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REVIEW **(Open Access)**

Metallothionein (MT): a good biomarker in marine sentinel species like sea bream (*Sparus aurata*)

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Abstract

This review presents an overview of the significance of the use of molecular biomarkers as diagnostic and prognostic tools for marine pollution monitoring. In order to assess the impact of highly persistent pollutants such as polychlorinated biphenyls (PCB), polychlorinated dibenzo–dioxins (PCDD), polychlorinated dibenzo–furans (PCDF), polynuclear aromatic hydrocarbons (PAH), tributyltin (TBT) and other toxic metals on the marine ecosystem a suite of biomarkers are being extensively used worldwide. Among the various types of biomarkers, like cytochrome P4501A induction, DNA integrity, and acetylcholinesterase activity, metallothionein induction represents an excellent biomarker. MTs are induced by toxic metals such as Cd, Hg, and Cu by chelation through cysteine residues and are used in both vertebrates and invertebrates as a biomarker of metal exposure. Sea bream is sentinel fish of its native sandy costal habitat as it is widely distributed throughout the entire Mediterranean Sea. Many studies, which results are further shown in this paper, has proposed metallothionein as a biomarker of heavy metal exposure in *Sparus aurata*. Recently MT expression profiles have been used as perfect diagnostic instruments to determine the physiological impact of aquaculture systems in *S. aurata*. All these knowledge could be very helpful to improve fish productivity and the aquaculture production system quality.

Keywords: marine pollution monitoring; heavy metals; oxidative stress.

1. MTs induction in aquatic organisms

MTs are cysteine rich peptides occurring mainly in the cytosol and in the nucleus and lysosomes. They are nonenzymatic proteins with a low molecular weight, high cysteine content, no aromatic amino acids and heat stability. The thiol groups (–SH) of cysteine residues enable MTs to bind particular heavy metals. MT-like proteins have been reported in many vertebrates including many species of fish [1, 2] and aquatic invertebrates mainly molluscs [3] and crustaceans. MTs can be induced by the essential metals Cu and Zn and the non-essential metals Cd, Ag and Hg in both vertebrates and invertebrates. Thus the exposure of marine organisms to toxic metals can lead to changes in several biochemical processes that have the potential to be used as biomarkers of exposure and therefore as ‘early warning’ signals of the presence of these particular contaminants. The induction of MTs was reported in the oyster (*Crassostrea virginica*) [4] and the mussel (*Mytilus galloprovincialis*, *Mullus barbatus*) [5]. About 50 different species of aquatic invertebrates, the majority of which are mollusks or crustaceans are found to show response to induction of MTs. Thus they are used for evaluation of pollution in the marine environment and are seen as potential biomarkers of metal exposure in mollusks and fish [2]. Tissues directly involved in metal uptake, storage and excretion have a high capacity to synthesize MTs. In aquatic organisms, these proteins have been identified in the digestive gland (also termed the midgut gland or hepatopancreas) and gills of molluscs and crustaceans [6, 7]. The role(s) of MTs in the physiological processes can be explained by the fact that it is primarily involved in essential and non-essential metal pathways. The activities of MT can be observed in elements of both homeostasis and detoxification. MT binds to excess of essential or pollutant metals. Thus it protects the organism against toxicity by restricting the availability of these cations at detrimental sites. Some toxic metals such as Ag, Cd, Cu, Hg and Zn have a high binding affinity for cysteine. In some cases, MTs have high cysteine content (30%), low molecular weight, heat-stability, and a strong affinity for binding metals. In the case of mollusks MT have a high glycine content

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differentiating them from most of the other MT isoforms known today [2]. MTs are distinctively identified in only a few species of marine molluscs (*Patella vulgata*, *Crassostrea virginica*, *Patella granularis*, *Mytilus edulis*) [8, 9] and recently in *Ruditapes decussates* [10] and fish [11]. The induction of MT has been detected in organisms from contaminated areas or in vitro experiments with exposure to metals such as Ag, Cd, Cu, Hg and Zn in laboratory. The extent of MT induction can vary between species and between tissues. The sequestration of metals by MT is clearly evident in the gills, digestive gland and kidney, indicating the significance of these tissues in uptake, storage and elimination of metals [12]. The use of MT as biomarker has been validated in many in situ studies [13-16]. The results are generally positive, in particular when the metal gradient of pollution is quite real. More and more of studies in situ combine the quantification of several biomarkers, of which MT is only one [17-22].

Numerous studies demonstrate Cd induced MT expression in a variety of fish species including the turbot [23], carp [24], goldfish [25], sole [26], zebrafish [27], rainbow trout [28] and tilapia [29]. MT transcript and protein are induced in fish by other bivalent metals, including Zn, Cu, Pb and Hg [30-35]. Therefore, MTs have become of great interest for assessing pollution in the marine environment and are seen as potential biomarkers of metal exposure in fish [36-39].

2. MT in sea bream

S. aurata is a cosmopolitan coastal demersal species of the NE Atlantic and the Mediterranean that often inhabits estuarine areas, at least during early life stages (Figure 1).

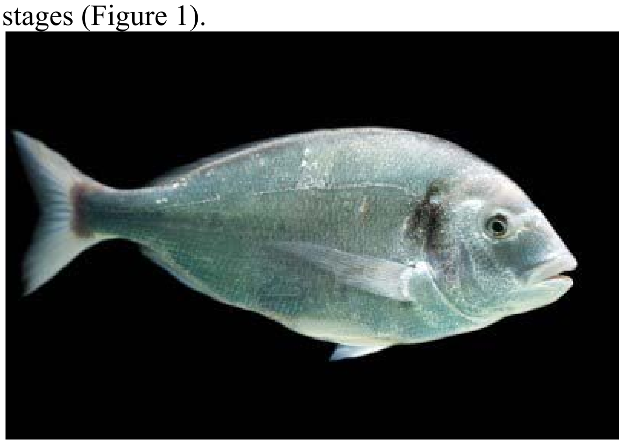

Figure 1. Sparus aurata

This species has a high economical value and is one of the most important closed-cycle mariculture productions in Mediterranean countries. Sea bream culture has increased considerably in the last few years, reaching a high production and a high commercial value. This intensive production has raised concerns over the quality of cultured fish in comparison to wild fish [40]. Thus, evaluating pollutant loads and biological responses in cultured and wild fish is a major need. As a consequence of indiscriminate exploitation of sea resources, as well as of the laws of conservation issued to assure a smaller environmental impact, fishing is no more able to satisfy the growing needs of consumers. As a result, during the past 30 years, aquaculture's great worldwide expansion has occurred, although aquatic farming is recorded in antiquity. Aquaculture production systems may be described either as extensive systems employing low animal density in relation to water volume or intensive systems in which higher animal density are used [41]. In the intensive system, fish are bred in tanks and fed with special feed fitting each single species. In the extensive system, fish grow in lagoons or brackish waters, naturally fed. When the natural diet is supplemented with special feed, the system is defined semi-intensive. It has long been recognized that heavy metals in the marine environment have a particular significance in the ecotoxicology, since they are highly persistent and can be toxic in traces. Metals as Pb and Cd are in fact potentially toxic and pose a serial risk for human health when they enter the food chain [42]. Fish are exposed to the various transition metal species at different intensities. There are two major routes of metals exposure. Metal ions dissolved in the environmental water are absorbed through the gills and other permeable body surfaces [34]. Metals bound to solid particles are ingested, detached from their carrier particles in the digestive system and absorbed through the gut epithelium [43]. Coastal fish have been proposed as sentinel species to assess the possible effect of anthropogenic activities on a coastal area and for monitoring marine environment pollution [44, 45]. Sea bream was selected as sentinel fish of its native sandy costal habitat as it is widely distributed throughout the entire Mediterranean Sea [46, 47].

S. aurata MT has a molecular weight of 5.966 kDa, contains 20 metalbinding cysteine residues and has already been proposed as a biomarker of heavy metal exposure [48]. Previous studies results [49] suggested that there is considerable interaction among MT and glucose-6-phosphate dehydrogenase (G6PDH) induction by metals in *Sparus sp.* (*Sparus sarba*). Both the Cd-induced MT and G6PDH responses may work together to protect cells against oxidative damage, since MT and GSH (generated by the G6PDH reaction) are both cellular thiols capable of protectin cells from Cd toxicity by virtue of their ability to sequester metals. Indeed, Schlenk and Rice [50] clearly demonstrated that concomitant induction of MT and GSH by metals may provide better protection against oxidative damage than MT induction alone.

Ghedira and colleagues [51] determined the value of MT induction using the assay of Viarengo [52] and cadmium and copper accumulation in liver, gills and kidney of *S. aurata* after short term exposure (48 hours). They noted that MT level induction related to the higher metal accumulation in three organs revealed the main role of MT in metal homeostasis and detoxification in *S. aurata*. Similar results were previously reported by Filipovic and Raspor [53], who found a high correlation between Zn and Cu accumulation and MT induction in liver of *M. surmuletus* and *L. aurata*.

For farmed fish, growing conditions (food and water chemistry) may determine metal composition of fish tissues as well as the response that these fish exhibit to metal toxicity. For this reason, Cretì and colleagues [54] examined the concentration of the heavy metals, Cd and Pb, in tissues of *S. aurata* collected from different types of fish farming (S.T.A.T.—Torremozza Ugento (Lecce), intensive system; HYDRA COOP-ACQUATINA—Frigole (Lecce), extensive and semi-intensive systems) (Figure 2).

Figure 2. Aerial image of the Acquatina basin (from Google Earth).

Heavy metal concentrations in feed used in fish farming were measured to determine the principal source of metals in fish tissues. In addition, the relationship between metal concentration and MT content in tissues was investigated. Other studies of heavy metal concentrations in tissues from different types of breeding allowed them to identify and define the potential impacts of the different aquaculture systems on the welfare of farmed fish. Data relating to muscle tissues demonstrate that in general, metal concentrations were lower in muscle compared to gills, liver, gut and kidney.

This is an important fact, because muscle constitutes the greatest mass of the fish that is consumed, and this result was in good agreement with observations carried out on several wild and cultured fish species [55-59].

Furthermore, it was noteworthy the fact that in the fish tissues, the content of both Cd and Pb was not strictly correlated with that of metallothionein. Indeed, the marked accumulation of both metals in liver, as well as the high Pb content in gills and kidney, was not accompanied by a concomitant accumulation of MT in these tissues. The results clearly demonstrated that MT levels were also tissue-specific, with the highest levels in the muscle, followed by gut, liver, kidney and gills. However, the absence of a correlation between MT content and metal accumulation in tissues has been already reported for many biological systems [60-62]. Trinchella and colleagues [61] have postulated that metal ions are mostly present in tissues not as free ions but possibly as metal–protein complexes, so the amount of free ions could be too low to bring about a measurable induction of MT expression and synthesis. The high MT level found in muscle could be explained considering that this tissue is the site of an intense oxidative metabolism [63] with the consequent production of a high amount of free radicals which, in turn, could induce the MT synthesis [64].

Another important consideration is that the highest MT content in the muscle of fish breed in the extensive system was in agreement with the high metabolic rate of these fish that, as stated above, could be considered as wild fish. The high MT level found in the muscle of fish from the intensive breeding could be associated with the high stocking density generally present in this type of breeding, which is the main cause of stress for fish.

Taken together, the results presented in this study performed by Cretì and colleagues [54], indicated that: (1) Cd and Pb concentrations measured in muscle, the edible part of the fishes, were below 0.01 and 0.1 μg/g tissue, respectively, for all the examined fish; (2) the MT content in muscle was probably due to environmental stressors other than heavy metals; (3) the low contents of Cd, Pb and MTs founded in fish from the semi-intensive farming method demonstrated that at least for *S. aurata*, this aquaculture practice guarantees the overall best quality products.

All the information presented in this review could help us to further understand the precious role of metallothioneins as biomarker in ecotoxicological studies and suggest one of scientific approach to use in ecosystem biomonitoring programs in Albania, in the future, in order to have an healthy ecosystem with healthy beings like humans. Using metallothionein as marine pollution biomarker could be extremely helpful to improve fish productivity and the aquaculture production system quality.

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