Nutritive and Xenobiotic Compounds in the Alien Algae *Undaria pinnatifida* From Argentine Patagonia

**M. N. Gil · A. I. Torres · M. G. Commendatore ·
C. Marinho · A. Arias · E. Giarratano ·
G. N. Casas**

Abstract Seaweeds have been used as food since ancient times. The edible brown algae *Undaria pinnatifida* is native to northeast Asia; however, in 1992, the first specimens in Patagonian environments were found and, since then, have rapidly expanded. The main object of this study was to determine, for the first time in Argentina, the nutritive composition and concentrations of trace elements and hydrocarbons in these alien algae and evaluate their usefulness as food. Sexually mature *U. pinnatifida* samples were collected at 10-m depth in the Nuevo and San José gulfs. The first site is influenced by activities from Puerto Madryn city, and the latter place was considered as the control. Protein, dietary fiber, and mineral concentrations were similar in both gulfs and in the same order as in eastern countries. Crude protein, indigestible fiber, and calcium and magnesium concentrations were greatest in blade; lipid concentration was greatest in sporophyll; and sodium and potassium concentrations were greatest in midrib. Amino acids showed the greatest concentrations in blades, and these were greater than those reported in kelp from Japan. Cadmium (Cd), arsenic, mercury, and hydrocarbons were detected, but only Cd showed concentrations that could be a risk for consumption. In Argentina, maximum acceptable levels of these contaminants in seaweeds are not established.

M. N. Gil (✉) · A. I. Torres · M. G. Commendatore ·
C. Marinho · E. Giarratano · G. N. Casas
Centro Nacional Patagónico, CONICET, Blvd. Brown 2915 -
9120, Puerto Madryn, Chubut, Argentina
e-mail: monicagil@cenpat-conicet.gob.ar

A. Arias
Instituto Argentino de Oceanografía, CONICET, Camino de la
Carrindanga Km 7.5, CC 804, Bahía Blanca 8000, Buenos Aires,
Argentina

The introduction of exotic seaweed species in coastal marine environments is one of many anthropogenic impacts that often produce irreversible changes in the functioning and structure of an ecosystem. The algae *Undaria pinnatifida* (Harvey) suringar (Phaeophyta) is native of northeast Asia (China, Japan and South Korea) where it is commercially cultivated for human consumption. Nowadays, as a result of accidental introductions, *U. pinnatifida* is widely expanded in Europe, Australia, New Zealand, the western United States, as well as in Argentinean Patagonia (Casas et al. 2004; Irigoyen et al. 2011; Piriz and Casas 1994),where it may be found in infralittoral areas (≤22 m depth) as well as in tidal pools. Although in eastern countries its main uses are related to food (fresh or dried), in western countries is primarily used as a thickener and gelling agent (due to high phycocolloids concentrations) in industrial applications, including food (Wong and Cheung 2000).It is also used as feedstock for the production of agar, alginates, and carrageenan (Sánchez-Machado et al. 2004a). For the Japanese population, the daily mean and maximum intake in their diet at 2–3 g and 12 g dry weight, respectively (Almela et al. 2006; Sakurai and Fujioka 1997).

The first specimens of the alien algae *U. pinnatifida* in the northern Patagonian gulfs were found in December 1992 in Nuevo Gulf (NG). Introduction was human-assisted from ballast water of cargo ships or fishing vessels from Asian ports (Piriz and Casas 1994). Since then, it rapidly expanded via spore dispersion by marine currents and drifting of sporophytes within NG, thus showing good acclimatization to its environmental conditions and high capacity to colonize different kinds of substrates (Casas and Piriz 1996). At the present time, the government of the Chubut province is carrying out manual removal of *U. pinnatifida* only in San José Gulf (SJG) to preserve bivalve

mollusks stocks, which have a great economic interest for the local community. Taking into account that the efforts to eradicate this kelp have been considered useless, its importance as a valuable marine resource could be attractive to local industries interested in its commercialization (Casas and Piriz 1996). Since that initial sighting in NG, research has been focused on its biology (Casas and Piriz 1996; Casas et al. 2004), phenology and ecology (Casas et al. 2008; Irigoyen et al. 2011; Raffo et al. 2009), nutrient kinetics uptake (Torres et al. 2004), and use as soil fertilizer (Eyras 2002).

Like other edible algae, *U. pinnatifida* are sources of vitamins, polysaccharides, dietary fiber, minerals, and proteins, and it has low lipid content (Perez et al. 2010; Rupérez and Saura-Calixto 2001; Urbano and Goñi 2002). However, due to its known ability to accumulate pollutants (Almela et al. 2006; Pavoni et al. 2003; Yamada et al. 2007), not only its quality but also its human health safety are of interest when assessing its potential use as food. From a legislative point of view, France, the United States, New Zealand, and Australia have established specific regulations for the use of edible seaweeds in human diet (Almela et al. 2006; Rupérez and Saura-Calixto 2001). In Argentina, seaweeds are not presently a common component in the diet, and thus no regulations have been developed.

Several investigators have studied nutritive and toxic components in *U. pinnatifida* growing in different countries [Japan (Murata and Nakazoe 2001, Spain (Rupérez et al. 2002; Rupérez and Saura-Calixto 2001; Sánchez-Machado et al. 2004a), France (Lahaye and Kaeffer 1997), Germany (Dawczynski et al. 2007), and India (Prabhasankar et al. 2009)], but no related information exists for *U. pinnatifida* growing in Patagonia. Taking into account that the concentrations of these components vary with geographical location, season, and temperature (Sánchez-Machado et al. 2004a), we considered it necessary to evaluate for the first time the nutritional and chemical composition of kelps from NG and SJG. These data will provide a baseline for future legislative regulations.

Materials and Methods

Study Sites and Environmental Parameters

The samplings were carried out between September and November 2009 in the western edge of NG and in the southwest coast of SJG (Fig. 1). Human influence in SJG is restricted to fishermen activities, whereas NG is influenced by Puerto Madryn city, which has approximately 80,000 inhabitants (Instituto Nacional de Estadísticas y Censos 2010). Sampling site in the latter was located near Almirante Storni Port and fishery effluents discharges.

Temperature and dissolved oxygen were measured in situ in bottom and surface seawater using a multi-probe sensor (model 58; YSI, Yellow Springs, OH, USA). Seawater samples from both depths were collected with a Niskin bottle. Once in the laboratory, samples were fractionated for determination of salinity (8410-A salinometer; Portasal, Smiths Falls, ON, Canada), chlorophyll *a*, phaeopigments, and ammonium according to Strickland and Parsons (1972). Nitrate + nitrite, phosphate, and silicate concentrations were determined using a Skalar San Plus auto-analyzer (Breda, The Netherlands) according to Skalar methods (Skalar 2005a, b, c).

Collection and Measurement of Morphological Characteristics of the Plants

Sexually mature specimens were manually collected at ≥10-m depth by SCUBA diving. They were placed in black nylon bags, transported to the laboratory, and processed immediately. Total length (cm) without holdfast, wet weight (g), and width of sporophyll (cm) and midrib
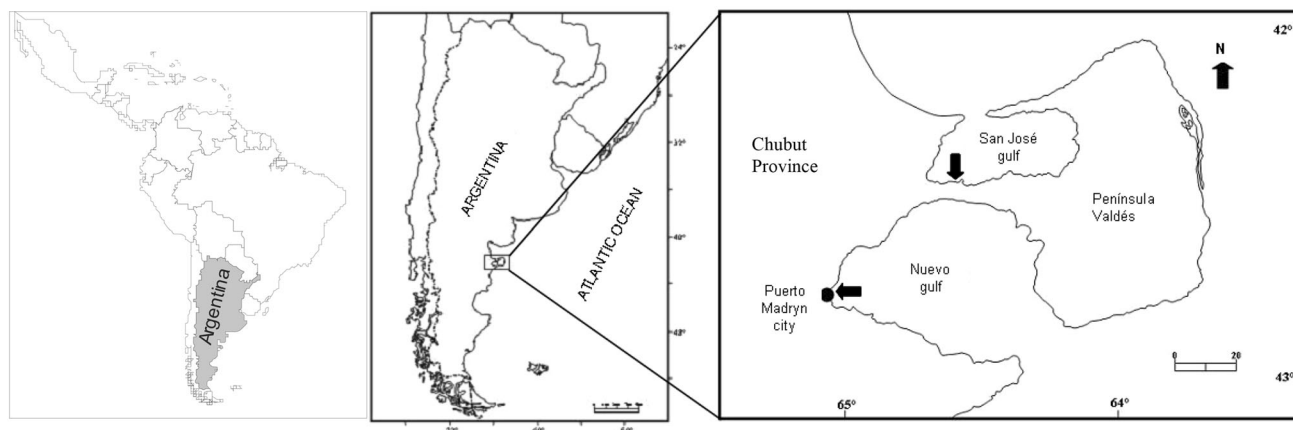

Fig. 1 Location of sampling sites of *U. pinnatifida* in NG and SJG from Argentine Patagonia

(cm) were measured in each plant. The three tissues were separated in plastic trays and rinsed twice with fresh water and distilled water. Afterward they were air-dried under dark conditions on nylon net at room temperature. Then they were crushed in a mill and stored in previously decontaminated glass flasks (calcined and acid-washed) until further analysis. Weight of total crushed air-dried biomass (TB) was calculated as the arithmetic sum of air-dried blade, midrib, and sporophyll weights.

Normality was evaluated using Lilliefors test, and when this assumption was not verified logarithmic transformation was applied (Sokal and Rohlf 1981). Homogeneity of variance was tested by Levene's test, and when this was not verified Mann–Whitney U test was applied (Siegel 1980). Morphometric differences between seaweeds from NG and SJG were tested by Student t test. Differences were considered significant at $p < 0.05$. All statistical analyses were conducted using Statistica software package version 6.0 (StatSoft, Tulsa, OK).

Humidity of Fresh Tissues

Immediately after washing and draining for a few minutes, aliquots of each fresh tissue were oven-dried at 105 °C until constant weight. Humidity was estimated by subtracting the oven-dried weight from the fresh weight.

Analytical Methods Performed on Air-Dried Crushed Tissues

Residual Humidity and Proximal Composition

Residual humidity was determined by oven-drying aliquots of air-dried crushed sample tissue at 105 °C until constant weight and subtracting the oven-dried weight from the air-dried weight. Total ashes was determined gravimetrically after heating oven-dried sample at 550 °C for 12 h in a muffle furnace (Isotemp model 186; Fisher). Total nitrogen was determined by micro-Kjeldahl method. Proteins were determined by multiplying the nitrogen content by a factor of 6.25 (Bremmer and Mulvaney 1982). Insoluble fiber was determined by quantifying the content of lignin, cellulose, and hemicellulose (Van Söest and Robertson 1985). Lipids were determined by applying the modified method of Erickson (1993). Nitrogen-free extract (NFE) was determined by indirect estimation of carbohydrate content, excluding insoluble fiber; it is obtained by subtracting from 100 the sum of the percentages of ashes, proteins, humidity, lipids, and insoluble fiber (Olvera Novoa et al. 1993).

To compare with literature data where results are reported for whole algae, the content of each component in TB was calculated as the weighted sum of each tissue contribution using the following equation:

$$X = \sum (X_t \cdot W_t) \,/\, \text{TBW},$$

where, X is the component concentration in TB; W_t is the weight of tissue t crushed biomass; t is the tissue (midrib, blade, sporophyll); and TBW is the weight of the total crushed biomass.

Free Amino Acids and Vitamins

Samples of blades and sporophylls of plants from SJG were selected for the analysis of free amino acids (AAs) and vitamins. High-performance liquid chromatography was employed for the determination of alanine, glycine, arginine, aspartic acid, glutamic acid, cysteine, histidine + glutamine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, asparagine + serine, threonine, tyrosine, valine, and ornithine (Waters AccQ-Tag Chemistry Package; Manual No. WAT052874, Rev 0, Milford, MA, USA). The B-group vitamins (B1, B2, B3, B6, and B12) were determined according to the method of R-Biopharm (microbiological microtiter plate test); meanwhile, vitamin E and carotene were determined according to the Association of Official Analytical Chemists (2005).

Macrominerals and Trace Elements

Minerals (potassium [K], sodium [Na], calcium [Ca], and magnesium [Mg]) and trace metals (iron [Fe], manganese [Mn], zinc [Zn], copper [Cu], nickel [Ni], cadmium [Cd], and lead [Pb]) were extracted by acid digestion of air-dried sample and measured by flame atomic absorption spectrophotometry (AAS; Instrumentation Laboratory IL-457) (Fleurence and La Coeur 1994). Hg was determined according to modified standard United States Environmental Protection Agency (USEPA) 3050 using an atomic absorption spectrophotometer (Zeeman Z-6100; Hitachi) equipped with a cold vapor-generation system (Standard SM 3500). Total As was analyzed applying modified standard USEPA 3050 and measured by inductively coupled plasma-atomic emission spectroscopy (model 9000; Shimadzu). At least two replicates were run for each sample. Two blanks were run with every sample set and were processed in an identical manner to tissue samples. Reagents of analytical grade were used for the blanks, calibration curves, and samples processing. The standard addition method was employed for control of matrix effect and recovery estimations ($\geq$90 % in all cases). The certified reference material of the aquatic plant *Lagarosiphon major* BCR 060 was used for quality control of trace-metal analysis. The precision for all metals, expressed as coefficient of variation, was between 0.8 and 5.6 %. The accuracy for all metals, expressed as percentage of recovery, was between 87 and 106 %. Detection limits were (μg/g) as

follows: Fe 2.00, Mn 0.13, Zn 0.05, Cu 0.05, Ni 0.50, Cd 0.05, Pb 3.00, As 0.05, and Hg 0.0014. The variation coefficients tested for five replicates of the same samples were always ≤10 %.

Aliphatic and Aromatic Hydrocarbons

Hydrocarbon Soxhlet extraction from algae tissues was carried out according to United Nations Environment Programme/Intergovernmental Oceanographic Commission/International Atomic Energy Agency (UNEP/IOC/IAEA) (1992) protocol, and separation into aliphatic and aromatic fractions in alumina microcolumn was based on United Nations Organization for Education, Science and Culture (1982). An additional clean-up step was performed in alumina column (10 g totally activated) eluting with 100 ml of dichloromethane. Fractions were analyzed by gas chromatography/flame ionization detection (trace gas chromatographer; Ultra-Thermo Scientific). Hydrocarbons were identified by retention time (RT) of authentic external standard mix (Chem Service >95 % pure) and quantified by means of the response factor of each individual compound. PAH identification was confirmed by gas chromatography (GC)-mass spectrometry (MS; HP68906C-HP5972). The percentage of relative deviation (RD), considering both individual aliphatic and aromatic hydrocarbons, varied from 0.4 to 9.0 %. Recovery was 95 ± 12 % for n-alkanes in the nC2 to nC35 range and was 84 ± 15 % for PAH. Blank procedures were performed with each batch of five samples. Chromatographic calibration with the standard mixture (five points) was made for both hydrocarbon fractions, and a control standard (5 ng/μl) was injected with each chromatographic sequence. The detection limit for individual compounds ranged from 5 to 10 ng/g dry weight. The following parameters were determined: n-alkanes (n-alk) from n-C10 to n-C35, isoprenoids pristane (Pr) and phytane (Ph), resolved aliphatic hydrocarbons (RAli; n-alk + Pr + Ph), aliphatic unresolved complex mix (AliUCM), total aliphatic hydrocarbons (TAliH; RAli + AliUCM), polyaromatic hydrocarbons (PAH; 16 USEPA priority + 1-methyl naphthalene + methyl phenanthrenes), aromatic unresolved complex mixture (AroUCM), and total aromatic hydrocarbons (TAroH; PAH + AroUCM).

Results and Discussion

Physical and Chemical Parameters in Seawater

Dissolved oxygen in surface and bottom seawater was at saturation level in the two sampled sites. For the other parameters, values in bottom and surface water were, respectively, the following: Temperature: 10.8–11.1 °C in NG and 12.5–13.0 °C in SJG. Salinity was 33.312–33.300 in NG and 33.498–33.310 in SJG. Nutrient concentrations in NG were 0.52–0.14, 0.38–0.13, 1.08–0.99, and 1.85–0.84 μM for ammonium, nitrate + nitrite, phosphate, and silicate, respectively, whereas those in SJG were 0.59–0.67, 0.19–0.13, 0.81–0.85, and 1.06–1.16 μM, respectively. Chlorophyll a concentrations were 2.8 mg/m^3 in NG and 1.9 mg/m^3 in SJG. All measurements of chemical and physical parameters were found within the range of previously reported data for this area and season (Gil and Esteves 2000).

Morphological Characteristics

The number of collected plants was 99 in SJG and 117 in NG. Most of the latter had damaged blades and were strongly epiphyted by bryozoans *Membranipora isabelleana*; on the contrary, SJG plants looked healthy. Average weights of the plants were not statistically different between gulfs. The greatest weight per plant was 1.117 g in NG and 983 g in SJG, with both of them related to the greatest sporophyll width (10–10.5 cm, respectively). Significant differences were observed in mean values of total length (SJG 104.4 ± 10 cm, NG 97.3 ± 18 cm, $p < 0.01$), midrib width (SJG 2.10 ± 0.6 cm, NG 1.44 ± 0.5 cm, $p < 0.05$), and sporophyll size (SJG 5.5 ± 1.7 cm, NG 6.11 ± 1.54 cm, $p < 0.05$). The width of sporophylls in plants from NG suggested a greater degree of maturity.

The total obtained TB was 3.0 kg in NG and 3.3 kg in SJG. Tissues contributions in TB from NG were 57 % of blade, 14 % of midrib, and 29 % of sporophyll, whereas those from SJG were 63, 1, and 20 %, respectively.

Humidity, Residual Humidity, and Proximal Composition

Water in fresh tissues ranged from 91 to 98 %, whereas residual humidity of TB was 10 % in blade and between 5 to 8 % in midrib and sporophyll. The contents of ash, protein, lipid, fiber, and NFE in each tissue and in TB are shown in Fig. 2a, b, respectively. The same distribution patterns among tissues were observed in both gulfs: the greatest of ash in midrib, greatest of protein and fiber in blade, and greatest of lipid and NFE in sporophyll. Most of the obtained results are within the ranges reported for brown seaweeds in the literature. For example, high contents of ash were found by other investigators in *U. pinnatifida* (≤40 %), which was attributed to ions associated with charged polysaccharides and large amounts of sulphate derived from fucans (Rupérez and Saura-Calixto 2001).

In relation to proteins, contents in red and green seaweeds (15–30 %) are typically greater than in brown
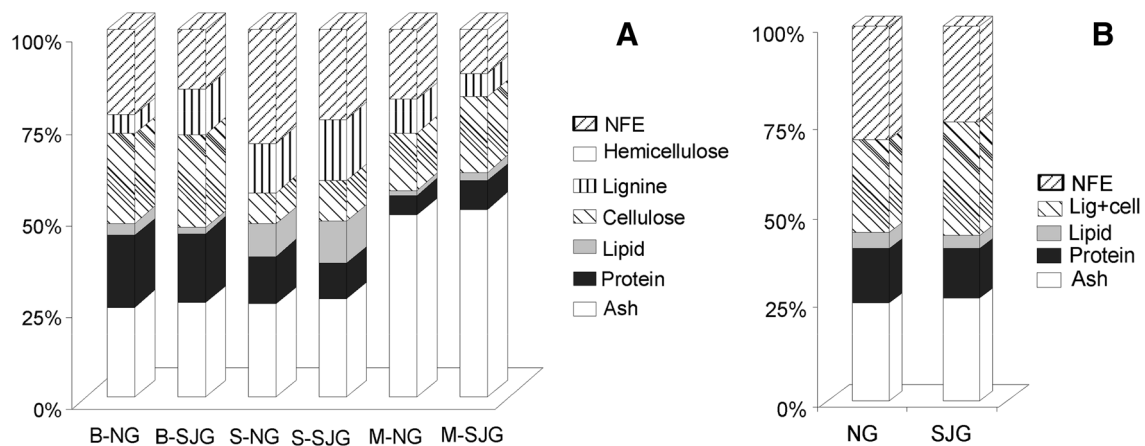
Fig. 2 Nutritive composition (%) in each tissue (*B* blade, *S* sporophyll, *M* midrib) of *U. pinnatifida* from NG and SJG (**a**) and in total biomass (**b**) are shown. Averages in g/100 g are presented

seaweeds. The maximum levels reported for *U. pinnatifida* have been 10–15 % and ≤24 % in plants from aquaculture (Burtin 2003). Quality of protein in this species has been classified as good by comparison with the essential AA contents in reference protein [Food and Agriculture Organization/World Health Organization (FAO/WHO/UNU) 1985]. Digestibility level is still unclear due to the known presence of polyphenols in brown algae (Burtin 2003). According to this study, a daily intake of 2 g TB of *U. pinnatifida* from Patagonian gulfs would be supplying approximately 0.3 g of protein, which is in agreement with the estimation of Kolb et al. (2004).

Compared with other plants, the total amount of lipids in marine algae is relatively low. Nevertheless, these lipids are equally important because they are significant constituents of omega-3 fatty acids, phytosterol, and fat soluble-vitamins (Li 2013). The biological functions of lipids are energy storage, signaling, and structure cell membrane (Fahy et al. 2009). The percentages of lipids were in good agreement with the low expected concentrations in seaweeds. Pérez-Cirera et al. (1997) found 1.6–1.3 % in blade, 0.9–1.2 % in midrib, and 5.9–6.1 % in sporophyll of plants from Galicia, whereas Dawczynski et al. (2007) found <5 % in specimens from Japan and Korea. Fatty acids in marine seaweeds are important for human and animal health because of their particular polyunsaturated components, which have an important role in preventing cardiovascular diseases, osteoarthritis, and diabetes. According to Sánchez-Machado et al. (2004a) and Dawczynski et al. (2007), the major fatty acids constituents in lipids of *U. pinnatifida* are palmitic (16:0) and octadecatetraenoic (18:4 $n-3$) acids. In terms of sterols, the content and kind in seaweeds vary with the species. The predominant sterol in *U. pinnatifida* is fucosterol (84.98 %) followed by 24-methylenecholesterol (14.81 %) (Sánchez-Machado

et al. 2004b). Some researchers have indicated that phytosterols might be able to block cholesterol-absorption sites in the human body (Murata and Nakazoe 2001). This is because the absorption of phytosterols in human diets can result in the increase of hydrophobicity, which will redistribute the equilibrium between phytosterol and micelles, thus leading to the decrease in cholesterol absorption (Lagarda et al. 2006). Further studies are necessary to characterize lipids in *U. pinnatifida* growing in Patagonia.

With regard to insoluble fiber, we found the presence of cellulose and lignin, but hemicellulose was not detected. Dietary fiber is defined as components of vegetal diet that are indigestible for mammals. It consists principally of non-starch polysaccharide. It may also include polyphenols, such as hydrolysable tanines and phenylpropanoids, particularly lignin. According to the literature, insoluble fiber in brown seaweeds is mainly composed of cellulose, whereas soluble fiber is composed of alginates, fucans, and laminarans (Dawczynski et al. 2007; Rupérez and Saura-Calixto 2001). The fiber content of seaweed varieties is greater than those found in most fruits and vegetables. The human consumption of algal fiber has been proven to be health-promoting (Dawczynski et al. 2007). In this study, the highest cellulose levels were found in blade (NG 22 %, SJG 23 %) followed by midrib (NG 19 %, SJG 15 %) and sporophyll (NG 8 %, SJG 11 %). Lignin content was greater than cellulose content only in sporophyll. Estimates of total content of insoluble fiber in TB were 25 and 30 % in NG and SJG, respectively. Rupérez and Saura-Calixto (2001) reported 34 % of total dietary fiber (16 % corresponding to the insoluble fraction) for *U. pinnatifida,* whereas other investigators informed concentrations <7 % (Pérez-Cirera et al. 1997). Our results are rather high compared with these literature data suggesting lower digestibility of the studied plants. Some fibers show certain fermentative capacity in the lower intestine, but

the nature of soluble seaweed fibers is such that their passage through the gastrointestinal tract occurs largely without digestion. In addition, the fibers can increase feelings of satiety and aid digestive transit through their bulking capacity (Brownlee et al. 2005).

The highest content of NFE was measured in sporophylls (NG 29 %, SJG 23 %) and the lowest in midrib from NG (11 %) and blade from SJG (14 %). In TB it ranged between 30 % in NG and 25 % in SJG. These results are on the same order as those reported by Nisizawa et al. (1987). Carbohydrates in brown algae are related to stored carbon, cell wall, and extracellular polysaccharides. The first major storage is the monosaccharide mannitol, and the second one is laminarian (Dawczynski et al. 2007; Murata and Nakazoe 2001). Their cell wall components contain a mixture of sulfated and branched-chain polysaccharides (40–50 %), which are strongly associated with proteins, metal ions, and various other cell wall compounds (Tabarsa et al. 2012). Principal extracellular components are the mucopolysaccharides fucoidan and alginates (Davis et al. 2003). Fucan is a rich sulfated polysaccharide with many biomedical uses such as anticoagulant, antioxidant, and protection against toxic trace metals. Alginates represent a special class of polysaccharides found only in brown seaweeds and constitute >70 % of total *U. pinnatifida* polysaccharides (Billakanti et al. 2013). Alginates are polyurinoids commonly used as thickeners and gelling in food and cosmetic industries. Fucans and alginates abundance is dependent on depth, season, and growth stages. Further research is required to characterize polysaccharides present in *U. pinnatifida* growing in Patagonia.

Free AAs

Free AAs in seaweeds are important in relation to the seaweeds' taste. Results showed that most of them were the highest in blade, with the exception of glycine (similar concentrations in both tissues) and proline (sporophyll content doubled that in blade). Ornithine and AAs containing sulfur (cysteine and methionine) were not detected. The greatest concentrations in both tissues corresponded to alanine followed by glutamic acid. The latter is particularly important because palatability is mainly based on its presence. Our results are in the same order as those found by Nisizawa et al. (1987), except for glycine and proline, which were lower than in plants from Japan; meanwhile, histidine + glutamine and aspartic acid were greater in plants from this study than in those from Japan (Table 1).

Vitamins

Water-soluble vitamins in seaweeds include B group vitamins B1, B2, B3, B6, and B12) and vitamin C (not analyzed

Table 1 Free AA concentrations (mg/100 g) in blade and sporophyll of *U. pinnatifida* from SJG

AAs	Blade	Sporophyll	Nisizawa et al. (1987)
Alanine	745.8	285.3	612.0
Glycine	33.6	34.5	455.0
Arginine	48.7	17.1	36.5
Aspartic	34.8	20.2	5.4
Glutamic acid	140.8	92.2	89.8
Cystine	<0.3	<0.3	3.4
Histidine[a] + glutamine	120.7	67.6	2.1
Isoleucine	22.6	1.7	11.2
Leucine	63	2.2	19.6
Lysine[a]	57.2	2.9	34.6
Methionine	<0.3	<0.3	1.7
Phenylalanine[a]	52.4	6.6	9.2
Proline	6.3	13.8	156.0
Serine + asparagine	127.7	34.2	131.4
Threonine[a]	70.9	16.1	90.3
Tyrosine	26.9	9.7	10.1
Valine[a]	42.3	9.6	11.1
Ornithine	<0.3	<0.3	–

[a] Essential AAs

in this study). Liposoluble vitamins are mainly vitamin E and carotenes. B2 and carotenes predominated in blade, whereas B12 (cobalamine) and vitamin E predominated in sporophyll (Table 2). The other vitamins were similar in both tissues. Vitamin B6 in both tissues and vitamin E in blade were undetected. The concentrations of vitamins B1, B2, B12, and vitamin E in sporophyll were greater in this study than in those reported by other investigators (Table 2). According to Nisizawa et al. (1987), as opposed to processed products, when *U. pinnatifida* is air-dried it retains all vitamins. Among the B group, the B12 vitamin is particularly important considering that higher plants do not contain it. It is recommended in vegetarian diets and in the treatment of the effects of ageing, chronic fatigue syndrome, and anemia (Burtin 2003). Vitamin E is important in relation to its strong antioxidant capability.

Carotenoids are split into two classes, xanthophylls (which contain oxygen) and carotenes (which are purely hydrocarbons without oxygen). They possess an essential role in brown algae as photosynthetic pigments and also can act as antioxidants (Murata and Nakazoe 2001). In humans, they are important sources of retinol (vitamin A).

Macrominerals

Edible marine seaweeds are considered an important source of macrominerals. They are particularly rich in K, Na, Ca, Mg, P, and Fe in comparison with land vegetables

Table 2 Vitamin concentrations in blade and sporophyll of *U. pinnatifida* from SJG and values reported by others investigators (mg/100 g)

Vitamin	Blade SJG	Sporophyll SJG	Vinot and Durand (1986)	Nisizawa et al. (1987)	Kolb et al. (2004)	Recommended dose (mg)[a]	Covered proportion (%)
Vitamin B1	1.2	1.3	0.9	0.3	0.3	1.5[a]	1.6
Vitamin B2	4.7	2.4	1.2	1.1	1.3	1.7[a]	5.5
Vitamin B3	2.2	2.4	2.5	8.0	2.6	19.0[a]	0.2
Vitamin B6	<0.7	<0.7	0.3	ND	0.2	2.0[a]	0.7
Vitamin B12	2.5	3.2	0.4–1.0	ND	ND	2.0[b]	2.5
Carotenes	3.0	2.3	ND	3.3	1.3		
Vitamin E	<3.0	4.1	1.4	ND	ND		

Daily doses of recommended vitamins for adults between 25 and 50 years of age based on daily intake of 2 g of blade are given

ND no data

[a] Kolb et al. (2004)

[b] Handbook on Human Nutritional Requirements (1974)

(Kolb et al. 2004; Rupérez 2002). The concentration of macrominerals K, Na, Ca, and Mg were similar in both gulfs and were in the same order as those reported by other investigators (Table 3). K was present in the greatest concentration followed by Na, Ca, and Mg. Maximum concentrations of Na and K were found in midrib, whereas those of Mg and Ca were grastest in blade. The Na-to-K ratios ranged between 0.22 and 1.11, which is interesting from the nutritional point of view because the intake of sodium chloride as well as diets with a high N:K ratio have been related to the incidence of hypertension (Rupérez 2002). *U. pinnatifida* contains approximately eight times more Ca than milk (Moreiras et al. 2003) and thus is an excellent source of Ca for growing children and for the prevention and treatment of osteoporosis in premenopausal and postmenopausal women (Cofrades et al. 2010). Mg is usually in deficit in the human body (Fernández-Saá 2002). It helps to maintain the electrical potential of nerve and muscle cells; it is needed to synthesize various protein substances; it regulates the heartbeat; and a shortage of it can increase blood pressure (Cofrades et al. 2010). Contributions to the recommended dose of macrominerals assuming a daily intake of 2 g of TB are listed in Table 4.

Nevertheless, Na:K ratios were <1.5 in all of the seaweeds studied (0.33–1.34), which is interesting from the point of view of nutrition because the intake of sodium chloride as well as diets with a high Na:K ratio have been related to the incidence of hypertension.

Trace Elements

Trace elements concentration in seaweeds is the result of their accumulation capacity, which mainly depends on environmental exposure and species (Yamada et al. 2007). In brown seaweeds, this has been associated with the

ability of the negatively charged polysaccharides (alginates and sulfated fucans found in the mucilaginous matrix of the cell wall) to chelate these elements, adsorb and partially exclude mobile anions, and act as cation exchanger. The ultrastructure of alginates (sponge's form) and fucans (polyhedrical form) are responsible for these capacities (Andrade et al. 2004).

Although trace metals have different routes of entry into human body, the most common after occupational exposure is oral intake. In this study, we evaluated some elements that are essential for life but potentially toxic at high concentration, as well as others without biological function but toxic even at low exposure levels (Fig. 3). The maximum values of essential metals, such as Fe, Mn, Zn and Cu, were always measured in blades and in samples from NG. Ni remained within a narrow range (1.7–2.3 µg/g) independently of tissue and site. Concentrations obtained for these five elements were similar to those reported for plants from Spain (Rupérez 2002) and Japan (Kolb et al. 2004).

Elements of particular concern because their toxicities (Pb, Hg, As, and Cd) showed different behavior among each other. Pb was lower than the detection level (3 mg/kg) in all samples and was also lower than allowed levels for human consumption (3–5 mg/kg) (European Commission 2008) and animal foods (5–40 mg/kg) (European Commission 2003). Hg concentrations were ≤0.05 mg/kg, except for that in blade of NG (0.11 mg/kg), which was slightly greater than suggested regulations for human consumption (0.1 mg/kg) (European Commission 2008). The metalloid as presented similar concentrations in both gulfs for each tissue. The mean lowest concentration was observed in midrib (23 mg/kg), and mean levels in blade and sporophyll were 47 and 59 mg/kg, respectively. These values are similar to those reported for *U. pinnatifida* by

Table 3 Average mineral concentrations (g/kg dw), variation coefficient (VC %, $n = 3$), and relative difference (RD %, $n = 2$)

Mineral	NG			SJG			Murata and Nakazoe (2001)[a]	Kolb et al. (2004)[b]	Vinot and Durand (1986)[c]
	Blade	Midrib	Sporophyll	Blade	Midrib	Sporophyll			
K									
Average	46.9	167.8	82.2	53.5	163.9	84.1	55.0	56.9	55.2
VC %	1	2	11	3	3	3	–	–	–
Na									
Average	23.4	37.0	24.4	15.6	39.4	25.5	61.0	64.9	27.4
VC %	5	4	8	4	4	3	–	–	–
Ca									
Average	11.3	8.5	9.9	13.4	9.7	10.3	9.6	9.5	11.9
VC %	3	4	11	4	3	1	–	–	–
Mg									
Average	8.9	6.2	6.2	9.0	5.8	5.6	n/d	4.0	n/d
RD %	1	2	2	3	3	1	–	–	–

[a] *U. pinnatifida* from Japan

[b] *U. pinnatifida* from Japan

[c] *U. pinnatifida* from France

Table 4 Contribution to the recommended dose of macrominerals considering an intake of 2 g/day of *U. pinnatifida* from both gulfs

Mineral	Recommended dose (%)						Recommended dose for adult (mg/day)
	NG			SJG			
	Blade	Midrib	Sporophyll	Blade	Midrib	Sporophyll	
K	3.1–2.3	11.2–8.4	5.5–4.1	3.5–2.7	10.9–8.2	5.6–4.2	3,000–4,000[a]
Na	8.3	13.2	8.7	5.6	14.1	9.1	560[a]
Ca	3.8–2.3	2.8–1.7	3.3–2.0	4.5–2.7	3.3–1.9	3.4–2.0	600–1,000[a]
Mg	5.1	3.5	3.6	5.1	3.3	3.2	350[b]

[a] Kolb et al. (2004)

[b] Vinot and Durand (1986)

Besada et al. (2009). The European Commission (2003) sets a limit of total and inorganic As of 40 and 2 mg/kg dry weight, respectively, in *Hizikia fusiforme* for animal consumption. In France, a limit of 3 mg/kg of inorganic As has been set for the human diet (Burtin 2003). Contrary to organic forms, inorganic As (III and V) is toxic with carcinogenic effects in humans. Almela et al. (2006) determined the following total As content and percentage of inorganic forms in different seaweeds: 89.2–149 mg/kg and 47–80 % for *H. fusiforme*, 4.1 mg/kg and 0.2 % for *Laminaria japonica*, 116.0 mg/kg and 7.1 % for *Eisenia bicyclis*, and 2.4 % for *U. pinnatifida*. Applying this latter value on obtained concentrations in this study, one could estimate an inorganic As content between 0.6 mg/kg (sporophyll) and 1.5 mg/kg (blade), which are lower than the above-mentioned limits. The Joint FAO/WHO Expert Committee on Food Additives (JECFA 1996) indicated a tolerable weekly intake of inorganic As of 15 μg/kg of body weight. Subsequently this dose was decreased to 8 μg by the European Food Safety Authority (EFSA 2009) indicating that an exposure level of 15 μg/kg is the cause of lung cancer among other effects. Assuming an average daily intake of 2 g of TB, an adult of 60 kg would incorporate between 2 and 4 % of the tolerable weekly dose according to the kind of tissue consumed. Inorganic As in plants from Patagonia must be determined in future researches.

Distribution patterns of Cd among tissues were the same in both gulfs. Concentrations ranged between 0.4 and 7.6 mg/kg with the minimum in midrib. In most cases they exceeded the limits established by the European Commission (2008) (0.5 to 3 mg/kg for human and 0 to −10 mg/kg for animals), mainly in SJG samples where results were between 6 (blade and midrib) and 8 (sporophyll) times greater than those in NG. Comparing with literature data, our records were generally greater. Kolb et al. (2004), for example, found 0.28 mg/kg in *U. pinnatifida* from Japan, whereas Almela et al. (2006) reported 1.55 mg/kg in plants from Spain. We did not find regulations about this metal in seaweeds used for animal consumption; for other kinds of food limits vary between 0.5 and 10 mg/kg (European Commission 2003). JECFA (1996) suggests a tolerable
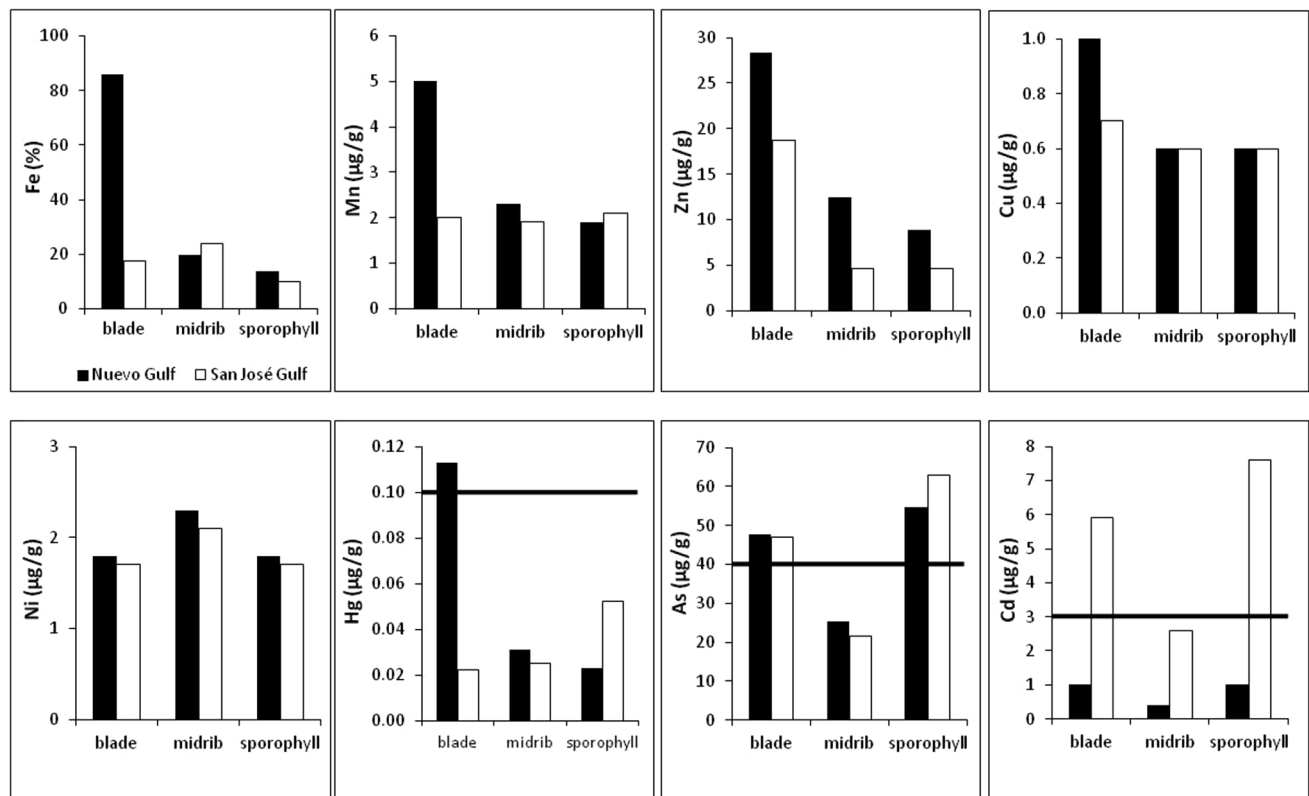
Fig. 3 Trace-element concentrations found in blade, midrib, and sporophyll of *U. pinnatifida* from NG and SJG. The permitted limits of Hg, Cd (European Commission 2008), and As (European Commission 2003) for consumption are shown

weekly Cd intake of 7 µg/kg of body weight. Assuming a mean consumption of 2 g of TB/day, an adult human weighing 60 kg would incorporate between 3 and 25 % of the tolerable dose depending on the tissue and the sampling site (NG: midrib 1 %, blade 3 %, sporophyll 3 %; SJG: midrib 9 %, blade 20 %, sporophyll 25 %). In particular, the contribution of blade and sporophyll from SJG would be very high for a single food product. Rather high Cd contents in bivalve mollusks from Patagonian areas without recognized anthropogenic sources have been previously reported (Giarratano and Amin 2010; Gil et al. 2006). This has been considered to be of natural origin and requires permanent monitoring of edible marine products.

Aliphatic Hydrocarbons

Most marine organisms contain an *n*-hydrocarbon series ranging from C13 to C33 with odd chain predominance. In marine algae, odd low molecular weight (LMW) hydrocarbons C15, C17, and C19 are generally the predominant n-alkanes (European Food Safety Authority 2012). The aliphatic hydrocarbon levels, without considering the contribution of normal alkane *n*-C15, were low for both gulfs and for the three analyzed tissues (2.7 ± 1.2 µg/g dw)

(Table 5). The presence of normal alkane *n*-C15 in different environmental substrates can result from both anthropogenic and biogenic inputs. The high concentration of LMW *n*-C15 in *U. pinnatifida* is likely because this macroalga is capable of synthesizing this compound. The highest percentage of *n*-C15 was found in blade tissue from individuals sampled in both gulfs where the contribution to AliR concentration reached 94 % (Table 5). Another LMW odd hydrocarbon (*n*-C17) was also determined in the blades at concentrations approximately one order of magnitude greater than compounds of the homologous series. This suggests the synthesis of *n*-C17, in addition to *n*-C15, by *U. pinnatifida*. A group of unidentified peaks with an RT value ranging from *n*-C20 to *n*-C22 was observed in sporophyll tissues from both gulfs with the greatest contribution corresponding to a compound with an RT value identical to that of *n*-C20. However, the presence of *n*-C20 in isolation is not normally observed in marine substrates. In agreement, the identification of this peak by GC/MS did not show a positive result for *n*-C20. Millie et al. (1992) referred to three compounds that elute just before *n*-C21 with RT and mass spectrum indicative of a group of branched alkenes from C25 and monoenes from C21. In addition to *n*-C15, *n*-C17, and *n*-C19, compounds around *n*-C21 RT are known to be synthesized by

**Table 5** Aliphatic hydrocarbons concentrations (µg/g dw) and contribution (%) of biogenic majority hydrocarbons

Hydrocarbons aliphatic	NG			SJG		
	Blade	Midrib	Sporophyll	Blade	Midrib	Sporophyll
Total aliphatics	41.8	4.3	44.3	63.6	3.3	73.6
n-C15	39.3	2.1	1.5	60.0	2.6	8.2
% n-C15	94.0	49.0	3.0	94.0	77.0	11.0
Biogenic group ni[a]	ND	ND	39.9	ND	ND	61.2
% biogenic ni	NA	NA	90.0	NA	NA	83.0
Total aliphatics[b]	2.5	2.2	2.9	3.7	0.8	4.1
UCM	ND	ND	ND	ND	ND	ND

UCM unresolved complex mixture, *ND* not detectable, *NA* not applicable

[a] Aliphatic hydrocarbons not identified constituents of biogenic group in sporophyll

[b] Total aliphatic hydrocarbons excluded n-C15 and the biogenic group in the case of sporophyll

Fig. 4 Aliphatic hydrocarbon concentrations as the sum of values of each tissue (µg/g dw) from NG and SJG (**a**) and excluding n-C15 and n-C17 in blade of *U. pinnatifida* from NG (**b**)
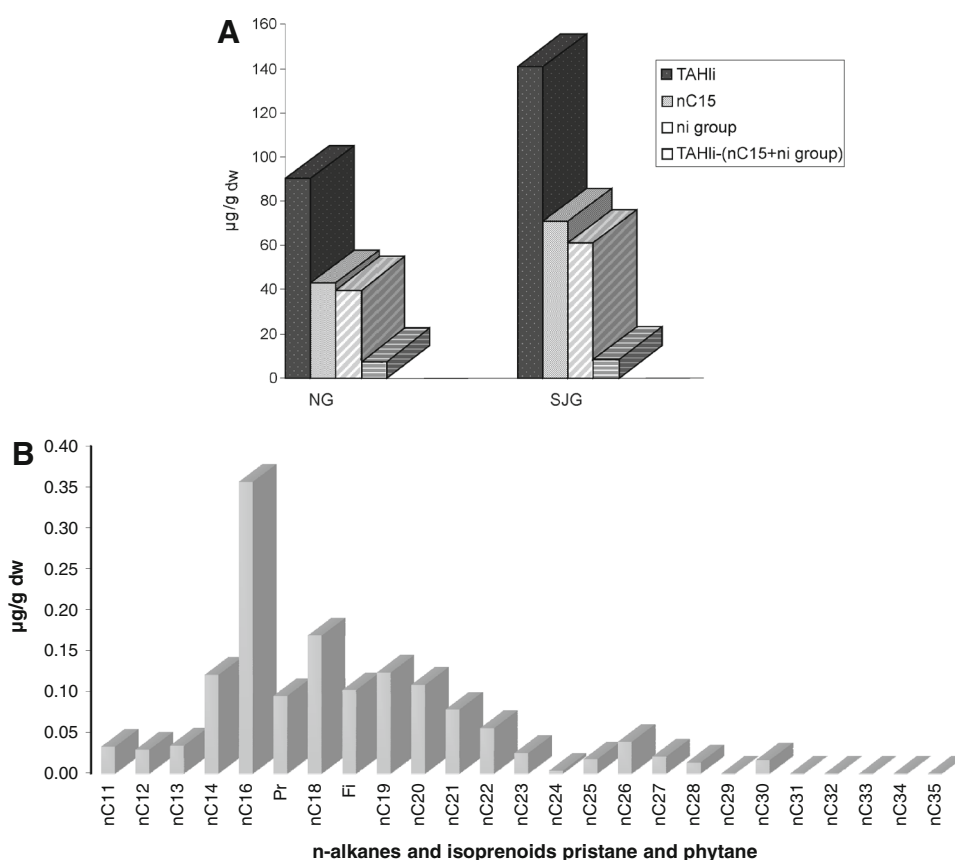
phytoplankton (UNEP/IOC/IAEA 1992). In a previous study carried out on sediments and bivalve mollusks (*Aulacomya atra atra*) of Nueva bay, Massara Paletto et al. (2008) found a group of unidentified compounds that were associated with diatoms. This kind of compound may be also synthesized by *U. pinnatifida*. The contribution to TAliH of this "biogenic group" in sporophyll tissues was 90 and 83 % in NG and SJG, respectively (Table 5). Greater TAliH concentrations found in SJG are related to a greater contribution of biogenic

hydrocarbons (Fig. 4a). Excluding the predominant n-C15 and the biogenic group, the distribution of n-alkanes in *U. pinnatifida* tissues ranged from n-C11 to n-C35/n-C32/n-C30 (Fig. 4b) as would correspond to the homologous series of n-alkanes, which is an indicator of hydrocarbon pollution (Commendatore and Esteves 2004; Commendatore et al. 2000). However, the absence of AliUCM and the low levels of TAliH (<4 µg/g) found (Table 5) suggest that petroleum hydrocarbons are not a threat for human consumption of *U*.

pinnatifida because the predominant hydrocarbons are naturally occurring in the algae. Information on hydrocarbon levels in *U. pinnatifida* and other seaweed species is scarce. Punín Crespo and Yusty (2006) assessed *n*-alkane (C18, C20, C22, C24, and C28) concentrations in samples from the Galician coast and found values <7.9 μg/g dw for these compounds and values ranging from 13.6 to 21.7 μg/g dw for total hydrocarbons. These values were greater than those found in this study excluding biogenic hydrocarbons.

Aromatic Hydrocarbons

Aromatic hydrocarbons and AroUCMs in tissues of *U. pinnatifida* from both gulfs were undetectable. Pavoni et al. (2003) studied organic micropollutants, including PAH, in Venice Lagoon. These investigators found that *U. pinnatifida* was characterized by a greater PAH concentration than other seaweed species, and argued that this is because *U. pinnatifida* floated partially in a film enriched with PAH during low tides and because its mucilaginous surface is able to easily take pollutants. Therefore, they recommended that the harvesting of this seaweed to be used in cosmetics and human consumption should be made in the subtidal zone. In our study, samples were obtained in the subtidal zone, which would decrease the possibility of hydrocarbon incorporation via surface waters, although this could happen through suspended particles, resuspension of bottom sediments, and/or dissolved hydrocarbons in the water column. In addition, methylates derivates of phenanthene, which are normally present in substrates polluted with petroleum and/or its derived products, were not detected. Some of the compounds identified in the aromatic fraction were ethyl ester pentadecanoic acid, methyl ester hexadecanoic acid, ethyl ester hexadecanoic acid, ethyl oleate, ethyl ester octadecanoic acid, 3 alpha 5 cholestane, and 3 alpha 4 cholestan-3-ol. These short-chain compounds, particularly alkanoic acids, are the resulting products from algal lipid synthesis. These investigators report that marine phytoplankton can synthesize ethyl cholesterols and that some lakes seaweeds seem to have the same capacity. Cholestanes found in *U. pinnatifida* in our study could be sterols derived from cholesterol. Further research is needed to characterize natural lipids in *U. pinnatifida* growing in Patagonia.

Conclusion

The analysis of morphometric and general characteristics of *U. pinnatifida* indicated that at the sampling, the specimens collected in NG had a greater degree of maturity and greater epiphytic level than those from SJG. Those from SJG looked healthier and presented lower total length and sporophyll diameters as is expected in younger plants. However, no differences were observed in mean fresh weights. Total TB per plant was lower in NG (3 kg/117 specimens) than in SJG (3.3 kg/99 specimens) due to greater discard of the former.

Chemical analysis showed that the studied seaweeds are important sources of total proteins, dietary insoluble fiber, lipids, carbohydrates (others than structural polysaccharides), vitamins, macrominerals, and essential trace elements at levels similar to those recorded in plants from eastern countries. This suggests that *U. pinnatifida* from NG and SJG may be potentially suitable for human and animal intake as a natural dietary supplement. The Na:K ratios were low enough to avoid incidence of hypertension while measured levels of glutamic acid ensure the expected palatability of this species. Further efforts would be important to better characterize polysaccharides and lipids.

Regarding the analyzed toxicities, PAH were undetectable, and aliphatic hydrocarbons were present in very low concentrations presenting no human health risks. Regular checks are needed anyway. Pb concentration was undetectable in all samples. Hg was slightly greater than suggested limits for human consumption only in blades from NG probably due to human influence from Puerto Madryn city. Total As levels in blade and sporophyll from both gulfs were greater than limits proposed for an animal diet. Determination of inorganic as should be included in future studies. The content of Cd always exceeded regulated levels for human and animal consumption mainly in samples from SJG. Both As and Cd would derive from natural sources within these gulfs. In spite of measured concentrations, taking into account probable human intakes, health risk was not expected at the time of this study. Nevertheless, the use of *U. pinnatifida* from NG and SJG in human or animal diets requires periodic controls of these elements in order to ensure food safety.

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