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Baseline

PAHs contamination in edible gastropods from north Patagonian harbor areas

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ABSTRACT

PAHs are persistent pollutants released into the environment by fossil fuels burning and leak during petroleum operations. Associated with suspended particles upon entering marine ecosystem are accumulated by benthic fauna. Human exposure occurs mainly from ingestion such as gastropods consumption. The objective was to determine PAHs in sediments and in the marine gastropod *Buccinanops globulosus* in sites with different maritime and urban influences. In sampling sites located 20 km from the harbor, PAHs were non-detected; while in harbor gastropods, the level of PAH4 was exceeded according to international normative. Level of dibenzo[*a*,*h*]anthracene in sediments was between the ISQG and PEL. Since these are the first results of PAHs in edible gastropods in South America, we concluded that PAHs can be dangerous for consumers according to ingestion frequency. Integrative studies are necessary to evaluate the interaction among pollutants in maritime areas and the incidence in human health due to shellfish consumption.

The contamination caused by persistent organic pollutants (POPs) is relevant, especially for public health (Smith, 1999; Jacob, 2013). A variety of these compounds have been intensively studied in environmental compartments (McElroy et al., 2010) while carcinogenic and mutagenic properties have been demonstrated for many of them (WHO, 2010). Anthropogenic sources provide a larger volume of POPs release, and pollution generated by industries, harbor areas, and human activities are of special interest due to effects caused on the marine organisms and human health.

Among POPs, polyaromatic hydrocarbons (PAHs) are important components of crude oil and its by-products, and their incorporation into the environment can occur through spills or as consequence of incomplete combustion of organic materials. These chemicals constitute a relatively stable group of organic compounds which may persist in sediments for long time (Wenning and Martello, 2014), can be bioaccumulated by different marine organisms, and biomagnified in the food webs. The pollutants bioaccumulation depends on many factors, mainly from the metabolic capacity of the biota (Meador et al., 1995). Particularly, mollusks have limited ability to metabolize PAHs due to its physiological characteristics, so it is frequent to detect concentrations of these and others POPs in different organ tissues (Oehlmann and Schulte-Oehlmann, 2003). A list of 16 PAHs has been established as priority pollutants by the Environmental Protection Agency of United States (USEPA), as they are detrimental to biota while carcinogenic effects of some of them have been demonstrated (IARC, 2010). Marine organisms destined to human consume may accumulate pollutants, and depending on the frequency and amount consumed it may be dangerous to human health. In Atlantic Patagonia (Argentina), marine gastropods from a variety of species are being consumed and commercialized by coastal populations, although no official regulations are established yet (Bigatti and Ciocco, 2008; Bigatti et al., 2015; Cumplido, 2016). In Argentina, many studies have been carried out to determine PAH concentrations in different environmental compartments (i.e. Colombo et al., 2005; Commendatore et al., 2012) including marine biota (Massara Paletto et al., 2008; Arias et al., 2009; Commendatore et al., 2015; Oliva et al., 2015) but effects on marine organisms and the potential risk for human health for its consumption has been scarcely evaluated. Regarding marine organisms from the Patagonian coastal zone inhabiting mainly in ports, the imposex incidence in gastropods is a sub-lethal effect that has been associated with its exposition to

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tributyltin (TBT) (Del Brio et al., 2016). Although imposex is principally related to TBT presence, a compound highly toxic and persistent in the environment, interactions (i.e. synergism) with other compounds common in harbors, such as PAHs, cannot be discarded. In field situations where pollutants occur as an undefined and complex mixture, results are not easily interpreted; interactions among them and with the biological systems will influence the effect of other environmental pollutants on the organism's response (Solé, 2000; Holmstrup et al., 2010). Therefore, it is important to identify where synergistic, addition or antagonistic effects exist (including effects of abiotic environmental factors), that might complicate the general interpretation or mask the biomarkers responses (Cravo et al., 2012).

Moderated levels of TBT and their metabolites (DBT and MBT) have been detected in sediments of the Nuevo gulf (Patagonia) (Bigatti et al., 2009) and in gastropods (Del Brio et al., 2016). The incidence of imposex up to 100%, has been demonstrated in the edible gastropod *Buccinanops globulosus* inhabiting harbor areas contaminated with TBT, as well as oxidative stress, shell malformations, reproductive alterations and acute toxicity in affected populations (Primost et al., 2015a; Primost et al., 2015b; Primost et al., 2015c; Primost et al., 2016; Averbuj et al., 2017; Márquez et al., 2017). Studies carried out in the Patagonian coastal zone of Argentina, related to organic compounds contamination have reported presence of polyaromatic hydrocarbons (PAHs), TBTs and organochlorines (OCPs and PCBs) (Commendatore et al., 2012; Commendatore et al., 2015).

The edible gastropod *B. globulosus* inhabits soft bottoms and lives buried in fine sediments; it feeds on carrion and can live up to 8 years (Bökenhans et al., 2016; Bökenhans et al., 2017). Regarding human health, fishermen and coastal dwellers may be even more at risk through more frequent consumption of fish and other marine resources (Fattore et al., 2002). In the last years is of special concern to determine contaminants in edible species that may affect human health. The objective of this work was to determine for the first time the presence of PAH in *B. globulosus* tissues and surrounding sediments in two sites with different anthropic activities to determine safety consumption and human health.

For this study, two sites within the Nuevo gulf, according to different anthropogenic activities developed in each one, were selected for sampling. One site was located in the harbor area of Puerto Madryn city given maritime traffic and proximity to urban and industrial zones as sources of pollution. The harbor area includes Luis Piedrabuena and Storni ports which have high maritime activity and are located nearby an industrial area. Maritime traffic reaches > 700 vessels per year (APPM, 2017) transporting materials for aluminum production, porphyry, and products for fishing industries as well as large touristic cruisers and sport vessels. In this area pollutants as hydrocarbons and TBTs have been reported (Commendatore et al., 2000; Massara Paletto et al., 2008; Del Brio et al., 2016). The other site, Cerro Avanzado (CA), located 20 km away from the city, has not large ships traffic and industries or urban populations are absent (Bökenhans et al., 2017). At this site, different pollutants such as TBTs and heavy metals in sediments and organisms have been in most samples non-detectable (Del Brio et al., 2016; Primost et al., 2017).

Sediment samples (n = 10) were randomly taken from the surface layer (0–5 cm) and homogenized in a composite sample representative of each sampling site. Then, sediment samples were stored in clean glass flask in freezer (−20 °C) until laboratory analysis. Gastropods (n = 25 per site) were collected by scuba diving in surrounding sediments. Complete soft tissues of gastropod were homogenized in a composite sample representative of each sampling site and stored in the same way than sediments until their analysis in the laboratory.

Sediments (~10 g) and gastropod tissues (~3 g) were chemical dried with anh. Na_2SO_4 and Soxhlet extracted with dichloromethane for 10 h. Dichloromethane extracts were reduce in volume in rotavapor to near 2 ml and transferred to a centrifuge tube where they were newly concentrated, solvent exchanged from dichloromethane to hexane, and then evaporated to 1 ml with a soft flow of ultra-high purity N_2 gas. Clean-up of concentrated extracts was done in a glass chromatographic column filled with alumina totally activated (10 g) eluting with 100 ml of dichloromethane. The solvent was reduced in volume, dichloromethane exchanged to hexane and then evaporated to reach 0.2 ml. Hexane extracts were seeded in alumina micro-column to isolate aromatic hydrocarbons. PAHs fraction was placed in glass vials to chromatographic injection. Hydrocarbon analysis was carried out in a Thermo Trace gas chromatograph equipped with a Triplus Autosampler (Thermo Electron Corporation, Whaltman, MA, USA) a J&W DB5 fused silica column (30 × 0.25 × 25), Split-less (1.2 min) capillary injection system (250 °C), and flame ionization detector (FID, 320 °C). Carrier gas was N_2 (1 ml min^{-1}). Chromatographic conditions were: oven temperature programmed from 60 °C (0 min) to 150 °C (0 min) at 15 °C min^{-1}, then 3 °C min^{-1} to 220 °C (0 min), and finally at 10 °C min^{-1} to 300 °C (15 min). PAH concentrations were calculated by comparison with response factors of individual target compounds contained in an external standard mix (16 EPA priority + 1-Methylnaphtalene; Accu Standard Z-014J-0.5X). PAHs analyzed were: Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo[*b*]fluoranthene, Benzo[*k*]fluoranthene, Benzo[*a*]anthracene, Chrysene, Benzo[*a*]pyrene, Dibenzo[*a,h*]anthracene, Indenopyrene, and Benzo[*ghi*]perilene. The identification of target compounds was confirmed by Gas Chromatography/Mass Spectrometry (GC/MS, Thermo Scientific Fucus GC/ISQ). PAH concentrations were expressed on a dry weight basis. Blank procedures were done with each batch of five samples to confirm the absence of contaminants. Duplicate analysis (two complete procedures) resulted in values of percent relative deviation (RDP %) from 0.4% to 9.0%. Standard Reference Material was analyzed to examine the PAHs analytical method performance; the recoveries in certified marine sediment (NIST 1944) were 84 ± 15%. Results were not corrected for recoveries. Chromatographic calibration was made with the standard mixture (five points, $R^2 \geq 0.995$) and a control standard (5 μg ml^{-1}) was injected with each chromatographic sequence. The detection limit of the method (LOD) was close to 5 μg kg^{-1} for individual aromatic hydrocarbons.

Concentrations of total PAHs (ΣPAHs) in the harbor area were higher in gastropod tissues (560 μg kg^{-1} dw) than in sediments (270 μg kg^{-1} dw), while in CA beach PAH levels were lower than the LOD in both snails and sediments (Table 1). As *Buccinanops globulosus*

Table 1
PAH levels (μg kg^{-} dw) in sediments and gastropods from LPB harbor and Cerro Avanzado (CA) beach.

	LPB harbor		CA beach	
PAHs	Sediments	Gastropods	Sediments	Gastropods
Naphthalene	Nd	Nd	Nd	Nd
1-methylnaphthalene	Nd	Nd	Nd	Nd
Acenaphthene	Nd	Nd	Nd	Nd
Acenaphthilene	Nd	Nd	Nd	Nd
Anthracene	Nd	174	Nd	Nd
Phenanthrene	Nd	Nd	Nd	Nd
Fluorene	Nd	Nd	Nd	Nd
Fluoranthene	30	141	Nd	Nd
Pyrene	20	28	Nd	Nd
Benzo[*a*]anthracene	50	22	Nd	Nd
Chrysene	30	Nd	Nd	Nd
Benzo[*b*]fluoranthene	30	151	Nd	Nd
Benzo[*k*]fluoranthene	40	44	Nd	Nd
Benzo[*a*]pyrene	30	Nd	Nd	Nd
Indenepyrene	Nd	Nd	Nd	Nd
Dibenzo[*a,h*]anthracene	20	Nd	Nd	Nd
Benzo[*ghi*]perilene	20	Nd	Nd	Nd
Σ PAHs	**270**	**560**	–	–

Bold represents the sum of all PAHs determined.

lives buried in fine sediments, which constitute a reservoir for lipophilic pollutant such as PAHs, bioaccumulation of these compounds trough carrion feeding and other snail activities on sediment is expected. In Puerto Madryn harbor area, the sediments can be considered as chronically contaminated for PAHs as these compounds were reported in previous studies (e.g. Massara Paletto et al., 2008). In the current study, individual PAHs detected in sediments ranged from four (Fluoranthene) to five (Dibenzo[*a*,*h*]anthracene) aromatic rings showing slight predominance of low molecular weight (LMW) over high molecular weight (HMW) hydrocarbons (Table 1).

In marine gastropods, PAHs pattern was different; while Anthracene was the major hydrocarbon registered; this compound was not present in sediments. This fact could be related with the ability of gastropods to metabolize PAH and sediment characteristics. In addition, relatively HMW compounds found in sediments such as Chrysene, Benzo[*a*]pyrene, and Dibenzo[*a*,*h*]anthracene were not detected in organisms. However, a bioaccumulation process of PAHs from sediments was clear in *B. globulosus*. The relatively different composition pattern for sediment and organisms can be related to a higher sediment bioavailability of LMW than HMW PAHs.

In sediments, the concentration of dibenzo[*a*,*h*]anthracene ($20\,\mu g\,kg^{-1}$ dw) exceeded the level established by the Canadian Guidelines for Marine Sediment Quality (ISQG = $6.22\,\mu g\,kg^{-1}$ dw), although was lower than the level of probable effects for biota (PEL = $135\,\mu g\,kg^{-1}$ dw). In CA beach, PAHs were non-detected whereby this site could be used as reference for future studies.

Regarding edible gastropods (e.g. *B. globulosus*) as well as other shellfish species, several worldwide organizations (USEPA, IACR, SCF, FAO/WHO JECFA, IPCS, EFSA, among other) and different countries (e.g. convention OSPAR between countries of the European Union, China, etc.) have adopted regulations, in which PAHs are included, establishing limits of concentration in food. The Scientific Committee on Food (SCF) highlighted various PAHs to be carcinogenic and recommended further investigation of their relative levels in certain foods. The normative generally needs to be interpreted through exhaustive explanatory guides for values analysis and its correct application to study cases. For PAHs the last trend is to follow simultaneously two considerations regarding compounds concentrations: (1) Benzo[*a*]pyrene which could be used as marker for the occurrence and effect of carcinogenic PAH in food (SCF) and (2) the sum of four PAHs (PAH4: benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*a*]pyrene, and chrysene) (EU commission, 2011). PAH4 were introduced, while maintaining a separate maximum level for benzo(a)pyrene to ensure comparability of temporal data (Zelinkova and Wenzl, 2015).

According to PAH4 maximum levels recommended in the Regulation (EU) N° 835/2011 in bivalves for human consumption ($30\,\mu g\,kg^{-1}$ dw), in our study level of PAH4 have been exceeded in gastropod tissues, indicating that special attention should be focused on monitoring of these compounds in *B. globulosus*. Although regulations are still lack in Argentina, and other regulations cited previously do not apply specifically for gastropods, it is evident that PAH concentrations registered in this work exceeded some criteria established worldwide. For instance, Fluoranthene had higher concentration than the EAC value established ($110\,\mu g\,kg^{-1}$ dw) for PAHs in mussels and oysters (OSPAR Commission, 2009). The data reported in this study indicates that human consumption of *B. globulosus* collected from contaminated areas should be of concern due to the finding of carcinogenic compounds in their tissues. Previous studies performed in the same harbor zone, have demonstrated imposex presence in *B. globulosus* (Bigatti et al., 2009), reproductive alterations (Primost et al., 2015a), oxidative stress (Primost et al., 2015c) and bioaccumulation of heavy metals as Cd and Pb (Primost et al., 2017) making the situation more complex from the point of view of the health of the organisms and their consumers, including the human population. Regarding B[*a*]P, this had concentrations lower than the limit of detection in gastropods although was present in sediments (Table 1). European Commission regulation (EC) N° 1881/2006 stated maximum levels for certain contaminants in food stuffs, establishing a maximum level of B[*a*]P of $10\,\mu g\,kg^{-1}$ ww for mollusk bivalves (fresh, chilled or frozen). The finding of B[*a*]P in sediment of the harbor zone may be a risk of future bioaccumulation in marine organisms.

It is know that imposex occurrence is related to the presence of TBTs in the environment. In fact, various studies have reported TBT pollution in sediments and gastropods in Puerto Madryn's harbors nearby areas (Bigatti et al., 2009; Del Brio et al., 2016), and gastropods studied in these zones presented 100% imposex incidence. Although experiments of imposex induction in gastropods exposed only to PAH have shown that these compounds have not been effective in the development of this phenomenon (Maran et al., 2006), it should not be discarded a synergic effect between PAHs and TBTs affecting the health of gastropods and their consumers. TBT may affect the mixed function oxygenase (MFO) system that plays a key role in the metabolism of xenobiotics (e.g. PAHs), as well as endogenous compounds such as fatty acids and hormones. Therefore, any interaction with this system may have consequences on the animal's reproductive capacity and its ability to deal with other contaminants (Cravo et al., 2012). TBT may interact with PAHs, providing lower biomarker response than what could be anticipated, due to a blockage response (Solé, 2000). Regarding gastropods studied here, in which levels of PAH4 in its tissues have been exceeded international regulations, and TBT acting concurrently, adverse effect could be expected. In addition, multiples stressors can be combined with pollutants to compromise the immune system of marine biota and deleterious effects can be observed in future populations inhabiting harbor areas (Holmstrup et al., 2010; Lawes et al., 2016).

The results obtained indicate that gastropods (and other fishing resources) inhabiting near urbanized areas should not be consumed without an adequate monitoring, particularly in areas where PAHs and TBT pollution is highly probable. Future work must be performed to determine if any interaction between both contaminants could be negative to the biota. In addition, is mandatory that clear rules about marine food and food quality for human consumption are created in Argentina and other countries lacking it, performing studies on contaminants and monitoring areas where recreational, artisanal, and commercial fishing is performed.

In conclusion, as the marine gastropod *Buccinanops globulosus* is a fishery resource that is consumed since pre-Hispanic times and is actually commercialized internally and exported to Asia, special consideration should be made in relation to PAHs levels; particularly, monitoring of Benzo[*a*]pyrene and PAH4 in gastropods collected in harbor areas. Bearing in mind that this species and other with similar characteristics are consumed with a major frequency and in greater quantity in other regions of the world, integral studies of potential pollutants and its interaction (e.g. PAHs and TBTs) should be taken into account when, regulations for the management of fishery resources with relevance to health human, are established. These are the first results reporting levels of PAHs in edible gastropods of South America.

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