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Protein and amino acid profile of *Ruditapes decussatus* and *Ruditapes philippinarum* from Çanakkale Strait, Türkiye

by

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Abstract

Protein content was lowest in April 2018 and highest in May 2018 for *Ruditapes decussatus* (Linnaeus 1758), and was lowest in September 2017 and highest in December 2017 for *Ruditapes philippinarum* (Adams, Reeve 1850). Amino acid content is particularly important to determine such variations every month to develop consumption strategies for these species. Leucine was the most abundant amino acid in both species, followed by isoleucine, arginine, methionine, and serine. Leucine plays a critical role in protein synthesis and muscle repair. Leucine levels were consistently high throughout the year for both species, but the recommended optimal consumption periods are all months except September and April for *R. philippinarum*, and all months except spring for *R. decussatus*. Arginine is essential for children, and it has been shown to enhance growth hormone synthesis. Arginine concentrations were high in winter and spring for *R. philippinarum* and autumn for *R. decussatus*. Methionine levels were highest in spring for *R. philippinarum*, autumn for *R. decussatus*. The essential amino acid to non-essential amino acid ratio was >1 for both species throughout the year, indicating high protein quality.

Key words: Manila clam, grooved carpet shell, leucine, isoleucine, arginine, serine, alanine

1. Introduction

Bivalves possess a nutritionally rich composition due to their high protein content, abundance of essential amino acids, and low lipid levels (Acarli et al., 2023; Prato et al., 2019; Vural, 2022; Vural & Acarli, 2021; Yildiz et al., 2021). These mollusks incorporate all amino acids essential for regulating lipid metabolism, supporting growth and repair processes, and preventing hepatic disorders and renal failure in the human body (Koral & Süleyman, 2017; Varlik et al., 2004). Among these, amino acids such as aspartic acid, glycine, and glutamic acid play a pivotal role in wound healing (Vural & Acarli, 2021; Zuraini et al., 2006).

Türkiye harbors two clam species with significant production potential among bivalve taxa. These are the native grooved carpet shell, *Ruditapes decussatus*, and the Manila clam, *Ruditapes philippinarum*. The origin of *R. philippinarum* is traced to the western Pacific coasts, with its natural distribution encompassing the Philippines, South and East China Seas, Yellow Sea, and Sea of Japan (Scarlato, 1981). Its introduction to the Adriatic and Aegean Seas was reported in 2004–2005 (Jensen et al., 2004, 2005). On the other hand, *R. decussatus* exhibits a distribution extending from Norway to Somalia, including the Iberian Peninsula and the Mediterranean Sea (Parache, 1982).

Various studies have been conducted in Turkish marine waters focusing on *R. decussatus* and *R. philippinarum*. These include investigations on the biochemical composition of both species (Çelik et al., 2014), heavy metal accumulation in *R. decussatus* (Bilgin & Uluturhan, 2015; Ulusoy, 2010), PAH contamination in *R. philippinarum* (Künili et al., 2021), spat collection in *R. decussatus* (Serdar et al., 2009), juvenile rearing in *R. decussatus* (Serdar et al., 2007), reproduction in *R. decussatus* (Serdar et al., 2010a), and morphological assessments of both species (Acarli et al., 2015; Doğan, 2005; Doğan et al., 2016). Additionally, research has addressed genetic traits and hybridization in *R. decussatus* and *R. philippinarum* (Acarli et al., 2015, 2018).

Monthly protein levels of *R. decussatus* in Türkiye have been analyzed in the Sufa Lagoon (Serdar & Lök, 2009; Serdar et al., 2009) and Çakalburnu Lagoon (Serdar et al., 2010a, 2010b) in the Aegean Sea. Additionally, Çelik et al. (2014) examined the seasonal protein levels of *R. decussatus* and *R. philippinarum* in the Marmara Sea (Bandırma). However, there is no research available on the extent of monthly variations in amino acid content for these two clam species in Türkiye. Determining such variations every month is particularly important for developing consumption strategies for these species.

This study aims to evaluate the monthly protein and amino acid contents of *R. decussatus* and *R. philippinarum* from the Çardak Lagoon, to identify their nutritional values, and to assess the extent to which these species meet human requirements for essential amino acids and protein. The findings will also help establish consumption recommendations and encourage the aquaculture of these species in the region based on the results obtained.

2. Materials and methods

2.1. Location and sampling

This study was conducted in Çardak Lagoon (40°22′56″ N, 21°42′58″ E), located in Lapseki District, Çanakkale Province, Türkiye (Fig. 1). The Çardak Lagoon is situated at the junction of the Çanakkale Strait and the Marmara Sea and is considered an important wetland for Çanakkale (Acar & Ateş, 2023). It hosts various commercially valuable bivalve species, including the striped venus clam *Chamelea gallina* (Linnaeus 1758), *R. philippinarum*, *R. decussatus*, the European flat oyster *Ostrea edulis* (Linnaeus 1758), and the smooth scallop *Flexopecten glaber* (Linnaeus 1758) (Vural & Acarli, 2018). The lagoon has a depth of 4.5 m and a muddy–sandy substrate.

During the study, samples of *R. decussatus* and *R. philippinarum* were collected monthly from July 2017 to June 2018, with 30 specimens per species brought to the laboratory each month. The samples were cleaned of fouling organisms using a knife. After separating the meat from the shells, the tissues were freeze-dried in a lyophilizer. The dried and ground tissues were subsequently used for protein and amino acid analyses.

2.2. Macroscopic gonad analysis

The gonads of the bivalves were macroscopically classified into four stages: developing, mature (ripe), spawning, and the fully spent stage.

2.3. Crude protein analysis

Crude protein analysis was performed using the Kjeldahl method (AOAC, 2000). A 0.5 g sample was digested in 98% H_2SO_4 with a Kjeldahl catalyst tablet for 1 hr in a Kjeldahl digestion unit. The digested samples were then distilled using a distillation apparatus with 0.1 M NaOH and 0.5 M boric acid. The distillates were titrated with 0.5 N HCl, and the protein content was

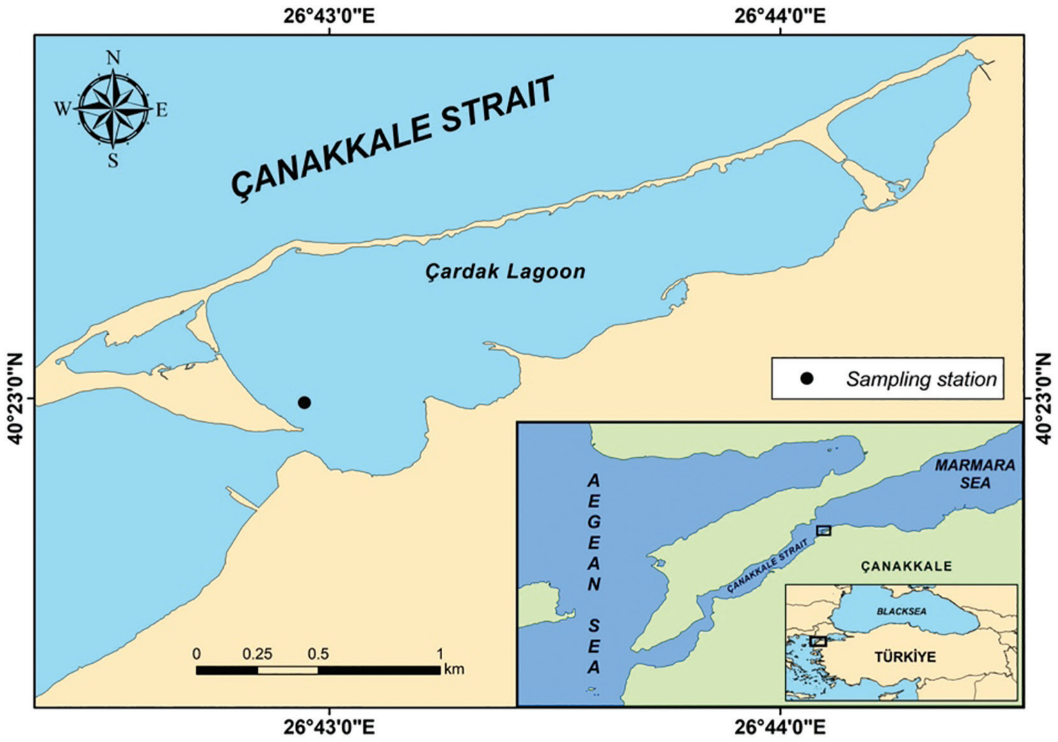

Figure 1

Sampling area.

calculated based on the volume of HCl consumed during titration. Calculations utilized a nitrogen-to-protein conversion factor of 6.25.

2.4. Amino acid analysis

Amino acid composition was determined using the HPLC method (Köse et al., 2011). Dried tissue samples were hydrolyzed in 6.0 M HCl at 110°C for 24 hr in an oven. The hydrolysates were filtered using a 0.20 μm PTFE syringe filter, evaporated with HCl, and derivatized. The amino acid concentrations were measured using an HPLC system with EZ:faast kits (EZ:faast GC/FID Amino Acid Kit).

2.5. Statistical analysis

Statistical analysis was performed using SPSS 29. One-way analysis of variance (ANOVA) was used to evaluate monthly variations in protein and amino acid composition. Levene's test was employed to assess the homogeneity of variance, while the Kolmogorov–Smirnov test was used to check for normality. Post hoc comparisons were conducted using the Tukey test, and differences were considered statistically significant at $p < 0.05$ (Homack, 2001; Kucuk et al., 2016; Sümbüloğlu & Sümbüloğlu, 2002).

Principal component analysis (PCA) was performed using R Version 3.6.1 (R Core Team, 2023) to identify the amino acids contributing most significantly to monthly variations in amino acid content. The FactoMineR (Lê et al., 2008), Factoextra (Kassambra & Mundt, 2020), and corrplot (Wei & Simko, 2017) packages were used for PCA computation and visualization of the correlation matrix based on Spearman correlation coefficients.

3. Results

3.1. Macroscopic gonad analysis

When examining gonad maturation macroscopically, maturation in *R. decussatus* occurred in all months except March and May, with ripening in all months except autumn and June, and spawning in the autumn and winter months. For *R. philippinarum*, gonad maturation occurred in all months except spring, and ripening occurred in all months except September. Spawning took place in the autumn and winter months (Fig. 2).

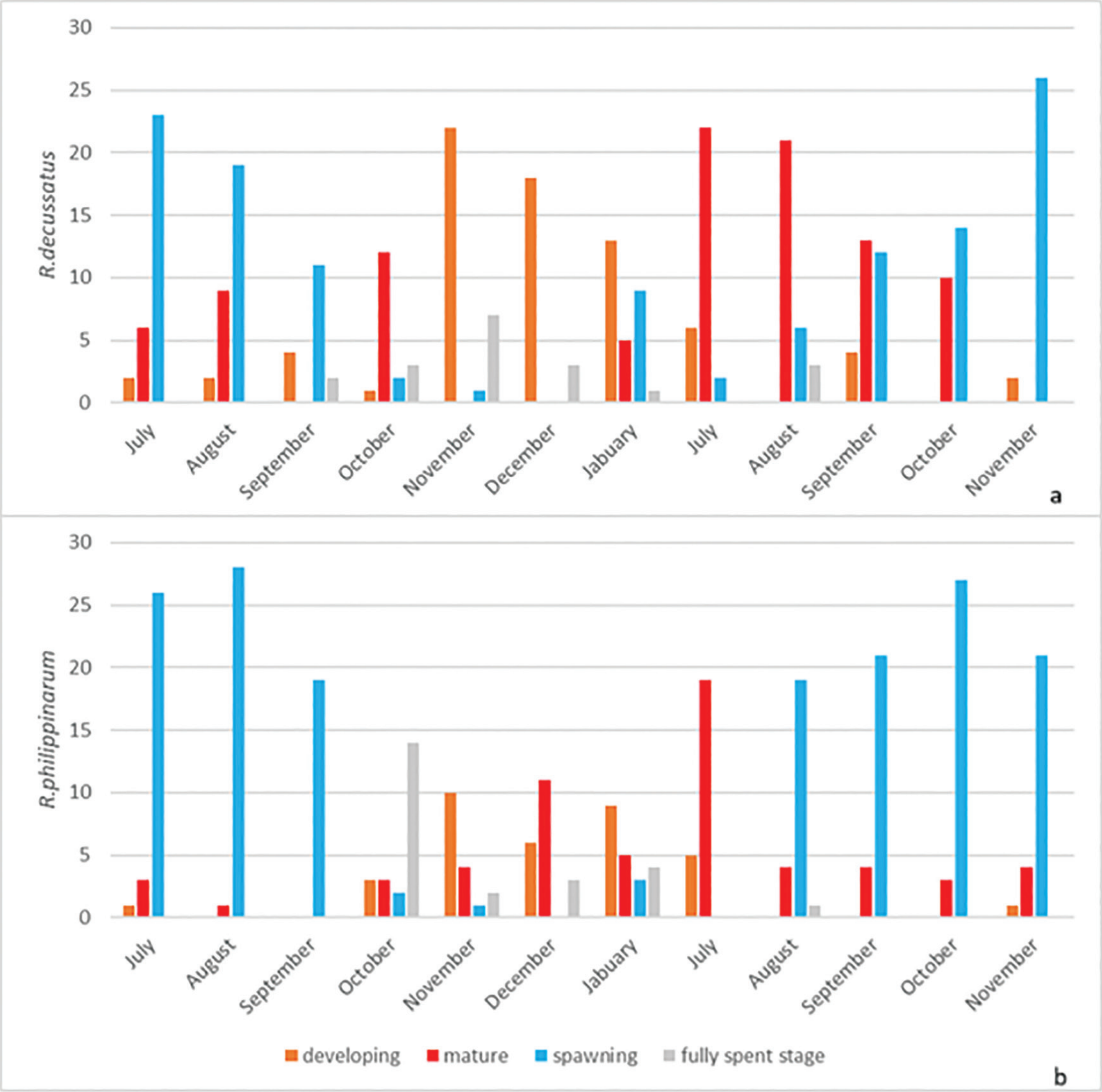

Figure 2

Gonad developing stage (July 2017–June 2018) of *R. decussatus* and *R. philippinarum*.

3.2. Crude protein content

The protein content of *R. philippinarum* ranged from 60.84 ± 0.16% in September to 72.91 ± 0.24% in December. For *R. decussatus*, the lowest protein content was observed in April at 63.44 ± 0.32%, while the highest was recorded in May at 80.00 ± 0.00% (Fig. 3).

3.3. Amino acid content

The wet-to-dry conversion ratios for *R. philippinarum* and *R. decussatus* are presented in Table 1. The amino acid compositions of *R. philippinarum* and *R. decussatus* are shown in Tables 2 and 3, respectively.

For *R. philippinarum*, the essential amino acids with the highest concentrations were leucine, isoleucine, methionine, and phenylalanine. Isoleucine ranged from 405.1 ± 3.7 mg · 100 g^{-1} dry weight (dw) in December to 920.4 ± 4.3 mg · 100 g^{-1} dw in August. Leucine was found at its lowest concentration in September (347.3 ± 3.3 mg · 100 g^{-1} dw) and highest in July (1004.3 ± 13.4 mg · 100 g^{-1} dw). Lysine had the lowest concentration in December (1.7 ± 0.0 mg · 100 g^{-1} dw) and the highest in November (8.2 ± 0.0 mg · 100 g^{-1} dw). Valine levels were lower in autumn and higher in spring (Table 2).

In *R. philippinarum*, a positive correlation was found between leucine and phenylalanine, while a negative correlation was observed between leucine and methionine, threonine, proline, and arginine ($p < 0.05$). Isoleucine showed positive correlations with methionine, threonine, and valine, but negative correlations with alanine and protein ($p < 0.05$). Methionine was positively correlated with histidine, isoleucine, threonine, proline, and arginine, but negatively with leucine, alanine, and serine ($p < 0.05$). Serine had a positive correlation with tryptophan and a negative correlation with methionine and phenylalanine ($p < 0.05$). Lysine was positively correlated with histidine, tryptophan, and tyrosine,

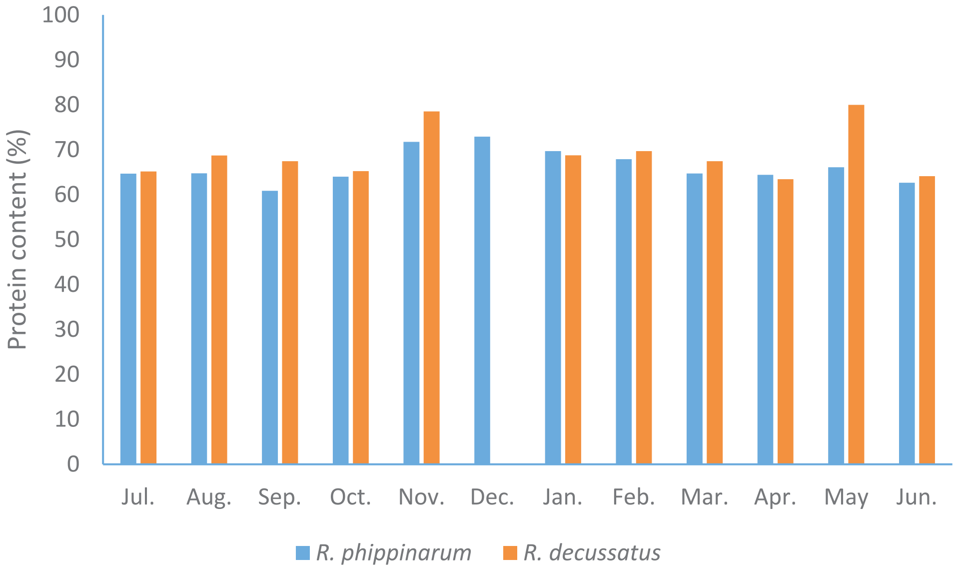

Figure 3

Protein content of *R. decussatus* and *R. philippinarum* (%) (July 2017–June 2018).

Table 1

Freeze dried to wet meat conversation coefficients of *R. philippinarum* and *R. decussatus*

	R. decussatus				***R. philippinarum***			
Month	**Meat Yield (%)**	**CI**	**Moisture (%)**	**Wet meat conversation coefficient**	**Meat Yield (%)**	**CI**	**Moisture (%)**	**Wet meat conversation coefficient**
Jul. 2017	22.23	9.61	79.90	0.20	21.75	7.08	79.93	0.20
Aug.	22.53	9.45	80.01	0.20	17.70	6.87	79.66	0.20
Sep.	21.25	9.79	78.57	0.21	16.60	6.29	79.51	0.20
Oct.	26.04	10.63	81.34	0.19	16.74	5.42	82.94	0.17
Nov.	22.70	9.61	80.49	0.20	19.03	7.23	80.89	0.19
Dec.	22.45	8.51	83.06	0.17	21.02	7.31	81.55	0.18
Jan. 2018	26.97	10.06	83.38	0.17	24.37	7.89	82.82	0.17
Feb.	23.92	9.90	80.69	0.19	18.64	6.97	80.97	0.19
Mar.	25.95	10.38	81.99	0.18	21.14	9.25	78.15	0.22
Apr.	26.11	12.69	77.67	0.22	22.95	10.00	78.26	0.22
May	24.26	10.94	79.46	0.21	20.93	8.21	79.77	0.20
Jun.	30.96	13.37	79.93	0.20	14.28	8.48	78.33	0.20

Table 2

Monthly variations in amino acid composition of *R. philippinarum* (mg/100 g^{-1} dw, mean ± SD)

	Jul. 2017	Aug.	Sep.	Oct.	Nov.	Dec.	Jan. 2018	Feb.	Mar.	Apr.	May	Jun.	Mean	Summer	Autumn	Winter	Spring
% Protein	64.6 ± 0.4	64.8 ± 0.3	60.8 ± 0.2	64.0 ± 0.6	71.8 ± 0.3	72.9 ± 0.2	69.7 ± 0.6	67.9 ± 0.3	64.7 ± 0.4	64.4 ± 0.2	66.1 ± 0.0	62.7 ± 0.3	66.2 ± 0.2	64.0 ± 0.0	65.5 ± 0.2	70.2 ± 0.2	65.1 ± 0.2
Histidine	3.8 ± 0.1	4.4 ± 0.0	4.8 ± 0.0	5.2 ± 0.0	6.7 ± 0.0	2.9 ± 0.0	5.9 ± 0.1	10.7 ± 0.4	3.7 ± 0.1	8.6 ± 0.2	7.3 ± 0.0	5.5 ± 0.0	5.8 ± 0.1	4.6 ± 0.0	5.6 ± 0.0	6.5 ± 0.2	6.5 ± 0.1
Isoleucine	473.7 ± 6.3	920.4 ± 4.3	618.3 ± 5.9	587.5 ± 5.6	494.2 ± 2.3	405.1 ± 3.7	441.7 ± 5.9	746.8 ± 28.2	835.2 ± 18.2	720.6 ± 16.4	636.3 ± 2.9	710.7 ± 6.8	632.5 ± 7.9	701.7 ± 1.3	566.7 ± 2.0	531.2 ± 13.5	730.7 ± 8.4
Leucine	1004.3 ± 13.4	565.1 ± 2.6	347.3 ± 3.3	622.8 ± 6.0	758.9 ± 3.6	672.8 ± 6.2	686.8 ± 9.2	634.4 ± 23.9	893.0 ± 19.5	493.8 ± 11.3	616.2 ± 2.8	826.0 ± 7.9	676.8 ± 6.8	798.5 ± 5.4	576.3 ± 1.5	664.8 ± 9.5	667.7 ± 8.3
Lycine	3.1 ± 0.1	3.8 ± 0.0	2.3 ± 0.0	6.5 ± 0.1	8.2 ± 0.0	1.7 ± 0.0	3.9 ± 0.1	7.7 ± 0.4	3.0 ± ± 0.1	7.7 ± 0.3	4.6 ± 0.0	4.8 ± 0.1	4.8 ± 0.1	3.9 ± 0.0	5.7 ± 0.0	4.4 ± 0.2	5.1 ± 0.1
Methionine	56.5 ± 0.7	42.4 ± 0.2	92.5 ± 0.1	53.5 ± 0.5	32.9 ± 0.2	23.6 ± 0.1	18.4 ± 0.2	100.0 ± 3.8	65.8 ± 1.4	100.5 ± 2.3	57.9 ± 0.3	10.1 ± 0.1	54.5 ± 1.1	36.3 ± 0.3	59.6 ± 0.4	47.3 ± 2.1	74.7 ± 1.0
Phenylalanine	21.2 ± 0.3	11.6 ± 0.0	8.1 ± 0.1	12.6 ± 0.1	9.2 ± 0.00	14.4 ± 0.1	42.3 ± 0.6	19.3 ± 0.7	22.0 ± 0.5	11.2 ± 0.3	17.6 ± 0.1	19.0 ± 0.2	17.4 ± 0.2	17.3 ± 0.1	9.9 ± 0.0	25.3 ± 0.3	16.9 ± 0.2
Throline	6.4 ± 0.1	18.3 ± 0.1	19.8 ± 0.2	5.5 ± 0.0	4.1 ± 0.0	2.3 ± 0.0	1.8 ± 0.02	14.0 ± 0.5	8.3 ± 0.2	22.8 ± 0.5	9.6 ± 0.0	8.3 ± 0.1	10.1 ± 0.2	11.0 ± 0.0	9.8 ± 0.1	6.0 ± 0.3	13.6 ± 0.2
Valine	3.6 ± 0.1	5.42 ± 0.0	3.9 ± 0.0	4.9 ± 0.0	2.7 ± 0.0	4.8 ± 0.0	4.3 ± 0.06	5.0 ± 0.2	8.3 ± 0.2	10.0 ± 0.2	6.5 ± 0.0	7.1 ± 0.1	5.6 ± 0.1	5.4 ± 0.0	3.9 ± 0.0	4.7 ± 0.1	8.3 ± 0.1
Triptofan	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	8.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.00	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.0 ± 0.0	01.0 ± 0.0	0.2 ± 0.0	3.0 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
NEAA	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Mean**	**Summer**	**Autumn**	**Winter**	**Spring**
Alanine	10.8 ± 0.1	9.5 ± 0.0	8.8 ± 0.1	7.9 ± 0.1	22.2 ± 0.1	16.2 ± 0.1	12.2 ± 0.2	8.3 ± 0.3	8.8 ± 0.2	11.1 ± 0.2	9.7 ± 0.0	11.5 ± 0.11	11.4 ± 0.1	10.6 ± 0.0	12.9 ± 0.0	12.3 ± 0.1	9.8 ± 0.1
Aspartic acid	2.4 ± 0.0	2.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.0	3.7 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	2.1 ± 0.0	1.4 ± 0.0	2.0 ± 0.0	2.4 ± 0.0	1.2 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.3 ± 0.0	1.5 ± 0.0	2.0 ± 0.0
Glutamic acid	5.1 ± 0.1	5.2 ± 0.0	4.0 ± 0.0	3.7 ± 0.0	6.2 ± 0.0	2.3 ± 0.0	3.8 ± 0.0	6.5 ± 0.2	3.2 ± 0.1	5.8 ± 0.1	6.0 ± 0.0	3.1 ± 0.0	4.6 ± 0.0	4.5 ± 0.0	4.6 ± 0.0	4.2 ± 0.1	45.0 ± 0.0
Glycine	4.5 ± 0.1	6.3 ± 0.0	12.4 ± 0.1	2.8 ± 0.0	9.6 ± 0.0	9.6 ± 0.0	3.3 ± 0.0	3.5 ± 0.1	3.5 ± 0.1	2.4 ± 0.0	1.3 ± 0.0	4.3 ± 0.0	5.3 ± 0.0	5.0 ± 0.0	8.3 ± 0.0	5.4 ± 0.0	2.4 ± 0.0
Serine	7.9 ± 0.1	24.0 ± 0.1	22.7 ± 0.22	33.5 ± 0.3	35.9 ± 0.2	11.1 ± 0.0	27.3 ± 0.4	6.9 ± 0.3	15.0 ± 0.3	14.5 ± 0.3	8.6 ± 0.0	20.4 ± 0.2	19.0 ± 0.1	17.4 ± 0.0	30.7 ± 0.1	15.1 ± 0.2	12.7 ± 0.2
Tyrosine	3.5 ± 0.1	0.8 ± 0.0	2.7 ± 0.0	7.1 ± 0.1	4.5 ± 0.0	3.6 ± 0.0	1.6 ± 0.0	1.7 ± 0.1	1.1 ± 0.0	8.7 ± 0.3	0.9 ± 0.0	4.1 ± 0.0	3.4 ± 0.1	2.8 ± 0.0	4.8 ± 0.0	2.3 ± 0.0	3.6 ± 0.1
Pyroline	7.3 ± 0.1	1.3 ± 0.0	8.3 ± 0.1	7.5 ± 0.1	3.2 ± 0.0	2.2 ± 0.0	1.1 ± 0.0	2.5 ± 0.1	2.2 ± 0.0	8.1 ± 0.2	2.5 ± 0.0	1.8 ± 0.0	4.0 ± 0.0	3.4 ± 0.0	6.4 ± 0.0	1.9 ± 0.0	4.3 ± 0.1
Arginine	76.5 ± 1.0	67.7 ± 0.3	108.5 ± 1.0	81.6 ± 0.8	62.7 ± 0.3	97.1 ± 0.4	98.8 ± 1.3	102.0 ± 3.8	106.3 ± 2.5	116.9 ± 2.7	78.3 ± 0.4	51.5 ± 0.5	87.3 ± 1.1	65.2 ± 0.4	84.3 ± 0.4	99.3 ± 1.8	100.5 ± 1.3
EAA/NEAA	13.33	13.40	6.49	8.90	8.95	7.87	8.07	11.51	13.00	8.12	12.35	16.28	10.29	14.22	8.04	9.08	10.87

Table 3

Monthly variations in amino acid composition of *R. decussatus* (mg/100 g^{-1} dw, mean ± SD)

	Jul. 2017	Aug.	Sep.	Oct.	Nov.	Jan. 2018	Feb.	Mar.	Apr.	May	Jun.	Mean.	Summer	Autumn	Winter	Spring
% Protein	65.1 ± 0.1	68.7 ± 0.0	67.4 ± 0.2	65.2 ± 0.1	78.5 ± 0.3	68.8 ± 0.2	69.7 ± 0.3	67.4 ± 0.2	63.4 ± 0.3	80.0 ± 0.0	64.1 ± 0.4	69.0 ± 0.1	66.0 ± 0.2	70.4 ± 0.1	69.2 ± 0.06	70.3 ± 0.2
Histidine	11.1 ± 0.1	11.0 ± 0:1	6.4 ± 0.1	10.1 ± 0.0	6.4 ± 0.0	3.0 ± 0.0	7.6 ± 0.1	7.0 ± 0.1	5.2 ± 0.0	3.8 ± 0.0	11.0 ± 0.2	7.5 ± 0.0	11.0 ± 0.1	7.6 ± 0.0	5.3 ± 0.03	5.3 ± 0.0
Isoleucine	491.5 ± 4.7	626.6 ± 3.1	491.3 ± 4	408.6 ± 1.9	624.4 ± 2.9	385.8 ± 3.6	700.0 ± 0.0	651.6 ± 6.0	278.9 ± 2.6	397.0 ± 1.9	325.8 ± 4.6	489.2 ± 1.7	481.3 ± 0.9	508.1 ± 1.4	542.9 ± 2.57	442.5 ± 2.2
Leucine	1141.9 ± 10.9	1139.0 ± 5.6	999.7 ± 9	883.3 ± 4.1	827.0 ± 3.8	747.4 ± 7.0	977.9 ± 9.1	602.6 ± 5.5	646.4 ± 6.1	668.9 ± 3.1	755.1 ± 10.6	853.6 ± 2.8	1,011.9 ± 3.0	903.3 ± 3.2	862.6 ± 1.4	639.3 ± 1.6
Lycine	7.7 ± 0.1	7.0 ± 0.0	4.9 ± 0.1	5.3 ± 0.0	4.9 ± 0.0	2.8 ± 0.0	6.1 ± 0.1	5.1 ± 0.1	4.1 ± 0.0	2.8 ± 0.0	5.9 ± 0.1	5.1 ± 0.0	6.9 ± 0.0	5.0 ± 0.0	4.4 ± 0.0	4.0 ± 0.0
Methionine	41.8 ± 0.4	72.3 ± 0.3	57.7 ± 0.5	50.6 ± 0.7	42.9 ± 0.6	19.4 ± 0.2	42.8 ± 0.2	44.7 ± 0.4	22.8 ± 0.2	35.6 ± 0.2	23.7 ± 0.3	41.3 ± 0.2	45.9 ± 0.0	50.4 ± 0.1	31.1 ± 0.0	34.3 ± 0.1
Phenylalanine	13.1 ± 0.1	36.2 ± 0.2	35.4 ± 0.3	30.0 ± 0.1	41.8 ± 0.2	13.0 ± 0.1	10.1 ± 0.1	5.3 ± 0.0	12.3 ± 0.1	16.7 ± 0.1	12.5 ± 0.2	20.6 ± 0.1	20.6 ± 0.0	35.7 ± 0.1	11.5 ± 0.0	11.4 ± 0.0
Throline	14.9 ± 0.1	48.9 ± 0.9	18.5 ± 0.2	30.5 ± 0.4	21.9 ± 0.3	2.0 ± 0.0	5.8 ± 0.0	5.6 ± 0.0	6.9 ± 0.1	10.3 ± 0.0	10.5 ± 0.1	16.0 ± 0.3	24.8 ± 0.4	23.7 ± 0.1	3.9 ± 0.0	7.6 ± 0.0
Valine	3.5 ± 0.0	8.9 ± 0.2	18.3 ± 0.2	12.0 ± 0.2	13.2 ± 0.2	4.0 ± 0.0	3.8 ± 0.0	2.1 ± 0.0	2.7 ± 0.0	1.9 ± 0.0	1.5 ± 0.0	6.5 ± 0.1	4.6 ± 0.1	14.5 ± 0.0	3.9 ± 0:0	2.2 ± 0.0
Triptofan	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.8 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
NEAA	**Jul.**	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**	**Mean**	**Summer**	**Autumn**	**Winter**	**Spring**
Alanine	34.5 ± 0.3	45.2 ± 0.9	69.0 ± 0.6	46.3 ± 0.6	63.8 ± 0.9	6.5 ± 0.1	7.9 ± 0.0	18.0 ± 0.2	2.6 ± 0.0	13.5 ± 0.1	16.9 ± 0.2	29.5 ± 0.3	32.2 ± 0.3	59.7 ± 0.1	7.2 ± 0.0	11.4 ± 0.1
Aspartic acid	4.3 ± 0.0	1.4 ± 0.0	3.5 ± 0.0	3.9 ± 0.0	3.5 ± 0.0	0.7 ± 0.0	2.5 ± 0.0	2.8 ± 0.0	2.0 ± 0.0	4.5 ± 0.0	4.5 ± 0.1	3.1 ± 0.0	3.4 ± 0.0	3.6 ± 0.0	1.6 ± 0.0	3.1 ± 0.0
Glutamic acid	5.4 ± 0.0	4.2 ± 0.0	5.8 ± 0.0	9.4 ± 0.0	5.8 ± 0.0	1.3 ± 0.0	5.5 ± 0.0	6.4 ± 0.1	3.4 ± 0.0	2.2 ± 0.0	5.6 ± 0.0	5.0 ± 02	5.1 ± 0.0	7.0 ± 0.0	3.4 ± 0.0	4.0 ± 0.0
Glycine	10.9 ± 0.1	12.2 ± 0.2	23.3 ± 0.2	20.5 ± 0.3	24.8 ± 0.3	3.2 ± 0.0	3.0 ± 0.0	2.0 ± 0.0	2.8 ± 0.0	3.3 ± 0.0	3.6 ± 0.0	10.0 ± 0.1	8.9 ± 0.1	22.9 ± 0.1	3.1 ± 0.0	2.7 ± 0.0
Serine	28.4 ± 0.3	31.2 ± 0.6	26.9 ± 0.2	29.2 ± 0.4	23.4 ± 0.3	26.4 ± 0.2	21.6 ± 0.1	16.1 ± 0.1	6.1 ± 0.1	7.9 ± 0.0	10.8 ± 0.1	20.7 ± 0.2	23.5 ± 0.2	26.5 ± 0.1	24.00 ± 0.	10.0 ± 0.1
Tyrosine	9.1 ± 0.1	18.8 ± 0.1	8.5 ± 0.1	6.7 ± 0.0	16.4 ± 0.1	2.3 ± 0.0	9.2 ± 0.1	2.3 ± 0.0	0.8 ± 0.0	4.8 ± 0.0	7.9 ± 0.1	7.9 ± 0.0	11.9 ± 0.0	10.5 ± 0.0	5.77 ± 0.1	2.6 ± 0.0
Pyroline	48.4 ± 0.5	26.4 ± 0.5	33.4 ± 0.3	49.7 ± 0.7	55.4 ± 0.8	3.0 ± 0.0	4.8 ± 0.0	0.8 ± 0.0	2.5 ± 0.0	3.8 ± 0.0	37.0 ± 0.5	24.1 ± 0.3	37.3 ± 0.0	46.2 ± 0.2	3.91 ± 0.0	2.4 ± 0.0
Arginine	77.8 ± 0.7	108.9 ± 2.1	84.5 ± 0.8	95.2 ± 1.3	115.9 ± 1.6	75.6 ± 0.7	87.7 ± 0.4	78.5 ± 0.7	32.9 ± 0.3	27.7 ± 0.1	48.3 ± 0.7	75.7 ± 0.6	78.3 ± 0.8	98.5 ± 0.4	81.6 ± 0.2	46.4 ± 0.3
EAA/NEAA	7.88	7.85	6.40	5.48	9.89	9.89	12.61	10.44	18.50	16.79	8.51	8.20	8.01	5.63	11.37	13.90

but negatively with glycine ($p < 0.05$). Additionally, histidine showed a positive correlation with lysine, methionine, threonine, aspartic acid, and glutamic acid ($p < 0.01$, Fig. 4).

For *R. decussatus*, leucine, isoleucine, methionine, threonine, and phenylalanine were the essential amino acids found in higher concentrations. Leucine was highest in July, and isoleucine reached its peak in February. Leucine levels ranged from 602.6 ± 5.5 mg · 100 g^{-1} dw in March to 1141.9 ± 10.9 mg · 100 g^{-1} dw in July. Lysine had its lowest value in May (2.8 ± 0.0 mg · 100 g^{-1} dw) and highest in November (7.7 ± 0.1 mg · 100 g^{-1} dw). Isoleucine, leucine, and methionine were found at higher levels in winter, while arginine, methionine, and serine were higher in autumn (Table 3).

In *R. decussatus,* a positive correlation was found between isoleucine and lysine, leucine, methionine, alanine, glutamic acid, serine, tyrosine, arginine, and protein ($p < 0.05$). Leucine showed positive correlations with lysine, histidine, isoleucine, methionine, phenylalanine, threonine, valine, alanine, glycine, serine, tyrosine, proline, and arginine. Methionine was positively correlated with lysine, histidine, isoleucine, leucine, phenylalanine, threonine, valine, alanine, glutamic acid, glycine, serine, tyrosine, and arginine, but negatively with tryptophan. Serine was positively correlated with lysine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, valine, alanine, and glycine, but negatively with tryptophan ($p < 0.05$, Fig. 5).

In *R. philippinarum*, arginine was the most abundant non-essential amino acid, followed by serine and alanine. Arginine was highest in spring and lowest in summer. Positive correlations were found between arginine and methionine, threonine, and proline, while negative correlations were found between arginine and leucine, alanine, and aspartic acid ($p < 0.05$, Fig. 4). Serine was highest in autumn in the Manila clam (Table 2).

Similarly, in *R. decussatus*, arginine was the most abundant non-essential amino acid, followed by serine,

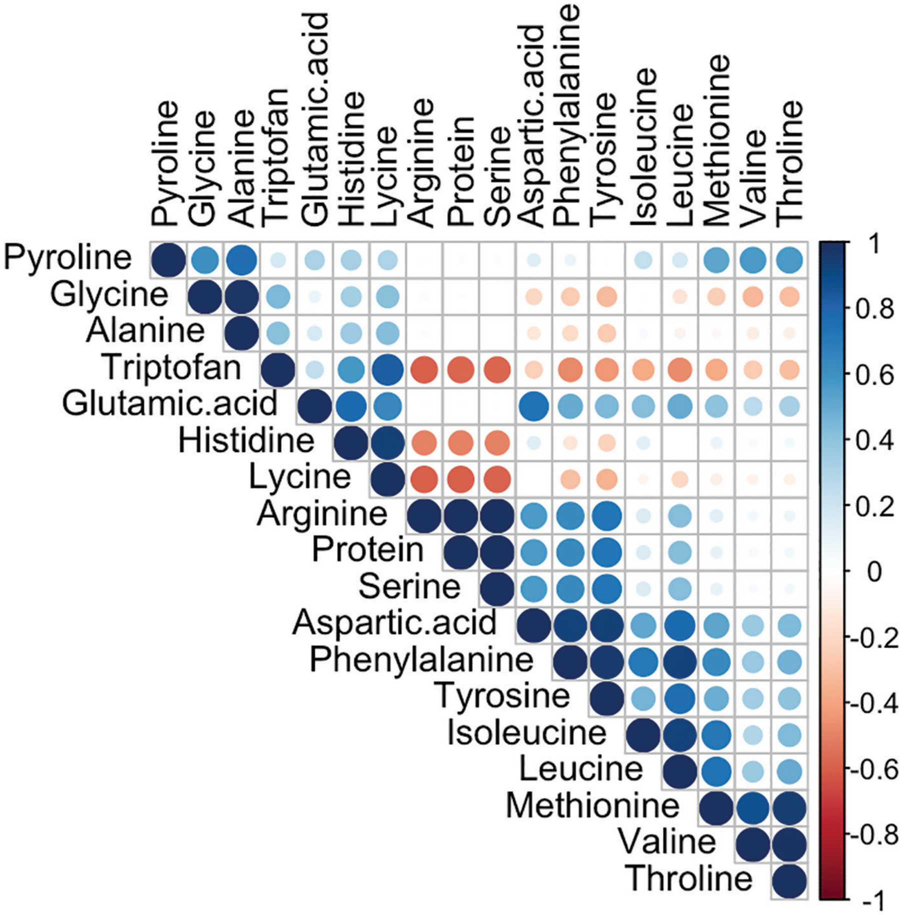

Figure 4

Pairwise correlation plot between amino acid and protein concentrations of *R. philippinarum.*

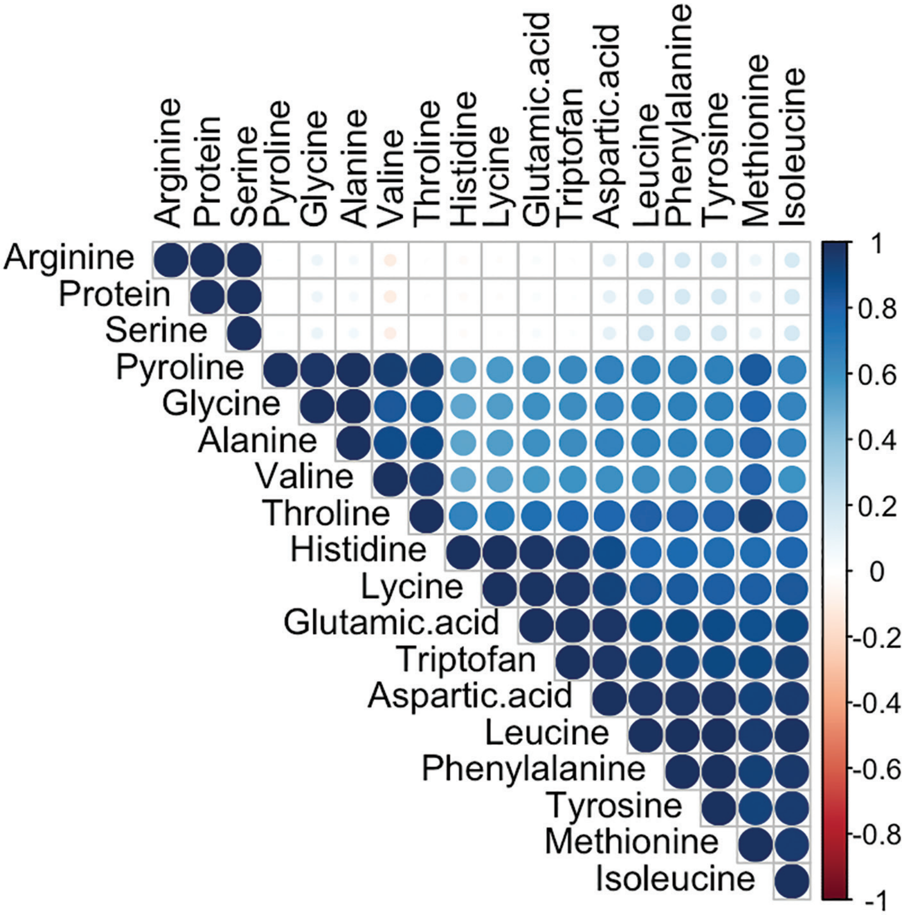

Figure 5

Pairwise correlation plot between amino acid and protein concentrations of *R. decussatus*.

alanine, and proline (Table 3). Positive correlations were observed between arginine and lysine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, valine, alanine, glutamic acid, glycine, serine, tyrosine, and proline ($p < 0.05$, Fig. 5).

PCA of *R. philippinarum* revealed that phenylalanine, lysine, histidine, methionine, leucine, tyrosine, and aspartic acid contributed significantly to the variations in amino acid content. Higher protein, serine, and arginine content were observed in January and December, while higher concentrations of glycine, tryptophan, lysine, alanine, and histidine were found in April, October, and September (Fig. 6). For *R. decussatus*, PCA indicated that arginine, serine, protein, and methionine had the most significant contributions to the amino acid composition. Higher amino acid concentrations, excluding protein, serine, and arginine, were observed in July, August, September, and October (Fig. 7).

4. Discussion

Proteins are the building blocks of life; they are essential for cell renewal, strengthening the immune system, protecting the body against external factors, and maintaining and strengthening muscle tissue. The nutritional value of certain bivalve species is superior to that of land animals such as beef, chicken, and pork in terms of protein content (Wright et al., 2018). The protein content in bivalves is affected by their reproductive period (Acarli et al., 2018; Vural & Acarli, 2021). During gonad maturation, an increase in protein content is observed in bivalves, continuing until spawning, after which it decreases (Berthelin et al., 2000; Gökoğlu, 2021). Additionally, protein can serve as an alternative energy source during gametogenesis in some bivalve species (Acarli et al., 2015; Beninger & Lucas, 1984; Galap et al., 1997). Vural and Acarli (2021) suggested that the decrease in

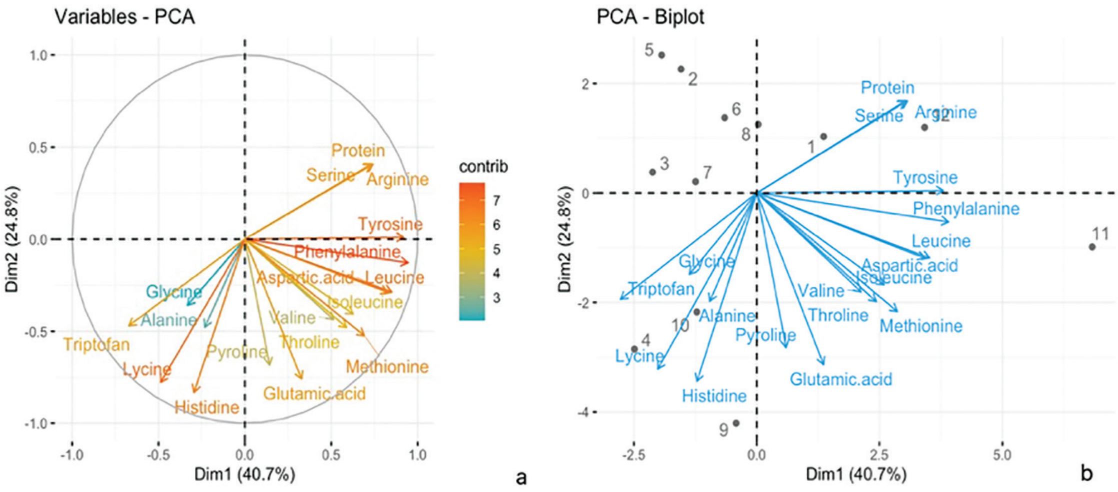

Figure 6

PCA of the composition of amino acids of *R. philippinarum*. (a) Ordination plot of the variables showing the contribution of each amino acid. (b) Ordination biplot. 1: January, 2: February, 3: March, 4: April, 5: May, 6: June, 7: July, 8: August, 9: September, 10: October, 11: November, 12: December. PCA, principal component analysis.

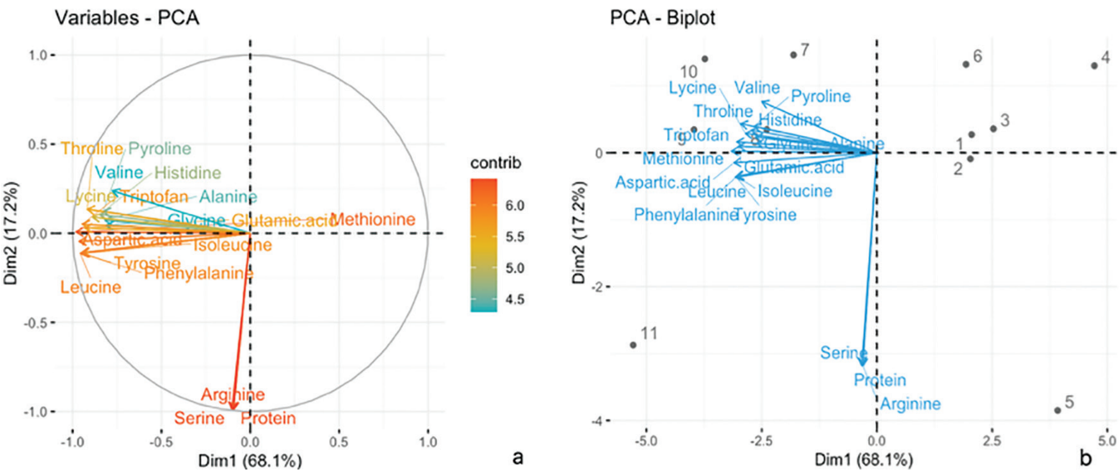

Figure 7

PCA of the composition of amino acids of *R. decussatus*. (a) Ordination plot of the variables showing the contribution of each amino acid. (b) Ordination biplot. 1: January, 2: February, 3: March, 4: April, 5: May, 6: June, 7: July, 8: August, 9: September, 10: October, 11: November, 12: December. PCA, principal component analysis.

protein levels in *F. glaber* from August to September could be related to the transition to the spawning and resting phases. Acarli et al. (2023) indicated that in *Modiolus barbatus*, protein could be used as an energy source during the onset and continuation of gametogenesis. Anibal et al. (2011) observed that in *R. decussatus*, gametogenesis began in January, ovulation continued from June to September, and the resting period lasted from October to December. Serdar and Lök (2009) reported that in *R. decussatus* from the Sufa Lagoon in Izmir, gametogenesis began in January, the peak reproductive period occurred between July and September, and gonad development ceased completely between October and December. Protein levels were highest in March, and decreased to their lowest in the month following gonad maturation. Lök et al. (2011) also reported that *R. decussatus* in both the Marmara and Aegean Seas had spawning in the summer months. Gözler and Tarkan (2000) observed that in *R. decussatus* from the Çardak

Lagoon in the Çanakkale Strait, gonad development began in March and ended in October, with a resting phase between November and March. Mature individuals with ripe eggs were found in June. This study was conducted in the same region as the one by Gözler and Tarkan (2000). In this study, *R. decussatus* showed high protein levels in July and August, which decreased in September and continued to decline in October. There was a sharp increase in November, followed by a decrease in January, with a subsequent increase in February. Protein levels decreased in March and April, and after a sharp increase in May, another decline was observed in June (Fig. 2). When examining gonad maturation macroscopically, maturation in *R. decussatus* occurred in all months except March and May, with ripening in all months except autumn and June, and spawning in the autumn and winter months. For *R. philippinarum*, gonad maturation occurred in all months except spring, and ripening occurred in all months except September. Spawning took place in the autumn and winter months (Fig. 2). In this study, protein content in *R. decussatus* ranged from 63.44 ± 0.32% in April to 80.00 ± 0.00% in May, and in *R. philippinarum*, it ranged from 60.84 ± 0.16% in September to 72.91 ± 0.24% in December. The protein content in *R. decussatus* was reported to range from 39.82% to 64.24% in the Çakalburnu Lagoon (Aegean Sea) by Serdar et al. (2009), from 44.7% (December) to 50.8% (June) in Galicia (Spain) by Ojea et al. (2004), from 34.3% to 42.7% in Izmir (Mersin Bay) by Lök et al. (2011), and from 36.55% to 50.20% in Musakça (Balıkesir). The species, water temperature, available nutrients, and reproductive period can influence protein levels in bivalves (Acarli et al., 2018; Vural & Acarli, 2021). The higher protein levels observed in this study may be attributed to regional differences, reproductive phases, temperature, salinity, and nutritional factors.

Bivalves typically contain essential amino acids and bioactive peptides (Venugopal & Gopakumar, 2017). The levels and proportions of amino acids are crucial for the proper functioning of the human body. Changes in amino acid levels play a significant role in the formation of various diseases, making it important to address deficiencies in these amino acids for treatment. A balanced ratio of EAA to NEAA (>1.0), with a higher quantity of sulfur-containing amino acids, indicates that these species provide well-balanced protein deposition (Joy & Chakraborty, 2016). The EAA/NEAA ratio for *Paphia malabarica* and *Villorita cyprinoides* (Joy & Chakraborty, 2016), *Senilia senilis* (Inyang & Effong, 2017), and *Crassostrea madrasensis* (Chakraborty et al., 2016) is above the threshold of 1.0, demonstrating that these bivalve species offer high-quality, well-adjusted protein. The EAA/NEAA ratio for razor clams, *Ensis siliqua*, and *F. glaber* was found to be <1 (Baptista et al., 2014; Vural & Acarli, 2021). In this study, the EAA/NEAA ratio was found to be high in both *R. decussatus* and *R. philippinarum* in all months, indicating that they possess high protein quality.

Bivalves are known to be rich in essential amino acids (Abirami et al., 2015; Inyang & Effong, 2017; Vural & Acarli, 2021). Amino acids such as lysine, methionine, threonine, tryptophan, isoleucine, leucine, phenylalanine, and valine are essential for humans (Vural & Acarli, 2021). Lysine, which is absent in cereals but present in adequate amounts in bivalves, can complement the limiting amino acids in our daily diets (Chakraborty et al., 2015). Lysine is necessary for fat metabolism, B vitamins, calcium absorption, muscle protein formation, and the synthesis of hormones, antibodies, and enzymes (Greenstein & Winitz, 1961; Özden & Erkan, 2011). *P. malabarica* and *V. cyprinoides* were found to have high levels of lysine, an essential amino acid (Joy & Chakraborty, 2016). The daily required intake of lysine for humans is 1–1.5 g (Özden & Erkan, 2011). In this study, the average lysine content was 6.9 ± 0.0 mg · 100 g^{-1} dw for *R. decussatus* and 4.8 ± 0.1 mg · 100 g^{-1} dw for *R. philippinarum*. Based on these results, consuming 100 g · day^{-1} of *R. decussatus* or 208 g · day^{-1} of *R. philippinarum* would provide the daily lysine requirement for a human.

Histidine and arginine are semi-essential amino acids, meaning they are essential for children but not for adults (Baykara et al., 2019). Arginine plays a role in the synthesis of creatine, ornithine, putrescine, nitric oxide, urea, protamine, and histone compounds (Keleşoğlu, 2012). In this study, histidine levels were found to be 7.5 ± 0.00 mg · 100 g^{-1} dw in *R. decussatus* and 5.8 ± 0.01 mg · 100 g^{-1} dw in *R. philippinarum*. The recommended daily intake of histidine for pregnant women is 33 mg · kg^{-1} (HMDB, 2019). Based on this study, 22 g · kg^{-1} dw of *R. decussatus* and 33 g · kg^{-1} dw of *R. philippinarum* would meet the recommended daily histidine requirement.

Threonine is another important amino acid, and its levels were found to be 16.0 ± 0.3 mg · 100 g^{-1} dw in *R. decussatus* and 10.1 ± 0.2 mg · 100 g^{-1} dw in *R. philippinarum* in this study. Threonine is recommended to be used at 1 g · day^{-1} for the treatment of genetic spasticity disorders (HMDB, 2019). Therefore, 50 g · kg^{-1} dw of *R. decussatus* and 100 g · kg^{-1} dw of *R. philippinarum* could meet the daily threonine requirement. Specifically, for *R. philippinarum*, August and September, and for *R. decussatus*, August, are the most important months due to their peak values.

Valine, leucine, and isoleucine are used in the treatment of liver diseases and kidney failure. They are also recommended for stress and severe burns (Aydogan & Ekici, 2012; Cengiz, 2019). Leucine plays a critical role in protein synthesis and muscle repair. It can increase resistance to diseases, especially under stressful conditions such as reproduction and migration periods (Machado et al., 2020). In this study, leucine content was found to be 853.6 ± 2.8 mg $\cdot$ 100 g^{-1} dw in *R. decussatus* and 676.8 ± 6.8 mg $\cdot$ 100 g^{-1} dw in *R. philippinarum*. Baptista et al. (2014) suggested that the increased leucine levels in *E. siliqua* during spawning may be due to its involvement in the formation of gonads post-spawning. Leucine, threonine, and lysine amino acids showed a strong correlation, increasing during spawning and decreasing afterward (Wu et al., 2013). In this study, a positive correlation between leucine and lysine, histidine, isoleucine, methionine, phenylalanine, threonine, valine, alanine, glycine, serine, tyrosine, proline, and arginine was observed in *R. decussatus* ($p < 0.05$). In *R. philippinarum*, positive correlations were found between leucine and phenylalanine, alanine, and negative correlations with methionine, threonine, proline, and arginine ($p < 0.05$). When evaluating the results in conjunction with Wu et al. (2013), it was observed that the leucine levels did not show complete synchronization, suggesting that other factors also influence the use of leucine.

Glycine is a non-essential amino acid synthesized from serine, threonine, choline, and hydroxyproline (Wang et al., 2014). This amino acid plays a vital role in metabolic regulation, antioxidant reactions, and neurological functions. In animals, glycine serves as a critical component of extracellular structural proteins such as collagen and elastin, which are essential for maintaining tissue integrity and resilience (Wang et al., 2014; Wu, 2009; Wu et al., 2013). Additionally, glycine has been shown to enhance sleep quality, contributing to its importance in physiological regulation (Bannai & Kawai, 2012). Although glycine is categorized as a non-essential amino acid, its endogenous synthesis may be insufficient under certain physiological demands, making dietary supplementation necessary. As such, it is often regarded as a conditionally essential amino acid (Wang et al., 2014).

Bivalves, including species from the genera *Ruditapes* and *Venerupis*, demonstrate physiological adaptations to salinity fluctuations through changes in hemolymph osmolality, transmembrane ion transport, and the concentration of free amino acids (Lin et al., 2021; Mo et al., 2020). Free amino acids play a crucial role in cell volume regulation and osmoregulatory processes (Pourmozaffar et al., 2020). Specifically, glycine, taurine, alanine, proline, and glutamic acid are essential for bivalves to withstand salinity changes and other environmental stressors (Cao et al., 2022; Zhou et al., 2023). Prior studies, such as those by Chakraborty et al. (2016) on *C. madrasensis* and Vural and Acarli (2021) on *F. glaber*, have reported lower glycine levels during summer months, potentially linked to environmental factors.

In this study, glycine concentrations in *R. philippinarum* and *R. decussatus* were highest in autumn and lowest in spring. A significant positive correlation between glycine and alanine levels ($p < 0.05$) was observed, supporting the role of glycine in physiological adaptations to environmental conditions.

Arginine, another non-essential amino acid, is critically important for various physiological functions in aquatic organisms. It has been shown to enhance growth hormone synthesis (Paddon-Jones et al., 2004) and is the sole substrate for nitric oxide synthase, which catalyzes the production of nitric oxide—a key molecule in vasodilation and immune responses (Palmer et al., 1987). Additionally, arginine is required for the synthesis of polyamines, which regulate DNA replication and cell division. It also plays a role in the synthesis of micronutrients such as iron, folate, zinc, and magnesium, as well as nucleic acids (Calder, 2020).

Arginine, along with glutamine, threonine, and tryptophan, is essential for modulating immune responses in bivalves (Wu, 2009). Seasonal variations in arginine levels were evident in this study. In *R. philippinarum*, arginine concentrations peaked in spring and were lowest in summer. Conversely, in *R. decussatus*, arginine levels were highest in autumn and lowest in spring. These findings highlight the dynamic role of arginine in supporting the physiological and immunological needs of bivalves during different seasonal and environmental conditions.

5. Conclusion

R. decussatus and *R. philippinarum*, collected from the Çardak Lagoon, Çanakkale Strait, exhibit a protein and amino acid composition that suggests they are beneficial for consumption. However, bivalve aquaculture in Türkiye is currently limited to mussels. The findings of this study can pave the way for clam aquaculture in Türkiye, potentially generating new employment opportunities, reducing unemployment, and contributing to the national economy. Thus, studies like this are significant for better economic utilization, promotion of consumption, and encouragement of production for these clam species.

The data obtained in this study may also guide the identification of optimal periods for harvesting these species based on their amino acid and protein content. Among essential amino acids, leucine and isoleucine were the most abundant, while arginine, serine, and alanine dominated the non-essential amino acid profile. Leucine levels were consistently high throughout the year for both species, but the recommended optimal consumption periods are all months except September and April for *R. philippinarum*, and all months except spring for *R. decussatus*.

Furthermore, the EAA/NEAA ratio exceeding 1.0 underscores the high protein quality of these species distributed in the region. These findings support the economic and nutritional potential of these clams and highlight the importance of further research and development in their aquaculture and market utilization.

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Author's contributions

Sample collection and laboratory work: PVB, SA, SB, SK, and PÇ. Article writing and evaluation of data: PVB and SA.

Conflict of interest

No potential conflict of interest was reported by the author(s).

Ethics approval and consent to participate

Ethics approval is not applicable to this article.

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