Manuscript submitted to: Volume 2, Issue 2, 333-344.

AIMS Environmental Science DOI: 10.3934/environsci.2015.2.333

Received date 22 January 2015, Accepted date 23 April 2015, Published date 27 April 2015

Research article

Modulation of metallothionein, pi-GST and Se-GPx mRNA expression in the freshwater bivalve *Dreissena polymorpha* transplanted into polluted areas

Périne Doyen [1], Etienne Morhain [2] and François Rodius [2,*]

[1] Université du Littoral Côte d'Opale, Institut Charles Viollette, Equipe Biochimie des Produits Aquatiques (BPA), Boulevard du Bassin Napoléon, 62327 Boulogne-sur-Mer, France

[2] Université de Lorraine, UMR CNRS 7360 : Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC), Rue Delestraint, 57070 Metz, France

* **Correspondence:** Email: francois.rodius@univ-lorraine.fr; Tel: +33-387-37-85-09.

Abstract: Glutathione S-transferases (GST), glutathione peroxidases (GPx) and metallothioneins (MT) are essential components of cellular detoxication systems. We studied the expression of pi-GST, Se-GPx, and MT transcripts in the digestive gland of *Dreissena polymorpha* exposed to organic and metallic pollutants. Mussels from a control site were transplanted during 3, 15 and 30 days into the Moselle River, upstream and downstream to the confluence with the Fensch River, a tributary highly polluted by polycyclic aromatic hydrocarbons and heavy metals. Se-GPx and pi-GST mRNA expression increased in mussels transplanted into the upstream site, Se-GPx response being the earliest. These genes were also induced after 3-days exposure at the downstream site. These inductions suggest an adaptative response to an alteration of the environment. Moreover, at this site, a significant decrease of the expression of MT, pi-GST and Se-GPx transcripts was observed after 30 days which could correspond to an inefficiency of detoxification mecanisms. The results are in correlation with the levels of pollutants in the sediments and their bioaccumulation in mussels, they confirm the environmental deleterious impact of the pollutants carried by the Fensch River.

Keywords: *Dreissena polymorpha*; pi-class GST; selenium-dependent GPx; metallothionein; mRNA levels; field study

1. Introduction

Industrial discharges are major sources of pollution for the freshwater aquatic environment.

Mixtures of pollutants produce numerous effects on organisms which can not be predicted exclusively with chemical analyses. To study the impact of chemical pollution the use of different biomarkers has been introduced into monitoring programs, in addition to chemical analyses, in order to evaluate the effects of pollutants on living species. To face pollutant effects, organisms possess cellular detoxication systems like glutathione S-transferases (GST), glutathione peroxidases (GPx) and metallothioneins (MT).

Bivalve molluscs are appropriate species to study the quality of the aquatic environment because they are sedentary and live in sediment or at the interface of water and sediment. They filter large amounts of water to cope with nutritional and respiratory needs. The zebra mussel *Dreissena polymorpha*, due to its abundance, distribution and functional role in ecosystems, had been often used to describe toxic effects in freshwater ecosystems. They are useful bioindicators to study the quality of an aquatic environment using active monitoring: mussels are sampled from a pristine area and caged in different sites for severals durations [1-6]. As transplanted mussels come from an homogeneous population, this approach allows to avoid the biological variability of organisms from various origins.

GST are cytosolic enzymes belonging to the most important phase II biotransformation system. They catalyse the conjugation of electrophilic compounds to glutathione. Among the enzymatic antioxidant system, the glutathione peroxidases can be divided into two types of enzymes: the selenium-dependent GPx (Se-GPx) and the selenium-independent GPx (non Se-GPx). Se-GPx catalyses the reduction of organic and inorganic peroxides like hydrogen peroxide (H_2O_2) while non Se-GPx reduces only organic peroxide [7].

Metallothioneins are low molecular weight proteins with high cystein content. The thiol groups (SH) of cystein residues enable MT to bind heavy metals. They are highly conserved and ubiquitously distributed throughout all organisms. They play a role in homeostatic control of essential metals (Cu, Zn) as they represent metal storage entities ready to fulfill enzymatic and metabolic demands [8]. MT are involved in metal detoxification and also protection against reactive oxygen species [9]. Monomeric (MT-10) and dimeric (MT-20) MT isoforms have been identified in mussels [10]. It was suggested that MT-10 takes part in the homeostasis of heavy metal cations, MT-20 being implicated in the defense against the effects of metals. Assessing pollution using MT is of great interest in the aquatic environment; indeed, invertebrate MT have been valided as useful biomarkers of exposure to metal contamination in molluscs and other aquatic organisms [11].

In order to evaluate the effect of industrial pollution on the detoxification performance of sentinel organisms, we studied the expression of pi-GST, Se-GPx and MT transcripts in the digestive gland of *D. polymorpha* transferred from a control site into the Moselle River, upstream and downstream from the confluence with the Fensch River. This river is located in a former industrial area in the Lorraine Region (France) and is polluted by high amounts of polycyclic aromatic hydrocarbons (PAHs) and heavy metals [12]. MT, pi-GST and Se-GPx coding sequences from the freshwater mussel *Dreissena polymorpha* have been previously identified [13,14]. These genes correspond to biomarkers widely used to evaluate exposure of aquatic organisms to numerous pollutants in the environment [15,16].

2. Materials and Methods

2.1. Experimental animals

Mussels, *D. polymorpha,* were sampled from a reference site, a small canal in the Meuse Basin (France). They were transplanted into the Moselle River upstream and downstream from the pollution source constituted by the confluence with the Fensch River (Figure 1). Mussels were kept in cages during 3, 15 or 30 days. Animals from the reference site served as controls. The digestive gland of control and transplanted mussels were removed immediately after collection, placed in 4 M guanidium isothiocyanate solution (Fermentas Life sciences, Vilnius, Lithuania) and conserved in liquid nitrogen to avoid RNA degradation. Five mussels were used at each site to measure the pi-GST, Se-GPx and MT mRNA expressions.

Figure 1. Location of the upstream and downstream sites in the Moselle River.

2.2. Chemical analysis

Analysis were performed on mussels soft tissues from control, upstream and downstream sites at each time of exposure, and on sediments samples collected at day 30 from upstream and downstream sites. Heavy metals were quantified by coupled plasma mass spectrometry (ICP-MS), atomic emission spectrometry (ICP-AES) or atomic absorption spectrometry (AAS). Polycyclic aromatic hydrocarbons were characterized by dichloromethane extraction followed by liquid chromatography and analysed by gas chromatography-mass spectrometry. The detection limits of these methods are indicated in Table 1.

Table 1. Concentrations PAHs and metals in the sediments of the sites where mussels were encaged (dw: dry weight). The thresholds of average quality sediments correspond to the system established by the French water authorities (Agences de l'Eau, 2003). N.A.: no data available.

Contaminants	Concentration(mg/kg dw)		Quantification limits	Thresholds of average quality sediments (mg/kg dw)
	Upstream	Downstream		
PAHs			(µg/kg dw)	
Naphtalene	0.10	0.80	0.95	
Acenaphthylene	0.52	1.84	0.17	
Acenaphtene	0.14	0.70	0.22	
Fluorene	0.45	1.27	0.12	
Phenanthrene	3.42	5.77	0.17	
Anthracene	1.16	2.61	0.15	
Fluoranthene	7.79	17.51	0.31	
Pyrene	4.76	11.41	0.49	
Benzo(a)anthracene	3.03	7.49	0.18	
Chrysene	2.78	6.98	0.24	
Indeno(1,2,3-cd)pyrene	1.94	5.80	30.00	
Benzo(b)fluoranthene	3.07	8.43	26.00	
Benzo(k)fluoranthene	1.16	3.30	28.00	
Benzo(a)pyrene	1.45	5.37	25.00	
Dibenzo(a,h)anthracene	0.75	2.20	81.00	
Benzo(g,h,i)perylene	1.55	4.67	0.34	
Total PAHs	34.07	86.15		7.5
Metals			(mg/kg dw)	
Chromium	111	758	5	110
Cooper	39	135	1	140
Lead	137	428	0.6	120
Nickel	37	103	5	48
Tin	10	741	0.5	N.A.
Zinc	498	2138	1	460

2.3. RNA isolation

RNA extraction was carried out on 30 mg of digestive gland using the RNeasy Mini Kit (Qiagen, Maryland, USA) following the manufactor instructions. An electrophoresis on agarose gel was carried out in order to check the integrity of the RNAs.

2.4. Primers design

Primers were obtained from Invitrogen (Carlsbad, USA), the sequences are given in Table 2.

They were choosen in the beta-actin, MT, pi-GST and Se-GPx coding sequences available in GenBank or identified in our laboratory (accession no: AF082863, DPU67347, EF194203, EF194204 respectively).

Table 2. Sequences of primers used for amplification of *D. polymorpha* cDNA.

Primers	Sequences (5'-3')
ACT.DF	GGATCTGGAATGTGCAAAG
ACT.DR	CATCCCAGTTGGTGACGATA
MT.DF	ACCTGACTTTACGCATTCAAC
MT.DR	AACTGTGTGACACCATCCCA
GST.DF	TG ACTTGATCAAGGACGCGA
GST.DR	GATAGCACGCACAGGATGTC
GPX.DF	GGAGTTGACGAACGGTCTA
GPX.DR	GATGGGATGGTACAGCTTCT

2.5. cDNA synthesis

Reverse-transcriptions were performed on 1 µg of total RNA using the Maxime RT oligo dT PreMix Kit (iNtRON Biotechnology, Seongnam, South Korea) according to the manufacturer's instructions. At the end of the reaction each cDNA were diluted 10 times in Tris HCl 10 mM (pH 8.3).

2.6. MT, pi-GST and Se-GPx mRNA expression

Four µL of cDNA were diluted from 1:2 to 1:64 to calculate PCR efficiencies which are the following: 1.081 (MT), 1.029 (pi-GST), 0.928 (Se-GPx) and 1.030 (beta-actin).

Amplification of MT, pi-GST, Se-GPx and beta-actin cDNA was carried out by real-time quantitative PCR in a MiniOpticon System (Bio-Rad, Hercules, USA) using GoTaq qPCR Master Mix containing SYBR Green (Promega, Madison, USA) according to the manufacturer's instructions. PCR amplifications were carried out on 4 µL of 8-folds diluted cDNA using 300 nM of beta-actin or MT primers, or 500 nM of pi-GST or Se-GPx primers. The PCR steps consisted of a 10-min initial denaturation at 95 °C followed by 40 cycles of heat denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, polymerisation at 72 °C for 30 s, and a 5-min final extension at 72 °C. Negative controls were performed on non reverse-transcribed RNA.

The normalized folds of MT, pi-GST and Se-GPx transcripts were calculated using the -ΔΔCt method [17].

2.7. Statistical analyses

Statistical analyses were carried out to compare MT, pi-GST and Se-GPx mRNA expression in control and transplanted mussels. The ratios of MT, pi-GST and Se-GPx levels/beta-actin levels were analysed by a t-test (comparison of means) using the R software [18]. As PCR is a geometric reaction, we considered differences as significant if the fold change was ≥ 1.5 and $P \leq 0.05$.

3. Results

3.1. Chemical analysis

The concentrations of pollutants in sediments from upstream and downstream sites are given in Table 1. Total PAHs concentration in the upstream and downstream sites are respectively around 34 and 86 mg/kg of dry sediment. These sites are also contaminated by heavy metals. For example, chromium, lead and zinc concentrations in the downstream site are respectively 6, 3 and 4 times higher than the thresholds of average quality sediments.

The analysis show a bioaccumulation of metals and PAHs in mussels in accordance with the time of exposure (Table 3). The highest levels of almost all metals and PAHs are pointed out in mussels exposed 30 days at the downstream site. For instance, total PAHs, copper and zinc concentrations are 2-times the values in control mussels whereas iron and lead levels are around 10-times higher compared to controls.

Table 3. Concentrations of total PAHs and metals in the soft tissues of control and transplanted mussels (dw: dry weight).

Pollutants	Concentrations in mussels (µg/g dw)						
	Control site	Upstream site			Downstream site		
		3 days	15 days	30 days	3 days	15 days	30 days
Total PAHs	32.93	32.99	50.50	45.60	35.27	67.43	65.22
Cd	0.40	0.33	0.31	0.66	0.40	0.48	0.40
Cu	8.97	9.14	16.68	17.82	12.06	16.99	20.32
Fe	118.30	227.91	374.89	584.96	410.10	638.38	1089.76
Ni	4.93	4.44	4.92	7.54	5.12	5.17	6.27
Pb	0.23	0.34	0.52	1.07	0.82	1.32	2.20
Zn	83.25	88.97	114.45	185.37	103.88	166.04	169.48

3.2. MT, pi-GST and Se-GPx expression patterns

We evaluated the expression pattern of MT, pi-GST and Se-GPx transcripts from *D. polymorpha* in the upstream, downstream sites (Figure 2). Amplifications performed on non reverse-transcribed RNA (negative control) did not produce any amplimer, indicating that PCR products were not amplified from any genomic DNA remaining in the samples.

Mussels transplanted into the upstream site showed an increase of pi-GST mRNA levels after 30 days of exposure, and as soon as 3 days in the case of the Se-GPx compare to the controls. Both

genes were induced after 3-days exposure at the downstream site. A significant decrease of MT, pi-GST and Se-GPx mRNA expression levels was observed at the downstream site after 30 days.

Considering the amounts of pollutants in *D. Polymorpha* soft tissues, no clear correlation was established between genes expression and PAHs concentrations. However, a binomial curve was observed in the case of metals (Figure 3), correlation coefficients of MT, pi-GST and Se-GPx expressions beeing respectively 0.921, 0.726 and 0.997.

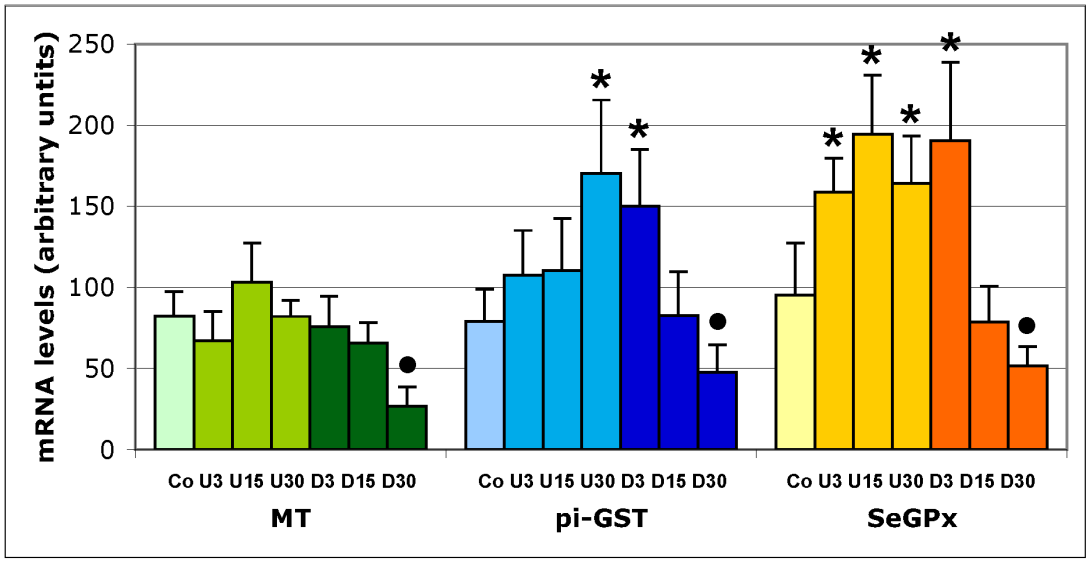

Figure 2. Expression levels of MT, pi-GST and Se-GPx mRNA in the digestive gland of *D. polymorpha* from control site (Co) and transplanted 3, 15 or 30 days into the Moselle River. U and D correspond to upstream and downstream sites. Stars (*) and dots (•) indicate respectively significant increase or decrease compared to control.

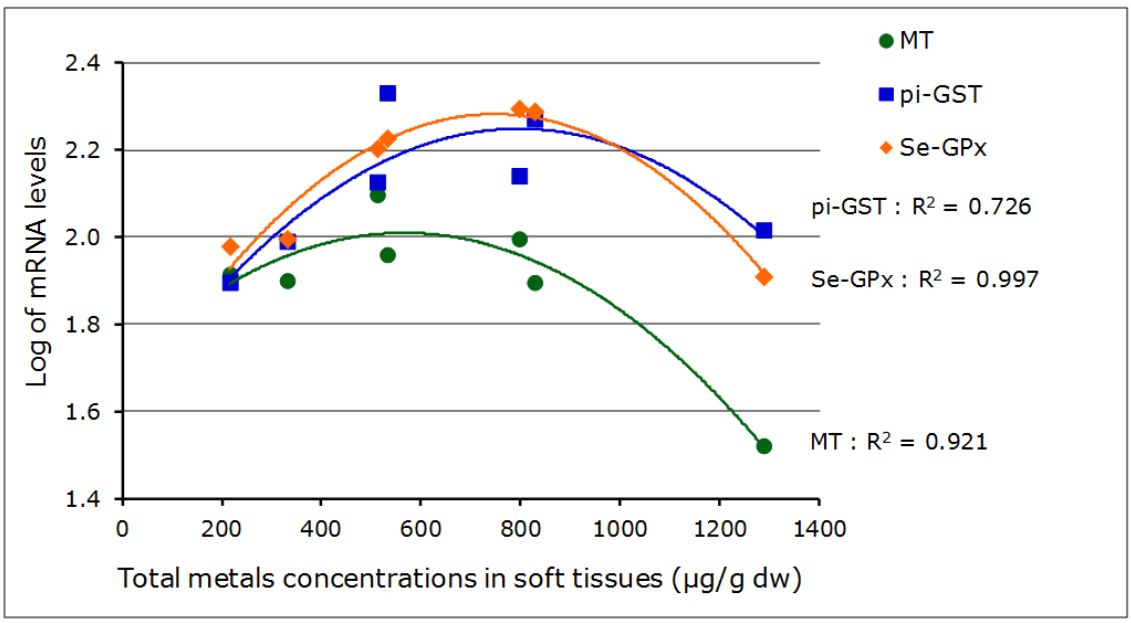

Figure 3. MT, pi-GST and Se-GPx mRNA expressions according to total metals concentrations in mussels soft tissues.

4. Discussion

The Fensch River is the major source of pollution in the present field study. The bed and the banks of this river are highly degraded by urbanisation and industrialisation [19]. A large part of the pollutants is rapidly transferred into the Moselle River. The pollutant concentrations in downstream site (Table 1) largely exceed the thresholds corresponding to sediments of average quality according to the river quality classification system established by the French Water Authorities [20].

MT is a scavenger of heavy metals and free radicals, Se-GPx is a major component of the antioxidant systems and pi-GST is a phase II biotransformation enzyme. As these proteins are involved in different detoxification process, their expression could be used as warning indicator of environmental contamination.

The significant increase of pi-GST and Se-GPx mRNA levels in mussels transplanted into upstream and downstream sites could correspond to an antioxidant stress response. However, MT, pi-GST and Se-GPx expression showed a decrease trend at the downstream site according to the time of exposure. We observed a similar hormesis effect on mussels, *C. fluminea*, exposed to copper and cadmium [21]. The transcript levels of the three genes were significantly lower after 30-days exposure compared to controls. Moreover, pi-GST and Se-GPx expressions at days 15 and 30 were lower compared to day 3. The results of a previous work performed in the Fensch River by our laboratory are in correlation with the present study: freshwater mussels, *U. tumidus*, encaged downstream from the outfall of a cocking plant exhibited lower Se-GPx activities in the digestive gland compared to values at the upstream site [22].

It has been demonstrated that GST and GPx activities of mussels were induced by environmental pollutants [23-28] and that MT synthesis was induced by metal contaminants [29]. However, other studies showed also a decrease of GST, GPx or MT at the protein or mRNA levels. This decrease of MT, pi-GST and Se-GPx transcripts levels in mussel transplanted into the downstream site could correspond to an overwhelming of the detoxification systems; il could also be due to the presence of inhibitors of their expression in higher proportions than activators in the pollutant mixture. Indeed, pi-GST gene expression is under the regulatory control of both enhancer and silencer elements [30].

A decrease of GST activity was observed in a freshwater mussel, *Anodonta cygnea*, exposed to pesticides [31]. Results similar to our study were obtained with the mollusc *Ruditapes decussatus* exposed to three concentrations of municipal effluents for 7 and 14 days: GST activity was induced at the lowest concentration and time-exposure, then decreased in animals exposed to the two other concentrations for 14 days [32]. Such biphasic response was pointed out in the digestive gland of snails exposed to several levels of metals [33].

An exposure to copper, performed in laboratory, showed a significant decrease of MT protein level in limpets *Patella vulgata* [34]. Mussels, *Mytilus galloprovincialis*, exposed to environmentally relevant concentrations of chromium showed a decrease of MT mRNA level [35]. An inhibition of MT expression was pointed out in the oyster *Crassostrea gigas* from an area higly polluted by metals compared to control organisms [36].

Several authors have shown a decrease of GPx activity in organisms exposed to multiple water pollutants. For example, the bivalves *Ruditapes decussatus* collected from sites contaminated by metals and PAHs showed a lower GPx activity compared to control organisms [37]. Heavy metals can also lead to a decrease of GPx activity. Mussels, *Mytilus galloprovincialis*, from the Izmir Bay

revealed a decrase of GPx activity in animals sampled in an heavily polluted area compared to a clean area [38]. These observations showed the relationship between the proximity of the pollution source and the responses of the organisms.

High toxicant concentrations may lead to the inhibition of enzyme activity [39]. For example, a reduced GPx activity could indicate that its antioxidant capacity was surpassed by the amount of hydroperoxides produced by lipid peroxidation [40]. Moreover, a deficiency of the Se-GPx activity reduces detoxification efficiency and reveals a precarious status in the exposed bivalves, suggesting that this inhibition was predictive of toxicity [22]. A reduced capability to neutralise radical oxidative species has been suggested to play a fundamental role in mediating oxidative toxicity and has been often associated with the presence of cellular alterations [41].

The present work showed the deleterious effects of an industrial pollution on the detoxification capacity of aquatic organisms. The results are in accordance with the amounts of PAHs and metals identified in the sediments and with the bioaccumulation of pollutants in transplanted mussels. Detoxification systems were induced in mussels transplanted into the upstream site wich showed moderate levels of pollutants. However, after an early increase, an inhibition of these systems was pointed out in dreissena encaged 30 days in the highly polluted downstream site. These results were likely to correspond to an overwhelming of MT, pi-GST and Se-GPx defense capabilities suggesting that mussels were not able to face the environmental perturbations. A biphasic response was observed according to metals concentration in mussels soft tissues, especially in the cases of MT and Se-GPx (Figure 3), suggesting that, in the present study, metallics pollutants have more impact on dreissena detoxification systems than PAHs. Therefore, it is also highly likely that transplanted mussels are mainly exposed to an oxidative stress. Indeed, previous studies performed at the same sites showed lipid peroxidation and depletion of antioxidant defense systems in transplanted mussels [42].

Our results showed that molecular methods, such as study of detoxification genes expression in sentinel organisms, could be an early warning of environmental perturbations. In the perspective of the present study, an other downstream site more distant to the confluence with the Fensch River could be chosen to assess if the modulation of gene expression is linked to the distance with the source of pollution.

5. Conclusion

We used *D. Polymorpha* to monitor the effects of an industrial pollution on the detoxification performance of sentinel organisms. Mussels were transplanted into the Moselle River upstream and downstream from the confluence with the Fensch River, a tributary polluted by PAHs and heavy metals. Modulation of MT, pi-GST and Se-GPx mRNA levels correlates with the amounts of pollutants (especially metals) and the exposure duration. These results confirm the environmental deleterious impact of the pollutants carried by the Fensch River.

The present study shows that molecular approaches performed on bioindicators such as *D. polymorpha* are essential tools to detect environmental perturbations. Expression studies of pi-GST, Se-GPx and MT at the transcriptional level will also contribute to a better insight of the xenobiotic toxicity in the aquatic environment.

Acknowledgements

This work was supported by the CPER of the Region Lorraine and the French Ministry of Research.

Conflict of interest

All authors declare no conflicts of interest in this paper.

References

1. Roméo M, Hoarau P, Garello G, et al. (2003) Mussel transplantation and biomarkers as useful tools for assessing water quality in the NW Mediterranean. *Environ Pollut* 122: 369-378.
2. Guerlet E, Ledy K, Meyer A, et al. (2007) Towards a validation of a cellular biomarker suite in native and transplanted zebra mussels: A 2-year integrative field study of seasonal and pollution-induced variations. *Aquat Toxicol* 81: 377-388.
3. Viarengo A, Lowe D, Bolognesi C, et al. (2007) The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. *Comp Biochem Physiol C Toxicol Pharmacol* 146: 281-300.
4. Borcherding J (2010) Steps from ecological and ecotoxicological research to the monitoring for water quality using the zebra mussel in a biological warning system, In: Van der Velde G, Rajagopal S and bij de Vaate, *A The Zebra Mussel in Europe*, Leiden/Margraf, Weikersheim: Backhuys, 279-283.
5. Voets J, Bervoets L, Smolders R, et al. (2010) Biomonitoring environmental pollution in freshwater ecosystems using Dreissena polymorpha, In: Van der Velde G, Rajagopal S and bij de Vaate, *A The Zebra Mussel in Europe*, Leiden/Margraf, Weikersheim: Backhuys, 301-321.
6. Chatel A, Faucet-Marquis V, Gourlay-Francé C, et al. (2015) Genotoxicity and activation of cellular defenses in transplanted zebra mussels Dreissena polymorpha along the Seine River. *Ecotoxicol Environ Saf* 114: 241-249.
7. Almar M, Otero L, Santos C, et al. (1998) Liver glutathione content and glutathione-dependent enzymes of two species of freshwater fish as bioindicators of chemical pollution. *J Environ Sci Health* 33: 769-783.
8. Viarengo A, Nott JA (1993) Mechanisms of heavy metal cation homeostasis in marine invertebrates. *Comp Biochem Physiol C Toxicol Pharmacol* 104: 355-372.
9. Roesijadi D (1996) Metallothionein and its role in toxic metal regulation. *Comp Biochem Physiol C Toxicol Pharmacol* 113: 117-123.
10. Lemoine S, Laulier M (2003) Potential use of the levels of the mRNA of a specific metallothionein isoform (MT-20) in mussel (*Mytilus edulis*) as a biomarker of cadmium contamination. *Mar Pollut Bull* 46: 1450-1455.
11. Mourgaud Y, Martinez E, Geffard A, et al. (2002) Metallothionein concentration in the mussel *Mytilus galloprovincialis* as a biomarker of response to metal contamination: validation in the field. *Biomarkers* 7: 479-490.
12. Rodius F, Hammer C, Vasseur P (2002). Use of RNA arbitrarily primed PCR to identify genomic alterations in the digestive gland of the freshwater bivalve *Unio tumidus* at a

contaminated site. *Environ Toxicol* 17: 538-546.
13. Engelken J, Hildebrandt A (1999) cDNA cloning and cadmium-induced expression of metallothionein mRNA in the zebra mussel *Dreissena polymorpha*. *Biochem Cell Biol* 77: 237-241.
14. Doyen P, Bigot A, Vasseur P, et al. (2008) Molecular cloning and expression study of pi-class glutathione S-transferase (pi-GST) and selenium-dependent glutathione peroxidase (Se-GPx) transcripts in the freshwater bivalve *Dreissena polymorpha*. *Comp Biochemi Physiol C Toxicol Pharmacol* 147: 69-77.
15. Snell TW, Brogdon SE, Morgan MB (2003) Gene expression profiling in ecotoxicology. *Ecotoxicology* 12: 475-483.
16. Sarkar A, Ray D, Shrivastava AN, et al. (2006) Molecular Biomarkers: their significance and application in marine pollution monitoring. *Ecotoxicology* 15: 333-340.
17. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* 25: 402-8.
18. R, Developement Core Team (2006) R: A 701 language and environment for statistical 702 computing. R foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0
19. Mazauer P, Matte JL (2001) Qualité du milieu physique de la Fensch – synthèse. Direction Régionale de l'Environnement de Lorraine, France.
20. Agences de l'Eau (2003) Seuils de qualité pour les micropolluants organiques et minéraux dans les eaux superficielles-synthèse, In: Agences de l'Eau, *Système d'Evaluation de la Qualité de l'Eau*, Paris, France.
21. Bigot A, Minguez L, Giamberini L, et al. (2011) Early defense responses in the freshwater bivalve Corbicula fluminea exposed to copper and cadmium: transcriptional and histochemical studies. *Environ Toxicol* 26: 623-632.
22. Cossu C, Doyotte A, Jacquin MC, et al. (1997) Glutathione reductase, selenium-dependent glutathione peroxidase, glutathione levels, and lipid peroxidation in freshwater bivalves, *Unio tumidus*, as biomarkers of aquatic contamination in field studies. *Ecotoxicol Environ Saf* 38: 122-131.
23. Suteau P, Daubeze M, Migaud ML, et al. (1988) PAH metabolising enzymes in whole mussel as biochemical tests for chemical pollution monitoring. *Mar Ecol Prog Ser* 46: 45-49.
24. Livingstone DR, Martinez PG, Michel X, et al. (1990) Oxyradical production as a pollution-mediated mechanism of toxicity in the common mussel, *Mytilus edulis L.*, and other molluscs. *Funct Ecol* 4: 415-424.
25. Livingstone DR (1998) The fate of organic xenobiotics in aquatic ecosystems: quantitative and qualitative differences in biotransformation by invertebrates and fish. *Comp Biochem Physiol A Mol Integr Physiol* 120: 43-49.
26. Sole M, Porte C, Albaiges J (1994) Mixed-function oxygenase system components and antioxidant enzymes in different marine bivalves: its relation with contaminants body burdens. *Aquat Toxicol* 30: 271-283.
27. Chatel A, Faucet-Marquis V, Perret M, et al. (2012) Genotoxicity assessement and detoxification induction in Dreissena polymorpha exposed to benzo[a]pyrene. *Mutagenesis* 27: 703-711.
28. Chatel A, Faucet-Marquis V, Pfohl-Leszkowicz A, et al. (2014) DNA adduct formation and induction of detoxification mechanisms in Dreissena polymorpha exposed to nitro-PAHs. *Mutagenesis* 29: 457-465.

29. Bebianno MJ, Langston WJ (1992) Cadmium induction of metallothionein synthesis in *Mytilus galloprovincialis*. *Comp Biochem Physiol C Toxicol Pharmacol* 103: 79-85.
30. Imagawa M, Osada S, Koyama Y, et al. (1991) SF-B that binds to a negative element in glutathions transferase P gene is similar or identical to trans-activator LAP/IL6-DBP. *Biochem Biophys Res Commun* 179: 293-300.
31. Robillard S, Beauchamp G, Laulier M (2003) The role of abiotic factors and pesticide levels on enzymatic activity in the freshwater mussel *Anodonta cygnea* at three different exposure sites. *Comp Biochem Physiol C Toxicol Pharmacol* 135: 49-59.
32. Kamel N, Jebali J, Banni M, et al. (2012) Biochemical responses and metals levels in Ruditapes decussatus after exposure to treated municipal effluents. *Ecotox Env Saf* 82: 40-46.
33. Radwan MA, El-Gendy KS, Gad AF (2010) Oxidative stress biomarkers in the digestive gland of Theba pisana exposed to heavy metals. *Arch Environ Contam Toxicol* 58: 828-835.
34. Brown RJ, Galloway TS, Lowe D, et al. (2004) Differential sensitivity of three marine invertebrates to copper assessed using multiple biomarkers. *Aquat Toxicol* 66: 267-278.
35. Ciacci C, Barmo C, Gallo G, et al. (2012) Effects of sublethal, environmentally relevant concentrations of hexavalent chromium in the gills of Mytilus galloprovincialis. *Aquat Toxicol* 120: 109-118.
36. David E, Tanguy A, Riso R, et al. (2012) Responses of Pacific oyster Crassostrea gigas populations to abiotic stress in environmentally contrasted estuaries along the Atlantic coast of France. *Aquat Toxicol* 109: 70-79.
37. Geret F, Serafim A, Bebianno J (2003) Antioxidant enzyme activities metallothioneins and lipid peroxidation as biomarkers in *Ruditapes decussatus*? *Ecotoxicology* 12: 417-426.
38. Kucuksezgin F, Kayatekin BM, Uluturhan E, et al. (2008) Preliminary investigation of sensitive biomarkers of trace metal pollution in mussel (*Mytilus galloprovincialis*) from Izmir Bay (Turkey). *Environ Monit Assess* 141: 339-345.
39. Cheung CCC, Zheng GJ, Li AM, et al. (2001) Relationships between tissue concentrations of polycyclic aromatic hydrocarbons and antioxidative responses of marine mussels, Perna viridis. *Aquat Toxicol* 52: 189-203.
40. Remacle J, Lambert D, Raes M, et al. (1992) Importance of various antioxidant enzymes for cell stability. Confrontation between theoretical and experimental data. *Biochem J* 286: 41-46.
41. Regoli F (2000) Total oxyradical scavenging capacity (TOSC) in polluted and translocated mussels: a predictive biomarker of oxidative stress. *Aquat Toxicol* 50: 351-61.
42. Doyotte A, Cossu C, Jacquin MC, et al. (1997) Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater bivalve Unio tumidus. *Aquatic Toxicol* 39: 93-110.