Table 1: Studies that evaluated MT protein or gene expression in response to heavy metal exposure in aquatic animals

Animal	Species	Study Aim	MT induction	Tissues	Heavy Metals	Key Findings	Reference
Teleosts	*Dicentrrchus labrax, Solea senegalensis*	Cd organotropism, accumulation and MT induction	MT protein expression	liver, muscle	Cd	MT concentrations failed to reflect Cd exposure in both species.	Croizier et al. 2018
	Oncorhynchus kisutch	Effect of cadmium on MT induction	qRT PCR	liver, gill, and olfactory tissues	Cd	Peripheral olfactory system may be of greater utility for assessing short-term environmental exposures to cadmium	Espinoza et al. 2012
	Salmo salar	Responses of biomarkers in Atlantic salmon (*Salmo salar*) following exposure to environmentally relevant concentrations of complex metal mixture	MT protein abundance	Liver, kidneys	Zn, Cu, Ni, Cr, Pb, Cd	Liver and kidneys MT did not show statistically significant differences between the control and exposed group (MIX). However, hepatic MT level increased 1.34-fold after MIX treatment. Consequently, no further investigation of MT content after treatment with MIX variants was performed.	Stankevičiūtė et al. 2018
	Salmo salar	Combined γ-irradiation and metal exposures in teleost	MT gene expression	liver	Al + Cd	No significant differences were seen between the metal exposure groups and control groups	Olsvik et al. 2010

Naso hexacanthus	MT expression as a biomarker of toxicity of heavy metals	Semi-Quantitative Analysis of MT Gene Expression	liver gills	Cd, Cu, Zn and Pb	MT expression increased in response to metal exposure compared to control	Montaser et al. 2010
Sparus aurata	MT content in tissues of fish from three different fish farming systems	MT content is determined by estimation of cysteine residues by the Ellman reaction	caudal muscle, gut, liver, kidneys and gills	Cd, Pb	No correlation is present between heavy metals and MT content in muscle tissue.	Creti et al. 2010
Clarias gariepinus	MT expression from wild African populations of	qRT PCR	liver	Cu, Zn	Significant correlations were found between Zn and Cu levels and MT expression in livers	M'kandawire et al. 2017
Nothobranchius rachovii	MT response to short-term exposure to physiological and chemical stressors	Northern blot	liver, gill, and intestine	Cd	Increase in gill MT mRNA induction was observed in non-spawning killifish exposed to 6 ppb of waterborne $CdCl_2$ compared with controls	Van Cleef-Toedt et al. 2001
Oreochromis niloticus	Seasonal assessment of MT gene expression pollution	qRT PCR	liver and muscle	Zn, Cu, Pb, Cd	A positive correlation occurred between MT levels and Cu and Pb concentrations	Girgis et al. 2019
Trematomus hansoni	Physiological response of fish against metal toxicity	Silver saturation and qRT PCR	gills and liver	Cu, Cd	MT expression in liver was increased in response to Cd, whereas there was a	Bakiu et al. 2022

						significant downregulation after Cu exposure	
Molluscs	*Lymnaea stagnalis* and *Dreissena polymorpha*	MT response to metal exposure in molluscs that have contrasting feeding behavoir and therefore metal accumulation profiles	Determination of cysteine residues	digestive gland	Al, Cd	MT is up regulated in molluscan responses to cadmium, but not aluminum, exposure	Desouky, 2012
	Crassostrea gigas and *Mytilus edulis*	Influence of metal exposure on metallothionein synthesis and lipid peroxidation in two bivalve mollusks:	pulse polarography	gills, digestive gland	Ag, Cd, Cu, Hg, Zn	Metal exposure decreased the total protein concentrations in the gills of oysters and the digestive gland of mussels and an increase on metallothionein concentrations in the gills of both mollusks were observed.	Géret et al. 2002
	Haliotis discus hannai	Transcriptional responses of metallothionein gene to different stress factors	qRT PCR	hemocyte muscle, gut tentacle, heart testis, eye hepatopancreas gill, ovary	Cd, Co, Cr,Cu, Mn,Ni, Zn	MT gene is heavy-metal inducible, and modulated by immune stimulation, hypoxia, heat shock, and xenobiotics exposure	Lee & Nam, 2016
	Crassostrea iredalei and *Crassostrea glomerata*	Relationship between levels of lead, cadmium and mercury, and metallothionein protein abundance	Polyclonal secondary antibody-based immunoprecipitation	gills, stomach	Pb, Cd, and Hg	MT levels were significantly associated with heavy metal levels	Hertiga et al. 2018
	Corbicula fluminea	Influence of hydrodynamic conditions on	The MT concentrations were calculated	visceral mass, foot, gill and mantle	Cd	The MT concentrations were barely affected by hydrodynamic conditions and were significantly	Geng et al. 2015

	metallothionein response	using a molar ratio of 7 for Cd/MT. The Cd bound to the MT was measured using an ICP-MS.			linearly related to the Cd concentration in the visceral mass	
Anodonta woodiana	Cadmium accumulation and MT biosynthesis	Indirect measurement by Cd estimation through atomic absorption spectrophotom etry	gills, mantle, foot, visceral mass, digestive gland	Cd	MT levels in the gills and mantle of the mussels increased significantly, which were in positive correlation with Cd accumulation	Li et al. 2015
Crassostrea angulata	Relationship between Cd accumulation, oxidative stress and MT	Cytosolic fraction using differential pulse polarography	gills, digestive gland	Cd	Cd accumulation induces MT-like protein but suppresses GSH levels to decrease and inhibits antioxidant enzymes.	Macías-Mayorga et al. 2015
Crassostrea hongkongensis	MT protein expression in response to Cd stress	Polyclonal antibody based immune-precipitation	gill, mantle, digestive gland	Cd	A striking induction of MT protein expression under Cd stress was observed	Xu et al. 2015
Biomphalaria glabrata	MT response patterns due to Cd exposure and temperature stress	qRT PCR	midgut gland	Cd	*MT* gene in the midgut gland of *Biomphalaria glabrata* did not show transcriptional upregulation upon exposure to Cd, in spite of the strong metal accumulation in this organ	Niederwanger et al. 2017

	Mytilus galloprovincialis and *Ruditapes decussatus*	Comparative evaluation of MT induction in response to Cd in two bivalves	Differential pulse polarography using rabbit liver MT as working standard	gills	Cd	MT concentration detected in the gills of M. galloprovincialis increase fourfold while for the clam R. decussatus MT concentrations increased twofold in response to Cd	Bebianno and Serafim, 1998
	Mytilus coruscus	Identification, functional domain study and tissue based, time-dependent expression of MT	qRT PCR	hemocyte, gill, hepatopancreas, gonad, adductor and mantle	Cu, Pb	The time-dependent expression pattern of MT was observed in each treated group, but the highest expression appeared at different times for various concentrations of Cu and Pb.	Ge et al. 2020
Crustaceans	*Litopenaeus vannamei*	Aqaufeed as a route of Hg toxicity of shrimp	qRT PCR	muscle, hepatopancreas and exoskeleton	Hg	MT showed no variation in expressionindicating that the contact with feed containing the observed Hg concentrations were not sufficient to activate gene transcription	Soares et al. 2011
	Litopenaeus vannamei	MT-like proteins and energy reserve levels after heavy metal exposure	Polarographic determination in heat-denaturated cytosol	hepatopancreas, muscle	Ni and Pb	No influence of Ni and/or Pb in MT induction in muscle	Nunez-Nogueira et al. 2010
	Exopalaemon carinicauda	MT-1 gene response to heavy metal ions challenge	qRT PCR	hepatopancreas	Cd, Cu	*EcMT-1* was up-regulated after challenge with Cd or Cu exposure	Liu et al. 2022

Sinopotamon henanense	MT response after exclusive and combined metal exposure	Indirect estimation through Cd determination by atomic absorption spectrophotometer	gills and hepatopancreas	Cd, Zn	MT contents increased after single Cd exposure and also showed a time- and dose-dependence, in a tissue-specific way. Zn showed a limited ability of MT induction both in gills and hepatopancreas	Li et al. 2013
Litopenaeus vannamei	Accumulation and depuration of Cd and its effect on the expressions of MT	real-time PCR	gills, hepatopancreas and muscles	Cd	MT expression in hepatopancreas increased in a time-dependent manner after Cd exposure	Li et al. 2021
Sinopotamon henanense	Bioaccumulation, morphological changes, and induction of MT expression in the digestive system of crab *Sinopotamon henanense* after exposure to Cd	real-time PCR	Hepatopancreas and digestive tract	Cd	The expression of MT mRNA increased with increasing Cd concentration, and MT mRNA level in acute exposure groups was significantly lower when compared to the subchronic exposure groups.	Wu et al. 2015
Macrobranchium malcolmsonni	Glutathione S-transferase and metallothionein levels in prawn exposed to heavy metal	^{203}Hg radio assay	Hepatopancreas, gills	Hg	The induction of MT level was higher compared with control	Yaamuna et al. 2012