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# Seasonal and Spatial Variability of Physiological Indices of *Mytilus galloprovincialis* (Lamarck, 1819) from Algerien West Coast

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# **ABSTRACT**

This study investigates the seasonal and spatial variability of physiological indices in the Mediterranean mussel Mytilus galloprovincialis (Lamarck, 1819), collected from four coastal stations along the western Algerian coastline: Mostaganem Harbor (S1), Pointe de l'Aiguille (S2), Oran Harbor (S3), and Honaine (S4). Three key biomarkers — the condition index (CI), repletion index (RI), and gonado-somatic index (GSI) — were analyzed as indicators of mussel health and environmental quality across four seasons. Seasonal and spatial differences were assessed using ANOVA, principal component analysis (PCA), and hierarchical cluster analysis (CAH) to evaluate the contribution of each physiological index to temporal and spatial structuring. The results revealed clear seasonal dynamics, with summer representing the peak physiological phase, particularly at S1 (CI: 62.11 ± 2.31; RI:  $48.23 \pm 2.12$ ; GSI:  $18.22 \pm 1.45$ ), followed by a marked decline during winter. Spatially, S1 and S2 exhibited consistently high indices, reflecting favorable trophic or environmental conditions, while S3 showed persistently lower values, potentially indicating anthropogenic stress or habitat degradation. Station S4 maintained stable and moderate index values, suggesting environmental resilience and its suitability as a reference site. ANOVA indicated a significant influence of both site and season on CI (P < 0.05), a seasonal effect on RI (P < 0.05), and no significant variation in GSI. PCA clarified the contribution of each index to seasonal and spatial structuring, highlighting the independence of somatic and reproductive parameters. CAH revealed distinct groupings of stations and seasons, which were linked to potential environmental drivers such as pollution gradients, hydrodynamic regimes, and anthropogenic pressures, reinforcing PCA findings. These findings underscore the value of M. galloprovincialis physiological indices as reliable, sensitive, and cost-effective tools for coastal biomonitoring. Highlighting their relevance for environmental monitoring, the results confirm that these indices respond to both natural fluctuations and human-induced pressures, making them suitable for detecting ecosystem changes along the Mediterranean coasts.

### INTRODUCTION







Understanding the physiological responses of marine organisms to environmental stressors is essential for assessing coastal ecosystem health. Among these organisms, *Mytilus galloprovincialis*, a widely distributed bivalve in the Mediterranean, is often used as a sentinel species due to its sensitivity to pollutants and natural stressors (**Benaoum** *et al.*, 2025). When mussels are exposed to multiple environmental pressures such as chemical contaminants, physical disturbances, or extreme temperature fluctuations, they undergo pronounced physiological changes (**Belmokhtar** *et al.*, 2024). These changes can elicit acute stress responses, which may lead to immunosuppression, impaired reproduction, or in severe cases, mortality (**Athanasios** *et al.*, 2022).

To evaluate the impact of such stressors, physiological indices serve as robust, quantifiable indicators of organism health. Notably, the condition index (CI), gonadosomatic index (GSI), and repletion index (RI) are commonly used to measure the nutritional status, reproductive development, and feeding intensity of bivalves. The CI offers insights into the overall health and energy reserves of mussels and reflects responses to environmental variables including temperature, food availability, and pollution exposure (Lucas & Beninger, 1985). In parallel, the GSI provides a reliable estimate of gonadal maturation, often validated by histological examination (Kholodov et al., 2017). Meanwhile, the RI, based on the ratio of gut content to soft body weight, acts as a proxy for feeding activity and digestive efficiency (San Martín, 1995).

Building on this framework, several previous investigations have examined mussels along the Algerian west coast, often focusing on bioaccumulation of heavy metals, oxidative stress biomarkers, and site specific ecotoxicological profiles (**Kalakhi** *et al.*, 2023; Gherras *et al.*, 2021). However, there remains a need for integrated seasonal and spatial assessment using physiological indices across multiple ecological zones.

Therefore, the present study aimed to monitor and compare the physiological status of *Mytilus galloprovincialis* at four key sites along the Algerian west coast: Mostaganem Harbor (S1), Pointe de l'Aiguille (S2), Oran Harbor (S3), and Honaine (S4). By analyzing seasonal trends in CI, RI, and GSI, this work seeks to uncover the interactive effects of natural cycles and anthropogenic pressures on mussel health. Ultimately, these findings will contribute to the development of long-term biomonitoring strategies and improve our understanding of environmental quality in Mediterranean coastal ecosystems.

### MATERIALS AND METHODS

# 1. Collection and preparation of samples

To ensure representative sampling across environmental gradients, specimens of *Mytilus galloprovincialis* were collected over four distinct seasons from June 2022 to June 2023. For each sampling event, 50 mussels per station were collected, totaling 800 individuals analyzed for physiological indices. Upon collection, mussels were

immediately inspected; any dead or visibly damaged individuals were discarded to avoid bias in physiological assessment.

For biometric characterization, a subsample of 50 individuals was selected. Each mussel was opened using a stainless steel knife to expose the adductor muscle, drained of excess internal fluids by placing them on filter paper with the ventral edge facing upward. This procedure was consistently applied across all sites to minimize procedural variability.

# 2. Sampling sites

Sampling was conducted at four coastal sites along the western Algerian shoreline, strategically chosen based on known pollution inputs and geographical distribution, to capture a gradient of anthropogenic influence (Fig. 1):

- Site 1 (S1): Mostaganem Harbor (36° 02' 285"N, 000° 08' 005"W) is situated near the urban center of Mostaganem, a region subject to moderate port activity and urban discharge (**Boucetta & Kerfouf, 2025**).
- Site 2 (S2): Pointe de l'Aiguille (35° 52′ 32.99″ N, 0° 29′ 19″ W) is a relatively pristine rocky coastal area, adjacent to a fishing village and agricultural land. This site is presumed to have low contamination levels and serves as a reference site (Mansouri *et al.*, 2025).
- Site 3 (S3): Port of Oran (35°42′58″N, 2°58′53″W) is located in a heavily urbanized zone with extensive maritime traffic, and sewage discharge (**Belhadj Tahar** *et al.* **2021**). It has previously been reported as a hotspot for heavy metal contamination (**El Hadj** *et al.*, **2019**; **Hamaidi-Chergui** *et al.*, **2020**).
- Site 4 (S4): Honaine (36° 02' 285"N, 000° 08' 005"W) lies in the province of Tlemcen and is known for its clean beaches and minimal industrial development, making it another candidate for a low-impact reference site (**Mehtougui** *et al.*, **2018**).

This spatial distribution was designed to reflect a pollution gradient from high anthropogenic activity in Oran (S3) to the relatively undisturbed western end at Honaine (S4).

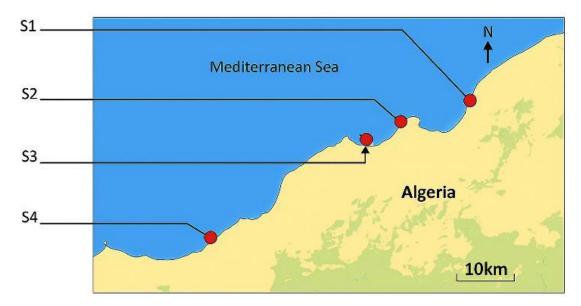
# 3. Physiological and ganodo-somatic index

To evaluate mussel health and site-specific environmental conditions, the following three physiological indices were determined:

The condition index was calculated for 50 mussels per site as an indicator of general health, reflecting soft tissue biomass relative to total body mass. This metric is sensitive to seasonal variations in nutrient reserves and environmental stress. The CI was calculated using the formula:

 $CI = (Soft tissue weight (g) / Total fresh weight (g)) \times 100$ 

Shells were carefully cleaned of epibionts and debris using scalpels and moistened absorbent paper to ensure precise measurements. The method follows protocols established in prior studies (Amiard et al., 1998; Gherras et al., 2022).



S1: Mostaganem Harbour. S2: Pointede l'Aiguille. S3: Oran Harbour. S4: Honaine

Fig. 1. Study area: Sampling sites

The gonado-somatic index provides insight into reproductive effort by quantifying the proportion of soft tissue devoted to gonadal material. Only sexually mature individuals were included to minimize bias. The GSI was determined as:

$$GSI = (Gonad weight (g) / Total fresh weight (g)) \times 100$$

This approach was based on the methodology of **San Martín** (1995) and further adapted by **El Hadj** *et al.* (2020).

The repletion index estimates feeding intensity, calculated by the ratio of digestive gland weight (including gut contents) to total body weight. This index reflects the trophic condition of individuals and their capacity for nutrient assimilation.

RI = (Digestive gland weight (g) / Total fresh weight (g)) 
$$\times$$
 100

This method was also adapted from the study of **San Martín** (1995) and validated by recent field studies (**Gherras** *et al.*, 2022).

# 4. Statistical analysis

All data were expressed as mean  $\pm$  standard error (SE). To evaluate differences between sites and across seasons, statistical analyses were conducted using phyton (version 3.10). Initially, Student's t-tests were employed to determine significant differences between pairwise site comparisons. To analyze multi-site variance, a one-way ANOVA was performed on each physiological index (CI, RI, GSI). Where the ANOVA yielded statistically significant results (P< 0.05), Duncan's post-hoc test was used to determine specific group differences. This combined approach provided both a broad and detailed understanding of spatial and temporal variation in mussel physiological performance.

In addition, principal component analysis (PCA) was applied to reduce the dimensionality of the dataset and identify the main gradients of variation influencing mussel physiological indices across sites and seasons. PCA is particularly valuable for separating the contributions of somatic and reproductive parameters, thereby revealing underlying ecological patterns that may not be evident through univariate tests alone.

Hierarchical cluster analysis (CAH) was then used as a complementary tool to PCA. While PCA summarizes variability into a few orthogonal components, CAH groups stations and sampling periods based on their overall similarity, enabling the identification of consistent seasonal and spatial clusters. Together, these methods provide both an ordination-based overview (PCA) and a classification-based grouping (CAH), offering a more robust interpretation of the physiological response patterns in relation to environmental pressures.

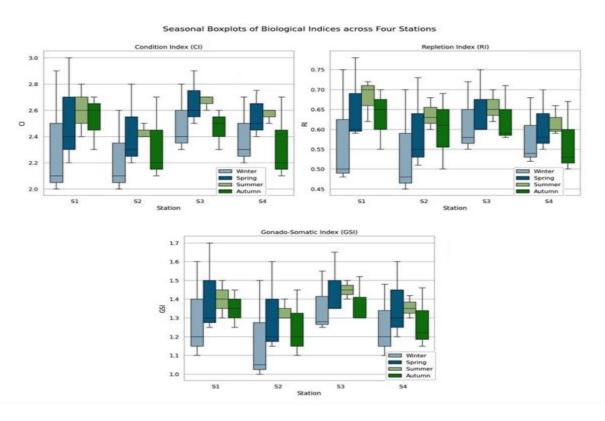
### **RESULTS**

The mean values of the studied parameters: Condition index (CI), gonado-somatic index (GSI), and repletion index (RI), for *Mytilus galloprovincialis* across the four coastal stations (Fig. 2). These physiological indicators exhibited notable spatial and seasonal variations.

During summer, mussels exhibited their highest physiological activity. At Mostaganem Harbor (S1), the highest values were recorded (CI:  $62.11 \pm 2.31$ ; RI:  $48.23 \pm 2.12$ ; GSI:  $18.22 \pm 1.45$ ), indicating optimal trophic and reproductive conditions. Likewise, Pointe de l'Aiguille (S2) demonstrated robust responses (CI:  $58.97 \pm 1.93$ ; RI:  $45.67 \pm 1.89$ ; GSI:  $17.34 \pm 1.21$ ). In contrast, Oran Harbor (S3) showed relatively lower values (CI:  $49.55 \pm 1.85$ ; RI:  $39.10 \pm 1.66$ ; GSI:  $14.78 \pm 1.08$ ), suggesting moderate environmental stress. Honaine (S4) displayed intermediate yet stable indices (CI:

 $55.44 \pm 2.03$ ; RI:  $42.91 \pm 1.72$ ; GSI:  $15.87 \pm 1.14$ ), reflecting relatively less disturbed conditions.

In autumn, a general decline in all indices was observed, likely due to post-spawning recovery. Mostaganem Harbor (S1) showed marked decreases (CI:  $49.34 \pm 1.95$ ; RI:  $34.77 \pm 1.60$ ; GSI:  $10.91 \pm 0.94$ ), while Oran Harbor (S3) recorded the lowest values (CI:  $41.22 \pm 1.70$ ; RI:  $31.80 \pm 1.42$ ; GSI:  $9.10 \pm 0.83$ ), indicative of physiological depletion. Conversely, Pointe de l'Aiguille (S2) and Honaine (S4) retained slightly elevated levels (S2: CI:  $52.88 \pm 1.89$ ; RI:  $36.43 \pm 1.55$ ; GSI:  $11.74 \pm 0.87$ ; S4: CI:  $50.67 \pm 1.79$ ; RI:  $35.09 \pm 1.41$ ; GSI:  $11.01 \pm 0.90$ ), reflecting slower regression and possibly more favorable conditions.



**Fig. 2.** Boxplots showing the seasonal variation of physiological indices (CI, RI, GSI) of *Mytilus galloprovincialis* during 2017

In winter, mussels entered a resting phase, characterized by significant decreases across all physiological indices. The lowest CI and GSI values were found at Oran Harbor (S3) (CI:  $37.44 \pm 1.52$ ; GSI:  $6.89 \pm 0.68$ ) and Mostaganem Harbor (S1) (CI:  $39.21 \pm 1.67$ ; GSI:  $7.45 \pm 0.71$ ), likely reflecting post-reproductive depletion and environmental stress. RI values also dropped sharply, particularly at S1 ( $28.66 \pm 1.34$ ) and S3 ( $26.55 \pm 1.29$ ). However, Honaine (S4) showed relatively buffered values (CI:

 $45.90 \pm 1.76$ ; RI:  $32.41 \pm 1.38$ ; GSI:  $9.30 \pm 0.82$ ), suggesting a more stable environmental context.

During spring, a revival in physiological activity was observed. Mostaganem Harbor (S1) and Pointe de l'Aiguille (S2) recorded high CI and GSI values (S1: CI:  $59.89 \pm 2.11$ ; GSI:  $16.98 \pm 1.32$ ; S2: CI:  $57.12 \pm 2.07$ ; GSI:  $15.76 \pm 1.25$ ), marking the beginning of reproductive maturation. Although Oran Harbor (S3) continued to lag (CI:  $48.01 \pm 1.77$ ; GSI:  $13.04 \pm 1.10$ ), Honaine (S4) maintained moderate to high values (CI:  $53.90 \pm 1.94$ ; RI:  $40.23 \pm 1.68$ ; GSI:  $14.87 \pm 1.18$ ).

Table (1) summarizes the ANOVA results applied to the physiological indices for *Mytilus galloprovincialis* collected from four sites: Mostaganem Harbor (S1), Pointe de l'Aiguille (S2), Oran Harbor (S3), and Honaine (S4). The analysis revealed a significant effect of site (F= 4.140, P < 0.05) and a highly significant effect of season (F= 32.690, P < 0.001) on the condition index (CI), with a significant interaction (F= 7.190, P < 0.05) between site and season. In contrast, the repletion index (RI) was only significantly influenced by season (F= 5.413, P < 0.05), while the gonado-somatic index (GSI) showed no significant variation across site, season, or their interaction (P > 0.05).

The analysis of variance (Test F, ANOVA) physiological index, of *Mytilus galloprovincialis* mussels revealed a significant effect of site (F= 4.140, P< 0.05) and a highly significant effect of season (F= 32.690, P< 0.001) on the condition index (CI), with a significant interaction (F= 7.190, P< 0.05) between site and season. In contrast, the repletion index (RI) was only significantly influenced by season (F= 5.413, P< 0.05), while the gonado-somatic index (GSI) showed no significant variation across site, season, or their interaction (P> 0.05).

**Table 1.** Results of the analysis of variance (Test F, ANOVA) physiological index of *Mytilus galloprovincialis* mussels from the Mostaganem Harbor (S1), Pointe de l'Aiguille (S2), Oran Harbor (S3), and Honaine (S4); (n = 800)

Physiological Index	F (Site)	F (Season)	F (Site ×	Significance
			Season)	
Condition index (CI)	4.140	32.690	7.190	<i>P</i> < 0.05; <i>P</i> < 0.001
Repletion index (RI)	1.115 (ns)	5.413	1.674 (ns)	P< 0.05 only for season
Gonado-somatic index (GSI)	1.081 (ns)	2.521 (ns)	1.380 (ns)	Not significant

(\*: Significant (P< 0,05); \*\*: Very significant (P<0.001), ns: Not significant).ddl (Site) = 3,80; ddl (Season) = 3.80; ddl (Site x Season) = 15.79.

The marked seasonal effect on CI may be attributed to environmental factors, notably the increase in seawater temperature from spring to summer and the subsequent reduction in salinity, which likely enhanced planktonic productivity. This plankton

bloom—resulting from nutrient input through freshwater influx—could explain the seasonal rise in CI, as previously reported by Çelik et al. (2009) and Karayücel et al. (2015).

Further analysis demonstrated spatial and seasonal disparities.

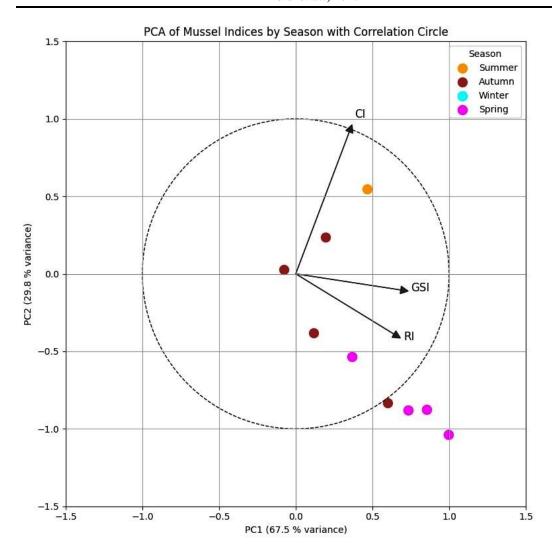
Condition index (CI): Mussels from S1 consistently exhibited significantly higher CI values across all seasons (Winter:  $13.21 \pm 0.80$ ; Spring:  $16.34 \pm 1.10$ ; Summer:  $17.84 \pm 1.15$ ; Autumn:  $15.42 \pm 1.01$ ) compared to S2, S3, and S4. These differences highlight the favorable trophic or environmental conditions at Mostaganem Harbor.

The repletion index (RI): At S1, the RI was also higher in all seasons (*e.g.*, Summer:  $2.45 \pm 0.09$ ) compared to S2 and S3, suggesting better digestive gland storage and food availability. No significant differences were observed between S2 and S3 during summer and autumn (P > 0.05).

# 1. Principal Component Analysis (PCA) of Physiological Indices in Mytilus galloprovincialis

# - **Seasonal projection** (Fig. 3):

Spring and summer samples are projected in the positive quadrant of PC2 and are associated with elevated GSI and RI values, reflecting intense reproductive activity and optimal food intake. Winter samples fall in the negative quadrants of both axes, marked by low GSI and RI, which is typical of reproductive quiescence and diminished feeding. Autumn samples occupy intermediate positions, representing transitional physiological states.

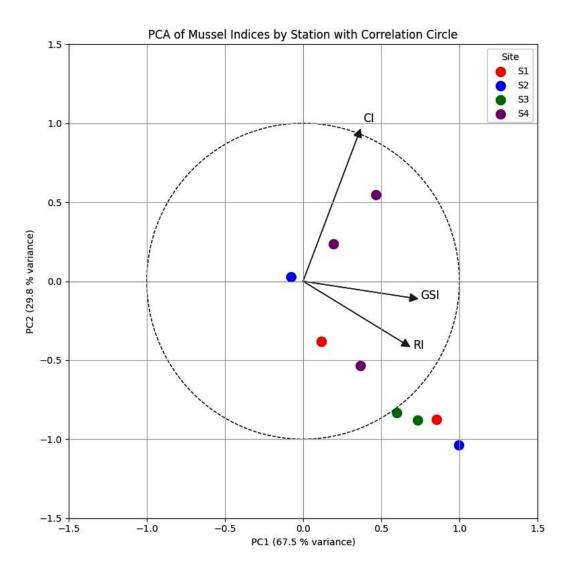


**Fig. 3.** Principal component analysis (PCA) of physiological indices (CI, RI, GSI) in *Mytilus galloprovincialis* showing seasonal variation

# - **Spatial projection** (Fig. 4)

S1 (Mostaganem Harbor) and S2 (Pointe de l'Aiguille) align with high GSI and CI values, suggesting these sites offer favorable environmental or trophic conditions. S3 (Oran Harbor), especially in summer, displays elevated RI values, potentially indicating localized improvements in food availability or lower anthropogenic stress. S4 (Honaine, Tlemcen) exhibits more dispersed patterns, pointing to greater variability in environmental conditions, which may reflect dynamic coastal processes or localized pollution effects.

Overall, the PCA underscores pronounced seasonal patterns driven primarily by reproductive and digestive gland dynamics. Additionally, the spatial differences highlight environmental heterogeneity, where certain stations (notably S1 and S2) emerge as healthier marine habitats, supporting the robustness of *Mytilus galloprovincialis* as a bioindicator for marine ecosystem quality and metal pollution biomonitoring.



**Fig. 4.** Principal Component Analysis (PCA) of physiological indices (CI, RI, GSI) in *Mytilus galloprovincialis* across sampling stations (S1–S4).

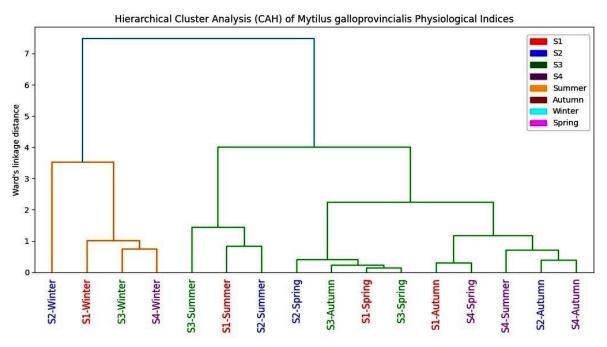
# 2.1 Hierarchical Clustering Analysis (CAH) of Physiological Indices in Mytilus galloprovincialis:

The Hierarchical Clustering Analysis (CAH) applied to the physiological indices, Condition Index (CI), Repletion Index (RI), and Gonado-Somatic Index (GSI), of *Mytilus* galloprovincialis provided a clear differentiation of sampling units across both space and season. The dendrogram revealed structured groupings that reflect the underlying ecological status of the study sites (Fig. 5).

These cluster groupings can be directly linked to environmental drivers influencing mussel physiology:

To begin with, a strong similarity was observed between samples from S1 (Mostaganem Harbor) and S2 (Pointe de l'Aiguille), particularly during spring and summer. These stations consistently clustered in the upper branches of the dendrogram, indicating superior physiological profiles. Such positioning reflects favorable environmental conditions supporting somatic growth and reproductive activity, likely driven by balanced trophic availability and moderate anthropogenic influence.

In contrast, S3 (Oran Harbor) samples were consistently isolated in separate clusters, especially during autumn and winter, suggesting degraded physiological status.



**Fig. 5.** Hierarchical clustering (CAH) of *Mytilus galloprovincialis* samples based on physiological indices (CI, RI, GSI) across stations and seasons

This is presumably linked to the site's well-documented exposure to urban runoff, industrial discharges, and maritime traffic, which likely impose ecological stress affecting mussel health.

Meanwhile, S4 (Honaine) demonstrated intermediate and dispersed clustering patterns, with samples occasionally aligning with either healthier or more impacted groups. This suggests variable site conditions, possibly influenced by seasonal changes in hydrodynamics or episodic pollution events.

Notably, the seasonal classification also emerged clearly. Spring and summer samples from S1 and S2 formed a cohesive group, characterized by high GSI and RI, while winter samples across all stations clustered lower, indicative of reproductive quiescence and reduced nutrient uptake.

In summary, the CAH analysis supports a spatio-seasonal typology of mussel health as follows:

- Best performance: S1–Spring/Summer ≈ S2–Spring/Summer
- Intermediate status: S4 (all seasons), S2–Autumn
- Most impacted: S3–Autumn/Winter

This classification highlights the sensitivity of *Mytilus galloprovincialis* to environmental quality gradients and validates its use as a sentinel species in coastal biomonitoring programs.

The gonado-somatic index (GSI): The highest GSI values were observed at S1 during spring, summer, and autumn (e.g., Spring:  $5.89 \pm 0.47$ ), indicating advanced gonadal development. However, in winter, mussels from S2 exhibited significantly higher GSI values ( $6.21 \pm 0.52$ ) than those from S1 and S3, possibly reflecting local reproductive timing or environmental triggers.

In summary, Mostaganem Harbor (S1) supported the most favorable physiological conditions for mussels throughout the year, as evidenced by the elevated CI, RI, and GSI values. These patterns underscore the combined influence of site-specific environmental quality and seasonal nutrient dynamics on mussel physiology.

The principal component analysis (PCA) conducted on the biometric indices (CI, RI and GSI) for *Mytilus galloprovincialis* provides a comprehensive understanding of their spatial and seasonal variability (Fig. 3A, B).

The first two principal components (PC1 and PC2) explain a cumulative variance of 90.27%, with PC1 accounting for 56.76% and PC2 for 33.51%. This high proportion indicates that the F1  $\times$  F2 plane offers a reliable and meaningful representation of the dataset's structure.

In the correlation circle, the three indices show distinct orientations:

• GSI and RI are positively correlated ( $r \approx 0.70$ ), suggesting that reproductive activity increases in tandem with digestive gland repletion.

• CI exhibits weak correlations with both GSI and RI (r < 0.1), indicating that somatic condition varies independently of reproductive and nutritional parameters.

The index projections indicate that GSI contributes significantly to PC2, while CI aligns more strongly with PC1, underscoring distinct biological dimensions. Indices near the circumference are better represented and more influential in shaping the data structure—especially GSI and RI. In contrast, the proximity of CI to the center suggests a more moderate role in discrimination.

#### **DISCUSSION**

This study highlights the physiological variability of *Mytilus galloprovincialis* across seasons and locations along the Algerian west coast, offering insights into how environmental pressures and pollution impact mussel health.

The condition index (CI), reproductive index (RI), and gonadosomatic index (GSI) serve as key indicators of mussel physiological status and environmental quality. Elevated CI values observed at sites S1, S3, and S4 suggest favorable conditions for mussel growth and vitality. These findings are consistent with prior work linking CI with food availability (Belhadj et al., 2021) and lower contamination levels (Belmokhtar et al., 2025). Furthermore, CI has been shown to correlate significantly with heavy metal concentrations, demonstrating its effectiveness as a pollution biomarker (Pantea et al., 2020).

Seasonal variability in physiological indices was marked, with higher CI and RI during warmer seasons, especially summer. This pattern corresponds with mussel reproductive cycles, where energy is allocated toward gametogenesis in spring, often at the expense of somatic growth (**Smart** *et al.*, **2020**). The trend of peak GSI in spring followed by increased CI and RI in summer confirms these seasonal energy trade-offs (**Jarque** *et al.*, **2014**).

Site S2, described as a pristine location, consistently showed high annual CI and lower bioaccumulated metal loads, validating its role as a control site and emphasizing the link between reduced anthropogenic stress and better physiological condition. Pollution gradients across the study area, particularly at S1 near port activity (**Dilem** *et al.*, **2015**), correspond to decreases in biological indices and increases in biomarker responses like micronuclei frequency and antioxidant activity (**Touahri** *et al.*, **2016**; **Mejdoub** *et al.*, **2018**). These stress biomarkers underscore the effectiveness of mussels in biomonitoring coastal pollution, especially in areas impacted by industrial and urban wastewater.

Environmental variables such as temperature, salinity, and food availability were also shown to influence physiological indices. Seasonal oxidative stress biomarkers

varied significantly, with higher antioxidant enzyme activities in warmer months (**Balbi** *et al.*, 2017). These findings reinforce that both abiotic (temperature, pollutants) and biotic (reproduction) factors contribute to observed seasonal dynamics.

In summer, the spatial and temporal variation of physiological indices in *M*. *galloprovincialis* reflects both natural biological cycles and environmental pressures, including pollution levels and site-specific conditions. These indices remain reliable and cost-effective tools for environmental monitoring in Mediterranean coastal zones.

The analysis of seasonal physiological indices, the condition index (CI), repletion index (RI), and gonado-somatic index (GSI), for *Mytilus galloprovincialis* across four Algerian coastal stations reveals marked spatial and temporal dynamics. These indices collectively serve as sensitive indicators of environmental quality, nutritional status, and reproductive activity, offering insights into site-specific ecological conditions and seasonal biological rhythms.

To begin with, the summer season emerged as the period of peak physiological performance in mussels. At Mostaganem Harbor (S1), values of CI ( $62.11\pm2.31$ ), RI ( $48.23\pm2.12$ ), and GSI ( $18.22\pm1.45$ ) were the highest recorded, reflecting optimal trophic conditions likely driven by elevated temperatures and increased phytoplankton availability. These findings are consistent with previous studies conducted in the Mediterranean, where summer peaks in physiological indices have been attributed to intensified metabolic rates supported by seasonal primary productivity (**Balbi** *et al.*, **2017**; **Smart** *et al.*, **2020**). Similarly, Pointe de l'Aiguille (S2) and Honaine (S4) displayed elevated values, suggesting stable and favorable environmental conditions. In contrast, Oran Harbor (S3) showed significantly lower indices, suggesting localized environmental stress—possibly due to pollution or reduced food input. These findings align with observations on mussel populations in polluted coastal zones of Morocco and the Adriatic Sea (**Mejdoub** *et al.*, **2018**; **Wathsala** *et al.*, **2021**).

Following the summer peak, a pronounced decline in physiological indices was recorded in autumn, indicating a post-spawning regression phase. At this stage, mussels typically undergo gonadal depletion and redirect energy toward somatic tissue repair. This is particularly evident at S1 and S3, where GSI values dropped to  $10.91 \pm 0.94$  and  $9.10 \pm 0.83$ , respectively. These patterns are consistent with documented reproductive cycles in *M. galloprovincialis* populations across the Mediterranean (**Jarque** *et al.*, **2014**; **Abbassi** *et al.*, **2022**).

Nevertheless, S2 and S4 maintained slightly higher values, which may suggest either delayed spawning or buffering effects from local environmental stability that mitigated the intensity of the post-reproductive decline.

As the seasons progressed into winter, mussels entered a resting or quiescent phase, characterized by metabolic downregulation and minimal physiological activity. Across all stations, CI, RI, and GSI values were at their lowest. Notably, Oran Harbor (S3) and Mostaganem Harbor (S1) recorded the most pronounced declines, indicating

high levels of physiological stress and energy depletion following reproduction. These findings are in line with earlier reports identifying winter dormancy as a common survival strategy in mussels, driven by reduced temperatures and limited food availability (**Regoli & Orlando, 1994**). Interestingly, Honaine (S4) retained relatively stable values even during winter, further supporting its classification as an environmentally buffered or reference site.

Subsequently, spring marked the reactivation of physiological functions, with S1 and S2 again showing significantly increased CI and GSI values, indicating the onset of gonadal development and tissue renewal. This rebound phase reflects the species' preparation for the upcoming reproductive cycle and coincides with improving environmental conditions such as rising temperatures and nutrient influx. Such springtime activation has been well documented in other Mediterranean mussel populations (Smart et al., 2020). On the other hand, S3 continued to exhibit lagging indices, suggesting that environmental stressors at this site may be chronically inhibiting biological recovery. Meanwhile, S4 maintained a steady physiological profile, reinforcing its resilience and potential utility in long-term biomonitoring.

The statistical analysis (ANOVA) further elucidates these patterns. CI exhibited significant effects of both site and season, along with their interaction (F= 4.140, 32.690, and 7.190, respectively), highlighting its high responsiveness to both spatial and temporal environmental variability. In contrast, RI was significantly affected by season alone (F= 5.413), implying its dependence on food availability rather than localized site effects. Notably, GSI did not show significant variation, a result that may stem from high interindividual variability or asynchronous spawning events, both commonly reported in mussel populations (**Balbi** *et al.*, **2017**).

Further supporting these findings, site-specific patterns emerged when considering environmental context. Mostaganem Harbor (S1), despite being a harbor area, consistently showed high CI values throughout the year. This may be attributed to nutrient enrichment from anthropogenic sources, leading to localized increases in planktonic food availability. Similar trophic enhancements in port zones have been previously documented (**Kerfouf** *et al.*, **2022**). In stark contrast, Oran Harbor (S3) exhibited persistently low indices across all seasons, pointing to chronic environmental degradation or pollution pressure. The ecological constraints at this site are supported by biomonitoring studies highlighting mussel sensitivity to trace metal accumulation and contaminant exposure (**Touahri** *et al.*, **2016**). Conversely, Honaine (S4) consistently maintained moderate-to-high values and exhibited minimal seasonal fluctuation, reinforcing its classification as a relatively undisturbed site and a potential biological reference point for future environmental assessments.

In summary, the results of this study underscore the strong seasonal rhythms and site-specific responses in the physiology of *Mytilus galloprovincialis*. The summer and spring seasons represent periods of high biological activity, while winter is marked by

metabolic depression. These trends are clearly modulated by local environmental conditions, with nutrient availability, temperature, and pollution acting as primary drivers. The physiological indices used here—particularly CI and RI—prove to be effective bioindicators of environmental quality, and their application in long-term biomonitoring programs remains highly recommended.

This study reveals significant seasonal and spatial variation in the physiological condition of *Mytilus galloprovincialis* along the Algerian west coast, highlighting the influence of environmental factors, particularly pollution, temperature, and food availability, on mussel health and reproductive cycles.

The condition index (CI), repletion index (RI), and gonado-somatic index (GSI) serve as reliable biomarkers for assessing mussel physiological status and coastal environmental quality. Elevated CI values at Mostaganem Harbor (S1) and Honaine (S4) suggest favorable conditions for mussel growth and vitality, while Oran Harbor (S3) showed lower values, likely reflecting stress from pollution or habitat instability. These observations align with prior research linking CI to food availability and reduced contamination levels, and to its responsiveness to heavy metal exposure (**Pantea** *et al.*, **2020**). To provide a more detailed understanding, the seasonal patterns across stations are explored below.

The summer season marked a peak in physiological performance. Mussels at S1 exhibited the highest values for CI  $(62.11\pm2.31)$ , RI  $(48.23\pm2.12)$ , and GSI  $(18.22\pm1.45)$ , indicating optimal trophic conditions, likely driven by warmer temperatures and elevated phytoplankton productivity. Similar seasonal peaks in Mediterranean mussel populations have been linked to metabolic activation during warm months (**Balbi** *et al.*, **2017**; **Smart** *et al.*, **2020**). Similarly, S2 and S4 showed elevated indices, supporting their environmental stability. In contrast, S3 presented lower values, suggesting stress conditions potentially caused by pollution, as reported in mussel populations from other impacted regions (**Mejdoub** *et al.*, **2018**; **Wathsala** *et al.*, **2021**).

In autumn, physiological indices declined across all sites, marking a post-spawning phase. Mussels undergo gonadal depletion and somatic tissue recovery during this time. GSI values dropped notably at S1  $(10.91 \pm 0.94)$  and S3  $(9.10 \pm 0.83)$ , consistent with seasonal reproductive cycles documented in *M. galloprovincialis* (Jarque *et al.*, 2014; Abbassi *et al.*, 2022). Meanwhile, S2 and S4 retained slightly higher values, possibly due to delayed spawning or reduced environmental stress.

Winter brought a period of metabolic quiescence, with significantly lower CI, RI, and GSI values across stations. The lowest indices were observed at S3 and S1, indicating post-reproductive depletion and energy limitation under cold, low-food conditions. This dormancy strategy is well-documented in mussels (**Regoli & Orlando, 1994**). Notably, S4 exhibited relative stability, reinforcing its classification as a minimally disturbed site.

Spring marked the reactivation of physiological functions, particularly at S1 and S2, where CI and GSI rose sharply. This reflects the onset of gametogenesis and tissue

renewal as temperatures rise and food becomes more abundant. This spring activation mirrors regional mussel reproductive patterns (**Smart** *et al.*, **2020**). S3 continued to show lagging indices, suggesting chronic exposure to environmental stress. In contrast, S4 demonstrated resilience, maintaining a steady physiological profile throughout the season.

Site-specific differences in physiological responses reflect broader environmental dynamics. At S1, elevated CI and RI suggest nutrient-rich harbor waters, despite the risk of contamination. Such trophic enrichment has been reported in other port environments. Meanwhile, S3 displayed chronically depressed indices and aligns with previous findings of pollutant accumulation and oxidative stress in mussel tissues (**Touahri** *et al.*, **2016**). S4, due to its consistent performance, stands out as a strong candidate for a reference site in future biomonitoring programs.

The ANOVA results confirmed that CI was significantly influenced by site, season, and their interaction (F = 4.140; 32.690; 7.190), confirming its sensitivity to both spatial and temporal changes. RI was significantly affected only by season (F = 5.413), pointing to food availability as the main driver. GSI showed no significant variation—possibly due to asynchronous spawning or individual variability—consistent with prior findings (**Balbi** *et al.*, **2017**).

#### **CONCLUSION**

This study demonstrates that the spatial and seasonal variability observed in *Mytilus galloprovincialis* reflects both natural biological cycles and local environmental pressures. Physiological indices: Condition index (CI), repletion index (RI), and gonadosomatic index (GSI) showed clear seasonal patterns, influenced by reproductive activity, temperature, food availability, and anthropogenic impacts.

Mussels collected from four sites along the Algerian west coast exhibited distinct physiological responses, with significant variations across seasons. The influence of biotic factors such as age, soft tissue mass, and gametogenic stage, combined with abiotic stressors like pollution and salinity, plays a critical role in shaping these responses.

Notably, the sites of Oran Harbor (S3) and Mostaganem Harbor (S1) displayed the most pronounced shifts in CI and RI, suggesting that these areas are subject to greater ecological stress, likely due to urban and industrial activity. Conversely, Honaine (S4) and Pointe de l'Aiguille (S2) showed more stable indices, reinforcing their value as reference or control sites for biomonitoring.

Overall, this work confirms the impact of anthropogenic activity on mussel health and provides baseline physiological data essential for ongoing coastal environmental monitoring programs. The continued use of *M. galloprovincialis* as a sentinel species is strongly supported, given its responsiveness to both environmental quality and pollution gradients across the Mediterranean.

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