# INFLUENCE OF BIOTIC FACTORS ON METALLOTHIONEIN LEVELS IN MULLUS BARBATUS COLLECTED FROM THE IBERIAN MEDITERRANEAN COAST.

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**Abstract**

The objective of this study was to determine the influence of some biotic factors (gender, size and sexual maturity) on the concentration of hepatic metallothionein (MT) in the demersal fish *Mullus barbatus*, as a initial step for the ultimate identification of basal levels of this biomarker. The influence of biotic factors was assessed applying polynomial regression models and one-way ANOVA. The results confirm previous findings (1) that the biotic factors considered must be taken into account in biomarker monitoring as they could affect the fish MT system.

*Key words: Mullus barbatus, bio-indicators, pollution, Western Mediterranean, coastal waters.*

**Introduction**

Metallothioneins (MTs) are a family of low-molecular-weight, cysteine-rich proteins that have a high affinity for divalent ions. MTs play important roles in the regulation of essential trace metals, such as zinc and copper, and in the detoxification of heavy metals, such as cadmium and mercury. Recently, MT level has become a major biomarker for monitoring metal pollution in fish, and it has been included in several Monitoring Programmes, such as the Joint Assessment Monitoring Programme (JAMP) or the Mediterranean Pollution Monitoring Programme (MEDPOL), as an early indicator of biological effects of heavy metals.

**Material and methods**

Specimens of *Mullus barbatus* were caught by trawling before (May 1999) and after (October 1999) the spawning season, in six areas along the Iberian Mediterranean coast (Fig. 1) exposed to different degrees of anthropogenic activities. Water temperature and salinity was recorded at each sampling. Individual striped mullet were sexed, weighed, length measured and liver removed. MTs content was measured applying the spectrophotometric assay, adapted from Viarengo et al (2, 3), based on the estimation of the sulfhydryl content of MT proteins using Ellman's reagent.


**Figure 1 : Map of sampling sites.**

The influence of size in hepatic MT was studied applying a curvilinear regression analysis in order to obtain the determination coefficients ($R^2$) for different models. The relationships between both variables were evaluated applying the F-test with regression ANOVA using specimens, with the same gender, captured in six areas, before and after spawning.

The influence of gender in hepatic MT was studied applying one-way ANOVA on log-transformed data. Specimens within the size range 14 to 18 cm, captured in three different areas before and after spawning, were used in this analysis.

The influence of maturation state in hepatic MT was assessed applying one-way ANOVA on log-transformed data. Specimens within the size range 14 to 18 cm, captured in two different areas before and after spawning, were used in this analysis.

**Results and Discussion**

The results of the regression analysis showed the existence of significant positive relationship between size and MT content in the six cases studied ($p < 0.05$; Fig. 2). Furthermore, variability of the data was better explained by the power model $[MT] = b_0 + size^{b1}$ ($R^2 = 0.203$; $p < 0.05$). Significant differences in MT levels between male and female specimens were found in all cases during prespawning but not during postspawning period ($p < 0.05$). Mean MT was always bigger in females (Table 1). We have also found significant differences in MT levels between specimens (belonging to the same gender) captured in prespawning and postspawning period in all cases studied. Mean MT was always higher during prespawning period (Table 1).

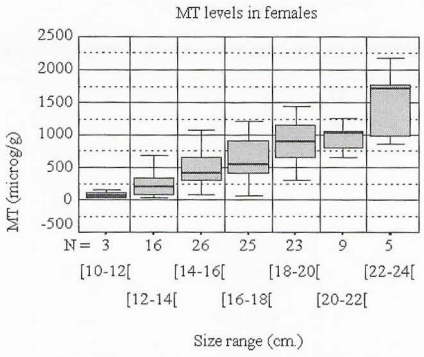


**Figure 2 : Influence of size on MT levels in *Mullus barbatus* collected in May 1999 at Portman. $R^2$= 0.373, $b_0$ = 0,8731, $b_1$ = 0,1118.**

**Table 1. Results of the influence of gender and sexual maturity on MT contents (µg /g hepatic tissue) in *Mullus barbatus*. (*) Statistically significant differences.**

| Gender | Date | Site | n | MT Mean | St. Error | F | p |
|---|---|---|---|---|---|---|---|
| Male | May | Segura river | 18 | 19,213 | 0,065 | 86,060 | 0,000* |
| Female | | Segura river | 61 | 25,828 | 0,025 | | |
| Male | May | Tabarca I. | 29 | 18,143 | 0,063 | 33,158 | 0,000* |
| Female | | Tabarca I. | 30 | 22,734 | 0,046 | | |
| Male | May | Columbretes I. | 10 | 18,256 | 0,055 | 62,545 | 0,000* |
| Female | | Columbretes I. | 18 | 23,637 | 0,036 | | |
| Male | October | Cabo de Palos | 36 | 12,689 | 0,057 | 4,591 | 0,037* |
| Female | | Cabo de Palos | 17 | 13,311 | 0,072 | | |
| Male | October | Tabarca I. | 16 | 13,896 | 0,031 | 0,244 | 0,624 |
| Female | | Tabarca I. | 26 | 14,013 | 0,043 | | |
| Male | October | Columbretes I. | 13 | 13,372 | 0,052 | 0,565 | 0,575 |
| Female | | Columbretes I. | 14 | 13,612 | 0,047 | | |
| **Date** | **Gender** | **Site** | **n** | **MT Mean** | **St. Error** | **F** | **p** |
| May | Female | Tabarca I. | 37 | 22,311 | 0,046 | 381,1 | 0,000* |
| October | | Tabarca I. | 40 | 14,019 | 0,035 | | |
| May | Female | Columbretes I. | 31 | 23,112 | 0,034 | 467,9 | 0,000* |
| October | | Columbretes I. | 14 | 13,612 | 0,047 | | |
| May | Male | Tabarca I. | 52 | 17,671 | 0,045 | 69,61 | 0,000* |
| October | | Tabarca I. | 17 | 13,880 | 0,030 | | |
| May | Male | Columbretes I. | 30 | 17,321 | 0,056 | 73,81 | 0,000* |
| October | | Columbretes I. | 14 | 13,416 | 0,050 | | |

**Conclusions**

Our results showed that the use of MT levels in *Mullus barbatus* necessitates the utilization of specimens within a same size range in order to reduce variability and allow standardized comparisons. Considering that specimens within a size range 14 to 18 cm were most abundant and their liver size was appropriate for analysis, we propose this size class for comparative purposes. The reported influence of gender and maturation state on MT levels have shown the necessity to establish the sampling time and to study independently specimens of different sex. Since variability of data was lower in postspawning periods than in prespawning, it is advisable to carry out the sampling outside the prespawning period.

**References**

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