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Comparative Assessment of Biochemical Composition, Microbial Contamination, and Growth Performance of *Perna perna* from Polluted and Protected Coastal Sites along the Gulf of Aden, Yemen

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ABSTRACT

Perna perna is an ecologically and nutritionally important bivalve that can accumulate microorganisms and reflect the environmental conditions of coastal ecosystems. This study evaluated the biochemical composition, microbial contamination, and growth-related characteristics of *P. perna* collected from two contrasting coastal sites, Al-Hami and Sharma, along the Gulf of Aden, Yemen. A total of 240 mussels were collected monthly from December 2022 to March 2023, with 30 individuals sampled from each site per month. Proximate composition was determined using standard analytical methods, while microbiological quality was assessed by measuring total bacterial counts, coliforms, and *Escherichia coli*, with *Salmonella* spp. examined as a major foodborne pathogen. Significant differences between sites were observed in several biochemical and microbiological parameters. Moisture content was significantly higher at Sharma (81.99 ± 4.60%) than at Al-Hami (70.26 ± 1.11%; $p = 0.008$), whereas protein content was relatively high at both sites. Microbiological analyses revealed substantially higher coliform contamination in seawater at Al-Hami (125.00 ± 12.50 CFU/100 mL) than at Sharma (1.67 ± 0.30 CFU/100 mL; $p < 0.001$). Similarly, *E. coli* counts in mussel tissues were significantly higher at Al-Hami (1.75 ± 0.21 CFU/g) than at Sharma (0.375 ± 0.08 CFU/g; $p = 0.003$). No *Salmonella* spp. were detected in any of the examined mussel samples. Monthly variation in microbial and biochemical parameters was generally limited, with most differences not reaching statistical significance. Overall, the findings demonstrate clear spatial differences in microbial contamination and biochemical composition between the two coastal sites and support the potential use of *P. perna* as a bioindicator of coastal environmental quality. The relatively high protein content and absence of detectable *Salmonella* further highlight the nutritional importance and microbiological status of this species within the conditions investigated.

Keywords: Bacterial contamination, bioindicator, *Escherichia coli*, Gulf of Aden, Yemen, *Perna perna*, proximate composition

INTRODUCTION

Marine bivalves are important components of coastal ecosystems and are widely consumed as seafood because of their nutritional value, relatively low production costs, and ecological importance (FAO 2022). Among them, the brown mussel, *Perna perna*, is widely distributed in tropical and subtropical marine environments and is recognized as a valuable source of high-quality protein, essential amino acids, minerals, and polyunsaturated fatty acids (Prato et al. 2019). In addition to its nutritional importance, *P. perna* has considerable potential as a bioindicator of coastal environmental quality because its filter-feeding activity facilitates the accumulation of microorganisms and other contaminants from surrounding seawater (Gosling 2015; Smaal et al. 2019).

The continuous filtration of large volumes of seawater enables mussels to integrate environmental conditions over time, making them useful for monitoring microbial contamination and coastal ecosystem health (Gifford et al. 2007; Smaal et al., 2019). Accordingly, an integrated assessment of proximate composition, microbial contamination, and morphometric characteristics can provide complementary information on mussel nutritional quality, food safety, and responses to prevailing environmental conditions. Protein, lipid, moisture, ash, and carbohydrate contents reflect nutritional and physiological condition, whereas microbial indicators provide information on environmental contamination and potential food-safety concerns. Morphometric characteristics, in turn, provide useful information on growth and biological performance under different environmental conditions (Gosling 2015; FAO 2022).

The growth and physiological condition of *P. perna* may vary according to environmental factors such as temperature, salinity, food availability, and hydrodynamic conditions, which influence metabolism, energy allocation, and tissue composition (Orban et al., 2002; Krešić et al., 2020). Consequently, differences in environmental quality between coastal habitats may be reflected in the biochemical, microbiological, and morphometric characteristics of mussels. In the present study, it was hypothesized that differences in anthropogenic pressure between coastal sites would be associated with site-specific variation in the microbiological quality, proximate composition, and morphometric characteristics of *P. perna*.

Despite the ecological and economic importance of *P. perna*, information on its biochemical composition and microbiological quality along the Yemeni coastline remains limited. Although the nutritional quality and microbiological safety of edible bivalves have been investigated in many regions, studies integrating biochemical, microbiological, and morphometric characteristics of *P. perna* remain scarce in the northwestern Indian Ocean, particularly along the Yemeni coastline (Masanja et al. 2023). To the best of our knowledge, no previous study has simultaneously evaluated these characteristics in *P. perna* collected from contrasting coastal habitats in Hadhramout Governorate, Yemen. This gap limits understanding of how local environmental conditions influence mussel quality and seafood safety.

Therefore, the present study aimed to comparatively assess the biochemical composition, microbial contamination, and growth-related characteristics of *P. perna* collected from polluted and protected coastal sites along the Gulf of Aden, Yemen. The findings provide baseline information that may support the use of *P. perna* in coastal environmental monitoring, seafood safety assessment, and sustainable management of marine resources in Yemen.

METHOD

Study Area

This study evaluates the impact of anthropogenic stress on Perna perna along Hadhramout's Gulf of Aden coast in Yemen by contrasting two ecologically distinct sites (Figure 1). Al-Hami served as the impacted site, characterized by domestic and fishing-related pressures, whereas Sharma represented a sheltered reference site with minimal human impact. This comparative design allowed for assessing site-specific variations in the species' biochemical composition, microbial contamination profile, and growth-related parameters.

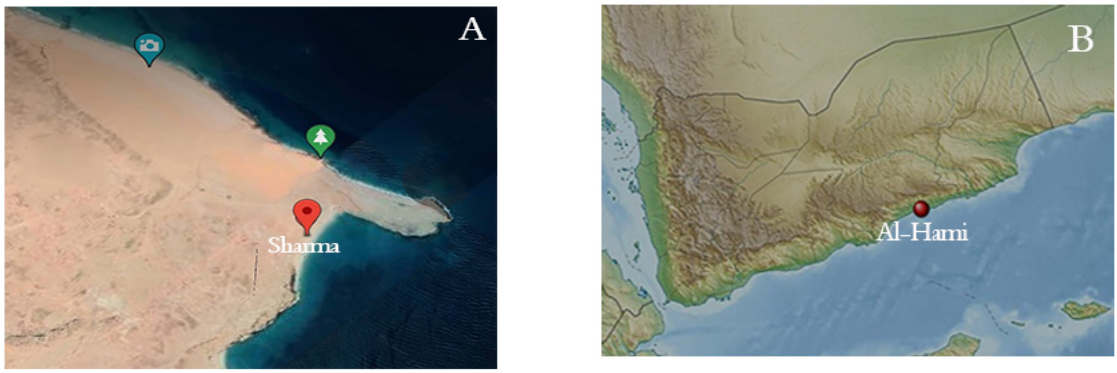


Figure 1: Map of sampling locations (El-Hami vs. Sharma)

Sample Collection and Experimental Design

To evaluate spatial and temporal variation, 240 *Perna perna* mussels were sampled from Al-Hami and Sharma in Yemen across four monthly sampling cycles from December 2022 to March 2023, comprising 30 specimens per site each month. To minimize size- and age-dependent physiological variability, specimens were standardized to a 4–5 cm shell length. Following collection, mussels were placed in sterile containers, refrigerated during transport, and processed immediately for biochemical and microbiological testing. Standardized protocols were applied uniformly across all sampling events to ensure data consistency and inter-site comparability.

Proximate composition

The proximate composition of *Perna perna* tissues was evaluated to assess nutritional quality and stress indicators, using standard analytical protocols (AOAC 2016). The parameters assessed included water content, crude protein, crude fat, crude fiber, total ash, nitrogen-free extract (carbohydrates), and gross energy.

Water Content Determination

Approximately 2.0 g of homogenized tissue sample was placed in a pre-weighed porcelain crucible and dried in a laboratory oven (Binder GmbH, Germany) at 105°C until a constant weight was achieved. After drying, crucibles were cooled in a desiccator for 60 min and reweighed. Water content was calculated as:

$$Water\ content\ (\%) = \frac{W1 - (W2 - W0)}{W1} x100 \dots\dots\dots\dots (1)$$

Where:

W_0 = Weight of the empty crucible (g)
W_1 = Initial weight of the sample (g)
W_2 = Weight of the crucible + dried sample (g)

Protein Content

Crude protein was determined by quantifying total nitrogen using the Kjeldahl method with a Kjeltec analyzer (AOAC 2016). The procedure involved three sequential steps: acid digestion, distillation, and titration against standard HCl. Total nitrogen percentage was calculated using Equation (2):

$$Nitrogen\ (\%) = \frac{V\ x\ Acid\ normality\ \ x\ 14}{W1} x100 \quad \ldots\ldots\ldots\ldots (2)$$

Where:

W1 = Sample weight (g)

V = Volume of HCl (mL)

14 = Atomic weight of nitrogen.

Total nitrogen was converted to crude protein using a conversion factor of 6.25:

$$\text{Crude protein } (\%) = \text{ Nitrogen } (\%) \text{ x } 6.25 \quad \ldots\ldots\ldots\ldots (3)$$

Crude Lipid Content

Crude lipid content was determined via exhaustive Soxhlet extraction using petroleum ether in an automated extraction system (Model 2055, FOSS Tecator, Sweden). Homogenized samples (1.0 g) were transferred into pre-weighed extraction thimbles covered with cotton wool, with 100 mL of petroleum ether added per sample. The automated extraction comprised: boiling (20 min), rinsing (35 min), solvent recovery (10 min), and drying (5 min). The extraction cup was further dried at 105°C for 60 min, cooled in a desiccator, and weighed. Crude lipid content was calculated using the following formula:

$$Crude\ lipid\ (\%) = \frac{W3 - W1}{W2} x100 \quad \ldots\ldots\ldots\ldots (4)$$

Where:

W_1 = Container weight (g)

W_2 = Sample weight (g)

W_3 = Lipid + container weight (g)

Total Ash Determination

Ash content, representing inorganic mineral residue, was measured by high-temperature incineration. Pre-dried porcelain crucibles containing 1.0 g of homogenized tissue were placed in a muffle furnace (Carbolite, UK) at 550°C overnight. Crucibles were then cooled in a desiccator and reweighed. The total ash content was calculated using the following equation:

$$Total\ Ash\ (\%) = \frac{W3 - W1}{W2} x100 \quad \ldots\ldots\ldots\ldots (5)$$

Where:

W_1 = weight of the empty crucible (g)

W_2 = weight of the sample (g)

W_3 = weight of the crucible + ash residue (g)

Nitrogen-free extract (carbohydrate) determination

The nitrogen-free extract (NFE), which primarily represents the carbohydrate fraction of the sample, was determined by subtracting the sum of the moisture, crude protein, ether extract (fat), and ash contents from the total weight of the sample. The calculation was performed using the following equation:

$$NFE\ (\%) = [100 - WC\ \% + CP\ \% + CL\ \% + AS\ \% \quad \ldots\ldots\ldots\ldots (6)$$

Where:

NFE = Nitrogen-free extract
WC = Water content
CP = Crude protein
CL = Crude lipid
AS = Ash

Gross Energy Determination

Gross energy value was calculated based on macronutrient percentages using Atwater conversion factors (4.0 kcal/g for protein and carbohydrate, and 9.0 kcal/g for lipid), expressed per 100 g wet weight:

Microbiological Analysis

Total Bacterial Count

The total viable bacteria in muscle tissue samples were quantified using the standard plate count method. Muscle tissue samples (10 g) were aseptically homogenized in 90 mL of sterile buffered peptone water (10^{-1} dilution), serially diluted to 10^{-6}, and plated in duplicate onto nutrient agar plates. Plates were incubated at 37°C for 24 – 48 h. Colonies were counted and expressed as $\log_{10}$ CFU/g of tissue.

The limit of detection (LOD) was 10 CFU/g (1.0 $\log_{10}$ CFU/g). Quality control included uninoculated buffered peptone water and blank nutrient agar plates as negative controls, alongside Escherichia coli as a positive control. All analyses were performed in triplicate.

Coliform Enumeration via MPN Method

Fecal contamination was evaluated by enumerating coliform bacteria using the Most Probable Number (MPN) method. Homogenized tissue samples were serially diluted and inoculated into lauryl sulfate tryptose (LST) broth tubes, then incubated at 37°C for 24–48 h. Gas formation indicated presumptive positive coliform presence.

For confirmation, aliquots from positive tubes were streaked onto eosin methylene blue (EMB) agar and incubated under identical conditions. Characteristic metallic sheen colonies confirmed coliform presence. Final MPN values were determined using standard probability tables (Huss et al. 2000; Huss et al. 2014; APHA 2017; ISO 2018).

E. coli Detection and Confirmation

To identify *Escherichia coli*, presumptive coliform-positive samples were subcultured onto eosin methylene blue (EMB) or MacConkey agar and incubated at 37°C for 24 h. Typical E. coli colonies were identified by characteristic morphology, including a metallic sheen on EMB agar. Suspected colonies were confirmed using standard biochemical tests, including indole production, methyl red, citrate utilization, and motility assays (ISO 2018; Feng et al. 2020).

Statistical Analysis

Data were analyzed using SPSS v26 to evaluate variations in biochemical and microbiological parameters across sampling sites. Quantitative assessments included growth trends, seasonal reproductive patterns, and correlations with environmental factors. Statistical significance was defined as $P < 0.05$. The Effects of site (S), month (M), and their interaction (S×M) on biochemical parameters were identified using Two-way ANOVA.

RESULTS

Morphometric Variation and Size Distribution of *Perna perna*

Morphometric analysis of *Perna perna* collected from Al-Hami and Sharma revealed moderate spatial and temporal variation in shell length, shell width, and total body weight during the study period. The observed differences indicate variation in the size and condition of mussels between sampling periods and coastal habitats. The highest morphometric values were generally observed during February, whereas comparatively lower values were recorded during December. These temporal differences may reflect variation in local environmental conditions and food availability.

The observed changes represent temporal variation in morphometric characteristics rather than calculated growth rates, as the present study did not apply a formal growth model. Accordingly, the results are interpreted as differences in shell size and body condition among sampling periods rather than estimates of individual growth increments. The monthly variation in shell length is presented in Table 1.

Table 1: Monthly growth rate of *Perna perna*

Month	Avg. Shell Length (cm)
January	2.1 ± 0.3
February	2.5 ± 0.2
March	3.0 ± 0.4
April	3.5 ± 0.3
May	3.8 ± 0.4
June	4.0 ± 0.3
July	4.2 ± 0.2
August	4.5 ± 0.3
September	4.3 ± 0.2
October	4.0 ± 0.3
November	3.5 ± 04
December	3.0 ± 0.3

Spatial Variation in Bacterial Contamination in Seawater and Mussel Tissues

Significant spatial differences in bacterial indicators were observed between the two sampling sites. Total coliform concentrations in seawater were substantially higher at Al-Hami (125.00 ± 12.50 CFU/100 mL) than at Sharma (1.67 ± 0.30 CFU/100 mL), representing an approximately 75-fold difference between the sites ($t = 8.47$, $p < 0.001$). In contrast, *E. coli* concentrations in seawater were higher at Al-Hami (24.00 ± 3.20 CFU/100 mL) than at Sharma (1.33 ± 0.40 CFU/100 mL), although this difference was not statistically significant ($t = 1.86$, $p = 0.082$).

The spatial differences in bacterial indicators demonstrate a clear contrast in the microbiological characteristics of the two coastal habitats. The substantially higher total coliform concentration at Al-Hami indicates greater microbial contamination at this site, whereas the lower values observed at Sharma indicate comparatively lower contamination pressure. The corresponding bacterial indicators for seawater and mussel tissues are summarized in Table 2.

Table 2. Spatial variation of bacterial indicator levels in seawater and *Perna perna* tissues along the Hadhramout coast, Yemen

Seawater (CFU/100 mL)						
Parameter	**Site**	**Mean ± SE**	**Range**	**t-value**	***P*-value**	**Status**
Total coliforms	Al-Hami	125. ± 12.5[a]	98-156	8.47	<0.001	Exceeds YSMO limit (2.2)
	Sharma	1.67 ± 0.3[b]	0 – 4			Within limit
E. coli	Al-Hami	24.00 ± 3.2[a]	18–32	1.86	0.082	NS between sites
	Sharma	1.33 ± 0.4[a]	0–3			
Tissue (CFU/g wet weight)						
E. coli	Al-Hami	1.75 ± 0.21[a]	1.2- 2.4	4.23	0.003	Exceeds ISO 7251 (0)
	Sharma	0.375± 0.08[b]	0.1–0.6			Within standard

Different superscript letters ([a],[b]) indicate significant differences between sites (Independent Samples t-test, P <0.05).
YSMO = Yemen Standardization and Metrology Organization
ISO = International Organization for Standardization
NS = not significant; SE = standard error; n = 240 specimens (60 per site per month).

Bacterial Load in *Perna perna* Tissues

The density of *Escherichia coli* in *Perna perna* tissues differed significantly between the two sampling sites. Mussels collected from Al-Hami contained significantly higher *E. coli* counts (1.75 ± 0.21 CFU/g) than those collected from Sharma (0.375 ± 0.08 CFU/g), with a significant difference between sites (t = 4.23, p = 0.003) (**Figure 3**). Despite the significant spatial difference, the tissue counts recorded during the study remained within the microbiological safety criterion applied in the study.

E. coli concentrations in the surrounding seawater were also higher at Al-Hami (24.00 ± 3.20 CFU/100 mL) than at Sharma (1.33 ± 0.40 CFU/100 mL); however, the difference was not statistically significant (t = 1.86, p = 0.082). The tissue-to-water bioaccumulation ratio

was 0.073 at Al-Hami and 0.282 at Sharma, indicating site-specific differences in the relationship between bacterial concentrations in seawater and mussel tissues.

These findings demonstrate clear spatial variation in microbial contamination between the two coastal sites, with greater *E. coli* loads in mussel tissues at Al-Hami. The results provide evidence of localized differences in microbial environmental quality while indicating that the tissue concentrations remained within the safety criterion applied in the present study. Figure 2 shows *E. coli* abundance in *Perna perna* tissues collected from Sharma and Al-Hami coastal sites.

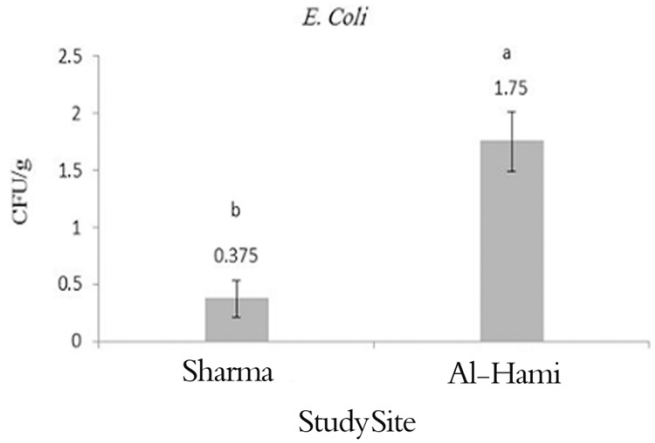


Fig 2: *E. coli* abundance in ***Perna perna*** **(Sharma Vs El-Hami)**

Monthly Variation in Bacterial Load

Temporal analysis of *Escherichia coli* concentrations in *Perna perna* tissues from December 2022 to March 2023 revealed relatively minor fluctuations in bacterial abundance. The highest mean bacterial load was recorded in December 2022 (approximately 1.73 CFU/g), followed by lower values in January (0.75 CFU/g) and February (0.75 CFU/g), with a slight increase in March (1.00 CFU/g). However, these monthly differences were not statistically significant ($P > 0.05$), indicating that *E. coli* levels in mussel tissues remained relatively stable throughout the sampling period. Monthly variation in *E. coli* abundance is presented in Figure 3, while the corresponding statistical results are summarized in Table 3.

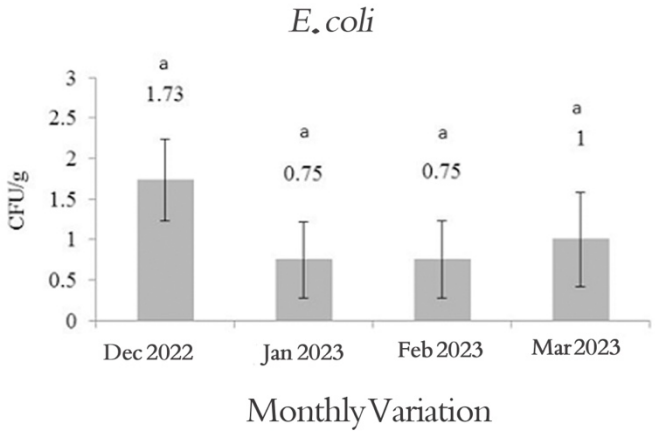


Fig 3: Monthly variation of *E. coli* in *Perna perna*

Table 3: Monthly variation in *Escherichia coli* load in *Perna perna* tissues from Hadhramout coast, Yemen.

Month	Al-Hami (CFU/g)	Sharma (CFU/g)	Combined Mean	F-value	*P*-value
December 2022	1.73 ± 0.18^{a}	0.42 ± 0.09^{a}	1.08 ± 0.15		
January 2023	1.20 ± 0.15^{ab}	0.35 ± 0.07^{a}	0.78 ± 0.12		
February 2023	1.85 ± 0.22^{a}	0.38 ± 0.08^{a}	1.12 ± 0.17	1.24	0.321
March 2023	1.22 ± 0.16^{ab}	0.30 ± 0.06^{a}	0.76 ± 0.13		
Grand Mean	**1.50 ± 0.18**	**0.36 ± 0.08**			

Note: One-way ANOVA revealed no significant monthly variation within sites ($P>0.05$). Different superscript letters indicate differences in temporal patterns (LSD post-hoc test). Data presented as Mean ± SE; n = 60 per month per site.

Absence of *Salmonella* spp.

No *Salmonella* spp. was detected in any of the examined *Perna perna* tissue samples collected from Al-Hami and Sharma during the study period. The analysis included all 240 mussel specimens collected from the two sampling sites. Thus, *Salmonella* was not detected in the mussel tissues examined throughout the four-month sampling period.

Proximate Composition and Nutritional Characteristics

Moisture Content

Moisture content constituted a major component of the soft tissues of *Perna perna*. Mussels collected from Sharma exhibited significantly higher moisture content (81.99 ± 4.60%) than those collected from Al-Hami (70.26 ± 1.11%), with a significant difference between sites (t = 3.42, p = 0.008). Two-way ANOVA also indicated a significant effect of sampling month on moisture content ($F_{3,228}$ = 3.78, p = 0.042), although the observed monthly variation was relatively limited. The results indicate that moisture content differed significantly between the two coastal sites during the study period (Table 4).

Crude Protein Content

Crude protein was a major biochemical component of *P. perna* tissues and showed significant differences between the two sampling sites. On a wet-weight basis, protein content was significantly higher at Al-Hami (15.36 ± 0.45%) than at Sharma (10.73 ± 0.94%; t = 4.87, $p < 0.001$). On a dry-weight basis, protein content was 51.65 ± 0.82% at Al-Hami and 60.46 ± 5.31% at Sharma, with a significant difference between sites (t = 2.18, p = 0.049). The difference between wet- and dry-weight values reflects the substantial difference in tissue moisture between the two sites (Table 4).

Lipid Content

The lipid content of *P. perna* tissues showed spatial and temporal variation during the study period (Table 4). On a wet-weight basis, lipid content was significantly higher in mussels from Al-Hami (6.07 ± 2.17%) than in those from Sharma (3.02 ± 0.31%; t = 2.79, p = 0.018). On a dry-weight basis, lipid content was 22.08 ± 4.60% at Al-Hami and 14.98 ±

1.54% at Sharma; however, this difference was not statistically significant (t = 2.05, p = 0.058). Two-way ANOVA indicated a significant effect of sampling site ($F_{1,228}$ = 6.34, p = 0.028) and sampling month ($F_{3,228}$ = 4.56, p = 0.024), whereas the site × month interaction was not significant.

Table 4. Proximate biochemical composition of *Perna perna* from polluted (Al-Hami) and pristine (Sharma) sites, Hadhramout, Yemen.

Component	Al-Hami (Polluted)	Sharma (Pristine)	Difference (%)	t-value	*P*-value
Dry matter basis					
Moisture (%)	70.26 ± 1.11[a]	81.99 ± 4.60[b]	+14.0	3.42	0.008
Dry matter (%)	29.74 ± 1.11[a]	18.01 ± 4.60[b]	-65.1	3.42	0.008
Crude protein (%)	51.65 ± 0.82[a]	60.46 ± 5.31[b]	+14.6	2.18	0.049
Lipid (%)	22.08 ± 4.60[a]	14.98 ± 1.54[b]	-47.4	2.05	0.058
Ash (%)	11.55 ± 0.57[a]	15.68 ± 3.97[a]	+26.3	1.24	0.243
Carbohydrate (%)	14.73 ± 4.29[a]	8.90 ± 6.23[b]	-65.5	2.89	0.014
Energy (kJ/g)	19.63 ± 5.27[a]	21.67 ± 2.18[a]	+9.4	0.72	0.489
Wet weight basis					
Crude protein (%)	15.36 ± 0.45[a]	10.73 ± 0.94[b]	-43.1	4.87	<0.001
Lipid (%)	6.07 ± 2.17[a]	3.02 ± 0.31[b]	-100.9	2.79	0.018
Ash (%)	3.75 ± 0.39[a]	2.72 ± 0.37[b]	-37.9	3.42	0.008
Carbohydrate (%)	4.38 ± 1.11[a]	1.81 ± 1.25[b]	-141.9	4.12	0.003

Values represent mean ± SE of four monthly samples (n=60 per site). Superscript letters indicate significant differences between sites (Independent Samples t-test, *P*<0.05). Energy calculated using conversion factors: protein 23.6 kJ/g, lipid 39.1 kJ/g, carbohydrate 17.6 kJ/g.

Ash Content

Ash content also differed between the two sampling sites. On a wet-weight basis, ash content was 3.75 ± 0.39% at Al-Hami and 2.72 ± 0.37% at Sharma, whereas the corresponding dry-weight values were 11.55 ± 0.57% and 15.68 ± 3.97%, respectively. The highest wet-weight ash value (4.33%) was recorded at Al-Hami in December, while the lowest value (2.30%) was observed at Sharma in February. Two-way ANOVA showed a significant effect of sampling site ($F_{1,228}$ = 8.92, p = 0.012), whereas sampling month and the site × month interaction were not statistically significant (Table 4).

Carbohydrate Content

Carbohydrate content represented a relatively small proportion of the measured biochemical composition of *P. perna* (Table 4). On a wet-weight basis, carbohydrate content was significantly higher in mussels from Al-Hami (4.38 ± 1.11%) than in those from Sharma (1.81 ± 1.25%; t = 4.12, p = 0.003). Although numerical differences were observed among sampling months, the monthly effect was not statistically significant according to the two-way ANOVA.

Energy Content

The calculated energy content showed limited spatial variation between the two sampling sites (Table 4). Energy values were 19.63 ± 5.27 kJ/g for Al-Hami and 21.67 ± 2.18 kJ/g for Sharma on a dry-weight basis. Although the mean energy value was slightly higher at Sharma, the difference was not statistically significant (t = 0.72, p = 0.489).

Two-way ANOVA of Biochemical Parameters

The two-way ANOVA demonstrated significant site effects for all evaluated biochemical parameters, including protein, lipid, ash, moisture, and carbohydrate contents. Sampling month had a significant effect on lipid ($F_{3,228}$ = 4.56, p = 0.024) and carbohydrate ($F_{3,228}$ = 3.78, p = 0.042), whereas monthly effects on protein, ash, and moisture were not statistically significant. None of the site × month interactions reached statistical significance (p > 0.05), indicating that the observed spatial differences were not significantly dependent on sampling month (Table 5).

Table 5: Two-way ANOVA: Effects of site (S), month (M), and their interaction (S×M) on biochemical parameters of *Perna perna*.

Source of Variation	Protein (%)		Lipid (%)		Ash (%)		Moisture (%)		NFE (%)	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Site (S)	18.47	<0.001	8.92	0.012	6.34	0.028	15.23	0.002	12.67	0.004
Month (M)	3.21	0.058	4.56	0.024	2.89	0.082	2.45	0.118	3.78	0.042
S × M	2.87	0.086	3.12	0.068	1.94	0.178	2.16	0.142	2.54	0.098
Error										
$LSD_{0.05}$	**1.62**		**2.17**		**0.43**		**2.68**		**0.57**	

DISCUSSION

Morphometric Variation and Growth Performance of *Perna perna*

The present study revealed moderate spatial and temporal variation in the morphometric characteristics of *Perna perna*, including shell length, shell width, and body weight. Growth and condition in marine bivalves are determined by the balance between energy acquisition and metabolic expenditure. They are influenced by environmental factors such as food availability, temperature, water quality, and hydrodynamic conditions

(Gosling 2015). The relatively moderate variation observed in the present study may therefore reflect differences in local environmental conditions and resource availability between the two coastal sites.

Food availability is an important determinant of bivalve growth because increased availability of suspended food particles can enhance filtration, energy assimilation, and tissue development. Previous studies have demonstrated that environmental productivity and food availability can contribute substantially to variation in bivalve growth and condition (Filgueira et al. 2014; Smaal et al. 2019). However, phytoplankton availability was not directly measured in the present study; therefore, its contribution to the observed morphometric differences cannot be confirmed.

Reproductive activity may also influence the allocation of assimilated energy between somatic growth and gonadal development. During gametogenesis, a proportion of available energy is directed toward reproductive tissues rather than shell and somatic growth, potentially reducing growth increments during periods of intensive reproductive investment. Similar reproductive–growth relationships have been reported in marine bivalves, including *P. perna* (Freites et al. 2002; Galimany et al. 2017). Nevertheless, reproductive stages were not directly assessed in the present study, and therefore reproductive condition should be considered a possible explanation rather than a demonstrated cause of the observed variation.

Temperature can additionally affect filtration, metabolism, and growth when conditions remain within the species' physiological tolerance range (Sarà et al. 2008). The moderate variation observed during the study period may indicate relatively stable environmental conditions; however, because temperature and other physicochemical variables were not continuously monitored, this interpretation should be treated cautiously.

The observed differences in shell dimensions and body weight are consistent with previous studies reporting strong relationships between environmental productivity and bivalve growth patterns (Rueda et al. 2005; García et al. 2015; Kroczek et al. 2017). Overall, the morphometric results indicate that *P. perna* populations at the two sites experienced measurable variation in size and condition, but the available data do not support attributing these differences to a single environmental factor. Continued monitoring of morphometric characteristics together with temperature, salinity, food availability, and other environmental variables would provide a stronger basis for evaluating growth performance and its ecological significance.

Bacterial Load in *Perna perna* Tissues

The present study demonstrated a significant difference in *Escherichia coli* abundance in *Perna perna* tissues between the two coastal sites, with mussels from Al-Hami showing higher bacterial loads than those from Sharma. This spatial pattern is consistent with the filter-feeding biology of bivalves, which allows them to remove suspended particles and microorganisms from surrounding seawater and retain a proportion of these microorganisms within their tissues (Gosling 2015; Smaal et al. 2019). Previous studies have also demonstrated that bivalves can accumulate fecal indicator bacteria from surrounding waters, supporting their use in the assessment of coastal microbial quality.

The higher tissue *E. coli* concentration observed at Al-Hami was consistent with the substantially higher bacterial contamination recorded in the surrounding seawater at this site. This relationship supports the interpretation that differences in the microbiological quality of the surrounding water contributed to the differences observed in mussel tissues. Similar accumulation of *E. coli* and other fecal indicator bacteria in mussel tissues has been

reported in bivalves exposed to contaminated waters (Gifford et al. 2007; Smaal et al. 2019).

The tissue-to-water bioaccumulation ratios observed in the present study also demonstrate that the relationship between bacterial concentrations in seawater and mussel tissues is not necessarily proportional. Bacterial accumulation in bivalves may be affected by filtration activity and by environmental and physiological factors, including temperature, salinity, suspended particles, and filtration efficiency (Gosling 2015; Smaal et al. 2019). Therefore, the lower ratio observed at Al-Hami compared with Sharma should not be interpreted simply as lower bacterial uptake at Al-Hami; rather, it may reflect differences in the relationship between ambient bacterial concentration and filtration or retention processes.

An important finding of the present study is that, despite the significant spatial difference, the *E. coli* concentrations detected in mussel tissues remained within the microbiological criterion applied in the study. This distinction is important because a statistically significant difference between sites does not necessarily indicate that the measured concentrations exceeded an established food-safety criterion. Thus, the results indicate spatial variation in microbial contamination rather than evidence of unacceptable contamination levels under the criterion applied in the present study.

The results further support the potential value of *P. perna* as a biological indicator of differences in coastal microbial quality. Bivalves are widely used in environmental monitoring because their filter-feeding behavior and ability to accumulate microorganisms and contaminants allow them to integrate environmental exposure over time (Gosling 2015; Smaal et al. 2019). However, tissue bacterial concentrations should be interpreted together with measurements of surrounding seawater and relevant environmental factors rather than used as a standalone measure of pollution.

Overall, the higher *E. coli* load observed in *P. perna* from Al-Hami, together with the higher bacterial indicators recorded in the surrounding seawater, indicates a clear spatial contrast between the two coastal environments. At the same time, the measured tissue concentrations remained within the criterion applied in this study, suggesting that the observed contamination was site-specific but did not exceed the safety criterion evaluated.

Monthly Variation in Bacterial Load

The present study showed relatively limited monthly variation in *Escherichia coli* abundance in *Perna perna* tissues during the four-month sampling period. Although differences in bacterial loads were observed among sampling months, these differences were not statistically significant (F = 1.24, p = 0.321). This finding indicates that the bacterial load in mussel tissues remained relatively stable over the study period despite numerical fluctuations among individual months.

Temporal variation in bacterial concentrations in bivalve tissues can be influenced by changes in environmental conditions, including temperature, salinity, suspended particulate matter, microbial abundance in the surrounding water, and the physiological condition of the mussels. Because bivalves continuously filter surrounding water, temporal changes in bacterial concentrations in the environment can potentially be reflected in their tissues (Gosling 2015; Smaal et al. 2019). However, the absence of a statistically significant monthly effect in the present study suggests that the observed fluctuations were insufficient to demonstrate a consistent temporal pattern.

Similar temporal and spatial variability in fecal indicator bacteria have been reported in *Perna perna*. Martinez and Oliveira (2010) found that bacterial densities accumulated in *P. perna* tissues could reflect chronic microbial contamination of the surrounding environment, although the magnitude of accumulation varied according to environmental conditions and clearance processes. Studies of *P. perna* from areas with different degrees of fecal pollution have likewise demonstrated that bacterial indicators in mussels can vary with the microbiological quality of the surrounding water (Neves et al. 2018; Silva et al. 2022). The relatively stable monthly bacterial load observed in the present study may therefore indicate that the microbial conditions affecting the mussels did not change sufficiently during the sampling period to produce a significant temporal response. Nevertheless, this interpretation should be considered cautiously because the study covered only four months and did not include continuous measurements of environmental variables such as temperature, salinity, or phytoplankton abundance.

Overall, the results indicate limited temporal variation rather than a significant monthly or seasonal change in *E. coli* abundance in *P. perna* tissues. The stronger difference observed between Al-Hami and Sharma therefore appears to represent a more important spatial pattern than the monthly variation detected during the study period.

Absence of *Salmonella* spp.

No *Salmonella* spp. was detected in the 240 *Perna perna* tissue samples examined from Al-Hami and Sharma during the study period. The absence of detectable *Salmonella* is a favorable microbiological finding, particularly because bivalve molluscs are recognized as potential vehicles for enteric pathogens due to their filter-feeding behavior and ability to concentrate microorganisms from surrounding waters (Lees, 2000; Iwamoto et al. 2010).

The non-detection of *Salmonella* in the present study is consistent with investigations in which *Salmonella* was not detected in mussel samples from areas with relatively favorable microbiological conditions. For example, studies of mussels and other bivalve molluscs have reported variable occurrence of *Salmonella*, demonstrating that its detection can differ according to geographical location, environmental conditions, sampling period, and degree of fecal contamination (Hernroth et al. 2009; Normanno et al. 2006).

The absence of *Salmonella* in the present samples should nevertheless be interpreted cautiously. Previous investigations have demonstrated that fecal indicator bacteria do not always show a direct relationship with the presence of *Salmonella* in aquatic environments, indicating that indicator organisms cannot be considered definitive evidence for the presence or absence of specific pathogens (Giusti et al. 2018).

From a food-safety perspective, the finding is nevertheless relevant because international microbiological criteria for live bivalve molluscs include the absence of *Salmonella* in 25 g of flesh as an important safety criterion. The absence of *Salmonella* from all samples examined in the present study therefore provides supportive evidence of the microbiological status of the mussels during the sampling period.

However, the result should not be interpreted as evidence that the study area was completely free of fecal contamination or that other pathogenic microorganisms were absent. The simultaneous detection of *E. coli* in mussel tissues demonstrates that microbial contamination was present, particularly at Al-Hami. Therefore, the absence of *Salmonella* should be considered a favorable finding within the specific sampling period and detection method used, rather than evidence of complete microbiological safety.

Overall, the non-detection of *Salmonella* spp. in all 240 examined *P. perna* specimens represents an important positive finding of the present study. When considered together with the measured *E. coli* concentrations, it indicates that although spatial differences in microbial contamination existed between Al-Hami and Sharma, *Salmonella* was not detected in the mussel tissues during the study period.

Proximate Composition and Nutritional Characteristics

The proximate composition of *Perna perna* tissues demonstrated clear differences between the Al-Hami and Sharma populations, particularly in moisture, protein, lipid, ash, and carbohydrate contents. Such variation is consistent with the dynamic biochemical composition of marine bivalves, which can respond to environmental conditions, food availability, physiological status, and reproductive activity (Gosling 2015; Smaal et al. 2019). Previous research on *P. perna* from the coastal waters of Yemen has also demonstrated seasonal changes in protein, lipid, carbohydrate, glycogen, and ash contents, highlighting the importance of environmental and reproductive processes in determining the biochemical condition of this species (Sokołowski et al. 2010).

Moisture Content

Moisture represented the major component of *P. perna* tissues, with significantly higher values recorded at Sharma than at Al-Hami. The difference in tissue moisture is important when interpreting the other biochemical constituents because concentrations expressed on a wet-weight basis are strongly influenced by water content. Similar differences in moisture content among bivalves have been associated with seasonal physiological condition, reproductive activity, and environmental factors (Gosling 2015; Smaal et al. 2019). Therefore, the higher moisture content observed at Sharma should be considered when comparing the wet-weight concentrations of protein, lipid, ash, and carbohydrate between the two sites.

Protein Content

Protein was one of the predominant biochemical constituents of *P. perna* tissues in the present study. The significantly higher wet-weight protein content observed at Al-Hami compared with Sharma indicates spatial variation in protein accumulation. Protein is an important structural and functional component of bivalve tissues and may also contribute substantially to energy metabolism under conditions of nutritional or reproductive demand (Gosling 2015; Smaal et al. 2019). The relatively high protein content observed in the present study is consistent with previous investigations showing that protein commonly constitutes a major fraction of bivalve soft tissues. Its concentration can vary according to species, habitat, food availability, season, and reproductive condition (Orban et al. 2004; Smaal et al. 2019). In *P. perna* from the Gulf of Aden, seasonal changes in protein content have likewise been associated with changes in physiological condition and reproductive activity (Sokołowski et al. 2010).

Lipid Content

Lipid content showed significant spatial variation on a wet-weight basis, with higher values recorded at Al-Hami. Lipids represent an important energy reserve in bivalves and can fluctuate in response to food availability, reproductive activity, and seasonal energy requirements (Gosling 2015; Smaal et al. 2019). Studies of *P. perna* have demonstrated that lipid reserves can vary with both food availability and reproductive condition, with reproductive processes influencing the mobilization and accumulation of stored lipids (Narváez et al., 2008). The significant effects of sampling site and month observed in the present study suggest that lipid content was more responsive to spatial and temporal variation than some other proximate components. Similar seasonal changes in lipid reserves have been reported in bivalves and have been linked to the interaction between food availability and gametogenic activity (Pazos et al. 1996; Dridi et al. 2007). However, because reproductive stages and food availability were not directly measured in the

present study, these factors should be regarded as possible explanations rather than confirmed causes of the observed lipid variation.

Ash Content

Ash content also differed between the two sampling sites. Ash represents the inorganic mineral fraction remaining after combustion of biological tissue and is commonly used as an indicator of the mineral component of the proximate composition of bivalves. Differences in ash content among bivalve populations may reflect variation in tissue composition and physiological condition (Gosling 2015; Smaal et al. 2019). The spatial differences observed in the present study may therefore reflect differences in the biochemical and physiological condition of the mussels. However, because specific mineral elements were not analyzed separately, the present results cannot identify which minerals were responsible for the observed difference.

Carbohydrate Content

Carbohydrate represented a relatively small proportion of the proximate composition but showed a significant difference between the two sampling sites. In bivalves, carbohydrates, particularly glycogen, function as important readily mobilized energy reserves and may fluctuate according to food availability, metabolic activity, and reproductive demands (Gosling 2015; Smaal et al. 2019). Seasonal studies of *P. perna* from the Gulf of Aden have shown marked changes in carbohydrate and glycogen reserves in association with periods of energy accumulation, nutritional stress, and reproductive activity (Sokołowski et al. 2010). The relatively higher carbohydrate content observed at Al-Hami may therefore reflect differences in energy storage between the two populations. Nevertheless, because glycogen was not measured separately and food availability was not directly assessed, the specific mechanism underlying the observed difference cannot be established from the present data.

Energy Value

The calculated energy values showed limited spatial variation, and the difference between Al-Hami and Sharma was not statistically significant. This suggests that, despite significant differences in some individual proximate components, the overall estimated energy contribution of protein, lipid, and carbohydrate remained relatively comparable between the two sites. Comparable observations have been reported in mussels, where changes in individual biochemical constituents do not necessarily produce equivalent changes in overall caloric value because protein, lipid, and carbohydrate contribute differently to total energy content (Pazos et al.,1996; Smaal et al. 2019). The caloric value reported for *P. perna* from the Gulf of Aden has also been described as being within the range reported for mussels from other subtropical regions, supporting its nutritional importance as a local food resource (Sokołowski et al. 2010).

Overall, the proximate composition results demonstrate that *P. perna* tissues contained substantial protein and moisture, with lower proportions of lipid, ash, and carbohydrate. The significant spatial differences observed for several biochemical components indicate that the biochemical profile of *P. perna* varies between the two coastal environments. Previous research on *P. perna* and other bivalves indicates that such variation may be associated with environmental productivity, food availability, and reproductive processes (Gosling 2015; Narváez et al. 2008; Smaal et al. 2019). Nevertheless, because food availability, reproductive stage, and continuous environmental parameters were not directly measured in the present study, these factors should be regarded as potential explanatory variables rather than demonstrated causal factors.

Environmental and Site-Related Interpretation

The differences observed between Al-Hami and Sharma across microbial and biochemical parameters indicate that site-specific conditions may have contributed to the observed variation in *Perna perna*. The higher bacterial indicators in seawater and mussel tissues at Al-Hami, together with differences in moisture, protein, lipid, ash, and carbohydrate contents, suggest that the two coastal environments differed in their biological and environmental characteristics. Previous studies have shown that the biochemical condition of *P. perna* can vary according to environmental productivity, food availability, reproductive condition, and other local environmental factors (Gosling 2015; Sokołowski et al. 2010; Smaal et al. 2019). The bigger spatial differences compared with the relatively limited monthly variation observed for bacterial load suggest that local site characteristics may have been more influential than short-term temporal variation during the study period. The higher microbial indicators recorded at Al-Hami may reflect greater exposure to localized sources of microbial contamination, whereas the lower values at Sharma are consistent with comparatively lower microbial pressure. However, specific pollution sources were not directly identified in the present study, and environmental variables such as nutrient concentrations, phytoplankton abundance, temperature, salinity, and hydrodynamic conditions were not continuously monitored. Therefore, the observed site-related differences should be interpreted as associations rather than evidence of a specific causal environmental factor.

The combined microbial and biochemical findings nevertheless demonstrate the usefulness of examining both biological composition and microbial quality when evaluating coastal mussel populations. *Perna perna* integrates environmental conditions through its filter-feeding activity, while its tissue composition reflects physiological and nutritional status. The simultaneous assessment of these characteristics therefore provides a more comprehensive indication of differences between coastal habitats than either microbial or biochemical measurements alone (Sokołowski et al. 2010; Gosling 2015; Smaal et al. 2019).

CONCLUSION

This study demonstrated clear spatial differences in the microbiological and biochemical characteristics of *Perna perna* collected from Al-Hami and Sharma along the Gulf of Aden coast, Yemen. Mussels from Al-Hami showed significantly higher E. coli loads than those from Sharma, consistent with the higher bacterial indicators detected in the surrounding seawater. Despite this spatial difference, the bacterial concentrations detected in mussel tissues remained within the microbiological criterion applied in the present study, and Salmonella spp. were not detected in any of the 240 examined specimens.

The proximate composition analysis showed that *P. perna* tissues were characterized by relatively high moisture and protein contents, with lower proportions of lipid, ash, and carbohydrate. Significant spatial differences were observed for several biochemical components, whereas monthly variation in bacterial load was not statistically significant. These findings indicate that site-related differences were more evident than short-term temporal variation during the study period.

Overall, the results demonstrate that *P. perna* can provide useful information on differences in coastal microbial quality while also representing a nutritionally valuable marine resource. The combined assessment of microbial contamination, biochemical composition, and morphometric characteristics provides a more comprehensive evaluation of the condition of *P. perna* populations along the Hadhramout coast. Further studies incorporating continuous environmental monitoring, food availability, reproductive stage,

and specific pollution sources are recommended to clarify the factors responsible for the spatial variation observed between the two coastal sites.

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