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Blackfordia virginica in Non-Native Distribution Range: A Potential Food Source for Humans?

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Abstract: The seasonal occurrence of the Black Sea jellyfish *Blackfordia virginica* Mayer, 1910 blooms is a reason of concern in the Guadiana estuary in the South of Portugal (South-West Europe), causing considerable economic and ecological impacts to fisheries. Due to jellyfish biochemical properties, they may represent an opportunity as an alternative food source for humans. In this context, this work evaluated the nutritional profile of *B. virginica* (proximate composition, amino acids, minerals, and fatty acids methyl ester content). *Blackfordia virginica* biomass may be adequate for human consumption, as it has nutritional properties resembling other edible jellyfish species, with relevant levels of minerals, moderate content in crude protein, low-fat content, and a low energetic value. The high Cd levels in the biomass of *B. virginica* from the Guadiana Estuary may compromise its safety as a food source. Moreover, if these jellyfishes are proven as an edible invasive species, their management through fisheries should evaluate the cost effectiveness of investments.

Keywords: jellyfish; Guadiana estuary; non-indigenous species; nutritional composition

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1. Introduction

Biological invasions are considered one of the most important drivers of biodiversity loss in aquatic systems due to both direct biotic interactions with indigenous species, such as predation and competition, and indirect changes in habitat conditions, including turbidity and habitat structure [1,2]. Economic impacts are related to invasive species that have a strong impact on native species of high commercial value, which disrupt the functioning of an entire ecosystem and impact ecosystem services or cause damage to high-value infrastructure and/or require significant financial investment to eliminate and restore the operation of such infrastructure [3–6]. Public health issues, either directly caused by the species, or by parasites and pathogens that are introduced alongside [2,7,8], include issues related to venomous stinging species, such as in the Mediterranean Sea with the lionfish *Pterois miles* (Bennett, 1828), rabbitfishes *Siganus* spp. Forsskål, 1775, the schyphomedusa *Rhopilema nomadica* Galil, Spanier and Ferguson, 1990, or with poisonous species, such as the silver-cheeked toadfish *Lagocephalus sceleratus* (Gmelin, 1789) [9]. However, in some cases, aquatic invasive species can provide ecosystem services and positively impact the economy of the region, especially if their fisheries represent an important source of food and revenues [10–12], as it has been investigated in the Guadiana estuar (South-West Iberian Peninsula, South-West Europe) for different edible invasive species.

In the Guadiana estuary, biological invasions increased through the years as a result of the intense river modifications, including the construction of the Alqueva dam along

with the considerable shipping traffic in the Gulf of Cadiz [13–15]. Currently, more than ten non-indigenous species (NIS) have been identified in this estuary, including the Black Sea jellyfish *Blackfordia virginica* Mayer, 1910, *Corbicula fluminea* (Müller, 1774), *Cynoscion regalis* (Bloch and Schneider, 1801), *Callinectes sapidus* Rathbun, 1896, *Cordylophora caspia* (Pallas, 1771), *Arcuatula senhousia* (W. H. Benson, 1842) [10,16–20], among others. *Blackfordia virginica* was first observed in the Guadiana estuary in June 2001 (0.22 ind.m^{-3} with a maximum density of 31.5 ind.m^{-3} recorded in July 2008) [9], where it has probably been introduced by nautical activities [9,21]. Nowadays, it is one of the most widespread NIS in this estuary, tolerating a wide range of temperature (16.5 to 23.0 °C) and salinity values (2 to 35), with higher abundance in the middle estuary. Once established and occurring in high abundance, these populations represent a high risk to local zooplankton standing stocks, reducing the density of all zooplanktonic organisms, including fish eggs and larvae [9]. This effect can cause not only economic losses [9,22] but also changes in the food web structure and dynamics [23].

Despite the potential negative impacts on the ecosystem, non-indigenous jellyfish may be regarded as a new source of food for humans due to their usually high abundances and high regenerative and reproduction potential [24]. Indeed, innovative and sustainable food sources with high nutritional value have never been more critical than nowadays due to the exhaustion of several fish stocks [24]. Several processed (dried) scyphozoan jellyfish are appreciated in South-East Asia and Europe, such as *Rhopilema esculentum* Kishinouye, 1891 and *Aurelia aurita* Linnaeus, 1758, not only for their texture and taste but also because they ensure a low caloric diet due to their low contents in fat and cholesterol [25,26]. Indeed, generally, over 95% of a jellyfish's body weight is water, whereas dry weight (DW; 3–5% of fresh weight [FW]) is mainly represented by proteins (5–30% of DW) and minerals, rather than lipids (2–10% of DW) and carbohydrates (0.5–1.7% of DW) [25,27]. In addition, jellyfish contain bioactive compounds with important health-promoting properties, including antihypertensive and anticancer, thus emphasizing the possible utilization of jellyfish species not only as food but also in the medical field [27]. In fact, the valorization of biomass from NIS for commercial purposes is a relevant strategy in the mitigation and socio-economic adaptation efforts for reducing their impact on marine ecosystems [28].

Nevertheless, considered as "trophic dead ends" in the aquatic food webs [29,30], jellyfish can be an important food source for many aquatic predators [30]. Those predators may include not only indigenous species [23], but also NIS with jellyfish facilitating their colonization and establishment in a new ecosystem—invasion meltdown hypothesis [31]. Therefore, several opportunistic predators in the middle Guadiana estuary may benefit from food resource pulses that originated from the bloom events of *B. virginica*, which include several NIS, such as the Atlantic blue crab, as well as indigenous species, including the European green crab *Carcinus maenas* (Linnaeus, 1758). Thus, *B. virginica* can act as a threat, opportunity, or both, to the ecosystem. In this context, this study was designed to address the hypothesis that *B. virginica* biomass presents nutritional properties relevant for human consumption. Testing such hypothesis can help boost the valorization of *B. virginica*, turning a threat into an opportunity in this depressed economic area of South Iberia, the Guadiana catchment.

2. Materials and Methods

2.1. Study Site

The Guadiana estuary is in the South of Portugal (South-West Europe) (Figure 1), measures approximately 80 km long, and occupies a total area of 22 km^2, while the lower 50 km makes up the border between Portugal and Spain [15]. This area is a mesotidal estuary with tidal amplitudes ranging between 1.3 and 3.5 m, and the estuary has an average depth of 6.5 m [15]. The Guadiana basin has Mediterranean climatic characteristics, is listed as a Wetland of International Importance, and is included in the Natura 2000 Network, as it is an area of high ecological importance [13,15].

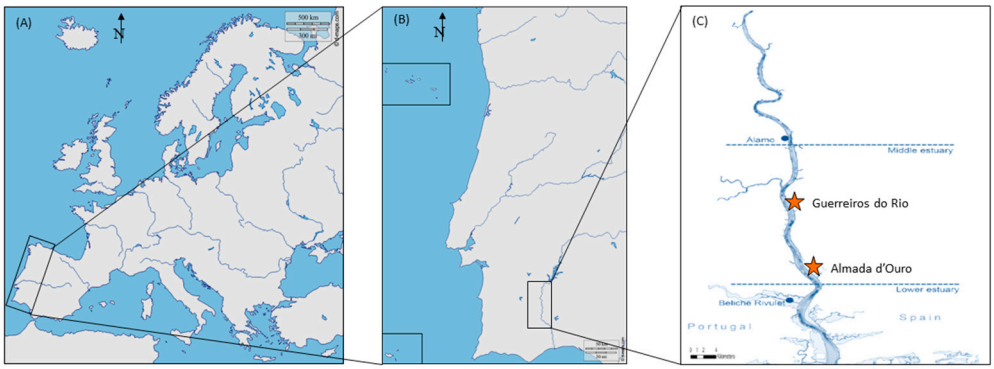

Figure 1. General (**A**,**B**) and details (**C**) of sampling sites at "Guerreiros do Rio" and "Almada d'Ouro" in the Guadiana river estuary, in the South of Portugal (South-West Europe) (adapted from Google Maps).

2.2. Sample Collection

Sampling was conducted in June 2019 in the middle area of the Guadiana estuary from Guerreiros do Rio (37°23′51.081″ N/7°26′47.782″ W) to Almada d'Ouro (37°18′49.654″ N/7°26′39.517″ W; Figure 1). Samples of *B. virginica* were collected by horizontal tows with a conical plankton net (200 µm mesh size, area = 0.13 m^2), equipped with a Hydro-Bios flow meter for 10 min. *Blackfordia virginica* samples were frozen and later freeze-dried for the evaluation of the nutritional profile.

2.3. Nutritional Profile

2.3.1. Proximate Composition

Moisture was determined by drying the samples in an oven (60 °C) until constant weight (12 h); crude protein content (N × 6.25) was estimated according to the AOAC Official Method 990.03 [32]; crude fat was determined gravimetrically by a modified protocol of the Bligh and Dyer method involving the homogenization of the dried biomass in a mixture of chloroform, methanol, and water (2:2:1), using an ultra sound bath (IKA-Werke GmbH, Staufen, Germany), as described previously [33]; ash contents were determined by incineration in a muffle furnace at 525 ± 15 °C until completely burned (5 h; AOAC, 1990) [34]; carbohydrates were calculated by difference. All the analyses were performed in triplicate. Results are expressed as g/100 g of DW biomass. Whenever needed, results were also expressed as g/100 g of fresh weight biomass (FW). Metabolizable energy (ME) was calculated using the Atwater specific factor for fish [35] according to the following equation: ME (Kcal) = 4.27 × (g protein) + 4.11 × (g carbohydrate) + 9.02 × (g lipid). ME was expressed as kcal/100 g of WW and DW.

2.3.2. Amino Acids

Total amino acids were determined in duplicates (30 mg each) by high pressure liquid chromatography (HPLC) with a reverse phase analytic system for amino acid (Waters ACQUITY UPLC H-Class System) using norvaline as an intern standard, after sample derivatization with a Waters AccQ Fluor Reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) according to the AcccQTag method (Waters, Milford, MA, USA). Samples (0.2 mg DW) were hydrolyzed in hydrochloric acid (HCL), 6 M for 48 h in vessels with a hydrogen atmosphere, and the mobile phase (polar) was applied to the column. The identification of amino acids was performed by comparing the retention time of the amino acid peaks in the sample with external standard peaks containing all 22 proteinogenic amino acids (Waters) using the Empower 3 software (Waters, Milford, MA, USA). For quantification purposes, separated calibration curves were generated for each amino acid in the standard (Empower 3 software). Results are expressed as mg/100 g DW and as the percentage of total amino acid content.

2.3.3. Fatty Acid Methyl Esters (FAMEs)

Lipids and free fatty acids (FAs) were converted to the corresponding FAMEs, by a direct transesterification method using acetyl chloride/methanol, followed by an extraction of the lipidic phase into hexane [36]. FAMEs were analyzed on an Agilent Gas Chromatography with mass spectrometry detection (GC-MS; Agilent Technologies 6890 Network GC System, 5973 Inert Mass Selective Detector, CCMAR, Faro, Portugal) equipped with a Bruker SCION TQ gas chromatograph fitted with a fused silica capillary column ZB-5MS (30 m × 0.25 mm internal diameter, 0.25 µm film thickness, Agilent Tech (Santa Clara, CA, USA) using nitrogen as the carrier gas (1 mL min^{-1}). Vials were injected on a column auto injector at 300 °C, and the oven temperature program was 60 °C (1 min), 30 °C min^{-1} to 120 °C, 4 °C min^{-1} to 250 °C, and 20 °C min^{-1} to 300 °C (4 min). For the identification and quantification of FAMEs, the total ion mode was used. The identification of FAMEs was performed by comparing the retention times of samples with an external standard (Supelco® 37 Component FAME Mix; Sigma-Aldrich, Sintra, Portugal) and further confirmed by a comparison of the MS spectra. For quantification purposes, calibration curves were generated for the standards. Assays were performed in triplicate, and, between each three replicates, the average, standard deviation, and coefficient of variation were calculated. Results were expressed as µg/100 g by dry weight (DW).

2.3.4. Minerals

Minerals were analyzed by the Agilent's (Santa Clara, CA, USA) Microwave Plasma-Atomic Emission Spectrometer (MP-AES; CCMAR, Faro, Portugal). Briefly, three replicates of approximately 500 mg each were microwave-digested in a closed-vessel microwave digestion system, Ethos 1, equipped with PTFE vessels with 6 mL of nitric acid (HNO_3). Mineralization was carried out by setting the following temperature program: 0–200 °C in 2 min (step 1), 200 °C held for 3 min (step 2), and 200–220 °C in 5 min (step 3) with a constant microwave power of 1000 W. Minerals were analyzed by the AES in three replicates along with blanks to check for any loss or contamination. Magnesium (Mg), sodium (Na), potassium (K), calcium (Ca), iron (Fe), manganese (Mn), and zinc (Zn) were analyzed by flame AAS with an air-acetylene flame. Cadmium (Cd), chromium (Cr), nickel (Ni), and lead (Pb) were analyzed with electrothermal atomization (GBC graphite furnace 3000) using an auto-sampler (PAL 3000). For quantitative proposes, the external calibration procedure was carried out with the help of multi-elemental standard solutions with a concentration ranging between 0.1 and 50 ppm. For method validation, a linear least-square regression analysis of the calibration graphs was performed to check for the linearity between the instrumental response and the nominal concentration of each elemental standard. Values were expressed as g/100 g DW (Ca, Mg, Na, and K) or µg/100 g of DW (Fe, Mn, Zn, Cr, Pb, Ni, and Cd).

3. Results

Nutritional Profile of B. virginica

The full body of *B. virginica* (1.25 ± 0.25 cm diameter) is mainly composed of water (98.6%; Table 1). The DW corresponds to 1.3% and is mainly composed of carbohydrates, (60.5%), ash (30.5%), and crude protein (7.62%). The total lipids were almost non-detectable (0.01%), resulting in a low energetic value (281 Kcal).

The mineral composition of *B. virginica* is summarized in Table 2. The MP-AES method showed good linearity for all the elements, with coefficients of correlation of 0.999. The most abundant macronutrient was sodium (Na: 728 mg/100 g, DW) followed by magnesium (Mg: 76.1 mg/100 g, DW), potassium (K: 56.9 mg/100 g, DW), and calcium (Ca: 17.4 mg/100 g, DW). The most abundant essential trace element was iron (Fe: 1208 µg/100 g, DW) followed by zinc (Zn: 110 µg/100 g, DW), manganese (Mn: 45.4 µg/100 g, DW), and copper (Cu: 26.3 µg/100 g, DW). Non-essential/toxic trace elements were also detected, especially cadmium (Cd: 337 µg/100 g, DW) and nickel (Ni: 68.5 µg/100 g, DW).

Table 1. Proximate composition (g/100 g) and energetic value of the body wall of the Black Sea jellyfish *Blackfordia virginica* Mayer, 1910, collected in the Guadiana estuary, in the South of Portugal (South-West Europe). Values are expressed as mean ± standard error of the mean (n = 3).

Proximate Composition	Fresh Weight (F)	Dry Weight (DW)
Moisture	98.6 ± 0.06	1.30 ± 0.01
Ash	0.40 ± 0.02	30.5 ± 1.55
Crude protein	0.10 ± 0.08	7.62 ± 0.62
Total fat	0.00 ± 0.00	0.01 ± 0.00
Carbohydrates	0.81 ± 0.18	60.5 ± 9.03
Energetic value (Kcal)	4.12 ± 0.73	281 ± 43.1

Table 2. Mineral composition of the body wall of Black Sea jellyfish *Blackfordia virginica* Mayer, 1910, collected in the Guadiana estuary, in the South of Portugal (South-West Europe). Results are expressed as mean ± standard deviation (SD) (n = 3) on a dry weight (DW) basis. n.d.: non-detected.

Minerals	Symbol	Contents
Essential macro elements (mg/100 g DW)		
Sodium	Na	728 ± 5.35
Magnesium	Mg	76.1 ± 3.29
Potassium	K	56.9 ± 8.87
Calcium	Ca	17.4 ± 0.23
Essential trace elements (µg/100 g DW)		
Iron	Fe	1208 ± 212
Zink	Zn	110 ± 16.3
Manganese	Mn	45.4 ± 4.33
Copper	Cu	26.3 ± 1.53
Selenium	Se	n.d.
Nonessential trace elements (µg/100 g DW)		
Cadmium	Cd	337.71 ± 9.16
Nickel	Ni	68.56 ± 6.55
Chromium	Cr	4.08 ± 0.58
Arsenic	As	2.31 ± 0.04
Lead	Pb	n.d.

Regarding amino acids (Table 3), the essential AA (EAA) histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), and valine (Val) were identified in the dry samples, representing 29% of the total AA detected. Tryptophan (Try) was not detected. Non-essential amino acids (NEAAs) represented 71% of the total amino acids. The most abundant amino acid in *B. virginica* was glutamic acid + glutamine (Glx: 16.8%), followed by glycine (Gly: 9.66%), alanine (Ala: 9.63%), aspartic acid + asparagine (Asx: 8.86%), proline (Pro: 6.70%), and tyrosine (Tyr: 6.15%).

The FAME profile of *B. virginica* is depicted in Table 4. The GC-MS method showed good linearity for all the calibration curves of all elements, with coefficients of correlation around 1, and allowed for the identification of 15 FAMEs in dried samples from *B. virginica*. Saturated fatty acids (SFAs) accounted for 76.9% of the total FAMEs followed by monounsaturated (MUFAs, 21%) and polyunsaturated fatty acids (PUFAs, 1.99%). The predominant FAMEs in *B. virginica* were methyl decanoate (SFA, capric acid, 19.9%), methyl tetradecanoate (SFA, myristic acid, 16.6%), methyl dodecanoate (SFA, lauric acid, 14.9%), methyl palmitate (SFA, palmitic acid, 14.8%), and methyl oleate (MUFA, oleic acid, 11.1%).

Table 3. Amino acid composition of the body wall of the Black Sea jellyfish *Blackfordia virginica* Mayer, 1910, collected in the Guadiana estuary, in the South of Portugal (South-West Europe). Data are expressed as the mean of two replicates as mg/100 g of dry weight (DW) ± standard deviation (SD) and as the percentage of total amino acids (n = 2).

Amino Acids	Symbol	Contents	
		mg/100 g	%
Glutamic acid + Glutamine	Glx	821 ± 8.40	16.8
Glycine	Gly	471 ± 10.20	9.66
Alanine	Ala	469 ± 4.80	9.63
Aspartic acid + Asparagine	Asx	432 ± 11.30	8.86
Arginine	Arg	353 ± 8.50	7.25
Proline	Pro	326 ± 6.00	6.70
Tyrosine	Tyr	300 ± 2.80	6.15
Serine	Ser	245 ± 4.30	5.04
Taurine	Tau	48.0 ± 0.10	0.98
Cystine	Cys	3.6 ± 0.10	0.07
Total non-essential AA	Σ NEAA	3471 ± 0.56	71.1
Lysine	Lys	281 ± 3.00	5.77
Leucine	Leu	265 ± 11.60	5.43
Valine	Val	265 ± 11.60	5.43
Threonine	Thr	227 ± 7.00	4.67
Isoleucine	Ile	158 ± 1.20	3.24
Phenylalanine	Phe	129 ± 0.80	2.66
Methionine	Met	68 ± 2.10	1.40
Histidine	His	10.2 ± 0.10	0.21
Total essential AA	Σ EAA	1405 ± 0.32	28.8
EAA/NEAA			0.41
EAA/TAA			0.30
LYS/ARG			0.79

Table 4. Fatty acid profile of the body wall of the Black Sea jellyfish *Blackfordia virginica* Mayer, 1910, collected in the Guadiana estuary, in the South of Portugal (South-West Europe). Data are reported in µg/100 g on a dry weight (DW) basis, as the mean ± standard deviation (SD) (n = 3). * source: PubChem.

FAME	Synonyms *	Chemical Formula	Fatty Acid	µg/100 g DW	%
Saturated Fatty Acids (SFAs)					
Methyl decanoate	Capric acid methyl ester	$C_{11}H_{22}O_2$	C11:0	1198 ± 2.53	19.9 ± 0.02
Dodecamethylcyclohexasiloxane	Cyclohexasiloxane, dodecamethyl	$C_{12}H_{36}O_6Si_6$	C12:0	40.5 ± 5.54	0.68 ± 0.05
Methyl dodecanoate	Lauric acid methyl ester	$C_{13}H_{26}O_2$	C13:0	899 ± 0.78	14.9 ± 0.01
Methyl tetradecanoate	Myristic acid methyl ester	$C_{15}H_{30}O_2$	C15:0	998 ± 0.73	16.6 ± 0.01
Methyl palmitate	Palmitic acid methyl ester	$C_{17}H_{34}O_2$	C17:0	887 ± 0.76	14.8 ± 0.01
Methyl heptadecanoate	Margaric acid methyl ester	$C_{18}H_{36}O_2$	C18:0	19.5 ± 0.38	0.32 ± 0.01
Methyl stearate	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	C19:0	421 ± 12.3	7.03 ± 0.12
Methyl arachidate	Arachidic acid methyl ester	$C_{21}H_{42}O_2$	C21:0	99.8 ± 0.18	1.66 ± 0.00
Tricosanoic acid	Tricosylic acid	$C_{23}H_{46}O_2$	C23:0	50.5 ± 0.37	0.84 ± 0.01
Total SFA				4616	76.9
Monounsaturated fatty acids (MUFAs)					
Methyl Cis-9-Tetradecenoate	Myristoleic acid methyl ester	$C_{15}H_{28}O_2$	C15:1 n-9	393 ± 10.1	6.56 ± 0.10
Methyl palmitoleate	Methyl (Z)-hexadec-9-enoate	$C_{17}H_{32}O_2$	C36:1 n-9	200 ± 0.01	3.34 ± 0.00
Methyl oleate	Oleic acid methyl ester	$C_{19}H_{36}O_2$	C19:1 n-9	666 ± 0.02	11.1 ± 0.00
Total MUFA				1260	21.0
Polyunsaturated fatty acids (PUFAs)					
Methyl linolenate	Linolenic acid methyl ester	$C_{19}H_{32}O_2$	C19:3 n-3	100 ± 0.21	1.67 ± 0.00
Squalene	Spinacene	$C_{30}H_{50}$	C30:6 n-2	19.4 ± 22.3	0.32 ± 0.22
Total PUFA				119	1.99

4. Discussion

This study reports for the first time the nutritional profile of *B. virginica*, targeting its commercial valorization as human food, based on fisheries on the Guadiana middle estuary.

The consumption of locally grown, minimally industrialized, and renewable foods is encouraged globally to achieve sustainable food production and healthier consumer behaviors [37–39]. In this context, the valorization of invasive jellyfish as food contributes to developing the blue economy and fostering sustainability, by identifying potential innovative healthy foods while minimizing the ecological and economic loss caused by jellyfish outbreaks [40]. Jellyfish exhibit a high-water content, with *B. virginica* containing 98.7%, which is consistent with the hydration levels observed in other jellyfish species (95–98%) [27]. Jellyfish are usually rich in protein and minerals, with low energetic value [27]. *Blackfordia virginica* followed this trend, presenting a high ash content (30.5%), similar to the values detected in other edible jellyfish, such as *R. esculentum* (33.2%) and *Acromitus hardenbergi* Stiasny, 1934 (31.1%) [41]. The high ash levels are likely attributed to their habitat, as marine and brackish waters are rich in minerals. The crude protein level was, however, low (7.62%), especially when compared with high protein-rich species, such as *Rhopilema hispidum* Vanhöffen (1888) (43.8%) and *R. esculentum* (53.8%) [41], but comparable to other edible species, including *Chrysaora pacifica* Goette (1886) (7.53%) [42], and *Rhizostoma luteum* Quoy and Gaimard (1827) (10%) [42,43]. These variations in the protein levels can be due to the amounts of structural collagen that is distributed throughout the mesoglea (about 60%), which is used to retain large amounts of water [44]. Total fat was almost non-detected in *B. virginica* (0.01%), resulting in low energetic value (281 kcal/100 g of DW), similar to or even lower than the values reported for other edible jellyfish [41]. Indeed, jellyfish contain no visible lipid deposits, except in relatively well-developed gonads during the reproductive cycle [45]. Overall, *B. virginica* has an adequate protein/lipid ratio (23:1), which is of particular interest for human nutritional purposes since proteins are valuable nitrogen and amino acid sources for the human body. The carbohydrate level (60.5%) was higher than the values reported for some jellyfish species (0.8–18%) [41], such as *Cyanea capillata* Linnaeus, 1758 and *Rhizostoma octopus* (Gmelin, 1791) [46] but similar to the large cannonball jellyfish, *Stomolophus nomurai* Kishinouye, 1922 that contains about 58% of carbohydrates [47].

The high ash contents observed in jellyfish translate into relevant amounts of beneficial minerals [27]. Indeed, *B. virginica* was rich in minerals, and the most abundant elements were Na, followed by Mg, K, and Ca, similar to other edible jellyfish species, including *Catostylus tagi* Haekel, 1869 [48], *Pelagia noctiluca* Forsskål (1775) [49], and *Aurelia* sp. [42]. Although Na is an essential nutrient, its excessive consumption is linked to several human pathologies, including hypertension and cardiovascular diseases, and, therefore, the World Health Organization (WHO) recommends that the daily intake of Na should not surpass 2000 mg. Considering *B. viriginica* biomass, a consumption of 100 g of dry tissue would represent an intake of 728 mg of Na; therefore, care must be taken so that the maximum allowed daily intake recommended by the WHO is not exceeded. The consumption of food rich in nutrients such as the trace elements Cu, Fe, Zn, and Mn can have positive health implications since they are co-factors of many key enzymes and play important roles as catalysts and antioxidants. However, these elements can become toxic at high concentrations, leading to damaging oxidative processes. *Blackfordia virginica* had lower values of trace elements than other edible jellyfish species, including *C. tagi* [48] and *P. noctiluca* [49]. Potentially toxic elements typically come from anthropogenic activities, such as mining, and have a negative impact on the aquatic environment. In general, considering the Commission Regulation (EC) N. 629/2008, amending the Regulation N. 1881/2006 fixing the maximum levels of heavy metals in food supplements, the mean concentration levels of toxic metals (Cr, Ni, As, Pb) in the investigated samples were below the legislated values for As (10 mg/Kg; 0.0231 mg/Kg for *B. virginica*) and Pb (5 mg/Kg; non detected in *B. virginica*). However, the Cd values were three times higher than those recommended (1.0 mg/Kg; 3.3 mg/Kg for *B. virginica*). High values for Cd are most probably related to old mine-related processes, i.e., acid mine drainage, from "Minas de São Domingos", located in the upper area of the Guadiana estuary [50]. Indeed, jellyfish can bioaccumulate toxic metals in varying degrees according to the species, reflecting a time-integrated measure of

their levels in the water [51]. However, mining activity in the Guadiana area ceased over five decades ago, and a progressive reduction in toxic levels is expected.

Similar to other edible jellyfish, *B. virginica* contained all the EAA except for tryptophan, since, generally, this AA is destroyed during the hydrolysis process with hydrochloric acid [49]. The percentage of EAAs out of the total AAs (28.82%) is similar to those from the edible *Aurelia coerulea* von Lendenfeld, 1884 (31.4%) [44]. The ratio values of TEAA/TAA (0.3) and EAA/NEAA (0.7) are in agreement with values established by FAO as proteins of good quality. The most abundant amino acids in *B. virginica* are Glx, Gly, Ala, Asx, Pro, Tyr, and Lys, resembling the amino acid profile of *R. esculentum* collagen [52], indicating that *B. virginica* may be a potential source of collagen. Additionally, the high Gly content is noteworthy, as evidence suggests that consuming Gly-rich foods may help reduce total serum cholesterol levels [53]. The low lysine/arginine ratio (0.8%) in *B. virginica* is associated with hypocholesterolemic effects, suggesting that biomass from this species may be beneficial for individuals with hyperlipidemia [54].

Usually, jellyfish present a rather saturated FA profile [27]. This trait was also observed in this work, where the FAMEs profile of *B. virginica* was largely dominated by SFAs, followed by MUFAs and PUFAS, similar to those from other edible jellyfish species, such as *P. nocticula*, *A. aurita*, and *Rhizostoma* sp., among others [27,44,49]. Saturated FAs consisting mostly of capric and myristic acid, followed by lauric and palmitic acid, are the most common FA in animal tissues [55]. Among MUFAs, oleic acid is the predominant FA and is essential to heterotrophic organisms [56]. It is the most common MUFA in human cells and is incorporated into cell membrane phospholipids, as it is important for proper membrane fluidity and the major energy source for cells [56]. The lower amount of PUFAs found in *B. virginica* when compared to other edible jellyfish, such as *C. tagi* (48–51%) [48] might be related to reduced levels of symbiotic microalgae, which are an important and significant source of essential ω-3 fatty acids [44]. Linolenic acid is a ω-3 FA and is the major component together with squalene in *B. virginica*'s biomass. The ω-3 types of FA are involved in several biological processes (e.g., growth, development, tissue, and cell homeostasis) and have a variety of health benefits, including hypo-triglyceridemic, anti-inflammatory, antihypertensive, anticancer, antioxidant, anti-depressive, antiaging, and antiarthritis effects [44].

Overall, *B. virginica* seems to have a potential nutritional profile suitable for human consumption and could be a way to control blooms that occur in estuaries where this jellyfish occurs. In the middle estuary of the Guadiana River, the abundance of *B. virginica* has been increasing since its appearance. Monthly samplings conducted in Foz de Odeleite from 2014 until 2021, have shown that this species is usually present between May and November, with average abundances of 26.2 $\pm$ 119.5 ind m^{-3}, reaching a peak of 976.1 ind m^{-3} in July 2021 [57]. These bloom abundances are comparable to the maximum levels recorded globally [58–60], indicating a high potential for disrupting the ecosystem's food web. Controlling *B. virginica* populations through human consumption could help mitigate its proliferation and impact on non-native estuaries worldwide.

5. Conclusions

This study showed that *B. virginica* is rich in carbohydrates and minerals, especially Na, Mg, K, and Ca, has a moderate total protein content, with a prevalence of the NEE Glx, Gly, Ala, Asx, Pro, and Tyr, a low-fat content, with the prevalence of SFAs, as well as a low energetic value. The high Cd levels in the biomass of *B. virginica* from the Guadiana Estuary may compromise its safety as a food source. However, further research is necessary to assess this issue and other relevant chemical components, including venoms and compounds with medical applications. Moreover, if these jellyfishes are proven as an edible invasive species, their management through fisheries should evaluate the cost effectiveness of investments while being aware of the counterproductive consequences of policies, which can induce unintended responses of stakeholders (e.g., triggering risk-increasing actions among economic sectors). Additionally, spatial strategies for invasion control should be

planned to mitigate long-term negative impacts and protect high-value economic resources or vast areas of ecological and economic value.

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