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Fatty acids and contaminants in edible marine gastropods from Patagonia

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Edible marine gastropods are consumed in northern Patagonia without regulations or knowledge of nutritional composition and food quality. We determined total lipids (TL), fatty acid (FA), paralytic shellfish poison (PSP) and metal contamination in six edible marine gastropods: Buccinanops globulosus, B. cochlidium, Trophon geversianus, Odontocymbiola magellanica, Tegula patagonica and Nacella magellanica. TL was lowest in the foot (0.29–0.56%) and maximum in organs (1.43–3.2%), presenting less TL than other edible species around the world. Saturated FA were similar to other consumed marine gastropods worldwide and dominant in all species studied (33.26–48.19%), while monounsaturated FA reached about 30% only in 2 species, but did not exceed 18% in the other species. Polyunsaturated FA reached up to 23.77%, but generally did not exceed 14%. Highly unsaturated FA (AA, EPA and DHA), reached about 27%. The dominant FA was palmitic acid (0.217 to 2.43 $\mu\text{g mg}^{-1}$). Differences in FA could be related to the different alimentation of the species. Limit of PSP consumption was exceeded in a few months only for two species. Lead was not detectable ($<4 \mu\text{g g}^{-1}$) while cadmium ranged from 0.07 to 15.32 $\mu\text{g g}^{-1}$. Our results can be useful to ensure the safety food of these resources, which are being consumed and exported in low quantities in Argentina, but may be commercialized massively in the near future. Monitoring these and other food resources is essential and should be performed in any established fishery of the species reported here to guarantee the safety human consumption.

Keywords: fatty acids, paralytic shellfish poison, edible gastropods, food quality, lipids, marine resources

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INTRODUCTION

The demand for high protein food is increasing, especially in developing countries (Woodcock & Benkendorff, 2008), encouraging the exploration of unconventional or underutilized resources. Several studies highlight the importance of marine resources and, particularly, marine gastropods on human diet (Manzano & Aranda, 1998; Leiva & Castilla, 2002; Vasconcelos *et al.*, 2008). Marine gastropods represent high values on international markets, playing an important social role in artisanal fisheries (Leiva & Castilla, 2002; Vasconcelos *et al.*, 2008) and contain low proportions of saturated fatty acids and high levels of unsaturated ones that are the most favourable for a healthy diet (Rudin, 1982; Isay & Busarova, 1984; Manzano & Aranda, 1998; Valenzuela & Nieto, 2003; D'Armas *et al.*, 2010). Nevertheless, many gastropods could be unsafe for human consumption due to their ability to accumulate contaminants.

In Argentina, official gastropod landings have been reported since 1936 (Sánchez *et al.*, 2012) but no catch

regulations have not yet been implemented. In Atlantic northern Patagonian coasts, gastropods consumption and exploitation occur locally (Bigatti & Ciocco, 2008), and commercialization to national and international markets is starting to develop but without any official regulation. Therefore, many efforts have been made in order to increase information and studies aimed to propose gastropod fishery policies in this region (Bigatti *et al.*, 2007, 2008; Bigatti & Ciocco, 2008; Penchaszadeh *et al.*, 2009; Averbuj *et al.*, 2010; Cumplido *et al.*, 2010, 2011; Zabala *et al.*, 2013; Averbuj *et al.*, 2014). Some gastropods, such as *Odontocymbiola magellanica*, *Buccinanops cochlidium*, *Buccinanops globulosus* and *Trophon geversianus*, are seen as potential species for consumption and massive commercialization (Narvarte, 2006; Bigatti & Ciocco, 2008; Narvarte *et al.*, 2008; Averbuj *et al.*, 2010; Cumplido *et al.*, 2010). Small gastropod species such as the genus *Tegula* and limpets of the genus *Nacella* are exploited in Chile and the latter is captured manually in the Atlantic coasts from 43°S (Conti *et al.*, 2012a). Although those resources are abundant in all the Patagonian coasts, gastropod fisheries are restricted by weather conditions, closures by red tide and fluctuations (Elías & Pereiro, 2003), while contaminants accumulated in the edible portion could lead to human intoxication or diseases.

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Oceans contain traces of more than 30 chemical elements resulting from anthropogenic activity (Pérez *et al.*, 2011), and molluscs are biological monitors of seawater metal pollution due to their capacity to accumulate (Rainbow, 1995; Ahn *et al.*, 1996; Gil *et al.*, 2006; Conti *et al.*, 2010; Giarratano *et al.*, 2013). Studies in northern Patagonian coasts showed high concentrations of cadmium (Cd) in mussels associated with natural sources (Gil *et al.*, 2006). Cd is a non-essential element and it may be highly toxic even at low concentrations. The effects of intoxication by Cd in humans can affect blood pressure, producing kidney damage, destruction of red blood cells and testicular tissues (Manahan, 2000; Pérez *et al.*, 2011; Conti *et al.*, 2012a). For this reason, metals determination in edible marine gastropod tissues becomes overriding.

Harmful algal blooms (HABs), being toxin producers, are one of the most important problems which affect the shellfishes' resources. Among the genera responsible for HABs, the dinoflagellate *Alexandrium* is one of the most important in terms of the severity, diversity and distribution of bloom impacts (Anderson *et al.*, 2012). *Alexandrium tamarensis* is the most frequently identified species in the north Patagonian coastal waters (Esteves *et al.*, 1992; Andrinolo *et al.*, 1999; Santinelli *et al.*, 2002), where it increases in density during spring and summer. This event is coupled with the accumulation of paralytic shellfish poison (PSP) in the molluscs, that can be fatal for people who consume them. The documented history of HABs occurrence in north Patagonian shores dates back to 1980, when there were two human fatalities due to PSP from an outbreak of *Gonyaulax excavata* (Braarud) Balech (= *Alexandrium tamarensis* (Lebour) Balech) in the tidal front of Valdés Peninsula (Carreto *et al.*, 1981). Since 1980, eight human fatalities and 17 human illnesses have been reported in Chubut coastal waters (Carreto *et al.*, 1981, 1986; Vecchio *et al.*, 1986; Esteves *et al.*, 1992; Andrade, 2001, 2002; Baulde, 2010a, b, 2011). Since 1985, the Ministry of Fisheries of Chubut province have a shellfish sampling programme in the coastal zone, principally in San José Gulf, which is one of the main suppliers of bivalve molluscs in Argentina (Ciocco, 1995; Orensanz *et al.*, unpublished data). Coastal bivalves and gastropods extraction is suspended during the spring and summer seasons due to blooms of *A. tamarensis* (Andrinolo *et al.*, 1999; Reguera, 2002; Gayoso & Fulco, 2006). Consequently, the beds are closed for harvesting when levels of PSP are above the maximum limit of consumption of 800 µg STX eq kg⁻¹ body weight established by the Argentine Food Code.

The aim of this work was to determine lipid concentration, fatty acid (FA) profile and some safety food parameters of six edible gastropod species that are being consumed without government regulation in Patagonian Argentina. Our results are useful to increase the knowledge of the food quality of a new marine resource.

MATERIALS AND METHODS

Sampling

From September 2010 to July 2011, 83 specimens of *Odontocymbiola magellanica*, 149 of *Buccinanops cochlidium* and 644 of *Trophon geversianus* were collected monthly in Playa Villarino (San José Gulf) (42°24'10"S 64°17'26"W) by

scuba diving, and 102 specimens of *Buccinanops globulosus* were captured by baited traps. Additionally, 170 specimens of *Tegula patagonica* and 50 of *Nacella patagonica* were caught monthly by hand-picking from January to December in 2012, in the rocky intertidal zone of Punta Ninfas (42°58'42"S 64°18'33"W) during low tides. Sampling was not done in August 2011 and in May 2012, due to adverse weather conditions (Figure 1). In San José Gulf artisanal fisheries of molluscs have been developed since the 1970s, capturing mainly scallops, but also gastropods of the species studied here. Punta Ninfas is an isolated place with very little human incidence. All the species studied were mature and are being consumed in the Patagonian coast or were established as potential fisheries resources (Bigatti *et al.*, 2015).

Q2

Total lipids

Total lipids were determined using the colorimetric method of Zöllner & Kirsch (1962), with cholesterol as standard. Samples were dried at 60°C to constant weight and ground to a powder in a mortar. About 10 mg samples were weighed and boiled for 10 min in concentrated sulphuric acid. After cooling to room temperature, 50 µl of the solution received 1 ml colour reagent (containing 11.9 mol l⁻¹ phosphoric acid and 8 mmol l⁻¹ vanillin). The absorbance at 530 nm was measured using a SmatSpec 3000 spectrophotometer. Results were expressed as per cent dry weight of tissues. Analyses were performed in triplicate over a composite sample for each species. Precision values, expressed as the coefficient of variation were: *T. geversianus*: 13.1%; *B. cochlidium*: 9.8%; *B. globulosus*: 26.2%; *O. magellanica*: 2.0%; *N. patagonica*: 10.9% and *T. patagonica*: 13.1%. In order to test accuracy, a combined sample of individuals of *O. magellanica* was submitted to addition of 5 ml (4.432 g) of olive oil to 50.0 g dried ground sample and the added sample was split into six replicates. The replicates were then analysed following the same method and the percentage of recovery was 110%.

Fatty acid profile

This analysis was performed over a composite sample for each species. About 30 mg of sample were subjected to methanolysis at 100°C for 1 h, with acetyl chloride/methanol reagent, according to Lepage & Roy (1986). Previously, a solution of tricosanoic acid (C_{23:0}) in toluene was added to each sample as internal standard, making 120 µg per sample. After cooling to room temperature, 5 ml of 6% K₂CO₃ aqueous solution was added. Fatty acid methyl esters (FAME) were extracted with n-hexane and separated by centrifugation, and the FAME samples were preserved in glass vials under N₂ at -80°C. They were quantified using a gas chromatograph (Trace GC Ultra, Thermo Electronic Corporation) equipped with a mass spectrometer detector, split-splitless injection system and automatic injector (Triplus AS). FAME peaks were identified by comparing their retention times with those of 37 authentic FAME standards (Supelco Inc.). The mass spectra of FAME not present in the standard mix were compared with those from the National Institute of Standards and Technology mass spectra library (NIST) and Lipid Library data (Christie, 2012). Quantification was done in SIM mode, using the Supelco mixture of FAME (CRM47885) making a calibration curve with the standards (5 points). To quantify recovery,

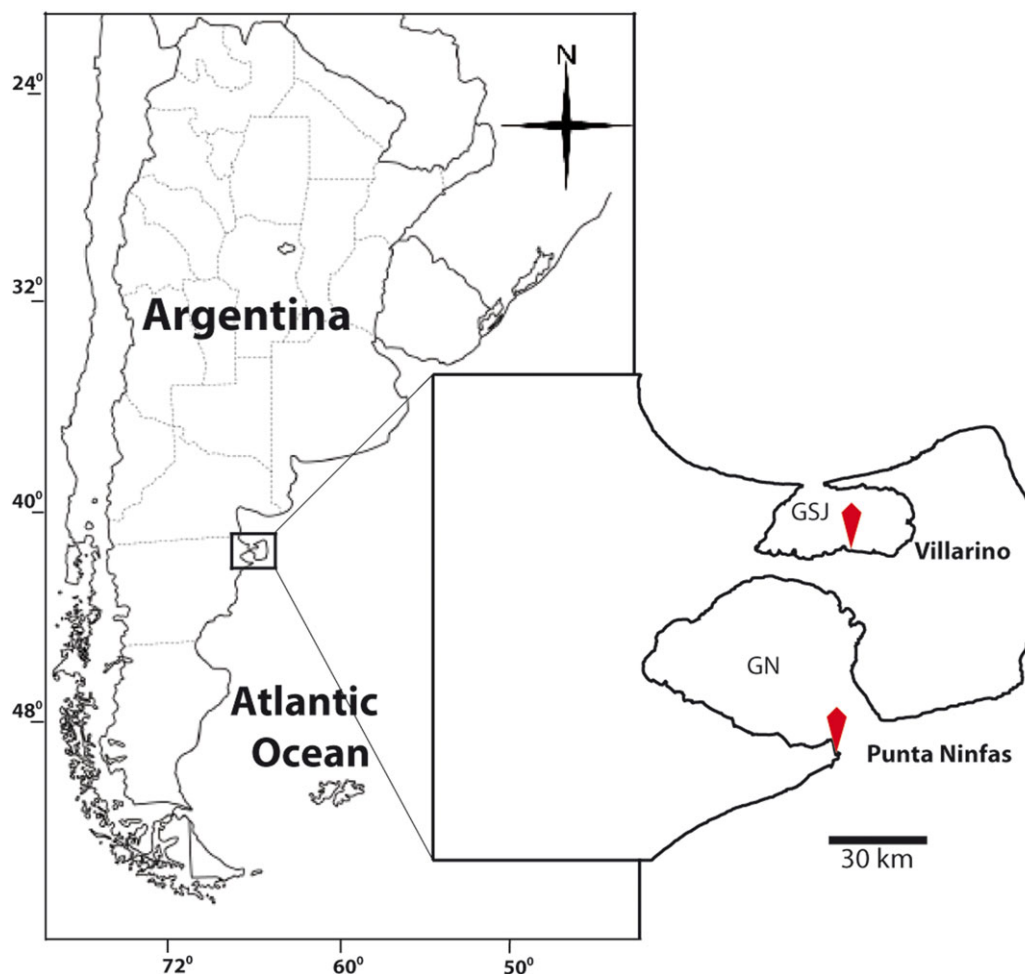


Fig. 1 - Colour online, B/W in print

Fig. 1. Sampling areas, Golfo Nuevo (GN): Punta Ninfa; Golfo San José (GSJ): Villarino.

2-Br-hexanoic acid methyl ester was added, and the recovery was 72%. Results are presented in per cent abundance of total FAME and in concentration in the tissues, in $\mu\text{g mg}^{-1}$. FA were grouped into saturated FA (SFA: FA with no double bond in the carbon chain), monounsaturated FA (MUFA: FA with one double bond in the carbon chain), polyunsaturated FA (PUFA: FA with two or three double bonds in the carbon chain) and highly unsaturated FA (HUFA: FA with four or more double bonds in the carbon chain).

Trace metals analysis

Before analysis, the gastropods were dried at 60°C to constant weight and carefully homogenized. Approximately 0.5 g of dried tissue was placed in a crucible in a muffle furnace and the temperature was slowly increased from room temperature up to 400°C for 6 h. After the sample was cooled down, 2 ml of concentrated HNO_3 (Merck, PA) were added and evaporated to dryness on a sand bath at 80°C . This procedure was repeated until white ashes were obtained and then were resuspended with a mixture of HNO_3 (3% v/v) and HCl (6% v/v) (Merck, PA) up to 10 ml (BOE, 1991). Two blanks were also prepared as samples. Measurements were performed in an IL-457 atomic absorption spectrophotometer with air-acetylene flame. Results are expressed in $\mu\text{g g}^{-1}$ wet weight ($\mu\text{g g}^{-1}$ ww).

Reference material NIST-SRM 1566a (oyster tissue) was used for the quality control of trace metal analysis. The precision for both metals, expressed as coefficient of variation, was below 7%. The accuracy, expressed as percentage of recovery, was 90 and 102% for Pb and Cd respectively. Detection limits were 0.8 (Pb) and 0.05 (Cd) $\mu\text{g g}^{-1}$ ww.

Paralytic shellfish poison

The presence and concentration of paralytic shellfish poison (PSP) was determined monthly by mouse bioassay following the protocol of the AOAC (1995) on whole organisms of each species. Each sample was analysed in the Laboratory of Food Science of the Provincial Department of Environmental Health (Secretaría de Salud, Chubut-Argentina). The bioassay requires more than 100 g of wet sample. Thus, the analysis could not be performed in *B. globulosus* because of the low catches.

RESULTS

The mean shell length for each species was 14.47 ± 1.23 cm for *O. magellanica*, 8.09 ± 0.76 cm for *B. cochlidium*, 3.14 ± 0.59 cm for *B. globulosus*, 3.34 ± 0.69 cm for *T. geversianus*, 2.78 ± 1.15 g for *T. patagonica* and 3.03 ± 0.31 cm

for *N. magellanica*. On the other hand, the mean weight for each species was 324.94 ± 91.37 g for *O. magellanica*, 60.41 ± 17.60 g for *B. cochlidium*, 6.13 ± 2.85 g for *B. globulosus*, 6.05 ± 3.78 g for *T. geversianus*, 3.5 ± 1.2 g for *T. patagonica* and 10.8 ± 3.8 g for *N. magellanica*.

Total lipids

Total lipid concentration was higher in the whole body of *N. magellanica* (9.06%) while the foot of the other species showed the lowest values (0.29–0.56%) and the organs, 1.43–3.2% (Table 1).

Fatty acid concentration

Species differed significantly in the concentration of the main FA. *Nacella magellanica* showed concentrations about five times higher than those of *T. geversianus*, *B. cochlidium*, *B. globulosus* and *O. magellanica*, and about twice higher than those of *T. patagonica*. Even when HUFA abundance was higher in *T. geversianus*, *B. cochlidium*, *B. globulosus* and *O. magellanica*, the concentrations of the nutritionally important HUFA AA and EPA were significantly higher in *N. magellanica* (Table 2).

Considering relative abundances over the total of FA, SFA were the dominant FA class in all the species, ranging from 33.26% in *N. magellanica* to 48.19% in *O. magellanica* (Table 2). The dominance of SFA was due to the high abundance of palmitic acid (C16:0), which was the major FA in the six species, ranging from 17.72% in *T. geversianus* to 28.12% in *T. patagonica*. The second SFA in abundance was stearic acid (C18:0) ranging from 13.49% in *T. geversianus* to 18.56 in *O. magellanica*. However, it reached low abundances in *N. magellanica* (4.01%) and in *T. patagonica* (6.41%).

MUFA were abundant in *N. magellanica* (30.70%) and in *T. patagonica* (28.68%) and showed the minimum value in *T. geversianus* (9.03%) (Table 2). In this FA class, the common oleic acid (C18:1(n-9c)) showed variable abundances: 1.80% in *O. magellanica*, 4% in *T. geversianus* and *B. cochlidium* and it reached 7.74% in *T. patagonica*. Strikingly, it was not found in *N. magellanica*, which, instead, showed a high abundance of its *trans*-isomer, elaidic acid (C18:1(n-9t)). Another remarkable MUFA was C20:1(n-9), which showed high abundances in *B. cochlidium* (6.13%), *B. globulosus* (9.46%) and *O. magellanica* (9.22%) but had low abundance in *T. patagonica* (2.54%) and was not detected in *N. magellanica*. C22:1(n-11) was detected only in *T. geversianus* (2.04%) and in very low abundance in *N. magellanica* (0.11%). Two of the three unidentified C22

MUFA (which differed in their retention times as eluted from the chromatographic column) were found in fairly high abundances (ranging from 4.51% in *B. globulosus* to 9.02% in *T. geversianus*) except in *N. magellanica*.

PUFA were abundant, 23.77% in *T. geversianus* and in *N. magellanica* 19.06%, and showed low values in the other species (Table 2). It was remarkable that the essential FA linolenic acid (C18:3(n-3)) was not detected in any of the species, whereas its precursor, linoleic acid (C18:2(n-6)) was present in low percentages in all the species (ranging from 1.14% in *O. magellanica* to 2.64% in *B. globulosus*).

HUFA were the second class in abundance in *T. geversianus*, *B. cochlidium*, *B. globulosus* and *O. magellanica* (around 27%) while they were lower in *N. magellanica* and *T. patagonica* (around 16%) (Table 2). The high values of HUFA were due to the fairly high abundances of arachidonic acid (AA, C20:4(n-6)) and eicosapentaenoic acid (EPA, C20:5(n-3)) in most species and of C22:5(n-3) in *B. cochlidium*, *B. globulosus* and *O. magellanica*. AA abundances were high in *T. geversianus* (11.64%, *B. globulosus* (9.25%) and *O. magellanica* (9.98%)), while they did not exceed 7% in the other two species. EPA abundances were about 9% in *T. geversianus*, *B. cochlidium* and *N. magellanica*, but very low in *O. magellanica* (2%). Only *T. geversianus* showed a fairly high abundance of docosahexaenoic acid (DHA, C22:6(n-3)).

As for families of FA, (n-6) FA were dominant in *T. geversianus*, while (n-3) FA were dominant in *B. cochlidium* and both families showed similar levels in the other species. (n-9) FA were dominant in *T. patagonica*, showed high values in *B. globulosus* and *N. magellanica* but showed low values in *T. geversianus*. (n-7) FA were relevant only in *T. patagonica* and only one (n-11) FA was present in *T. geversianus* and in very low values in *N. magellanica* and *T. patagonica* (Table 2).

Trace metals analysis

Lead (Pb) was not detectable ($<0.8 \mu\text{g g}^{-1}$ wet weight) in any gastropod species analysed. On the contrary, cadmium (Cd) was detected in all the species, being more concentrated in organs than in the foot (Figure 2).

A maximum weekly consumption of foot was established as 5800 g for *O. magellanica*, 1450 g for *B. cochlidium*, 193 g *B. globulosus* and 290 g for *T. geversianus*, relative to the trace metal concentration for each species.

Paralytic shellfish poison

Organs of *O. magellanica* accumulated PSP exceeded the maximum limit of consumption (MLC = $800 \mu\text{g STX eq kg}^{-1}$ snail flesh) in January ($1100 \mu\text{g STX eq kg}^{-1}$), February ($1020 \mu\text{g STX eq kg}^{-1}$) and June ($947 \mu\text{g STX eq kg}^{-1}$); while in the foot the limit was exceeded only in February ($838 \mu\text{g STX eq kg}^{-1}$). The accumulation of PSP in organs of *B. cochlidium* in spring and summer exceeded the limit in December with $4805 \mu\text{g STX eq kg}^{-1}$, while in the foot there was no accumulation of PSP, its consumption being suitable throughout the year. The whole body of *T. geversianus* accumulated PSP below the maximum limit in October, December, April and May, meaning it is suitable for consumption throughout the year. PSP was not detected by bioassay in *T. patagonica* and *N. magellanica*.

Table 1. Per cent total lipid concentration (dry weight \pm SD).

Species	% Lipids		
	Organs	Foot	Entire body
<i>T. geversianus</i>	1.43 \pm 0.06	0.29 \pm 0.23	
<i>B. cochlidium</i>	3.20 \pm 1.42	0.56 \pm 0.83	
<i>B. globulosus</i>	2.06 \pm 0.18	0.37 \pm 0.13	
<i>O. magellanica</i>	1.52 \pm 0.92	0.30 \pm 0.11	
<i>N. magellanica</i>			9.06 \pm 1.0
<i>T. patagonica</i>			3.61 \pm 0.47

Table 2. Percentage of FA classes and families, expressed over the total FA in a composite annual sample.

% Abundance FA	<i>T. geversianus</i>	<i>B. cochlidium</i>	<i>B. globulosus</i>	<i>O. magellanica</i>	<i>N. magellanica</i>	<i>T. patagonica</i>
SFA	38.47	45.76	44.48	48.19	33.26	47.25
MUFA	9.03	11.59	17.13	11.58	30.70	28.68
PUFA	23.77	13.61	12.55	13.78	19.06	8.62
HUFA	28.73	29.05	25.85	26.44	16.97	15.44
(n-3)	15.97	20.72	13.61	13.43	16.30	8.32
(n-6)	27.40	14.66	15.35	14.39	9.82	8.84
(n-7)	*	1.34	2.18	0.16	1.52	5.11
(n-9)	5.12	10.70	14.99	11.53	14.76	22.30
(n-11)	2.04	*	*	*	0.11	0.06

*Non detected.

DISCUSSION

Marine gastropods are consumed around the world, with England, France, Mexico and Korea being the countries where the largest catches were recorded in recent years (FAO, 2015). Although in Argentina the resource has been commercially captured and declared since 1936 (Sánchez *et al.*, 2012), it is consumed in low quantities in the local market but exports are reported. The implementation of new resources for the fishery is important to the regional economies, for the export of the resource and for human consumption.

Lipids plays an important role in an animal's life history, due to their structural function in cells as a substrate to catabolism (Wiegand, 1996). The percentage of body lipids are important in molluscs such as mussels because biochemical and gametogenic cycles are closely associated (Bayne, 1976). The percentage of lipids detected in the edible tissues of the species in this study (0.29–9.06%), was similar to that detected for commercial species worldwide (0.70–7.0%), such as *Strombus gigas*, *Strombus gracilior*, *Thais haemastoma*, *Nacella macquarensis*, *Haliotis varia*, *Bursa spinosa*, among others (Belisle & Stickle, 1978; Simpson, 1982; Aranda & Marrufo, 1999; Najmudeen, 2007; Babu *et al.*, 2011).

Lipid deposition and metabolism are closely connected with the biogenesis of some specialized membranes, such as myelin, clearly indicating a role for lipids in neural function (Sastry, 1985; Akhlaq *et al.*, 2000). The polyunsaturated and highly polyunsaturated fatty acids (PUFA and HUFA) have essential properties in human health, in particular ω -6, arachidonic acid, and ω -3, docosahexaenoic acid, are fundamental in the formation of the structure and the function of

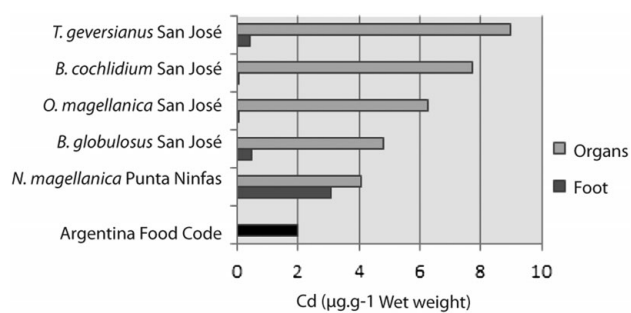
the human nervous and visual systems (Sastry, 1985; Valenzuela & Nieto, 2003). All species under study had SFA as the main FA class, as these FA accounted for no less than 33% of total FA. The most abundant FA in all species was palmitic acid. The FA profile of the species under study differed in the degree of unsaturation. *Trophon geversianus* had the most unsaturated profile, since PUFA plus HUFA accounted for more than half of the total FA. However, this species had one of the lowest total FA concentrations; although these FA were relatively abundant, their actual concentrations in the tissues were low and their importance as essential FA suppliers could be minimal for human health. On the other hand, the sum of the abundances of PUFA plus HUFA in *N. magellanica* hardly reached 36% of total FA. However, the concentration of AA nearly doubled that of *T. geversianus*, where AA was relatively more abundant. A similar situation was found for EPA: the highest relative abundances of this FA were found in *T. geversianus* and in *B. cochlidium*. However, the actual concentration of EPA in these species was nearly four times lower than that in *N. magellanica*. Other FA important for human food are the essential oleic, linoleic (ω -6 unsaturated) and α -linoleic acids (ω -3 unsaturated), because mammals cannot synthesize them from carbon precursors and they are precursors to the biosynthesis of the longer chained polyunsaturated fatty acids (Mahaffey, 2004). *Nacella magellanica* had the highest concentration value of linoleic acid and was the only species that had linolenic acid in the FA profile, however in very low concentration. On the other hand, oleic acid was not detected in this species, whereas it showed high concentration of the *trans* isomer, elaidic acid.

The phylum Mollusca displays a variety of lipid and fatty acid components (Ackman *et al.*, 1971; Joseph, 1982). Babu *et al.* (2010) found a similar distribution of FA classes to that reported in this work in the marine gastropod *Bursa spinosa*, with preponderance of SFA, followed by polyunsaturated FA (comprising what we consider PUFA plus HUFA). The dominant FA in that species was palmitic acid, with high abundances of EPA. A study reporting on *Patella depressa* found the same pattern, a preponderance of SFA (Morais *et al.*, 2003).

A dietary source of ω -3 fatty acids is needed, since human enzyme systems cannot insert a double bond closer than the ω -9 position of a fatty acid of 18 carbons in length (Mahaffey, 2004). Linoleic acid cannot be synthesized by humans and a lack of it results in adverse clinical symptoms, including a scaly rash and reduced growth (IOM, 2001).

Q3

Fig. 2 - B/W online, B/W in print

**Fig. 2.** Cadmium concentrations in gastropods from Golfo San José and Punta Ninfa.

Taking into account the high concentration of FA in *N. magellanica*, this species is the most suitable to be considered as a good human dietary supplement of essential FA.

Molluscs may accumulate different pollutants from the environment. Among them, Cd and Pb are two of the inorganic contaminants for which concentrations are limited by national and international regulations aimed at protecting biota and human health (SENASA, 2000; MERCOSUR, 2011). While the consumption of some species of marine gastropods has been growing in the region in recent years, the study of the presence of trace elements in their tissues is still limited (Primost, 2014). These authors have detected some metals (aluminium, iron, zinc, copper, cadmium and lead) in the edible marine gastropods *Adelomelon ancilla*, *Buccinanops globulosus* and *Trophon geversianus* and their surrounding sediments and prey in Nuevo Gulf. In this research we analysed Pb and Cd levels in five different species, four from San José Gulf and one from Punta Ninfa, where human activities are incipient or null. Pb was undetectable in all samples, indicating that regarding this element, consumption of this species is not harmful for humans. However, Cd exceeded the maximum allowable limit by the ACC ($2\mu\text{g g}^{-1}$ wet weight) in all organ samples and in foot samples of *N. magellanica* (Punta Ninfa). Bioavailability of this metal in Patagonian coastal waters has already been reported and attributed to natural sources from the upwelling process. Cd retained in biota is generally stored in metallothioneins (MT), due to their high cysteine content. These low molecular weight and non-enzymatic proteins play a key role in the homeostatic control of the essential metals Zn and Cu (necessary to fulfil metabolic demands), but Cd and other elements are able to displace them. In this way, MT is also thought to be involved in the detoxification of non-essential trace metals and excess amounts of essential ones. Expression of MT genes shows interspecific diversity and this would influence Cd storage. In mammals, Cd is sequestered almost exclusively in liver and kidney, but in invertebrates Cd-binding proteins appear to be distributed in several tissues, including the foot (Amiard *et al.*, 2006). Different authors have reported MT expression in the foot of different gastropods, such as the marine gastropod *Littorina littorea* (English & Storey, 2003), the freshwater gastropod *Melanopsis dufouri* (Ureña *et al.*, 2010) and the terrestrial gastropod *Helix pomatia* (Chabicoovsky *et al.*, 2003). The uptake of metals through the foot may be mainly via diffusion, however there is evidence of active take up by snails through the cells in the epithelial tissues of the foot. In general, the accumulation of Cd in the foot of gastropods is lower than in the visceral complex where organs such as the gills, digestive tract, digestive gland and the kidney comprise the entry, accumulation and detoxification sites of the metals (Bebiano & Langston, 1992, 1998; Langston *et al.*, 1998). The foot is composed of muscle and nervous tissue, so its participation in metal metabolism is much lower (Ureña *et al.*, 2010). In agreement with this, the ratio of Cd concentration for organ/foot obtained in this study was between ~ 10 and ~ 400 in species from San José Gulf and ~ 1 in gastropods from Punta Ninfa. The different patterns are probably related to the different environmental conditions of both sites and to specific characteristics of each species (animal size, growth rate, nutritional state, prey type and physiological condition). On the one hand, and contrary to San José Gulf stations, Punta Ninfa is directly influenced by

the open ocean. Gastropods exhibit a wide range of feeding behaviours, making a generalization difficult even within the same family. The herbivorous gastropods are dominant grazers in Patagonian rocky intertidals and present a characteristic radula which allows the penetration of hard substrates (Hawkins *et al.*, 1989). *Tegula patagonica* generally feeds on the biofouling adhered to the seaweed *Undaria pinnatifida* (Teso *et al.*, 2009) and other algae, whereas *N. magellanica* feed over the rocky shores biofilm (Nieto Vilela, 2013), like many intertidal herbivores (Hawkins *et al.*, 1989). Gastropods as *N. magellanica* could accumulate concentrations from tens (muscle) to hundreds of thousand times (organs) higher levels of metals than those present in the seawater (Conti *et al.*, 2012b). This is probably related with the alimentation of the species: it is known that *N. magellanica* feeds on the biofilm present on the hard bottoms this species inhabits (Nieto Vilela, 2013), and it is known that this is a source of concentration of many metals (Arribere *et al.*, 2010).

Beyond the reported distribution patterns between foot and organs, it is clear that human intake of gastropods may be related to the foot as well as to the whole soft tissues. Therefore, and on the basis of our results, the biomonitoring of Cd concentration in the analysed gastropods is highly recommended.

The accumulation of PSP in the tissues of gastropods coincided with the frequency of the harmful algal blooms, these being between late winter/early spring and late summer/early autumn in the southern hemisphere (Santinelli *et al.*, 2002). The feeding habits and habitat of the gastropods suggested that the primary source of the toxins was a benthic organism (Kotaki *et al.*, 1981). In Patagonia, PSP accumulation in carnivorous gastropods (*O. magellanica*, *B. cochlidium*, *B. globulosus* and *T. geversianus*) could be related to the ingestion of bivalve prey, such as *Aequipecten tehuelchus*, *Ensis macha*, *Leukoma antiqua*, *Mytilus edulis* and *Brachidontes* spp. In herbivorous gastropods (*N. magellanica* and *T. patagonica*), this accumulation occurs due to browsing over biofouling with accumulated cysts. Since *T. patagonica* scraped off biofouling, it could accumulate PSP by ingesting *A. tamarensis* cysts trapped in the biofilm. However, this toxin was not detected in *T. patagonica* during the period of sampling although closures were established for the harvesting of bivalves and gastropods in Chubut coastal waters during the sampling period. This could be related to the fact that *A. tamarensis* was not epiphytic, and it was therefore not associated with sheets of macroalgae, thus, cysts were not in contact with the biofilm. On the other hand, although *N. magellanica* feeds on the bottom, toxin was not detected in its body. Probably, the dynamic conditions of Punta Ninfa, exposed to a regime of constant waves (open sea), made cysts accumulation difficult.

Our results can be used to give guidelines to ensure the safety of these resources as food, since they are being consumed in the zone and may be commercialized massively in the near future. Monitoring these and other food resources is essential and should be performed in any established fishery of the species reported upon here prior to human consumption.

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