No statistically significant differences

Table 3
Values of TBT and their degradation products- DBT and MBT- in samples of *Brachidontes rodriguezii* mussels, expressed in ng Sn g^{-1} dry wt.

Season	Sites	TBT	DBT	MBT	TBts[a]	BDI[b]	CI[c]	BAF[d]	pH	T (°C)[e]	Salinity
Summer	S1	1.27 ± 0.12	3.40 ± 0.10	14.97 ± 1.05	19.64 ± 1.06	0.07	11.3	0.11	9.3	19.3	36.4
	S2	2.05 ± 0.15	2.67 ± 0.56	17.12 ± 1.52	21.84 ± 1.63	0.10	5.90	0.12	9.4	22.1	35.5
	S3	3.71 ± 0.50	7.00 ± 1.25	17.70 ± 0.86	28.41 ± 1.60	0.15	7.08	0.28	9.4	21.2	35.8
	S4	2.24 ± 0.40	4.66 ± 0.47	17.31 ± 0.75	24.21 ± 0.97	0.10	9.11	0.09	9.5	20.1	36.4
	S5	3.49 ± 0.17	4.50 ± 0.60	17.33 ± 0.15	25.32 ± 0.64	0.16	12.0	0.45	9.5	21.4	37.6
	S6	1.67 ± 0.17	2.64 ± 0.46	17.11 ± 0.32	21.42 ± 0.59	0.08	7.50	0.19	9.6	22.9	38.5
Autumn	S1	32.92 ± 6.76	24.90 ± 0.11	74.21 ± 16.10	132.03 ± 17.46	0.33	10.5	1.15	7.8	14.9	34.3
	S2	43.77 ± 0.97	24.79 ± 0.92	53.47 ± 4.13	122.03 ± 4.34	0.56	8.32	2.07	7.9	14.9	34.0
	S3	31.24 ± 1.99	33.06 ± 1.86	62.10 ± 5.17	126.40 ± 5.84	0.33	8.52	1.46	7.9	14.5	33.4
	S4	–	–	–	–	–	–	–	7.6	14.6	32.8
	S5	29.27 ± 2.55	41.38 ± 0.66	56.55 ± 4.96	127.20 ± 5.62	0.30	4.79	1.66	8.1	14.4	30.8
	S6	38.15 ± 3.58	50.08 ± 5.07	55.66 ± 4.12	143.89 ± 7.45	0.36	4.61	1.49	8.2	14.2	28.3
Winter	S1	44.66 ± 4.67	49.42 ± 2.10	n.c.	94.08 ± 5.12	0.90	20.2	2.27	8.3	9.5	35.6
	S2	52.70 ± 0.25	90.05 ± 5.92	9.39 ± 0.84	152.14 ± 5.98	0.53	6.74	4.21	8.3	8.7	34.8
	S3	50.18 ± 3.12	72.95 ± 3.98	36.69 ± 2.69	159.82 ± 5.73	0.46	6.86	5.94	8.4	8.2	34.5
	S4	66.53 ± 1.06	80.29 ± 1.54	8.18 ± 1.02	155.00 ± 2.13	0.75	6.33	8.63	8.5	8.1	34.5
	S5	60.37 ± 5.68	91.81 ± 0.82	8.50 ± 0.98	160.68 ± 5.82	0.60	6.73	3.86	8.5	8.0	32.1
	S6	42.77 ± 4.53	73.92 ± 6.35	n.c.	116.69 ± 7.80	0.58	5.71	4.16	8.7	9.7	30.7
Spring	S1	26.66 ± 4.24	73.90 ± 8.60	36.21 ± 3.05	136.77 ± 10.06	0.24	17.5	0.93	8.3	17.4	34.8
	S2	20.70 ± 0.82	42.49 ± 3.37	47.23 ± 1.99	110.42 ± 4.00	0.23	13.1	0.89	8.2	17.2	33.3
	S3	24.48 ± 2.65	58.78 ± 6.72	33.08 ± 2.94	116.34 ± 7.80	0.27	12.0	0.67	8.4	15.8	32.5
	S4	27.77 ± 2.27	132.59 ± 1.59	20.21 ± 2.32	180.57 ± 3.61	0.18	13.5	1.33	8.5	17.6	32.6
	S5	16.63 ± 3.78	21.37 ± 0.12	17.57 ± 1.65	55.57 ± 4.13	0.43	13.0	0.58	8.4	15.6	29.6
	S6	25.02 ± 4.29	129.79 ± 4.38	20.39 ± 1.99	175.20 ± 6.45	0.17	12.4	1.20	8.2	17.7	27.8

The values reported are the mean of three replicates along with their corresponding standard deviation. n.c.: Not quantified. -: Absence of samples.
a = TBts: TBT + DBT + MBT. b = BDI: Butyltin degradation index. c = CI: Condition Index. d = BAF: bioaccumulation factor. e = Temperature.

were found between the different sampling sites (Kruskal Wallis, $p > 0.05$), which would suggest that the organisms had a similar physiological condition independently of the studied site. However, statistically significant differences were noted between the CI values obtained in spring with those found in others seasons (Kruskal Wallis, $p < 0.05$). Some authors have postulated that a high CI is associated with high phytoplankton biomass since it influences in the reproductive cycle of mussels (Arrieche et al., 2002). In the area of study, the phytoplankton has shown a marked recurrent pattern with a winter- spring diatom bloom (Gayoso, 1999, 1998; Guinder et al., 2015; Popovich et al., 2008; Popovich and Marcovecchio, 2008). Then, following Arrieche et al., 2002 approach, this fact would probably be accounting for the highest CI values obtained in spring.

4.3. Butyltin degradation index

To assess whether organotin contamination derives from relatively fresh sources or not, it is useful to calculate butyltin degradation index (BDI), defined as the ratios between the parent compound and its breakdown products (Diez et al., 2002; Kim et al., 2008).

$$BDI = \frac{TBT}{MBT + DBT}$$

In general, the OTCs levels in mussels tissues are strongly influenced by several factors such as the sampling area, distance to possible sources, water temperature, pH, salinity, oxygen content and biological activity, among others (Diez et al., 2005; Sousa et al., 2009a). For this reason it is not an easy task to assess how recent an input is. However, the use of BDI seems to be adequate in view of the fact that the samples were collected at the same time, in a relatively small area and under similar environmental conditions. Although the BDI was firstly proposed for sediment studies, it has recently been used in gastropods and mussels tissues by many authors as TBT pollution indicator (Castro and Fillmann, 2012; Commendatore et al., 2015; Couceiro et al., 2009; Filipkowska and Lubecki, 2016; Kim et al., 2008; Ruiz et al., 2008, 1998; Sousa et al., 2009a; Wang et al., 2010).

A BDI > 1 would indicate recent inputs of TBT, on the contrary, BDI values less than 1 would mean that the parent compounds have degraded and thus the contamination should be considered "old".

As shown in Table 2, the calculated BDI varied from 0.07 to 0.90. In the light of these results, butyltin contamination in the Bahía Blanca Estuary could be classified as old, possibly under a general degradation process. On the one side, no statistically significant differences among the different sampling sites were observed (Kruskal-Wallis, $p > > 0.05$). At first, this would indicate that the degradation process was independent from the sampling site; however, a second reason could be attributed to a masking effect due to frequent sediment dredging activities at the area.

On the other side, statistically significant differences were noted between seasons (Kruskal-Wallis, $p \ll 0.05$). These differences were identical to those found for temperature values in the water column. BDI values, just like temperatures, increased seasonally from summer to winter and then declined again in spring. In agreement with existing literature, the low BDI values obtained in summer could be explained as a result of an increased capacity of *Brachidontes rodriguezii* either to degrade or to excrete TBT (Chandrinou et al., 2007; Hsia and Liu, 2003). Opposite to that, low temperatures during winter seemed to favor the TBT bioaccumulation in *Brachidontes rodriguezii,* probably due to a limited capacity to metabolize OTCs at low temperatures.

4.4. Butyltin levels

Butyltin compounds including TBT and its breakdown products, DBT and MBT, were detected in all bivalve samples analyzed, suggesting a widespread occurrence of these compounds at the area of study. From the 24 collected samples, a total of 23 composite samples could be analyzed, as for S4 all the valves were empty in autumn. Mean values for the concentrations of OTCs, average of three replicates (n = 3; mean value ± standard deviation), are presented in Table 3. The MBT levels ranged between unquantifiable values and 74.21 ng Sn g^{-1} dry wt. The levels of the first degradation product (DBT) were between 2.64 and 132.6 ng Sn g^{-1} dry wt. Finally, the TBT levels ranged from 1.27 to 66.53 ng Sn g^{-1}dry wt.

Considering the whole recorded data, the dominant compound in *Brachidontes rodriguezii* tissue was DBT, which accounted for 45% of the total butyltins (Table 3). The proportion of MBT was lower, reaching 29%, whereas TBT accounted for 26%. The higher DBT content in these bivalves denoted an efficient metabolization of TBT to DBT and simultaneously a deficient elimination or lower metabolization efficiency for DBT (Albalat et al., 2002; Chandrinou et al., 2007).

Considering the three OTCs levels, no statistically significant differences were found between the sampling sites (Kruskal Wallis, $p > > 0.05$). As mentioned before, actual differences could be masked by the periodically dredging performed in the Main Navigation Channel, as this activity generates artificial resuspension and subsequent transport and redistribution of sediments in the estuary. Despite this fact, statistically significant differences were noted between seasons (Kruskal-Wallis, $p \ll 0.05$). Interestingly, on the one side, TBT levels were inversely correlated to the water temperature values; for instance, at each site, TBT levels in mussels were higher in winter and lower in summer, suggesting that the water temperature acted as a key parameter for the control of TBT levels in *Brachidontes rodriguezii*. Similar results have also been shown in other studies (Tang et al., 2010; Tang and Wang, 2008). On the other side, TBT degradation products –DBT and MBT– did not show a clear seasonal trend for accumulation, probably due to their lower hydrophobicity (Rudel, 2003; Tang et al., 2010).

In 2004, the Oslo-Paris commission (OSPAR) updated the Ecotoxicological Assessment Criteria (EAC) values for TBT in water, sediments and biota, according to the most recent knowledge on the TBT concentrations that could cause possible adverse effects in mollusk populations (Furdek et al., 2012; OSPAR, 2004). The commission proposed for TBT in mussels a lower EAC value of 4.91 ng g^{-1} as Sn and an upper EAC value of 71.7 ng g^{-1} as Sn (OSPAR, 2004). By definition, values below the lower EAC would not cause adverse biological effects while values between the lower and the upper EAC thresholds could exert adverse effects such as biomarker response, reproduction, impaired growth, etc. Finally, concentrations above the upper EAC value would likely show long-term biological effects and possible acute biological effects on the survival of the population (OSPAR, 2004). As can be observed in Table 3, seasonal differences were observed in regards to EAC's thresholds. For instance, during summer, all the studied sites showed concentrations below the lower EAC; then, no biological effects were expected. Along the rest of the year, the TBT levels in tissue were found between the lower and the upper EAC values. This accounted for the 73.9% of the samples; therefore, a high risk of biological effects associated to contamination with TBT was assessed for *Brachidontes rodriguezii* communities.

Comparisons of OTCs concentrations with other worldwide reported should be carefully addressed due to species, sites, currents, microbial activity, etc. (Sousa et al., 2009a; Quintas et al., 2016). Beside this, in order to set the Bahía Blanca estuary levels in the worldwide context, the actual data was contrasted against the past ten years literature (Table 4). As it can be noted, local levels were considerably lower than those reported in Slovenia (Nemanič et al., 2009), Luermen Stream estuary, Taiwan (Tang et al., 2010), Busan Harbor, Korea (Choi et al., 2010) and Portugal (Sousa et al., 2009a). Concomitantly, the TBT concentration reported for Turkey (Kucuksezgin et al., 2011) and gulf of Gdańsk, Poland (Filipkowska and Lubecki, 2016) were slightly higher than the levels reported in this study. Furthermore, the levels found in Bahía Blanca estuary were comparable with those reported in Zealand (Höher et al., 2012) and Greek coasts (Chandrinou et al.,

Table 4
Tributyltin concentration in mussels (ng Sn g^{-1}) from different from worldwide locations and from this study.

Mussel	Locations	Sampling period	Range TBT ng Sn g^{-1} (d.w.)	Reference
Mytilus galloprovincialis	Aegean Sea, Greece	2001–2003	< 4–58	Chandrinou et al. (2007)
	From Viana do Castelo to Faro, Portugal	2006	1–720	Sousa et al. (2009a)
	Northern Adriatic Sea, Slovenia	2000–2006	36–6434	Nemanič et al. (2009)
	Aegean Sea, Turkey	2009	59–177	Kucuksezgin et al. (2011)
Perna viridis	Luermen Stream estuary,Taiwan	2003–2004	86.2–4329	Tang et al. (2010)
	Manila Bay, Philippine	2005	2.1–8.9	Olivares et al. (2013)
Mytilus trossulus	Gulf of Gdańsk, Poland	2008–2013	11–103	Filipkowska and Lubecki (2016)
Mytilus edulis	Busan Harbor, Korea,	2002–2007	84–600	Choi et al. (2010)
	Danish coastal, Zealand	2009	13.1–71.9	Höher et al. (2012)
Perumytilus purpuratus	San Vicente bay, Chile	2006	150	Pinochet et al. (2009)
Aulacomya atra atra; Mytilus edulis	San Jorge gulf, Argentina	2010	3.5–177	Commendatore et al. (2015)
Mytella Guyanensis	Todos os Santos Bay, Brazil	2010–2011	< 5–421	Artifon et al. (2016)
Anomalocardia brasiliana			12–67	
Brachidontes rodriguezii	Bahía Blanca Estuary, Argentina	2013	1.27–66.53	This study

2007). The overall lowest TBT pollution was reported for Manila Bay, Philippine (Olivares et al., 2013).

Particularly, within Latin America, a large number of studies have been reported for gastropods in coasts of Chile (Batista et al., 2016; Mattos et al., 2017); Venezuela (Paz-Villarraga et al., 2015); Brazil (Petracco et al., 2015; Rossato et al., 2016); Argentina (Del Brio et al., 2016) and Peru (Castro and Fillmann, 2012). Despite this fact, just a few studies performed monitoring programs of organotin contamination in mussels (Table 4). As shown in Table 4, the concentrations of TBT found in the Bahía Blanca estuary were similar to those recorded for bivalve tissues (*Anomalocardia brasiliana)* collected in Todos os Santos Bay (Brazil) (Artifon et al., 2016). In turn, the values reported in San Vicente bay (Chile) (Pinochet et al., 2009) and San Jorge gulf (Argentina) were slightly higher than those found in the study zone (Commendatore et al., 2015).

4.5. Bioacumulation of TBT in tissue

Bioaccumulation is a complex process of transferring chemicals between the environment and an organism (Laughlin, 1996). In order to estimate mussel TBT accumulation from sediment, the bioaccumulation factors (BAF) were calculated. The BAF is defined as the ratio between the concentration found in the mussel and the concentration found in the sediment (Cao et al., 2009; Laughlin, 1996). In cases where the total concentration of a pollutant in sediment is potentially bioavailable, the value of BAF is close to unity (OSPAR, 2009). Data of TBT level in sediments were obtained from a previous study (Quintas et al., 2016) and Table 3 shows the values of BAF. As can be seen, 56.5% of the samples appeared to have accumulated TBT through the sediments, with values of BAF > 1. This percentage clearly indicates that the bioavailability of TBT from sediments played an important role in the bioaccumulation process of TBT. The highest BAF values were recorded during winter, when both the highest concentration of TBT were found in sediments and the metabolization capacity of *Brachidontes rodriguezii* was probably set at its minimum (Furdek et al., 2012; Tang et al., 2010).

4.6. Butyltin distribution

Principal Component Analysis (PCA) was used to obtain a better portrait regarding the behavior of the OTCs, both temporally and spatially, in *Brachidontes rodriguezii* mussel samples. From PCA analysis, three main components collected the 99.12% of the total variance which accounted for 72.45%, 18.05% and 8.62% of it, respectively. The variables that contributed to the variance collected by the model were: temperature and TBT, DBT and MBT concentrations. The other variables (salinity, pH and IC) did not show such a variability which may have influence in the model (Fig. 2a–c). The first component or factor had a great positive contribution from the DBT concentration and, to a lesser extent, from the TBT concentration. At the same time, a small negative contribution from MBT concentration and temperature (MBT > temperature) was observed. The second factor had a great influence of the MBT concentration whereas TBT concentration contributed in a lesser extent. Finally, the third factor showed a positive and high contribution of TBT concentration and a negative contribution, considerably lower, due to the temperature and levels of MBT and DBT. Fig. 2d–f shows the scores plot of the first three principal components. As it can be seen from these plots, the samples appeared to be arranged depending on the season on which were collected.

In summer, the sites were grouped together indicating low variability among samples within that season (Fig. 2d–f). The position of the sites in the third quadrant corresponded to the lowest TBT and DBT levels obtained throughout the year of sampling. The low TBT concentrations found in summer may indicate that *Brachidontes rodriguezii*, like other bivalve mollusks, have a higher ability to remove TBT (either by degradation or excretion) as the temperature of the environment increase (Chandrinou et al., 2007; Hsia and Liu, 2003). In turn, the significant correlation obtained between TBT and DBT (Spearman coefficient: $r = 0.80$; $p = 0.05$) would indicate that the main source of DBT in mussels tissue was the TBT degradation.

In autumn, the location of the sites matched the highest MBT values, being S1 the site with the highest concentration of this compound (Fig. 2d and f). The lack of correlation obtained between MBT and DBT or TBT ($p > 0.05$) would indicate that the unique source of MBT in mussels tissue was not the OTCs degradation. The high MBT levels could be the result of direct exposure of mussels to additional sources of this compound (Kannan et al., 1995). This is important because for the first time, in Latin America it has been reported possible MBT sources beyond OTCs degradation. As mentioned before, one of the major applications of MBT is the stabilization of PVC; then, a hypothesis on MBT leaching from this material into the aquatic environment can be stated (Hoch, 2001). On the one hand, PVC is used in drinking water, wastewater and drainage water pipes. Looking at the surrounding area, wastewater discharges from the cities of Bahia Blanca (proximity to the site S2), General Daniel Cerri (proximity to the site S5) and Punta Alta (proximity to the site S1) are being continuously released. In addition, sites S3 and S4 include industrial discharges of one of the most important PVC manufacturing plants. Thus, it can be argued that MBT could be available in the system, then filtered and eventually accumulated by mussels living in the area. It was expected that site S2 in autumn would be positioned between the site S5 and the origin of coordinates defined by the factors 2 and 3; however, S2 showed a displacement due to its higher content of TBT with respect to the other sites (Fig. 2f). The site S2, located in the Ingeniero White Port, is characterized by the presence of large commercial vessels and general cargo ships, particularly in autumn, which pose a possible introduction

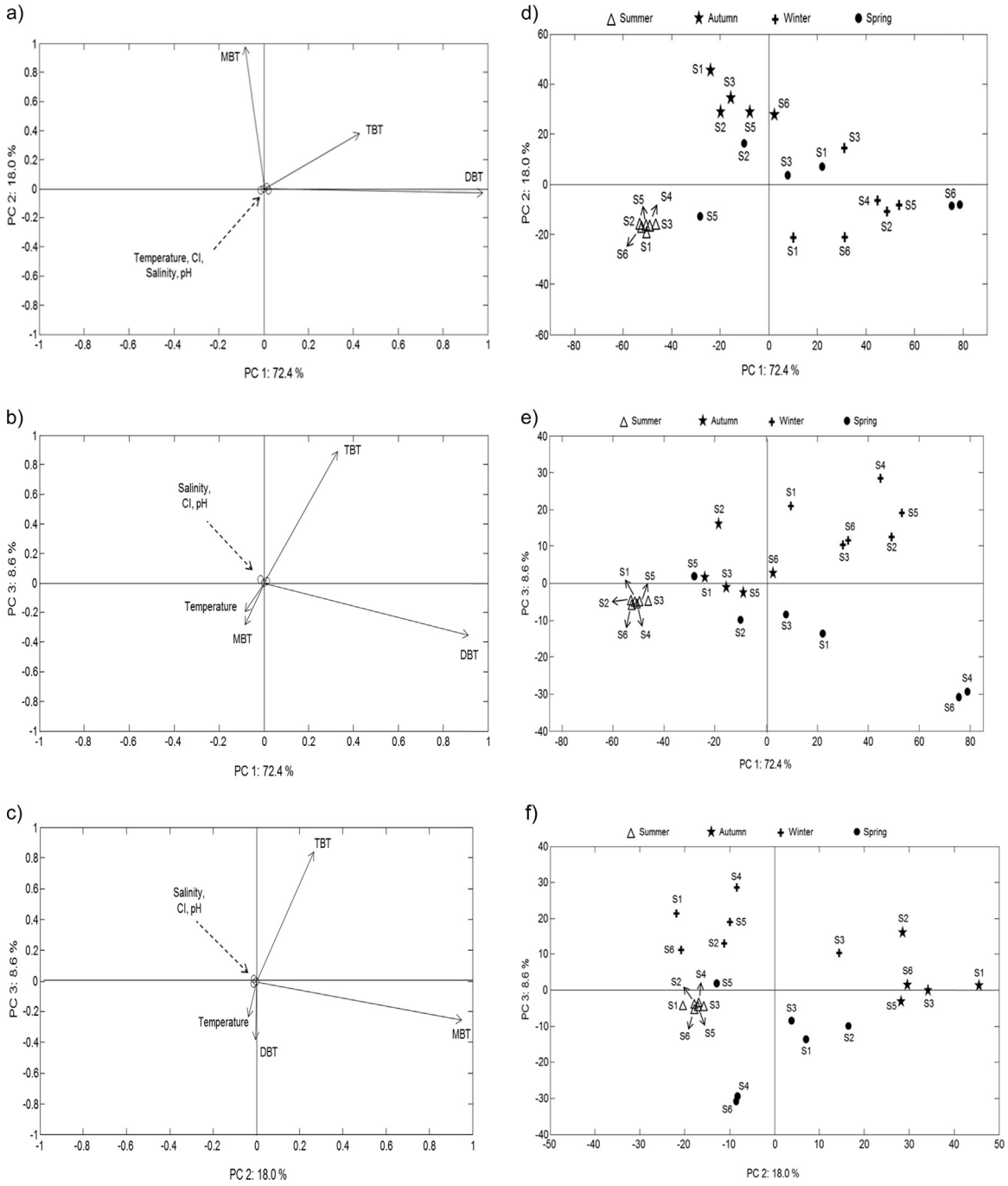

Fig. 2. a–c. Loadings plots, d–f scores plots for samples of *Brachidontes rodriguezii* mussels collected in 2013.

of TBT in the environment. Thus, it is highly plausible that TBT can be more available for mussels there.

Fig. 2d shows that, in winter, the sites are located in ascending order, from the origin of coordinates, according to the DBT concentration. Although it was expected that site S3 was located between the sites S1 and S6, it showed a displacement because it is the only site that has a considerable value of MBT in this season (can also be seen in the Fig. 2f). The high MBT concentration found at this site indicated an additional income of this compound. The site S3 is located in proximity to the industrial effluents collector, creating potential sources of MBT, mainly due to the discharge from PVC plastics factory in the study area. Thus, high MBT concentrations may be available to be filtered and/or accumulated by mussels there. Also in winter, the sites are positioned in high values of factor 3 coinciding with the highest TBT values (Fig. 2e and f). The site S4 has the highest concentration of this compound.

In winter, the metabolic activity of the microorganisms decreased as well as their TBT degradation process, probably owing to the low temperatures (Lee et al., 1989; Maguire et al., 1986; Tang et al., 2010). In general, this behavior causes the presence of high concentrations of this compound in the environmental and, thus, is available to be filtered by the mussels. Again, and due to the low temperatures of this season, bivalves have limited ability to metabolize OTCs, generating an effect of bioaccumulation (Chandrinou et al., 2007; Furdek et al., 2012). In turn, under these temperatures, the filtration rate of some mollusks decreases from TBT to MBT (Tang et al., 2010). This fact would explain the higher levels of TBT and DBT and, at the same time, the lowest MBT concentrations recorded in winter.

In spring, in the Fig. 2d can be seen that the sites were positioned in

accordance with their TBT and DBT levels (S5 < S2 < S3b < S1 < < S6 < S4). However, the site S2 had a small displacement due to its high MBT concentration, respect to the other sites. Finally, sites S4 and S6 were markedly away from the other study sites because they presented a high DBT concentration (the highest in the year sampled). This latter feature can also be evidenced in Fig. 2e.

TBT levels found in spring were one of the lowest in the year of sampling, after the summer. As already mentioned, in the temperate and eutrophic Bahia Blanca estuary, the phytoplankton annual cycle is characterized by a winter diatom bloom (June–September) (Guinder et al., 2015). The concentration of TBT in sediments during winter diminishes probably due to an accelerated degradation process caused by diatoms (Quintas et al., 2016; Seligman et al., 1996), a process that seems to be reflected in the mussels collected during the spring season. This occurs since they need a certain period of time to reflect environmental pollution around them. While this time is not clearly defined, it is influenced by several factors such as temperature, salinity, dissolved oxygen, food availability and speed of growth of mussels, among others. A significant correlation between the concentrations of TBT and DBT in spring (Spearman coefficient: $r = 0.94$; $p = 0.04$) was found, indicating that the source main of DBT was generated by TBT degradation in mussels tissue (Furdek et al., 2012; Ruiz et al., 2005; Sousa et al., 2009a).

The high content of MBT found at S2 (spring) probably resulted in a faster degradation of the TBT in the environment surrounding mussels samples. On the one hand, S2 is located in the proximity of "Luis Piedra Buena" thermoelectric facilities, where water at high temperature is discharged into the estuary, facilitating the thermal degradation of OTCs (Almeida et al., 2004). On the other hand, S2 is located nearby the main city hall sewage outlet, which drains domestic (black waters) without any treatment, where there is a significant biological activity (Baldini et al., 1999). Studies have shown that microalgae and bacteria can play an important role in the biodegradation of TBT in aquatic systems (Hoch, 2001); then, the biological generation of MBT from TBT could be present at the area and would explain the high MBT yields recorded.

DBT level at S6 (spring) was the highest of the sampling period. This site accounts for several special characteristics such as shallow water, low tidal energy and thinner layer of oxic sediments. The DBT accumulates in this site and the degradation process is less effective than in other sites of the estuary. As noted by Almeida et al. (2004), tidal currents are effective in conveying particulate matter carrying TBT and its degradation products from the main source areas to these remote zones, where settling of particles is favored by hydrodynamics. Then, results for this site outline the importance of transport and depositional behavior of particles at the Bahía Blanca Estuary (Quintas et al., 2016). At last, high levels of DBT reached in mussels samples at S4 in the spring season were probably due to an additional input of this compound (Chandrinou et al., 2007).

5. Conclusions

Bioindicators showed a general OCT pollution trend for the area under study, including the first record of MBT in history for Latin America. Mussels at all the sampling sites were shown to be impacted by TBT and their degradation products -DBT and MBT-. Moreover, a significant proportion of them showed TBT bioaccumulation vs. the sediment. In accordance with international criteria, mussels were classified under risks of biological effects associated to TBT pollution; despite this, TBT loads appeared to be old and subjected to a general degradation process enhanced by the high nutrient concentrations and phytoplankton biomass activity. Water column temperature was shown to be a key driver affecting both the ability to accumulate and to degrade organotin compounds by *Brachidontes rodriguezii*. Finally, evidence supported the occurrence of additional local sources of DBT and MBT beyond the natural degradation from TBT.

Capsule

Levels of MBT, DBT and TBT were determined for the first time in native mussels (*Brachidontes rodriguezii*) from Bahia Blanca estuary using CG-MS, after extraction/derivatization assisted by ultrasound.

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