# STUDY OF SEASONAL VARIATIONS OF THE CONDITION INDICES IN THE MUSSEL *Mytilus galloprovincialis*: RELATIONSHIP WITH PHYSICO-CHEMICAL PARAMETERS, BIOMARKERS AND METALS IN THE REGION OF SIDI IFNI (SOUTH OF MOROCCO)

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## ABSTRACT

The aim of this work is studying the correlation between seasonal variations of physiological condition indices, as physiological state and growth indicator, and biomarkers of pollution (Acetylcholinesterase, Glutathione S-transferase, Catalase, Malondialdehyde and Metallothioneins) in Mytilus galloprovincialis in three sites representative of Sidi Ifni's coastline (South Atlantic of Morocco): Mirleft site (S1), Cheikh Sidi Ali Ifni (S2) and Tazrout site (S3).

The study showed a pronounced variability in the biological and physiological behavior of mussel populations from Sidi Ifni coasts. The comparison of the results of physiological condition indices in Mytilus galloprovincialis with environmental factors and biochemical markers shows that the species is contaminated in the Ifni site compared to Mirleft and Tazrout.

**Keywords:** Acetylcholinesterase, Bioaccumulation, Biomarker, Catalase, Condition indices, Element Trace Metals, Glutathione S-transferase, Malondialdehyde, Metallothioneins, Morocco, Mytilus galloprovincialis, Sidi Ifni.

## 1. INTRODUCTION

Due to increasing anthropogenic activities, coastal cities in southern Morocco are being exposed to various contaminants that are a threat to the marine ecosystem. Due to its location on the edge of the Atlantic Ocean, Sidi Ifni is a tourist town located on the south Atlantic coast of Morocco.

In recent years, this region has experienced accelerated development and a very important demographic pressure concentrated on the coast. This population growth will undoubtedly have environmental consequences, in general, and on the marine environment, in particular, thus leading to problems of contamination of aquatic ecosystems involving many chemicals including metal elements released by industries, tourism projects and urban communities.

Marine environment of Sidi Ifni has become a complex environment, influenced by anthropogenic activity in addition to the effects of global warming. The main pollutants present in this environment include Metal Traces Elements (MTE) and persistent organic pollutants characterized by their high toxicity, persistence and tendency to bio-accumulate in the food chain, posing risks to humans and ecosystems.

For these reasons, some researchers recommend controlling pollutant concentrations in organisms to monitor the environment. The principle of bioaccumulation states that marine organisms concentrate pollutants, is an important factor to be investigated and their toxicity is a key parameter for assessing risks in polluted areas. Indeed, the exposure of marine organisms to trace metals can induce the formation of reactive internal metabolites that are more toxic than the original xenobiotics. It has also effects on biological systems through free radicals, which ultimately manifest as permanent oxidative stress on the body. When pro-oxidation levels exceed the antioxidant capacity of tissues, lipids and DNA are damaged (Cooke et al., 2003; Horton et al., 1987; Niki, 2009).

In this context, invertebrates, especially bivalves, such as mussels (*Mytilus galloprovincialis*), constitute excellent model's organisms for studying the biological effects of pollutants. These properties make them good candidates for environmental health monitoring in several programs (Goldberg, 1986; Michel *et al.,* 1994; Kurelec, 1995; Regoli & Principato, 1995; Bebianno & Serafim, 1998; Mora *et al.*, 1999a; Akcha *et al.,* 2000; Da Ros *et al.*, 2000; Lowe & Fossato, 2000; Melwani *et al.,* 2014)

In fact, physiological condition indices in these animals can inform their physiological state and growth. These indices allow the evaluation of seasonal metabolic changes related to the fluctuations of the environmental parameters (stress of the natural environment) and physiological states (Livingstone, 2001; Andral *et al.,* 2004; Schiedek *et al.,* 2006; Schmidt *et al.,* 2013). Otherwise, these indices are biometric indicators commonly used in shellfish farming to account for an animal's shell filling. It represents the succulent and physiological state of bivalve molluscs (Orban *et al.,* 2002 ; Cartier *et al.*, 2004; Lemaire *et al.*, 2006;).To characterize biomass, this index has the advantage of being a general condition index that includes multiple physiological factors (nutrition, reproduction). It can assess deviations from normal growth and show manifestations of biochemical and physiological changes expressed at the organism level in environmental monitoring studies like ours.

In fact, determining the physiological state index is a valuable method to assess the interaction of environmental stressors with various biological factors (Reproductive cycle, Growth, Age, Gender, etc.), and physicochemical ones (Temperature, pH, Salinity, etc.). These interactions can be used to assess the state of health of mussel populations in the different coastal habitats in which they have evolved.

In addition to the physiological indicators, biomarkers constitute also sensitive biological tools for measuring pollution-induced stress. These are measurements in biological tissues at lower levels and represent a sensitive early detection tool for measuring biological effects in assessing the quality of environmental health. In addition, the actual biological damage induced was only determined by biomarkers. They can provide more complete and biologically relevant information on the potential effects of pollutants accumulated in the body (Van der Oost *et al.*, 1996; Cajaraville *et al.,* 2000; Van der Oost *et al*., 2003; Nicholson & Lam, 2005;).

The aim of this work, which represents a continuation of several studies carried out in recent years in the framework of biomonitoring of the state of health of the marine environment of Sidi Ifni, is study the correlation between seasonal variations of physiological condition indices, as physiological state and growth indicator, and somme biomarkers of pollution in *Mytilus galloprovincialis*. Five biomarkers were considered: ***i)*** The activity of acetylcholinesterase (AChE) which is a biomarker of neurotoxicity sensitive to organophosphate pesticides and carbamates and inhibited by heavy metals and hydrocarbons (Payne *et al.,* 1996 ; *Mora et al.,* 1999b; Lionetto *et al.*, 2003; Kopecka *et al.*, 2004), ***ii)*** Glutathione S-transferase (GST) activity which is a phase II metabolic enzyme induced during the process of biotransformation and excretion of lipophilic compounds such as PCBs, PAHs and organochlorine and organophosphate pesticides (Narbonne *et al.*, 1991; Damiens *et al.*, 2007; Trisciani *et al.*, 2012), ***iii)*** Catalase activity (CAT) which is an intracellular antioxidant enzyme engaged in the defense system against free radicals produced by environmental oxidizing pollutants and responds to oxidative stress rather than a specific group of pollutants (Damiens *et al.*, 2004; Pampanin *et al.*, 2005; Valavanidis *et al.*, 2006), ***iv)*** Malondialdehyde forms which is an expression of lipo-peroxidation corresponding to the result of the attack of polyunsaturated lipids by reactive oxygen species generated under certain stress conditions, particularly with organic contaminants and metals (Sunderman, 1987) and ***v)*** Metallothioneins (MTs) which is used as a biomarker of exposure to metal contamination and involved in the regulation of metals spreading in the body (Viarengo *et al.,* 2000; Malins, 2018).

## 2.MATERIEL & METHODS

### Sampling sites

Sidi Ifni is one of the most important economic hubs of the Guelmim-Oued Noun region. This region, whose economy is based on fishing and tourism, is dependent on the richness of its coastline. However, these ecosystems receive wastewater from the city of Sidi Ifni, although from May 2012 a treatment plant was installed to mitigate their effects.
The study area stretches along the coast of the province of Sidi Ifni, from the coast of Mirleft to the coast of the city of Sidi Ifni (Figure 1).

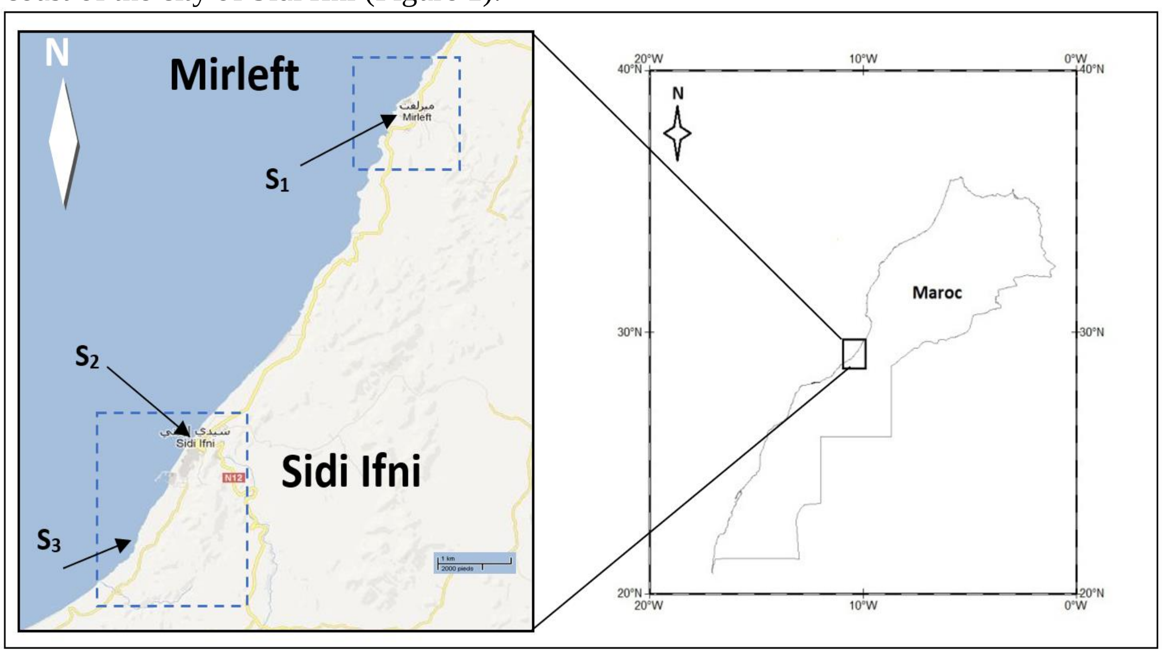



**Figure 1 :** Location of study sites in the Sidi Ifni coastline

The selection of sampling sites is based, on the one hand, on the quality of the site in terms of the remoteness and proximity of sources of pollution and, on the other hand, on the ease of access in order to be able to carry out sampling in the best conditions. To do so, a comparative study was carried out at three coastal sites in the province of Sidi Ifni:

- *Site 1 (S1)*: (29°58'41.68"N, 10°07'40.82"W), Mirleft site, which is located at the northern entrance to the center of Mirleft at 30km north of the city Sidi Ifni. This is a rocky area far from any source of pollution and considered as reference site. It is characterized by its intense hydrodynamics and inaccessibility, which protects it from the disruptive effects of the action of the inhabitants (low harvest of mussels).
- Site 2 (S2): (S1: 29°38'70.55"N, 10°17'29.54"W), Cheikh Sidi Ali Ifni Site which is located at the northern entrance of the city that still receives discharges of untreated domestic sewage from the city of Sidi Ifni and near the oued Ifni. This site is considered as polluted site
- Site S3: (S3: 29°17'37.39"N, 10°14'11.27"W), Tazrout site which is located at the southern end of the city and 15km away from the raw sewage discharge point and 5 km away from the point. This site is far from any source of pollution, but its beach is often visited by tourists because of its particular landscape. It corresponds to a pebble beach, with a low slope.

**Biological material**

*Mytilus galloprovincialis* was used in our study. This choice lies in the wide geographical distribution and abundance and representativeness of the environment of this species. It's easy harvesting, sedentary lifestyle and tolerance in addition to the biology and physiology better known reinforce this choice.

**Samples collected**

Coastal water and animals samples were taken from twelve companions, once a month and three times a season; July 2013 to June 2014, at low tide at three study sites.

Mussels of size 3 to 4 cm were collected at low tide and harvested at the medio-littoral level and then washed with seawater and then transported under isothermal conditions to the laboratory.

**Physicochemical analyses of water**

Three physico-chemical parameters were monitored in collected water samples: temperature, pH and salinity. Theses analyses were carried out in situ using multiparametric handheld devices (HANNA Instruments HI 9025 and Consort C5010).

**Determination of indice condition**

The condition indices were measured to accurately establish the physiological condition of *Mytilus galloprovincialis*. For their determination length, width and thickness and weight were measured for each sample according to the site and the season.

The first condition index ($CI_1$) is based on the weight of the individual, and is a commonly used biometric index for reporting the degree of shell filling in animals (Bodoy et al., 1986). This index is calculated according to the following ratio:

$$CI_1 = \frac{Fresh\,weight}{Total\,weight} \times 10^2 (g, g)$$

The second condition index ($CI_2$) is based on length testifies to the growth of the mass of the soft tissue as a function of the shell length (Bodoy et al., 1986). It is calculated according to the following equation:

$$CI_2 = \frac{Fresh\ weight(g)}{Length^3} \times 10^4 (g, mm)$$

The third condition index ($CI_3$) also based on the size of the individual, it takes into account the length, width and thickness of the shell(Bodoy et al., 1986). This index is calculated according to the following equation:

$$CI_3 = \frac{Fresh\ weight}{Length \times Width \times Thickness} \times 10^4 (g, mm)$$

**Biomarker determination**

Acetylcholinesterase activity (AChE) is determined according to the colorimetric method of Ellman et al. (1961), based on the reaction of Thiocholine, from the hydrolysis of acetylthiocholine, with 5,5'-dithio-bis (2-nitrobenzoate) (DTNB) followed at 412nm (Ellman *et al.*, 1961). The specific activities of AChE is expressed in nmol/min/mg protein in $S_9$ sample.

The determination of Glutathione S-transferase activity (GST) is based on the colorimetric method described by Habig *et al.* (1974), which consists in monitoring the appearance of the glutathione-substrate complex (GSX) by spectrophotometry at 340 nm. The specific activities of GST were expressed in nmol/min/mg protein in $S_9$ sample.

The determination of catalase activity (CAT) was adapted according to the method of Aebi (1983), based on the measurement of absorbance variations due to the degradation of hydrogen peroxide ($H_2O_2$) at 240 nm. The specific activities of CAT were expressed in nmol/min/mg protein in $S_9$ sample.

The determination of Malondialdehyde rate (MDA) is carried out according to the method of Sunderman (1985), based on the properties that certain compounds, such as MDA, have to react with thiobarbituric acid TBA to regenerate a colored product that absorbs at 532nm. The MDA rate was expressed in nmol/mg protein in the sample ($S_9$).

The total protein content of the $S_9$ fraction is determined by the method of Lowry *et al.,* (1951).

Metallothioneins in mussels are quantified by evaluating the residue content -SH by a spectrophotometric method using the Ellman reagent (DTNB: 5,5-dithiobis 2-acidnitro-benzonic) (Ellman, 1959). The determination is based on the detection of SH groups allowing a more selective evaluation of these metalloproteins.(Ellman, 1959;Viarengo *et al.*, 1997). The reaction between DTNB and the -SH groups of proteins produces stoichiometric amounts of TNB (thio-nitro-benzoate), a yellow compound that absorbs at 412 nm. The amount of metallothioneins was expressed as µg/mg of proteins of analysed tissues.

The Bradford method (1976), using the Coomassie Brilliant Blue, was used for quantitativedetermination of proteins using bovine serum albumin as the standard(Bradford, 1976).

**Determination of trace metals**

Animal samples, intended for the determination of metals, are cleaned and free of any impurities and are kept in housing for 48 hours to remove the contents of their digestive tract. Then they are frozen at -30 °C until they are used for the mineralization stage.

During use, the animals are thawed at room temperature and then shelled. The soft mass is dried at 70 °C in the oven for 48 hours and then crushed using a porcelain mortar until a fine powder is obtained. Aliquots of 200 mg of dry matter are placed in polyethylene tubes to which 4 ml of nitric acid (analytical quality) is added. The resulting mixture is incubated overnight and at room temperature, before being placed in a heating block at 80 °C for 4 hours. After cooling, freshly deionized water (MilliQ water) is added to have a final volume of 50 ml.

The technique used for the determination of metals (Cd, Cu, Zn and Ni) is atomic absorption spectrometry (AAS): The determination of cadmium (Cd) and nickel (Ni) concentrations was carried out by electrothermal atomic absorption spectrometry with Zeeman correction (SAAE, VARIAN 220 Z). For the concentrations of zinc (Zn) and copper (Cu), they were determined by flame atomic absorption spectrophotometry using the atomic absorption spectrophotometer (SAAF, VARIAN 20). The atomization of Zn and Cu is done in a burner with a flame resulting from the combustion of an air/acetylene mixture.

Spectral background noise correction for specific wavelengths (λ (Zn) = 213.9 nm, λ(Cu) = 324.8 nm) is achieved using a deuterium lamp. Results about trace metals are expressed as µg/g of dry tissues.

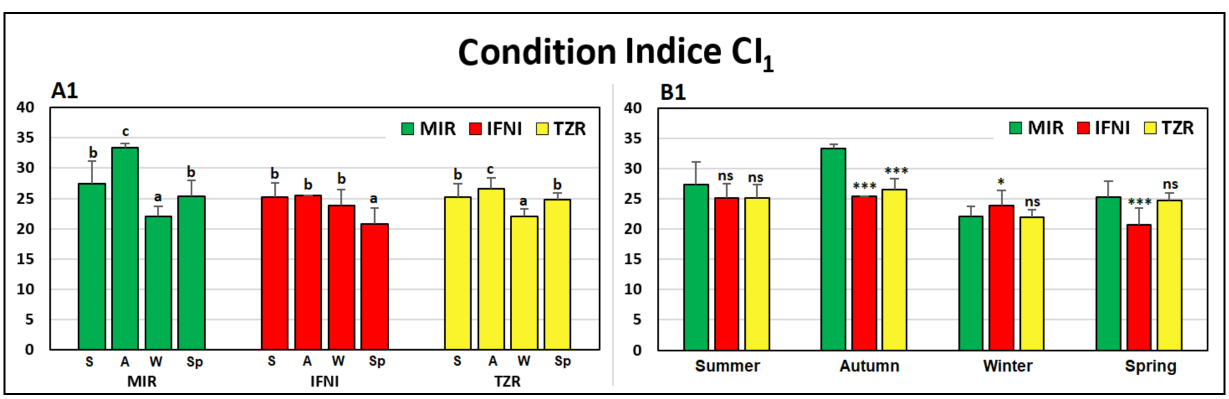
**Statistical analysis**

The results of the different elements are represented by the mean (m ± SD). All data were tested using the Analysis of Variance (ANOVA) and Tukey's DSH (Honest Significant Difference) test to analyze the effects of the "site" and "season" factors.Spearman's correlation coefficient is used to identify important patterns and relationships between variables. The statistical analysis was carried out using statistica software (Version 10 Statsoft®).

## 3.RESULTS AND DISCUSSION

### Seasonal variations in condition indices

Figure 2 shows the spatiotemporal variations in physiological indices at the three study sites: S1 [Mirleft (MIR)] S2 [Sidi Ifni (IFNI)] and S3 [Tazrout (TZR)].



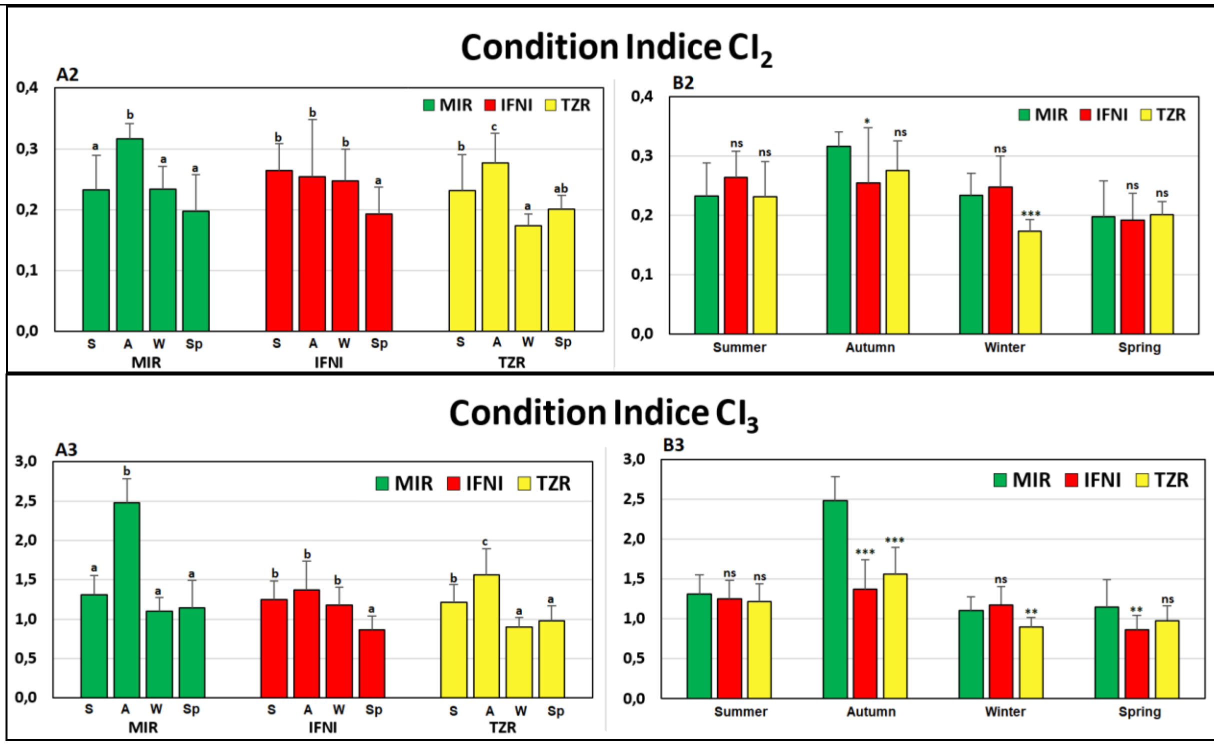



**Figure 2:** Variations in condition indices (CI) of *Mytilus galloprovincialis* collected at the Mirleft site (MIR), the Sidi Ifni site (IFNI) and the Tazrout site (TZR)

A: Seasonal variations by site; B: Inter-site comparison by season; S:Summer, A:Autumn, W:Winter, Sp: Spring; a, b, c: indicate significant homogeneous groups at p<0.05, using the Tukey test; ns: indicates a non-significant difference between the reference site and other study sites; (*)Indicates a significant difference at p <0.05; (**) significant at p <0.01; (***) significant at p <0.001, using Tukey's multiple comparison test

The results of the variations in the condition indices show a similar seasonal profile for the Mirleft reference site and the Tazrout site, the main difference is observed between autumn and winter (Figures 2-A1, 2-A2 and 2-A3). Indeed, the values recorded are significantly higher in autumn for all indices and significantly lower in winter for $CI_1$ and $CI_3$, while no significant difference was recorded between summer, winter and spring for $CI_2$. On the other hand, an inverse trend is observed for the Ifni site, the variations of the condition's indices remain more or less stable throughout the year with the exception of the spring period when significantly lower values were recorded.

In general, condition indices depend on biotic and abiotic factors, such as reproduction, food availability, temperature, salinity, etc.(Id Halla, 1997; Mourgaud *et al.*, 2002; Roméo *et al.*, 2003; Damiens *et al.*, 2007).

In autumn, the high values of indices of conditions indicate an increase in the weight of the flesh and the volume of the shell during this season. This may be related to the nutrient richness of the environment (Roméo *et al.*, 2003). Other work has shown that population density is the most important factor influencing shell growth in bivalves(Seed & Brown, 1978). Indeed, the shell is proportionally more elongated in the case of very dense mussel beds. However, previous work in the bay of Agadir, reported that the mussels have a contagious type distribution and forms very dense mussel beds especially in the site of Cap Ghir. This observation was also recorded during the sampling campaigns in Mirleft (Id Halla, 1997).

However, variations in physiological indices are related to the different phases of the reproductive cycle. The increase in weight reflects the storage of reserves and the restructuring of the gonad, while its fall corresponds to the periods of slopes (Id Halla, 1997; Schmidt *et al.*, 2013) .

However, the study of seasonal variations in condition indices, shows significantly lower values in spring, corresponds to the periods of main slopes. Indeed, the decrease in $CI_1$, $CI_2$ and $CI_3$ may be the consequence of possible gametogenesis and the diversion of metabolism to the benefit of reproductive function (Bitar & Hannach, 1987; Id Halla, 1997; Okumuş & Stirling, 1998; Roméo *et al.,* 2003; Bhaby *et al.,* 2014)**.** Similar work has stated a close relationship between the stage of gametogenesis, the condition index and the cycle of storage and consumption of energy reserves. Indeed, mussels can cushion the growth of their shell for a short time and use the accumulated glycogen reserves for gametogenesis (Bayne et al., 1983). In addition, the high rate of maturity of the gonads refers to a maximum laying coinciding with the collapse of conditions indices (Bhaby *et al.,* 2014).

Figures 2-B1, 2-B2 and 2-B3 represent the cross-site comparison, by season, of the condition indices in the mussel *Mytilus galloprovincialis*.

The study of the inter-site comparison of $CI_1$, $CI_2$ and $CI_3$, generally, show lower values in the population of Sidi Ifni compared to the reference population and Tazrout. However, the variations in $CI_1$ and $CI_3$ during autumn and spring in the impacted population of Sidi Ifni are less marked than those observed in the reference population (Figures 2-B1 and 2-B3), with a high significant ($p$<0.001) and highly significant ($p$<0.01) difference from the reference population, respectively, during the fall and spring. While the inter-site comparison of $CI_2$ indicates, a significant difference in autumn in favor of Ifni site and a second highly significant difference compared to the Tazrout site during winter. In summer and spring, the difference between the studied sites remains insignificant.

Several stressors may explain these results. The population of Sidi Ifni mussels evolves in a marine ecosystem, disturbed by anthropogenic pressure, where seasonality is much less marked than in open wilderness. Indeed, the monitoring of the physicochemical parameters of seawater at the Ifni site shows high temperatures, pH values, and low salinity values. This disturbance is mainly due to the presence of domestic wastewater outfall near our sampling point. The influence of these abiotic factors may be at the origin of a staggered reproductive cycle, reflected by the pattern of seasonal variations in (Abbassi *et al.*, 2015) physiological indices in relation to the Mirleft population. Indeed, several authors record a decrease in the condition index in wild mussels whose habitat is subject to effluents from anthropogenic activities (Amiard-Triquet *et al.,* 2013) ; which leads to the disruption of the implementation of gametogenesis and the displacement of the laying period. Similar phenomena were, clearly, observed in our study (Bodin *et al.,* 2004).

In general, the comparison of physiological indices in mussels shows very significant seasonal variations at the MIR and TZR sites compared to the Ifni polluted site. This would be one aspect of the adverse effects of pollution on mussel physiology. Indeed, Shafee (1992) reported that the low growth rate observed in the mussel is due to the environmental pollution. According to

Gosling, the growth of mussels varies with temperature (Gosling, 1992). Other authors suggest that the effect of temperature is usually combined with food. In addition, these results may be related to the presence of organic matter in the Ifni site. Indeed, the abundance of organic matter led to an increase in the carnal mass of*Mytilus galloprovincialis* (Schiedek *et al.*, 2006). In addition, some research has already reported that the growth of mussels can be correlated with the availability of nutrients in their living environment, in particular organic matter(Sarà & Pusceddu, 2008).

**Correlations between different condition indices**

Table 1 shows the analysis of correlations of physiological indices in mussels collected on the coasts of Sidi Ifni.

In the mussel *Mytilus galloprovincialis* collected from the three study sites MIR, IFNI and TZR, analysis of the results reveals that the indices of conditions $CI_1$, $CI_2$ and $CI_3$ were highly correlated (Table 1). These results were expected, since the three indices are generally based on the weight of the flesh and the length of the shell representing a similar indication that reflects the growth of mussels.

**Table 1: Spearman correlation analysis of seasonal data between physiological parameters in mussels collected at the Mirleft, Sidi Ifni and Tazrout.**
**$^{(**)}$ : The correlation is significant at the 0.01 level; $^{(*)}$ : The correlation is significant at the 0.05 level**

| Study site | Condition Index | Physiological Parameters | | |
|---|---|---|---|---|
| | | $CI_1$ | $CI_2$ | $CI_3$ |
| Mirleft | $CI_1$ | **-** | **0,572$^{(**)}$** | **0,817$^{(**)}$** |
| | $CI_2$ | **0,572$^{(**)}$** | **-** | **0,855$^{(**)}$** |
| | $CI_3$ | **0,817$^{(**)}$** | **0,855$^{(**)}$** | **-** |
| Ifni | $CI_1$ | **-** | **0,478$^{(**)}$** | **0,631$^{(**)}$** |
| | $CI_2$ | **0,478$^{(**)}$** | **-** | **0,865$^{(**)}$** |
| | $CI_3$ | **0,631$^{(**)}$** | **0,865$^{(**)}$** | **-** |
| Tazrout | $CI_1$ | **-** | **0,815$^{(**)}$** | **0,828$^{(**)}$** |
| | $CI_2$ | **0,815$^{(**)}$** | **-** | **0,855$^{(**)}$** |
| | $CI_3$ | **0,828$^{(**)}$** | **0,855$^{(**)}$** | **-** |
| Total | $CI_1$ | **-** | **0,581$^{(**)}$** | **0,762$^{(**)}$** |
| | $CI_2$ | **0,581$^{(**)}$** | **-** | **0,850$^{(**)}$** |
| | $CI_3$ | **0,762$^{**}$** | **0,850$^{**}$** | **-** |

In general, the $CI_1$, $CI_2$ and $CI_3$ index showed a significantly high correlation in all studied sites. However, the fluctuations in $CI_2$ and $CI_3$ were positively correlated with IC1, meaning that weight is proportionally related to the total length of the shell. Indeed, several studies confirm that growth is characterized by an increase in size and weight as a function of time and environmental variables (Burgeot & Galgani, 1998). In addition, other authors explain the speed of growth in length than that in width and thickness by the morphological heterogeneity of the shell (Bitar & Hannach, 1987).However, in mussels, reproductive processes disrupt growth through a temporary accumulation of large reserves that are subsequently converted into gametes and then expelled during egg-laying, causing a sudden loss of weight (Lubet, 1981 ; Cartier *et al.*, 2004).

**Correlations between condition indices and physicochemical parameters**

The physicochemical quality of the study environment is of great importance in determining the bioecological quality and the degree of pollution of the marine ecosystem. During the study period, three physicochemical parameters of seawater were monitored: temperature, pH and salinity.

The physicochemical study of the seawater of the coast of Sidi Ifni shows the existence of seasonal fluctuations (Abbassi *et al.*, 2017). Indeed, the variation of the temperature is related to the local climatic conditions and more particularly to the air temperature.

When it comes to pH, seawater is slightly alkaline. However, the salinity of seawater also shows seasonal fluctuations. It scored lower values in winter and higher in summer. This would be explained by the strong dilution of seawater caused by the high freshwater inputs resulting from heavy rainfall and the drop-in temperature accompanied by a low evaporation rater. The high values of water salinity recorded during hot periods are due to the combined action of high temperatures causing high evaporation and the decrease in precipitation, which is the cause of the decrease in fresh water supply. Table 2 presents the Spearman correlation between the condition's indices in *Mytilus galloprovincialis* and the physicochemical parameters of seawater at different study sites.

**Table 2: Spearman correlation analysis between condition indices in the mussel *Mytilus galloprovincialis* and physicochemical parameters of seawater in Mirleft, Sidi Ifni and Tazrout**

**(**) : The correlation is significant at the 0.01 level; (*) : The correlation is significant at the 0.05 level.**

| Study site | Condition Index | Physicochemical Parameters | | |
|---|---|---|---|---|
| | | T°C | pH | Salinity |
| Mirleft | $CI_1$ | **0,385(**)** | -0,187 | **0,596(**)** |
| | $CI_2$ | **0,389(**)** | 0,167 | **0,240(*)** |
| | $CI_3$ | **0,454(**)** | -0,036 | **0,483(**)** |
| Ifni | $CI_1$ | **0,406(**)** | **-0.273(*)** | **0,377(**)** |
| | $CI_2$ | **0,254(*)** | **-0.279(*)** | 0,180 |
| | $CI_3$ | **0,317(**)** | **-0.277(*)** | **0,318(**)** |
| Tazrout | $CI_1$ | **0,350(**)** | -0,113 | **0,548(**)** |
| | $CI_2$ | **0,367(**)** | -0,018 | **0,496(**)** |
| | $CI_3$ | **0,464(**)** | 0,012 | **0,599(**)** |
| Total | $CI_1$ | **0,379(**)** | -0,040 | **0,530(**)** |
| | $CI_2$ | **0,330(**)** | -0,039 | **0,250(**)** |
| | $CI_3$ | **0,387(**)** | 0,007 | **0,435(**)** |

The results show highly significant positive correlations between temperature and all condition indices in all study sites. Similar results were also recorded for the salinity of seawater. On the other hand, negatively significant correlations are reported between seawater pH and $CI_1$, $CI_2$

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and $CI_3$ indices in mussels collected at the Ifni site, while no relationship was reported between pH and condition indices in mussels collected at the MIR reference site and the TZR site.
In general, the Spearman correlation test, between the indices of conditions in *Mytilus galloprovincialis* and the physicochemical parameters of seawater at different study sites, confirms a strong link between physiological indices and environmental factors recorded such as temperature and salinity.
The condition index is related to the temperature of the water and corresponds to the filling rate of the soft tissues of the shell, which provide information about the nutritional and physiological status of the animal. Similar results suggest that temperature has an effect on triggering gametogenesis in mussels (Lubet, 1981; Lubet & Aloui, 1987).
Most growth variables, both length and weight are explained by temperature. Still, mussels showed strong growth during warm periods with high primary production and low growth in winter (Tomalin, 1995). In addition, the relatively high temperature, combined with the trophic speed of the environment, would act in favor of gonadal growth.
Many authors report that increasing the salinity of water reduces the rate of absorption by benthic organisms and may cause molluscs to reduce their ability to adapt to hypoxia (Namour, 1992; Bucheli & Fent, 1995; Stien *et al.*, 1998). According to other authors, the low index could be due to poor nutritional conditions or weight loss due to egg laying. Indeed, the growth of bivalve molluscs follows a complex process: in addition to temperature, food and breeding season, various other factors, such as substrate, depth and turbidity of water, affect the growth of these invertebrates. Nevertheless, temperature, organic matter and phytoplankton biomass are the main factors to be retained (Shafee, 1980; Heral *et al.*, 1987).

**Correlations between condition indices and biomarkers**

The present study demonstrated no significant correlation between biomarkers (AChE, CAT, and GST) and physiological indices ($CI_1$, $CI_2$ and $CI_3$) in mussels at the Tazrout site. On the other hand, at the MIR reference site, the results reveal a significant negative correlation between catalase activity and the two condition indices $CI_1$ and $CI_2$, and a significant positive correlation between AChE activity and the $CI_2$ index (Table 3). However, no significant correlation was detected between GST activity and physiological indices in mussels collected from all sites. However, these indices were positively influenced by the rate of MDA in Mirleft and Ifni. In addition, our study showed highly significant positive correlations between metallothioneins and physiological indices in *Mytilus galloprovincialis* harvested at the Mirleft and Ifni study sites, while at Tazrout, the results reveal a high significant negative correlation (Table 3). This study reports a positive significant correlation between STDs and physiological indices at all study sites. This suggests that metallothionein levels are growth-related and susceptible to interference with seasonal variation and the degree of contamination of study sites. Indeed, the comparison of the levels of MTs between the individuals of Mirleft and Ifni highlights a very clear influence of the season and the degree of contamination (Abbassi et al., 2021). These results were also endorsed by Amiard *et al. (1987)*, Banaoui *et al.* (2004); Amiard *et al.* (2006), and Khati *et al.* (2012).

**Table 3: Results of correlation analysis between condition indices and biomarkers in *Mytilus galloprovincialis*collected at Mirleft, Sidi Ifni and Tazrout**
**(**) : The correlation is significant at level 0.01; (*) : The correlation is significant at level 0.05.**

| Study site | Condition Index | Biomarkers | | | | |
|---|---|---|---|---|---|---|
| | | Ache | CAT | GST | MDA | Mts |
| Mirleft | $CI_1$ | 0,140 | **-0,471(**)** | 0,022 | **0,385(**)** | **0,367(**)** |
| | $CI_2$ | **0,297(*)** | -0,115 | 0,175 | **0,182** | **0,485(**)** |
| | $CI_3$ | 0,190 | **-0,308(**)** | 0,095 | **0,271(*)** | **0,421(**)** |
| Ifni | $CI_1$ | 0,007 | -0,187 | -0,082 | **0,309(**)** | **0,328(**)** |
| | $CI_2$ | 0,116 | -0,013 | -0,012 | **0,166(*)** | **0,294(*)** |
| | $CI_3$ | 0,088 | -0,127 | -0,205 | **0,417(**)** | **0,397(**)** |
| Tazrout | $CI_1$ | 0,074 | -0,031 | 0,070 | 0,042 | **-0.391(**)** |
| | $CI_2$ | 0,063 | -0,077 | 0,058 | 0,054 | **-0,319(**)** |
| | $CI_3$ | 0,137 | -0,177 | 0,185 | 0,149 | **-0,369(**)** |
| Total | $CI_1$ | **0,143(*)** | **-0,289(**)** | -0,090 | -0,032 | **0,143(*)** |
| | $CI_2$ | 0,092 | -0,052 | **0,139(*)** | -0,057 | **0,200(**)** |
| | $CI_3$ | **0,160(*)** | **-0,245(**)** | 0,024 | -0,053 | **0,194(**)** |

These results suggest that under unpolluted wild habitat conditions, there are no significant mutual influences between the biomarkers studied and the physiological indices. Thus giving us an indication of the influence of stress and endogenous factors on the stages of reproduction.
Some authors have discussed the close functional relationship between the digestive glands and gonadal development. Indeed, a possible sudden stress and antioxidant defenses of the digestive glands can act on the reproductive process (Wilhelm Filho *et al.*, 2001). Yet, biological factors and the different stages of the reproductive cycle must also be considered in studies interested to the antioxidant defenses of mussels. It should also be noted that the somatic gonadal and hepatic indices can inform us about the toxic effects of pollutants and therefore on energy reserves and capacities. Individuals tolerate the challenges of chemical pollution or other types of environmental stress (Wilhelm *Filho et al.*, 2001; Vidal-Liñán *et al.*, 2010).

**Correlations between condition indices and metallic trace elements**

In this section, we are interested in studying the influence of the degree of metal contamination of study sites on physiological indices in the mussel *M. galloprovincialis*. Measurements of trace metals Cd, Cu, Zn and Ni were carried out over four seasons at Mirleft, Ifni and Tazrout. The data were analyzed using Spearman correlation analysis with the condition indices $CI_1$, $CI_2$ and $CI_3$ (Table 4).

**Table 4: Results of correlation analysis between condition indices and trace metals in the mussel *Mytilus galloprovincialis* collected at Mirleft, Sidi Ifni and Tazrout**
**(**) : The correlation is significant at level 0.01; (*) : The correlation is significant at level 0.05.**

| Study site | Condition Index | Trace Metals | | | |
|---|---|---|---|---|---|
| | | Cd | Cu | Zn | Ni |

| | | | | | |
|---|---|---|---|---|---|
| Mirleft | $CI_1$ | 0,180 | **0,458(**)** | **0,372(**)** | 0,224 |
| | $CI_2$ | **0,268(*)** | **0,288(*)** | **0,408(**)** | -0,016 |
| | $CI_3$ | **0,266(*)** | **0,413(**)** | **0,446(**)** | 0,055 |
| Ifni | $CI_1$ | **0,406(**)** | **0,308(**)** | **0,338(**)** | **0,410(**)** |
| | $CI_2$ | 0,175 | **0,234(*)** | **0,364(**)** | **0,240(*)** |
| | $CI_3$ | **0,408(**)** | **0,363(**)** | **0,472(**)** | **0,464(**)** |
| Tazrout | $CI_1$ | **0,259(*)** | **-0,572(**)** | **-0,528(**)** | **-0,26(*)** |
| | $CI_2$ | **0,244(*)** | **-0,488(**)** | **-0,480(**)** | **-0,271(*)** |
| | $CI_3$ | **0,319(**)** | **-0,583(**)** | **-0,455(**)** | -0,213 |
| Total | $CI_1$ | **0,259(*)** | **-0,572(**)** | **-0,528(**)** | **-0,260(*)** |
| | $CI_2$ | **0,244(*)** | **-0,488(**)** | **-0,480(**)** | **-0,27(*)** |
| | $CI_3$ | **0,319(**)** | **-0,583(**)** | **-0,455(**)** | -0,213 |

The results related to the measurement of trace metals, in the dry tissues of the mussel taken from the study sites, show high significant positive correlations between physiological indices and copper, zinc and cadmium contents, except that the latter has no statistically significant link with $CI_1$ at Mirleft and $CI_2$ at Ifni. However, no significant correlation was reported between nickel (Ni) concentrations and all physiological indices in Mirleft mussels, as well as $CI_3$ at Tazrout. The latter characterized by the presence of a significant negative correlation between the indices ($CI_1$ and $CI_2$) and nickel (Ni), while this metallic element is positively associated with the indices recorded in the Ifni mussel.

Our previous work states that the levels of metal contamination in *Mytilus galloprovincialis*, record from a global perspective, a differentiation between the three study stations (*Abbassi et al.*, 2018 & 2021). Indeed, the result obtained shows very high metal contents in Ifni molds compared to those of Mirleft. However, significant correlations have also been revealed between element trace metals and biomarkers analyzed on mussels taken from the coasts of Sidi Ifni. Nevertheless, the influence of the activity of the antioxidant enzymes CAT and GST, and the levels of MDA and MTs could be explained by the toxic exposure to Zn and Cu.

Indeed, a significant increase in antioxidant enzymatic responses was observed with an improvement in oxidative stress in experiments exposed to pollutants and organometallics. In addition, it has been proven that some trace metals can cause oxidative stress and induce the formation of reactive oxygen elements (Kappus & Sies, 1981; Schaich, 1992; Roméo *et al.*, 2003;).

However, the responses of antioxidant enzymes are transient and variable for different aquatic species. Indeed, the results are sometimes contradictory *in vivo* with some authors showing an induction of activity and others an inhibition (di Giulio *et al.*, 1989 & 1993; Labrot *et al.*, 1996).

In fact, Cu and Zn concentrations were inversely correlated with CAT activity at our study site (Abbassi *et al.*, 2021). This suggests that the activity of the CAT enzyme is inhibited after high exposure of animals to these trace metals. In this sense, inhibition of antioxidant activity is considered a non-specific response to chemical challenges, such as heavy metal pollution (Regoli *et al.*, 2003) .

In general, the physiological conditions of mussels and their biological activities act synergistically during stress. In addition, the process of bioaccumulation of metal elements in mussels is related to biological factors (life history, age, nutrition, etc.) and the physicochemical conditions of the aquatic environment, which can significantly affect the evolution of metal contents (Buestel, 1997; Saha *et al.*, 2006).

## 4.CONCLUSION

The seasonal study carried out on three sites with extreme contrasts in the anthropogenic influence that characterizes them, showed a pronounced variability in the biological and physiological behavior of mussel populations from Sidi Ifni coasts. In general, biomarker responses to their habitat showed strong perturbations to contaminated Ifni sites, as expected.
The integration of biological responses with physiological indices focuses on interpreting the mussel's behavior in the face of stress and differentiating between stress caused by environmental changes and stress caused by anthropogenic activities. Thus, a more effective and detailed assessment of the health status of study sites can be carried out.
The cold season showed representative results in terms of biological response. This is why we decided to deepen our research on this season through a correlative study on the contamination and antioxidant biological responses of mussels from several sites along the Sidi Ifni Coast. Thus, the part of the study, which focuses on the levels of contamination of different coastal sites, allowed us to acquire data on the physicochemical characteristics of the environment, the bioaccumulation of metals and the study of biomarker responses in the species *Mytilus galloprovincialis* recognized for its commercial interest and its wide distribution along the coasts of Sidi Ifni.
Indeed, the comparison of the results of physiological indices in mussels with environmental factors and biochemical markers shows that the species is contaminated in the Ifni site compared to Mirleft and Tazrout.

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