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Donax trunculus L., 1758 (Bivalvia: Donacidae) from Bulgarian Waters of the Black Sea as a Sustainable Source of Macronutrients

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Abstract: The aim of the present study was to determine the chemical composition in terms of crude proteins, total lipids, carbohydrates, energy value and macroelements in *Donax trunculus* sampled from June to November from the Bulgarian coast of the Black Sea. Crude protein, carbohydrates and total lipids were determined using standard procedures. Macroelements (K, Ca, Mg and Na) were determined by ICP-OES. Analysed samples were characterised by high protein (15.2±1.0 to 17.3±0.2 g/100 g) and low lipid content (1.12±0.05 to 5.15±0.27 g/100 g). Lipid levels showed greater variations. Carbohydrates varied between 1.30 and 2.85 g/100 g and the energy values were between 97.7 and 116.0 kcal/100 g. Concentrations of the analysed macroelements varied between 1171 and 2820 mg/kg ww for K; 806 and 1946 mg/kg ww for Ca; 471 and 511 mg/kg ww for Mg; 2112 and 2223 mg/kg ww for Na. The present study revealed new data on the chemical composition of *D. trunculus* harvested from the Bulgarian Black Sea coast. Despite the variations in their composition, the results show that *D. trunculus* could be a healthy choice of low-energy dense food due to high protein and macroelement levels and low lipid, carbohydrate and energy contents.

Key words: *Donax trunculus*, proteins, lipids, macroelements

Introduction

Marine bivalves are important resources that provide basic and essential nutrients. They are also used as natural bioindicators for the status of the water environment. Worldwide, seafood products are the third major source of dietary proteins and they represent c. 17% of the global yield of animal meat, respectively lipids (FAO 2014, Kapranov et al. 2021). The most widespread bivalve species along the Bulgarian Black Sea coast is the Mediterranean mussel *Mytilus galloprovincialis*. Recently, more attention has been paid to other two bivalve species, the white clamps *Donax trunculus* and *Chamelea gallina*. Since 2012, both white clam species have been commercially exploited in the Bulgarian Black Sea part, with maximum catches reaching 506 tonnes in 2019 (Gumus et al. 2020). *Donax trunculus* is the dominant bivalve species of the Black Sea sandy shallow waters. It is well known that the proteins have a structural role in bivalve organisms, whereas lipids act as basic energy sources for sustaining mussel growth and development. Essential elements such as potassium, sodium, calcium and magnesium are crucial for vital physiological functions of organisms. Insufficient intake of macronutrients and essential elements can affect

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human health and lead to the development of different diseases such as CVD and osteoporosis (Biandolino et al. 2020). On the other hand, the variations of proximate composition, including macroelement content, in bivalve tissues may be important not only for the optimal survival and reproduction of the bivalve organism but for its nutritional quality. Thus, the evaluation of the chemical composition and the macronutrients content may increase consumer's interest and predict the market feasibility of the white clam *D. trunculus*. Limited information for *D. trunculus* nutritional quality characterised through proximate compositions and macroelement content (Na, K, Ca and Mg) was found in previous investigations. Few studies on *D. trunculus* from the Marmara Sea (Ozden et al. 2009), Algerian coast of the Mediterranean Sea (Sifi et al. 2013) and the Azov Sea (Bityutskaya et al. 2021) determined this species as protein-rich and low fat, with high nutritional quality and well-balanced elemental composition. The only research for the Black Sea white clam *D. trunculus* presented new information for the total concentration of seven trace elements (Cd, Cr, Cu, Fe, Ni, Pb and Zn) and essential polyunsaturated fatty acids (PUFAs, see Peycheva et al. 2021). Moreover, the chemical composition of bivalves can be affected by different abiotic factors, including those showing seasonality. However, studies of seasonal changes in the macronutrient contents of the wedge clam *D. trunculus* from the Bulgarian part of the Black Sea are lacking.

The aim of the present study is to determine the seasonal variations (June – November) in proximate composition (proteins, lipids and carbohydrates), energy value and macroelement contents (Na, K, Ca and Mg) of white clam *D. trunculus* harvested from the Bulgarian Black Sea coast.

Materials and Methods

Sample collection and preparation

For assessment of the abundance of *D. trunculus*, the study was carried out through dredging activities: 0.3 knots speed, 5 min dragging duration with drag in the coastal area within 1–3 m isobaths. Samples were collected from the area of Azalia Resort zone on two points (43.24642 N 28.01972 E, 43.24781N 28.02058 E, 43.24661N 28.02167E, 43.24569N 28.02111E). For each specimen, the main biometric parameters were measured individually: weight with the shell (TW, g) and length of the shell (SL, mm). The samples of wedge clams were provided on a monthly basis from June 2020 to November 2020. One kilogram of *D. trunculus* was brought to the laboratory each month. Only clams of mean size were taken for analyses. The specimens were washed, shucked and placed on a filter paper to absorb the excess moisture and then the flesh was removed.

Proximate composition analysis

The homogenised clam tissue (2.000±0.005g) was dried at 105±2°C in an air oven for 18–20 hours to a constant weight (AOAC 950.46). The crude protein content was determined by Kjeldahl's method (BDS 9374:1982). The total lipids (TL) were estimated according to the method of Bligh & Dyer (1959). The carbohydrates were determined according to BDS 13488:1976. The energy values based on fat, protein and carbohydrate contents were calculated according to WHO/FAO (2010) Atwater specific coefficients (4.0 kcal/g for proteins and carbohydrates, 9.0 kcal/g for lipids).

Macroelement analysis

One gram wet weight of homogenised *D. trunculus* tissue was mixed with 8 cm^3 HNO_3 (65% w/v) and 2 cm^3 H_2O_2 (30 %w/v), placed in Teflon vessels and digested in a microwave closed-vessel digestion system MARS 6 (CEM Corporation, USA). A 3-stage program was used according to the procedure given by Peycheva et al. (2021). The concentrations of Na, K, Ca and Mg in the samples were determined using ICP-OES Spectrometer (Optima 8000, Perkin Elmer, USA).

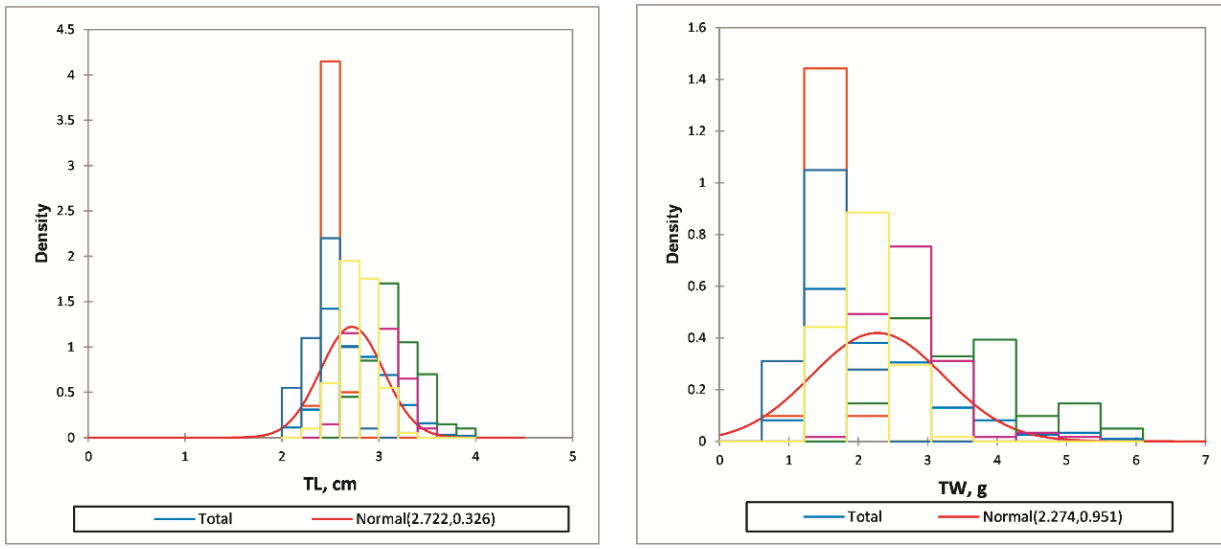
Statistical analysis

The XLSTAT software product was used to display the linear-weight histograms of the samples. The statistical data about the different length and weight classes, presented in the histograms, include lower and upper limits, frequency, relative frequency and density. Analyses for the chemical composition and macroelements were performed in six replicates and the results were expressed as mean values ± standard deviation (SD). Mean values were compared by one-way ANOVA followed by a post-hoc Tukey's test. Statistical significance was considered at $p \leq 0.05$ (Graph Pad Prism 6).

Results

Size and weight distribution

For the study period, the mean length of *D. trunculus* was 2.72 cm ± 0.33 SD (Fig. 1A) and the mean weight was 2.27 g ± 0.95 SD (Fig. 1B). Based on averaged sampling data, the dominant classes in the weight structure were of 1.22–3.05 g, forming 78 %



A B

Fig. 1. Histogram of the length (Tl, mm) (A) and weight (TW, g) classes distribution of *Donax trunculus*

Table 1. Descriptive statistics of the measured length classes of *Donax trunculus*

Lower bound	Upper bound	Frequency	Relative frequency	Density
2.0	2.2	11	0.022	0.110
2.2	2.4	31	0.062	0.310
2.4	2.6	142	0.284	1.420
2.6	2.8	101	0.202	1.010
2.8	3.0	89	0.178	0.890
3.0	3.2	69	0.138	0.690
3.2	3.4	36	0.072	0.360
3.4	3.6	16	0.032	0.160
3.6	3.8	3	0.006	0.030
3.8	4.0	2	0.004	0.020

Table 2. Descriptive statistics of the measured weight classes of *Donax trunculus*

Lower bound	Upper bound	Frequency	Relative frequency	Density
0.61	1.22	25	0.050	0.082
1.22	1.83	180	0.360	0.590
1.83	2.44	116	0.232	0.380
2.44	3.05	93	0.186	0.305
3.05	3.66	40	0.080	0.131
3.66	4.27	25	0.050	0.082
4.27	4.88	8	0.016	0.026
4.88	5.49	10	0.020	0.033
5.49	6.10	3	0.006	0.010

of all collected specimens. The length structure was dominated by the classes 2.4–3.2 cm, which formed 80 % of the total number of the measured specimens (Tables 1 and 2).

Proximate composition

More than 98% of the mussel tissues represent water, lipids (L) and proteins. The proximate composition of bivalves differs due to the specific abiotic factors across the water basins. Furthermore, the basic macronutrients proportions were species-specific and the greater inter- and intraspecies changes in chemical composition occur in energy reserves as lipids and carbohydrates (FAO 2010, 2018).

In this study, significant seasonal variations ($p<0.05$) were found for lipid content in wedge clams (Table 3). The total lipids (L) amounts were between 1.12 to 5.15 g/100 g ww. In summer months, *D. trunculus* contained twice-higher TL compared to autumn samples. In most of the analysed specimen, TL values were below 3 g/100 g ww, with the exception of June clams. Consequently, *D. trunculus* could be classified as "low fat" species (Regulation (EC) No 1924/2006).

Protein contents varied in a very narrow range, between 15.17 and 16.89 g/100 g ww. In all months of analysis, proteins were above 15 g/100 g ww, which, according to FAO (2010) and Regulation (EC) No 1924/2006 categorisation for the seafood had to be considered as protein-rich (threshold 15 g/100 g ww). Moreover, the insignificant seasonal variations in protein levels in *D. trunculus* charac-

Table 3. Seasonal variations in macronutrients composition of edible tissues of *Donax trunculus* from the Black Sea coast

Donax trunculus	**Lipids g.100^{-1}g ww**	**Proteins g.100^{-1}g ww**	**Carbohydrates g.100^{-1} g ww**	**Moisture**	**Energy values**
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**	**Mean±SD**
June	5.15±0.32	16.13±0.14	1.29±0.08	73.73±3.00	116.03±9.10
July	2.83±0.07	16.89±0.34	2.18±0.10	74.30±2.80	101.75±7.20
August	1.12±0.05	15.20±0.98	5.81±0.24	73.87±3.10	94.12±6.55
Mean, summer	3.03	16.07	3.09	73.97	103.97
September	1.42±0.06	15.17±0.13	5.68±0.20	75.60±2.46	96.18±8.25
October	1.74±0.14	16.49±0.69	3.77±0.27	76.10±3.15	96.70±7.39
November	1.69±0.11	15.99±0.22	4.65±0.23	75.95±3.20	97.77±8.40
Mean, autumn	1.62*	15.88*	4.70*	75.88*	96.88*

Values marked with * are significantly different ($p < 0.05$)

Table 4. Seasonal variations in macroelement contents (mg/kg ww) of edible tissues of *Donax trunculus* from the Black Sea coast

Donax trunculus	**K**	**Na**	**Ca**	**Mg**
June	2559.81±580.16	2223.96±136.6	1167.42±178.8	511.68±12.0
July	2820.50±132.47	2112.19±145.63	806.10±38.42	471.26±13.77
August	1187.15±29.40	2512.27±81.95	1477.57±90.50	710.31±19.15
Mean, summer	2189.15	2282.81	1150.36	564.41
September	1437.23±52.42	3370.11±137.73	819.21±100.63	717.15±32.83
October	1171.04±67.72	2134.75±122.13	1946.55±156.76	493.85±12.95
November	1702.34±30.12	2699.63±147.73	3905.96±266.80	742.57±52.13
Mean, autumn	1436.87*	2734.83*	2223.91*	651.19*

Values marked with * are significantly different ($p < 0.05$)

terised this species as a sustainable natural source of this macronutrient.

The most visible changes were found in carbohydrates levels. The highest amount was determined in August (5.81 g/100 g ww). In autumn months, high carbohydrate levels (av. 4.70 g/100 g ww) were also measured. Furthermore, opposite trends for higher carbohydrate levels and lower lipid amounts in autumn months in the edible tissues were found.

The analysed wedge clams demonstrated a seasonal variation of the energy values, which was mostly dependent on primary metabolites content and showed changes mainly related to the lipid amount decrease. The calculated energy levels of *D. trunculus* tissues were in the diapason 94.12–116 kcal/100 g ww. The lowest value was found in August (94.12 kcal/100 g ww), despite the fact that carbohydrates presented the highest amounts in this month. However, during the autumn, the energy values remained unchanged. Based on the results obtained throughout the study period, the wedge clam presented a low energy content.

Macroelement contents

The seasonal changes of macroelement content of *D. trunculus* soft tissues are presented in Table 4. Significant seasonal differences in element concentrations were observed. The maximum concentration of Na was found during September. More fluctuations were observed for the macroelements K and Ca. During summer seasons, K presented maximum concentration (av. 2189.15 mg/kg ww), with a decrease of over 34.4 % during autumn. Opposite trends were found for the elemental Ca (during autumn seasons, its concentration increased twice). The most stable macroelement among all the analysed ones was Mg. It could be summarised that, during the summer period, the macroelements showed the following pattern of distribution: Na> K> Ca> Mg; in autumn months, it changed as follows: Na> Ca> K > Mg.

Discussion

One of the biggest databases (FAO/INFOODS 2016) presented information for the chemical composition of more than 110 mollusc species. Generally, bivalves contain higher protein levels compared to fish. According to Venogupal & Gopakumar (2017), the average protein content in clams from the Indian Ocean varied between 9 and 14.5 g/100 g ww. Variations were reported: 8.20g/100 g ww protein content for *D. trunculus* from the Marmara Sea (Ozden et al. 2009) and 11.0g/100 g ww for *D. trunculus* from the Azov Sea (Bityutskaya et al. 2021). Protein content of the Black Sea *D. trunculus* was significantly higher, up to 16.89 g/100 g ww.

The lipid content shows the opposite trend. Bivalve species accumulate lipids as energy reserves prior gametogenesis. In this study, the highest levels of lipids have been determined in June–July, followed by a rapid decrease. It can be assumed that, in this period, the spawning peak of *D. trunculus* in the Black Sea occurs. No significant variations in the lipid levels have been observed in the autumn months. According to Zeichen et al**.** (2002), the sexual resting period for *D. trunculus* from the South Adriatic coast extends from the summer (August) to the end of the winter (February), which may explain the observed relatively constant contents of lipids in our study (Table 3). For wedge clams from different regions as the Marmara Sea (Ozden et al. 2009) and the Azov Sea (Bityutskaya et al. 2021), up to twice lower lipid levels (av. 0.8–0.9 g/100 g ww) have been reported.

In general, the carbohydrate contents in shellfish are low. They can vary between 1–3 % in green mussel (Venogupal & Gopakumar 2017). In the present study, the seasonal pattern in the carbohydrate contents was one-and-a-half times higher in the autumn period (up to 5.68 g/100 g ww) in comparison with the summer months. One possible explanation is that the carbohydrate levels (as energy reserves) can vary, being either utilised or accumulated in response to changes in environmental conditions (Biandolino et al. 2020). Significantly lower carbohydrate amounts were reported for wedge clam from the Azov Sea (Bityutskaya et al. 2021) whereas the data presented about the seasonal changes carbohydrate contents of *D. trunculus* in the Marmara Sea (Ozden et al. 2009) are similar to the present results. We provide the first data on the carbohydrate content of the wedge clam *D. trunculus* from the Bulgarian Black Sea coast.

Tissues of bivalves can deposit different minerals, which may have physiological significance to the human organism. These elements are studied as part of assessment of nutritional quality of seafood. Malnutrition or deficiency of these elements can provoke specific diseases (Lu & Wang 2021). Moreover, it is supposed that consumption of bivalves can reduce the different cardiovascular diseases (CDV), including hypertension, because of being a valuable source of K, Na, Ca and Mg, which are essential in the CDV prevention. The studies are usually focused on the toxic element concentration and, in contrast, the data regarding contents of macroelements as K, Na, Ca and Mg in white clam tissues are very limited. Having in mind these facts and the increased commercial significance of Black Sea *D. trunculus*, there is a need to update and enrich knowledge regarding macroelement contents of this resource of arising importance.

Sodium is the extracellular cation, which is important for osmoregulation, cell membrane potential and acid-base balance. Potassium is one of the vital elements for humans and an intracellular cation participating in the regulation of the osmotic pressure in cells. Magnesium is a critical intracellular divalent cation playing an essential physiological role in many functions in the human organism; it forms a key complex with ATP and participates in many important biological processes such as protein synthesis, cell replication and energy metabolism (Lall & Kaushik 2021). Calcium as well as phosphorus play a major role in the development and maintenance of the skeletal system and perform many other physiological functions, including the maintenance of acid–base equilibrium (Zimmer et al. 2019). Marine organisms absorb Ca and P from the aquatic environment via gills, gastrointestinal tract and integument; however, the gills represent the major site of Ca uptake (Lall & Kaushik 2021). From a nutritional point of view, the inadequate intake of these elements can provoke diverse disorders (Biandolino et al. 2020, Kapranov et al. 2021).

In this study, the dominant macroelement in *D. trunculus* tissues is Na. Similar concentrations of sodium was reported for *D. trunculus* from Azov Sea (Bityutskaya et al. 2021) and from the Chinese coastal waters mussels (Lu & Wang 2021). All these studies have reported a distribution pattern of macroelements: Na>Ca>K>Mg. Ozden et al. (2009) reported insignificant seasonal differences in elemental contents in the Marmara Sea wedge clam tissues, in both seasons the arrangement was Na>Ca>K>Mg.

In our study, a different pattern of the macroelement concentration was determined during the summer and autumn seasons, with K having higher amounts than Ca in the autumn period. One possible explanation is that marine bivalves are in osmotic equilibrium with the seawater and the observed differences are related with the specific salinity of the

localities. Sodium is the major component, whereas K has lower amounts in the seawater (Lu & Wang 2018), which is also reflected in the relevant concentrations in bivalve tissues. Marine invertebrates can obtain elements from both seawater and food as well as by assimilating inorganic particles. The observed differences in the macroelement contents depend of biotic (reproductive cycle, age, sex) and abiotic (salinity, temperature) factors as well as by the chemical form of the elements (Viral & Acelik 2021).

In conclusion, the significance of the presented study is associated with the lack of information about the seasonal changes in proximate composition and macroelement contents in wedge clam *Donax trunculus* from the Bulgarian Black Sea coast. The primary metabolites have shown significant seasonal dynamics but this species is a valuable food source, with high proteins and low lipids and carbohydrate contents and well-balanced macroelement composition, regardless the season. The results from this study can be useful in promoting the consumer acceptance of wedge clam as a promising new source of health-beneficial macronutrients in a country with relatively low consumption of seafood.

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