Academy of Romanian Scientists | *Volume 13, No. 1, 2024, pp. 7-23*
Annals Series on Biological Sciences | Online ISSN 2285 – 4177

ORIGINAL PAPERS

FOOD AVAILABILITY ON INFLUENCE MUSSEL *Mytilus galloprovincialis* (Lamarck, 1819) ON PHYSIOLOGICAL AND BIOCHEMICAL STATUS

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Abstract *Phytoplankton, as the primary food source for suspension-feeding bivalves, can significantly impact the growth and survival of bivalves. To investigate the influence of food availability on the condition index and biochemical composition of mussels Mytilus galloprovincialis from the Romanian Black Sea coast, phytoplankton and mussels samples were collected from four sites between November 2017 and November 2018. The phytoplankton quality and quantity varied across locations and seasons. The ports had the highest diversity and abundance of phytoplankton, while the area with low trophic conditions had the lowest. The most dominant phytoplankton groups observed were diatoms and dinoflagellates. The condition index values were higher in sites with greater food availability, reaching the peak in spring. The lipid and carbohydrate content peaked in spring when the food availability was high. The protein content was higher in winter and autumn. Condition index was positively correlated with phytoplankton abundance and biomass ($p < 0.05$). Several significant correlations were found between the biological parameters of mussels, such as proteins, lipids, carbohydrates, tissue dry weight, moisture, ash free dry weight, and ash. In conclusion, the results indicated that higher food availability and increasing seawater temperatures led to greater condition index and reserve accumulation, primarily in the form of proteins, carbohydrates, and lipids, providing mussels with enough energy to withstand stressful conditions.*

Keywords: mussels, phytoplankton, condition index, biochemical composition, Romanian Black Sea coast.

DOI 10.56082/annalsarscibio.2024.1.7

INTRODUCTION

Suspension-feeding bivalves have a significant ecological role as primary consumers of plankton in marine ecosystems and play an essential role in the energy transfer between trophic levels [27, 22]. Mussels *Mytilus galloprovincialis* (Lamarck, 1819) are widely distributed in the Black Sea, from shallow to deep waters. They inhabit hard coastal substrates, rocks, artificial constructions, and deep-water silts across various habitats and environmental conditions [19].

Various biological and environmental factors influence the success of bivalve populations in their natural habitats [41]. Mussels' physiological condition and biochemical composition vary seasonally and are strongly related to seawater temperatures, food availability, and gametogenic cycle [2].

Condition index (CI) is a physiological biomarker that reflects bivalves' ability to withstand natural and anthropogenic stress and indicates their physiological state [2]. It is also closely related to storage-consumption cycles of nutrients and gametogenesis [2]. Complex interactions among food availability, environmental conditions, growth, and the gametogenic cycle influence the metabolic activities in bivalves. Glycogen, lipids, and proteins are stored as energy before gametogenesis when food is abundant [10, 8]. The carbohydrate of bivalves is mainly composed of glycogen, which plays an essential role in the physiology of bivalves, particularly during their reproductive process. Lipids have a fundamental role in the metabolism of bivalves as energy reserves and membrane components in the form of phospholipids. Protein is the main component in the soft tissue of bivalves, and it also serves as an energy reserve supporting gametogenesis [8].

Coastal marine environments undergo large fluctuations in phytoplankton composition and abundance over both seasonal and diel periods, which affects food availability [22, 38]. Numerous studies have shown that food quality and quantity are the principal factors influencing bivalves' growth and survival performance [29, 19, 4, 38]. Phytoplankton plays a critical role in the marine biological ecosystem, representing the primary food source for suspension feeders [22]. Food availability strongly affects mussels' condition index (CI) and biochemical composition [10]. Food availability influences the energy storage and production of ripe gametes during spawning [31, 11]. Food availability can also affect biological responses to environmental pollution, supporting a detoxification mechanism against oxidative stress [32].

Understanding how food availability affects bivalve feeding behaviour is essential for predicting their growth and interactions with the ecosystem [38]. Therefore, the study aims to evaluate the influence of food availability on the condition index and biochemical composition of mussels *Mytilus galloprovincialis* in different seasons and trophic conditions.

MATERIAL AND METHODS

Study area and sampling

The field sampling was carried out along the Romanian Black Sea coast between November 2017 and November 2018. Four sampling sites representing different environmental conditions were chosen (Fig. 1). The locations were selected based on their various trophic conditions. Midia Port (S1), Constanta Port (S2), and Mangalia Port (S3) are semi-enclosed water bodies exposed to varying degrees of anthropogenic pressures (e.g.

eutrophication, pollution, sewage discharge, etc.), making these areas prone to frequent phytoplanktonic blooms [21]. The fourth site, 2 Mai Bay (S4), is an open-sea area exposed to much lower levels of anthropogenic pressures and reduced trophic conditions. The map of the sampling sites was plotted using the Ocean Data View software [37].

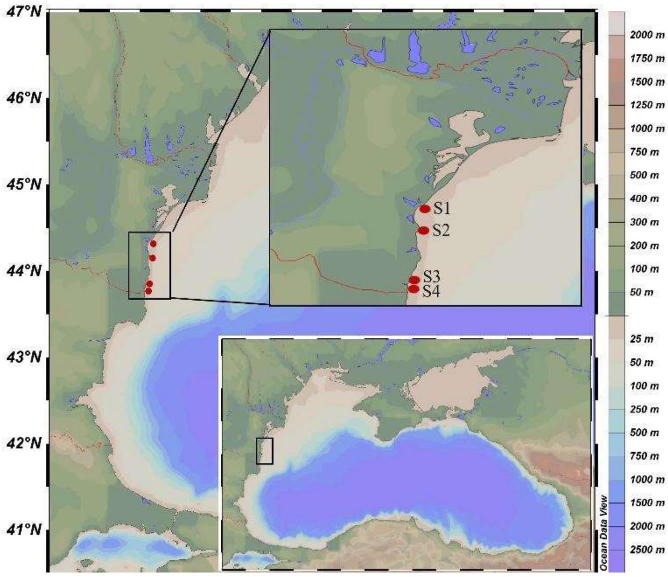

Fig. 1. Map of the sampling area. S1 : Midia Port, S2 : Constanta Port, S3 : Mangalia Port, S4 : 2 Mai Bay

Seawater temperature and salinity were measured *in situ* using a multi-parametric probe (HANNA HI 7698194). To assess the food availability (phytoplankton quality and quantity), 1L of seawater was collected at each sampling site from the seawater surface (0–0.5 m). The phytoplankton samples were immediately preserved with 14 ml of formaldehyde 37%.

Wild *M. galloprovincialis* specimens (80-100 individuals) were randomly collected from different depths (0.5–2 m) using a metal rake or by hand. After collection, samples were immediately transported to the laboratory in cool boxes and then processed. Thirty individuals were randomly selected for the condition index, and the remaining mussels were stored at −20 °C for further biochemical analysis.

Phytoplankton quality and quantity analysis

After homogenising the sample, a 10 ml sample fraction was poured into a Utermöhl sedimentation chamber and left to settle for 24 h [16]. The sample was then analysed under an inverted microscope (OLYMPUS IX51) at a magnification of 200x and 400x. The phytoplankton species were identified to the closest taxonomic level (genus, species, or subspecies), and the cells of each species were counted. To identify the species, we used various manuals for marine phytoplankton identification [36, 20, 33, 9]. The phytoplankton abundance (cells/L) and biomass (mg/m^3) were calculated using the Ecology Database software (NIMRD software).

Condition index and biochemical analysis

Thirty mussels were randomly selected from each sampling site to measure the condition index (CI), moisture (M) content of the tissue and ash (A). The mussels' shells were cleaned of encrusting organisms, such as barnacles, epifauna, and seaweeds. The soft tissue of mussels was dissected from the shells and dried with filter paper.

To determine the condition index (CI), mussels' soft tissues and valves were oven-dried at 105 °C for 24 h to obtain their dry weight. The dried tissues and valves were weighed individually using an electronic balance (KERN KB, d = 0.01 g). The condition index was calculated by the following equation [14]:

$$CI\ (\%) = (\text{dry weight of soft tissue (g)}/\text{dry weight of shell (g)}) \times 100 \quad (1)$$

Ash (A) weight and ash free dry weight (AFDW) were determined by combusting a known dry weight of tissue at 550 °C for 5 h in a muffle furnace and reweighing the tissue [3].

To determine the biochemical composition of the soft tissues (e.g. proteins, carbohydrates, and lipids), 25−30 mussels were pooled in one sample. The pooled tissues were dried at 60 °C and homogenized. For each biochemical assay, three sub-samples of the dried tissue powder were used.

The protein content was determined using the modified Lowry method [34]. A calibration curve was done using bovine albumin serum as a standard. Carbohydrates were determined by the modified Dubois method [34]. The lipid content was determined by the Soxhlet method, which involves ether extraction with a Soxhlet apparatus.

Data analysis

All data was tested for normal distribution (Shapiro-Wilk test) and homogeneity of variances (Levene's test). The Kruskal-Wallis ANOVA on ranks was used because the normality assumption was not met. The non-parametric Kruskal-Wallis test was used to test differences in phytoplankton abundance and biomass, CI, biochemical composition of mussels and other biological measurements (moisture, AFDW, ash, dry tissue weight) among sites and seasons. When significant differences were found, multiple pairwise comparisons among means were performed using the post-hoc non-parametric Dunn's test.

Spearman's correlation coefficients were calculated to investigate the statistical relationship between biochemical components, biological measurements, CI, phytoplankton abundance and biomass, and environmental parameters (temperature and salinity).

Multivariate statistical analysis (Principal Component Analysis, PCA) was used to interpret data variability. PCA was conducted to assess the relationship between mean values of CI, biochemical composition, phytoplankton abundance and biomass, and environmental parameters. PCA was based on the Euclidean distance matrix of the fourth root transformed and normalised data. The variables show high similarity if the distance between them is low [13]. We applied hierarchical cluster analysis using the fourth root transformed and normalised data to represent the similarity between sites and seasons in the PCA. In the analysis, we considered only principal components with eigenvalues greater than one.

The shade plot, a visual display of the data matrix, was used to show which species mainly influenced the multivariate results [13]. It was based on the Bray-Curtis similarity matrix of fourth root transformed and normalised variables. The shade plot displays the data pattern through varying colour scale intensities.

Statistical analyses of data were carried out with XLSTAT 2023.3.1 software (New York, NY, USA) [1]. and PRIMER v7.0.21 (PRIMER-E Ltd., Plymouth, UK) [13]. The significance level was set at $p < 0.05$ for all analyses.

RESULTS AND DISCUSSIONS

Environmental conditions

Seawater temperature showed a clear seasonal pattern and ranged from 9.15 °C (winter) to 24.74 °C (summer) (Fig. 2). Salinity recorded the highest value in S4 in autumn (16.58 PSU) and the lowest in spring in S3 (9.01 PSU). Overall, the highest mean salinity was observed in S4 (16.30±0.19 PSU) and the lowest in S3 (13.30±2.90 PSU), followed by S1 (14.17±0.81 PSU).

The salinity values observed in the S2 and S4 fall within the typical annual variation of salinity (15−18 PSU) in the southern part of the Romanian Black Sea coast [24]. The salinity in S1 and S3 is generally lower due to freshwater input, wastewater treatment plants (WWTPs) and industrial sources discharging into their waters [21]. The salinity level in the S3 dropped to 9.01 PSU during spring due to heavy rainfall, which caused an increase in stormwater runoff discharged within the port (field observations).

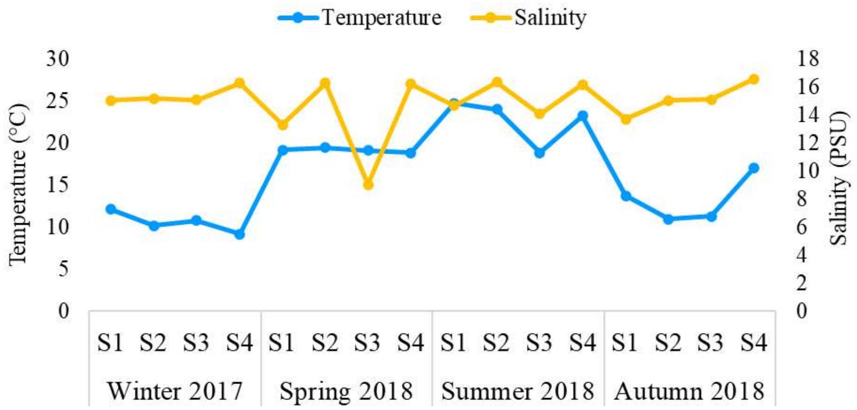

Fig. 2. Seasonal variation of seawater temperature and salinity in the Romanian Black Sea coast during 2017−2018. S1 : Midia Port, S2 : Constanta Port, S3 : Mangalia Port, S4 : 2 Mai Bay

Food availability - phytoplankton composition and quantity

During the study, a total of 135 phytoplankton species belonging to 9 phyla were identified. The number of species varied across locations and seasons (Fig. 3). The highest species diversity was observed in S1, S2 and S3 throughout all seasons. S1 recorded the highest number of species in autumn (67) and winter (50). S3 had the highest species diversity in spring (56) and summer (51). S4 had the lowest number of species in all seasons, with a maximum of 28 species in summer and a minimum of 14 in autumn.

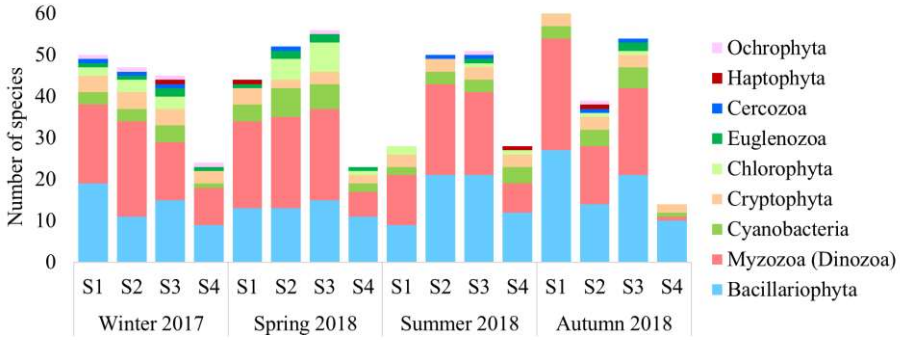

Fig. 3. Number of phytoplankton species variation in the Romanian Black Sea coast during 2017–2018. S1 : Midia Port, S2 : Constanta Port, S3 : Mangalia Port, S4 : 2 Mai Bay

On average, diatoms (Bacillariophyta) were the most represented in summer and autumn, especially in S4 (49.90%). In contrast, dinoflagellates (phylum Myzozoa, subphylum Dinozoa) were the most common in winter and spring, mainly in the sites S1, S2 and S3 (42.22%, 42.79% and 37.13%, respectively) (Fig. 4). Cyanobacteria was the most represented in spring and summer, mainly in S2 and S3 (9.03% and 8.69%, respectively). Cryptophytes (Cryptophyta) were the most common group in winter, summer, and autumn, accounting for 9.47%, 8.33%, and 8.00%, respectively. They were mainly present in S4 and S1, representing 11.55% and 8.07%, respectively. Euglenozoa, Cercozoa, Haptophyta, and Ochrophyta constituted the smallest proportion of species recorded, accounting for only 1.49%–4.44% of all species.

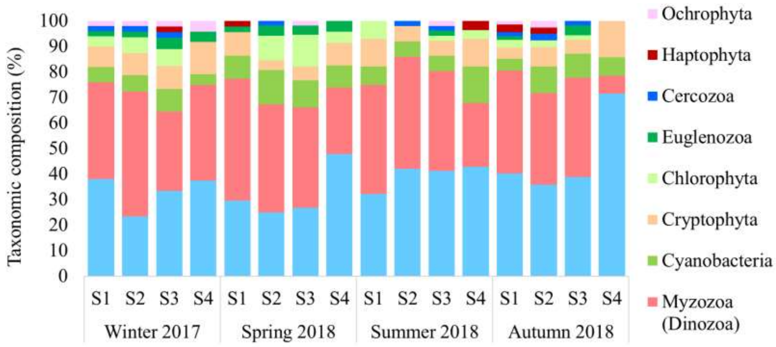

Fig. 4. Phytoplankton taxonomic composition in the Romanian Black Sea coast during 2017–2018. S1 : Midia Port, S2 : Constanta Port, S3 : Mangalia Port, S4 : 2 Mai Bay

The total phytoplankton abundance (cells/L) and biomass (mg/m^3) varied across seasons and sites (Fig. 5a and 5b). Generally, the total abundance and biomass follow a similar trend between seasons, with higher values in sites S1, S2 and S3. Higher abundances and biomasses were observed in spring (0.01–2.91×10^6 cells/L and 20.25–4248.87 mg/m^3), summer (0.06–3.42×10^6 cells/L and 39.21–15965.58 mg/m^3), and autumn (0.01–11.03×10^6 cells/L and 13.03–15778.24 mg/m^3). Lower abundances were recorded in winter (0.02–0.34×10^6

cells/L and 28.65−1241.81 mg/m^3). The highest abundance was recorded in S1 (in autumn), followed by S2 (in summer) and S3 (in spring). As for biomass, S2 had the highest value in summer, followed by S1 in autumn and S2 in spring.

Diatoms were the most dominant group in winter (in S1, 57.39%), spring (in S2, 55.95%), summer (in S2, 87.41%), and autumn (in S4, 54.32%) (Fig. 5a). Dinoflagellates were the most abundant in summer (in S3, 46.76%). Cyanobacteria were the most abundant group, particularly in S3 (47.54%) and S4 (55.86%) during spring and in S4 (69.61%) during summer. Cryptophytes dominated the phytoplankton community in almost all sites and seasons. Higher proportions for this group were observed in winter in S2 (45.09%) and S4 (50%), spring in S1 (58.45%), summer in S1 (89.90%) and S4 (69.61%), and autumn in S3 (82.36%).

Overall, the dinoflagellates were the most dominant group in terms of biomass. High biomass values for this group were observed in winter, in S1 (79.68%) and S2 (84.90%), in spring in S1 (85.36%) and S4 (79.06%), in summer in S1 (60.26%) and S3 (90.44%), and in autumn in S3 (57.76%) (Fig. 5b). High biomasses were observed for diatoms particularly in spring (63.43%), summer (97.44%), and autumn (81.90%). Cryptophytes had a more significant proportion, especially in summer in S1 (38.07%). Chlorophyta recorded the highest biomass value in autumn in S2 (47.74%). Euglenozoa, Cercozoa, Haptophyta, and Ochrophyta were less abundant and had lower biomass across all seasons and sites.

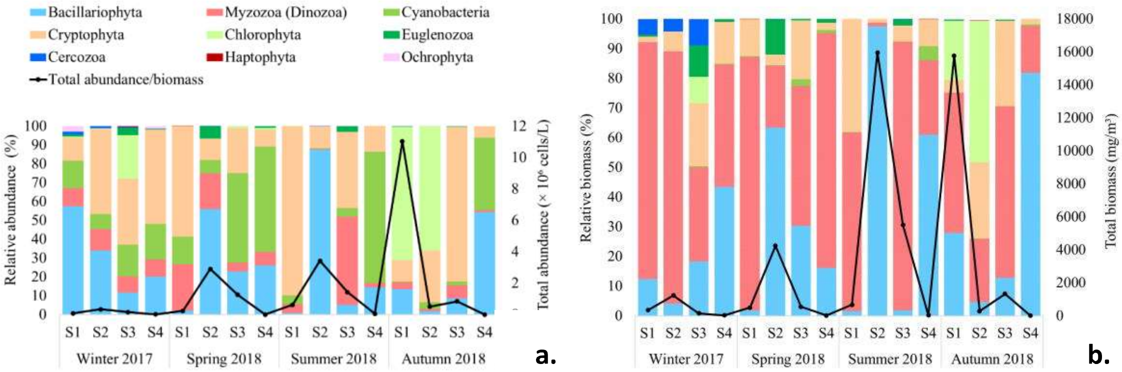

Fig. 5. Seasonal variation of relative abundance and total abundance of phytoplankton (a) and relative biomass and total biomass of phytoplankton (b) in the Romanian Black Sea coast during 2017−2018. S1 : Midia Port, S2 : Constanta Port, S3 : Mangalia Port, S4 : 2 Mai Bay

Different combinations of dominant species occurred in different seasons and sites (Fig. 6). In winter, *Komma caudata* (Cryptophyceae) reached the highest abundance (0.097×10^6 cells/L) in S2. Several species reached high abundances in spring, mainly in S2 and S3. These species include the diatoms *Cerataulina bergonii* (0.66×10^6 cells/L, in S2), *Pseudo-nitzschia delicatissima* (0.55×10^6 cells/L, in S2), *Diatoma tenue* var. *elongatum* (0.26×10^6 cells/L, in S3) *Cyclotella caspia* (0.18×10^6 cells/L, in S2) and *Skeletonema costatum* (0.14×10^6 cells/L, in S2), the dinoflagellate *Prorocentrum cordatum* (0.47×10^6 cells/L, in S2), the cyanobacteria *Pseudanabaena limnetica* (0.37×10^6 cells/L, in S3) and cryptophyte *Hillea fusiformis* (0.30×10^6 cells/L, in S3). The diatom *Dactyliosolen fragilissimus* bloomed in summer (in S2), reaching a maximum density of 2.56×10^6 cells/L. In autumn, two bloom events were observed in S1 caused by the development of the chlorophyte *Pyramimonas tetrarhynchus* (7.8×10^6 cells/L) and diatom *Cerataulina bergonii* (1.02×10^6 cells/L). In addition, high densities of the cryptophytes *Komma* caudata (0.82×10^6 cells/L) and *Hillea fusiformis* (0.44×10^6 cells/L), diatoms *Leptocylindrus minimus* (0.24×10^6 cells/L) and *Skeletonema costatum* (0.14×10^6 cells/L), as well as dinoflagellates *Prorocentrum cordatum* (0.15×10^6 cells/L) and *Prorocentrum micans* (0.11×10^6 cells/L) were observed.

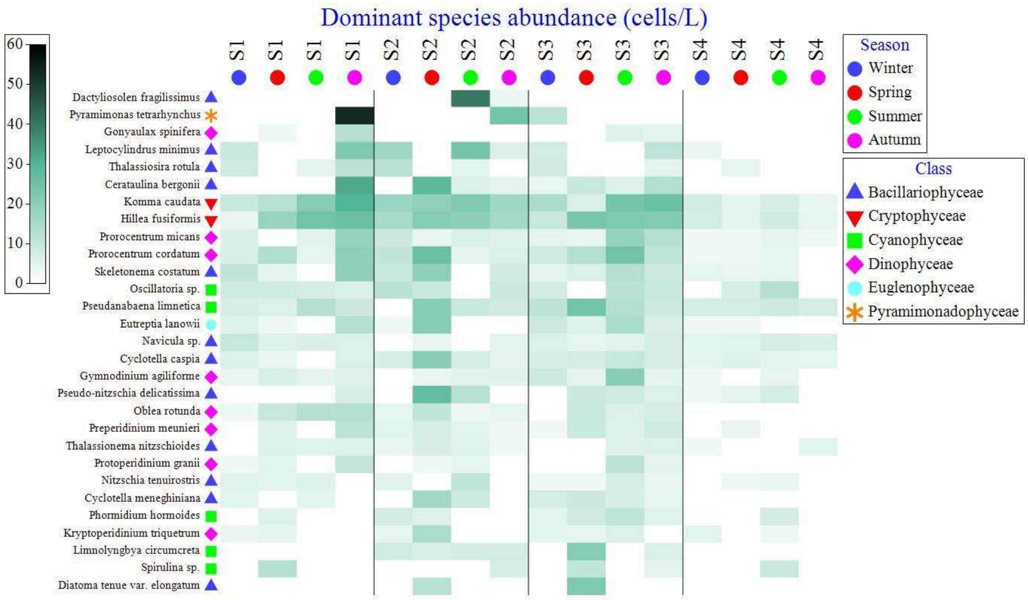

Fig. 6. Dominant phytoplankton species matrix (cells/L) per site and season in the Romanian Black Sea coast during 2017–2018. S1 : Midia Port, S2 : Constanta Port, S3 : Mangalia Port, S4 : 2 Mai Bay. Data was fourth root transformed. White space shows the absence of the species at that site, and the intensity of the colour scale is linearly proportional to the abundance of the species.

Phytoplankton dynamics influence food availability in the water [22]. Coastal waters experience significant fluctuations in food supply, which are influenced not only by food quantity but also by the species composition. The phytoplankton composition varies spatially and temporally due to various physical and biological factors [6]. Our study showed that ports (sites S1-S3) have a higher species diversity and cell abundance than S4 in all seasons. These ports are characterized by high levels of nutrients due to the sewage discharge from WWTPs [21]. This nutrient-rich environment creates favourable conditions for phytoplankton growth. In line with our findings, another study has shown that ports exhibit high species diversity and cell abundance [21].

Condition index of mussels

The condition index (CI) varied significantly across seasons and sites (Fig. 7). The highest CI values (mean ± SD) were observed during spring, with values ranging between

12.05 ± 2.52 (in S4) and 29.49 ± 6.03 (in S3). The lowest values were observed in winter, ranging from 4.96 ± 1.60 (in S4) to 9.88 ± 2.70 (in S1).

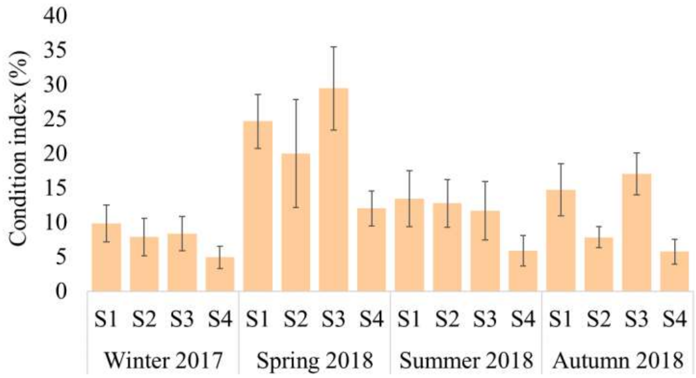

Fig. 7. Seasonal variation of condition index (mean ± SD) of mussels (*Mytilus galloprovincialis*) (n = 30) on the Romanian Black Sea coast during 2017−2018. S1: Midia Port, S2: Constanta Port, S3: Mangalia Port, S4: 2 Mai Bay, SD: standard deviation

Condition index and biochemical composition are strongly related to seawater temperature, food availability and gametogenic cycle [7, 5, 28, 30, 39, 10]. Temperature and food availability are the main factors affecting bivalve growth. However, the influence of these variables is complex and depends on how each species acquires and consumes energy in their natural environment [6].

The CI values were higher in sites with greater food availability, such as S1, S2, and S3, and lower in S4. These specific sampling sites have higher nutrient availability than other oligotrophic sites, creating favourable conditions for phytoplankton growth. The constant high phytoplankton abundance in these sites ensured a consistent food supply for mussels, resulting in higher CI values. However, the low phytoplankton abundance in S4 was a limiting factor for the growth of mussels, leading to lower CI values. Our results are similar to a study showing that higher CI of hard clam *Mercenaria mercenaria* seems to be associated with sites having greater food quantity [41]. According to Martínez-Gómez et al. [23], low food availability results in nutrient deprivation. Under such conditions, mussels tend to catabolise their tissue as an adaptative response to low food availability to generate energy and survive [18]. This research has also documented the highest CI values in the polluted sampling sites, which are located near commercial ports, as in our study.

The CI values showed an increasing trend from winter to spring, reaching their highest point during spring. The values started to decline during summer but increased again in autumn. According to Sahin et al. [35], gradually decreasing CI from spring to summer indicates a significant loss in tissue weight or energy reserves due to spawning. The highest values of CI in bivalves reflect maturity or physiological fitness, while the decline in the value is linked to the onset of spawning or the release of gametes [40, 12]. We observed that the increase in food availability during spring coincided with the highest levels of CI. The levels of food remained high during summer and started to increase again in autumn due to autumn blooms, resulting in the gradual increase of CI values. In agreement with our study, several authors have shown that the highest CI values were recorded in spring, coinciding with high phytoplankton abundance [25, 42, 10].

Biochemical composition of mussels

The carbohydrate content ranged from 0.66 ± 0.03% (in S4) to 20.31 ± 0.60% (S3), and lipid content from 1.29 ± 0.59% (in S4) to 13.23 ± % 0.85 (in S1) (Fig. 8a). The average carbohydrate content was high in spring (18.55 ± 1.22%) and low in summer 2.35 ± 2.02%. The mean lipid content showed higher values in winter (9.23 ± 3.84%) and spring (8.86 ± 0.96%), and lower values in summer (3.76 ± 2.63%) and autumn (2.57 ± 1.21%) (Fig. 8b). The protein content in mussels varied from 31.42 ± 7.01% (in S2) to 41.57 ± 1.95% (in S2) (Fig. 8c). The highest mean value of protein content was recorded in winter (40.38 ± 1.01%) and autumn (35.53 ± 1.65%), and the lowest in spring (34.09 ± 3.14%) and summer (34.38 ± 2.20%).

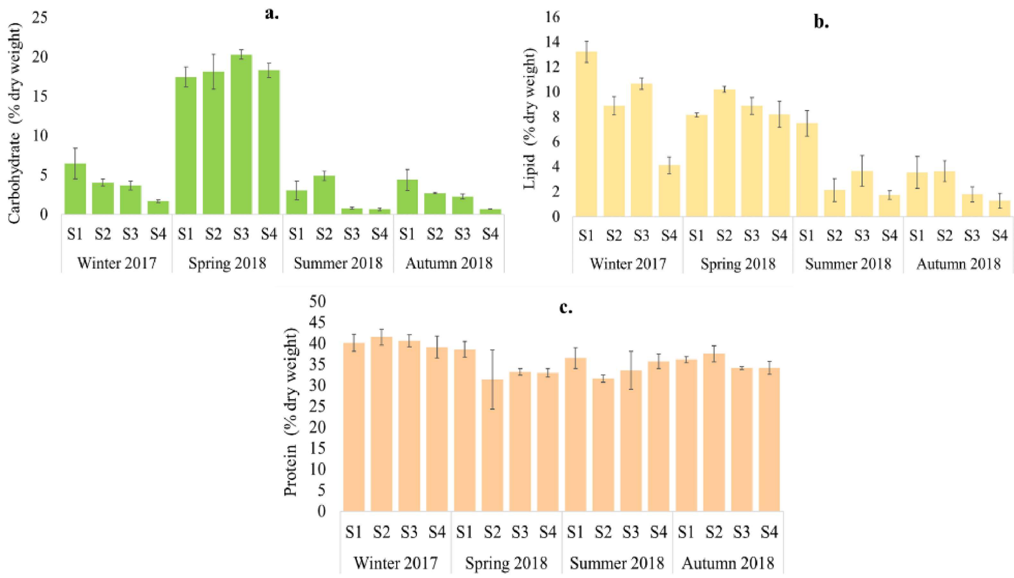

Fig. 8. Seasonal variation of, carbohydrate (b), lipid (c) and protein content (c) in mussels *Mytilus galloprovincialis* (mean ± SD, *n* = 3) from the Romanian Black Sea coast during 2017−2018. S1: Midia Port, S2: Constanta Port, S3: Mangalia Port, S4: 2 Mai Bay, SD: standard deviation

The highest mean value for moisture was recorded in winter (86.89 ± 0.24%), and the lowest in spring (78.86 ± 3.31%) (Fig. 9a). The tissue dry weight and AFDW showed the highest mean value in spring (21.14 ± 3.31% and 94.11 ± 0.40%, respectively). Ash content was higher in autumn (10.10 ± 2.78%) and lower in spring (5.89 ± 0.40%) (Fig. 9b).

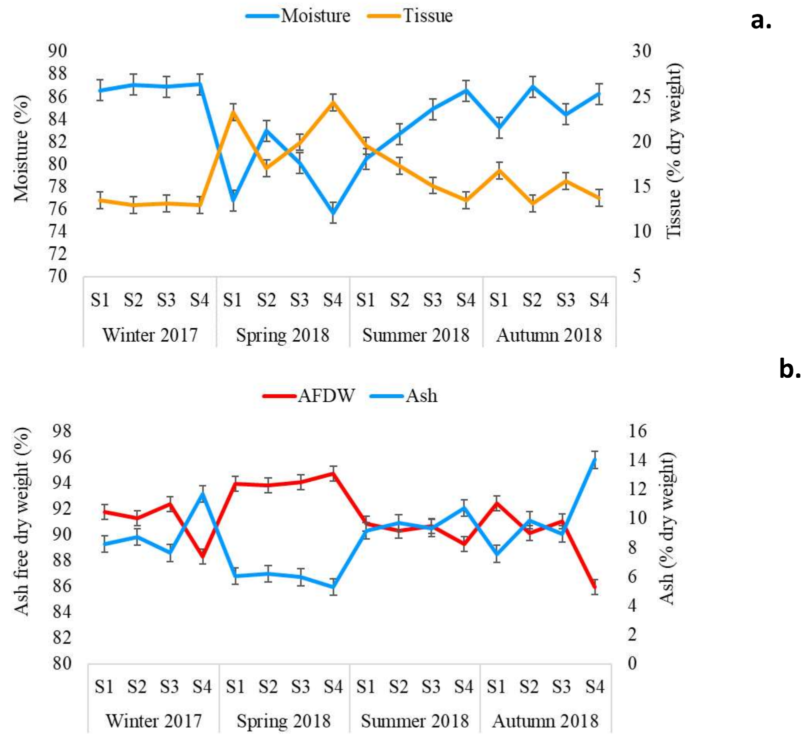

Fig. 9. Seasonal variation of moisture and tissue dry weight (a) and of ash-free dry weight and ash (b) of mussels *Mytilus galloprovincialis* from the Romanian Black Sea coast during 2017−2018 (mean ± SD, *n* = 30). S1: Midia Port, S2: Constanta Port, S3: Mangalia Port, S4: 2 Mai Bay, SD: standard deviation

Accumulated energy reserves, particularly glycogen and proteins, and gonadal development reflect good condition index values [35]. The metabolic activity of bivalves is characterised by phases of accumulation and consumption of body reserves [26]. This process is influenced by phytoplankton availability, environmental conditions, and the gametogenic cycle [10, 30]. Dridi et al. [15] reported that when food availability is high, mussels accumulate energy reserves in the form of glycogen, lipid, and protein before gametogenesis. These substrates are later utilised to produce gametes when the metabolic demand is high. Accumulated energy reserves are primarily used for basic metabolism. The surplus of energy is then allocated to tissue and/or shell somatic growth and storage glycogen [26, 25]. This glycogen is available as an energy source for reproduction maturation [26].

Our results showed that the lipid and carbohydrate content peaked in spring and decreased in the summer, probably because it was used in gametogenesis. The highest carbohydrate and lipid content recorded may be related to increased food availability. Freites et al. [17] reported that the lipid content of bivalves was at maximum when the organic matter in water was higher in spring and summer. Energy accumulates in the form of lipids and carbohydrates simultaneously in the early stages of gametogenesis [25]. During spawning, mussels deplete their energy reserves in the form of carbohydrates and lipids [25]. The protein content recorded higher values in winter and autumn and lower values in spring and summer. The lowest value coincided with the highest values for lipid and carbohydrate content, and the highest values are reached when these compounds are utilised. Our findings are similar to the results of Mladineo et al. [25].

Statistical analysis

Spearman's correlation coefficients between different biological parameters and measurements performed in mussels and environmental parameters are presented in Table 1.

CI was positively correlated with phytoplankton abundance (p = 0.009) and phytoplankton biomass (p = 0.018), carbohydrates (p = 0.004) and tissue dry weight (p = 0.0001). An inverse relationship was seen between CI and moisture (p = 0.0001).

Protein has shown a significant correlation with moisture (p = 0.009), tissue dry weight (p = 0.009) and seawater temperature (p = 0.013). Lipid correlated with carbohydrates (p = 0.008), AFDW (p = 0.008) and ash (p = 0.008). Carbohydrates have shown a significantly positive correlation with tissue dry weight (p = 0.018) and AFDW (p = 0.0001) and a negative one with moisture (p = 0.008) and ash (p = 0.0001).

Tissue dry weight correlated with temperature (p = 0.002), moisture (p = 0.0001), ash (p = 0.015) and AFDW (p = 0.015). Moisture also correlated with AFDW (p = 0.015) and temperature (p = 0.002). In our study, we found no correlation between moisture and ash, in contrast to the findings of Çelik et al. [10].

Table 1. Spearman's correlation coefficient between biological parameters evaluated in *M. galloprovincialis*, phytoplankton abundance and biomass, and environmental parameters (n = 16). L: lipids, C: carbohydrates, P: proteins, CI: condition index, AFDW: ash free dry weight, A: ash, M: moisture, TDW: tissue dry weight, ShDW: shell dry weight, PA: phytoplankton abundance, PB: phytoplankton biomass, T: seawater temperature, S: salinity. Values in bold are statistically significant at $p < 0.05$.

Variables	L	C	AFDW	A	CI	M	TDW	T	P	PA	PB	S	ShDW
L	**1**	**0.650**	**0.647**	**-0.647**	0.241	-0.003	0.003	-0.182	0.309	-0.026	-0.006	-0.124	-0.097
C	**0.650**	**1**	**0.871**	**-0.871**	**0.688**	**-0.591**	**0.591**	0.215	-0.229	0.276	0.232	-0.106	-0.041
AFDW	**0.647**	**0.871**	**1**	**-1.000**	**0.744**	**-0.603**	**0.603**	0.132	-0.150	0.241	0.203	-0.379	-0.003
A	**-0.647**	**-0.871**	**-1.000**	**1**	**-0.744**	**0.603**	**-0.603**	-0.132	0.150	-0.241	-0.203	0.379	0.003
CI	0.241	**0.688**	**0.744**	**-0.744**	**1**	**-0.791**	**0.791**	0.481	-0.447	**0.641**	**0.591**	-0.500	0.053
M	-0.003	**-0.591**	**-0.603**	**0.603**	**-0.791**	**1**	**-1.000**	**-0.739**	**0.641**	-0.300	-0.253	0.226	-0.074
TDW	0.003	**0.591**	**0.603**	**-0.603**	**0.791**	**-1.000**	**1**	**0.739**	**-0.641**	0.300	0.253	-0.226	0.074
T	-0.182	0.215	0.132	-0.132	0.481	**-0.739**	**0.739**	**1**	**-0.618**	0.305	0.280	0.001	0.230
P	0.309	-0.229	-0.150	0.150	-0.447	**0.641**	**-0.641**	**-0.618**	**1**	-0.394	-0.303	-0.188	0.288
PA	-0.026	0.276	0.241	-0.241	**0.641**	-0.300	0.300	0.305	-0.394	**1**	**0.944**	-0.391	0.018
PB	-0.006	0.232	0.203	-0.203	**0.591**	-0.253	0.253	0.280	-0.303	**0.944**	**1**	-0.376	-0.103
S	-0.124	-0.106	-0.379	0.379	-0.500	0.226	-0.226	0.001	-0.188	-0.391	-0.376	**1**	-0.141
ShDW	-0.097	-0.041	-0.003	0.003	0.053	-0.074	0.074	0.230	0.288	0.018	-0.103	-0.141	**1**

The Kruskal-Wallis test results showed significant differences between sites for phytoplankton abundance (K = 9.715, p = 0.021), phytoplankton biomass (K = 8.735, p = 0.033) and condition index (K = 148.550, p = 0.0001). Statistically significant differences were observed between seasons for condition index (K = 182.475, p = 0.0001), protein content (K = 9.419, p = 0.024), carbohydrate content (K = 9.287, p = 0.026), lipid content (K

= 11.007, p = 0.012), moisture (K = 11.228, p = 0.011), dry tissue (K = 11.228, p = 0.011), AFDW (K = 9.154, p = 0.027), and ash (K = 9.154, p = 0.027).

The post hoc analysis (Dunn's test) showed that statistically significant differences were detected for phytoplankton abundance and biomass between S4 and port sites: S1 (p = 0.049 and p =0.026, respectively), S2 (p = 0.003 and p =0.08, respectively), S3 (p = 0.031 and p =0.026, respectively). Significant differences were also found for CI between S4 (with low trophic level) and port sites (with high trophic level): S1 (p = 0.0001), S2 (p = 0.0001), S3 (p = 0.0001). Significative differences (p = 0.0001) were found for CI between winter, spring, summer, and autumn but not between summer and autumn (p = 0.963).

There were significant differences in the protein, carbohydrate, and lipid content across the seasons. The protein content was significantly different between winter, spring (p = 0.005), and summer (p = 0.014). The carbohydrate content was significantly different between spring, summer (p = 0.008), and autumn (p = 0.009). Meanwhile, the lipid content was significantly different between winter, summer (p = 0.026), and autumn (p = 0.006), as well as between spring and autumn (p = 0.017).

The PCA of the mean values of biological parameters evaluated in *M. galloprovincialis*, phytoplankton abundance and biomass, environmental parameters, and the related variability of the site distribution are shown in Figure 10. The first two principal components have eigenvalues of 3.89 and 2.31, respectively, and explain 68.9% of the total variance in the data matrix. Principal component 1 (PC1) explained 43.2% of the total variance and showed significant positive loading of the carbohydrates (0.42), CI (0.48), AFDW (0.43), and phytoplankton abundance (0.32). Principal component 2 (PC2) explained 25.7% of the data variability and was mainly characterised by the positive loading of proteins (0.46) and lipids (0.50).

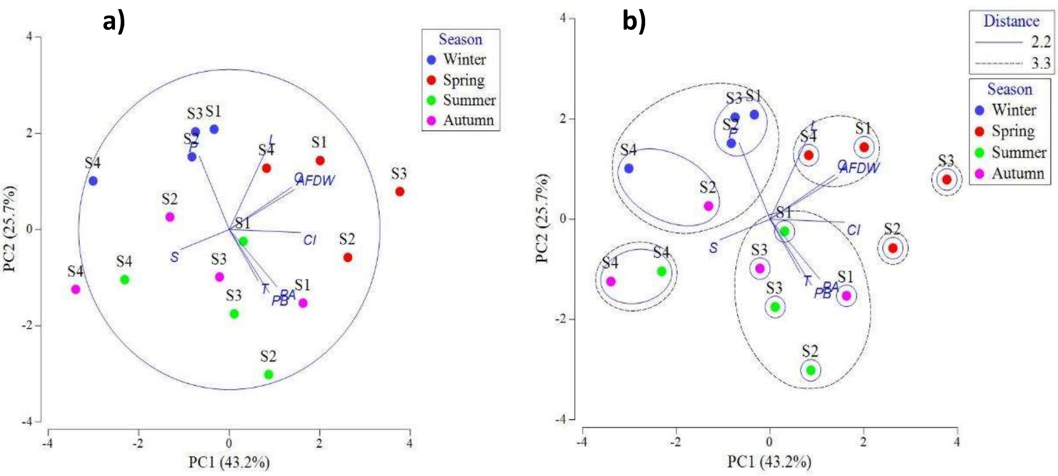

Fig. 10. Principal Component Analysis (PCA) of the CI, biochemical components, AFDW, phytoplankton abundance and biomass, and environmental parameters (temperature and salinity) (a) and clustering of sites (b), based on Euclidean distance matrix of fourth root transformed and normalised data. S1 : Midia Port, S2 : Constanta Port, S3 : Mangalia Port, S4 : 2 Mai Bay.

Significant correlations were found between several biological parameters. However, only a few correlations between biological parameters and environmental variables were statistically significant.

The positive relationship between CI of *M. galloprovincialis* and food availability was also demonstrated by Çelik et al. [10]. In agreement with our study, several authors have shown a strong correlation between food availability and CI of other bivalves [35, 39, 42]. In contrast to our study, the results of Çelik et al. [10] have shown a positive correlation between the CI and moisture of mussels. We found no correlation between protein and lipid levels in our study, which contradicts the findings of Çelik et al. [10].

The relationship between environmental parameters (physico-chemical and food availability), mussel CI and biochemical compositions was analysed using PCA. PC1 clearly separates the sites into three groups: group 1 (S1−S4 in spring), group 2 (S2−S3 in spring), and group 3 (S1−S3 in summer and S1 and S3 in autumn). On PC2, three groups were differentiated. The most similar groups were S1−S3 in winter, S4 (winter) and S2 (autumn), and S4 in summer and autumn.

CONCLUSIONS

Mussels can adjust physiologically to food quality and quantity variation in response to changing environmental conditions. Our results indicated that higher food availability and increasing seawater temperatures led to greater condition index and reserve accumulation, primarily in the form of proteins, carbohydrates, and lipids. Overall, mussels in the area with low food availability showed poor condition and low energy reserves. The seasonal cycle of mussel *M. galloprovincialis* from the Romanian Black Sea coast is marked by reserve accumulation and depletion phases, reflecting gonadal development and food availability. Higher food availability and accumulated reserves provide mussels with enough energy to withstand stressful conditions, even in heavily impacted environments due to human activities, such as ports. Our study highlights the importance of monitoring changes in both abiotic and biotic ecosystem components to understand potential impacts on mussel populations, providing valuable insights for resource management.

Acknowledgement

The study has been supported by the NUCLEU Program (INTELMAR), funded by the Ministry of Research, Innovation and Digitization, financing contract no. 45N/14.02.2019, project PN19260202.

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