

# Use of metallothioneins as biomarkers for environmental quality assessment on Atlantic coast & Harbour sites (France)



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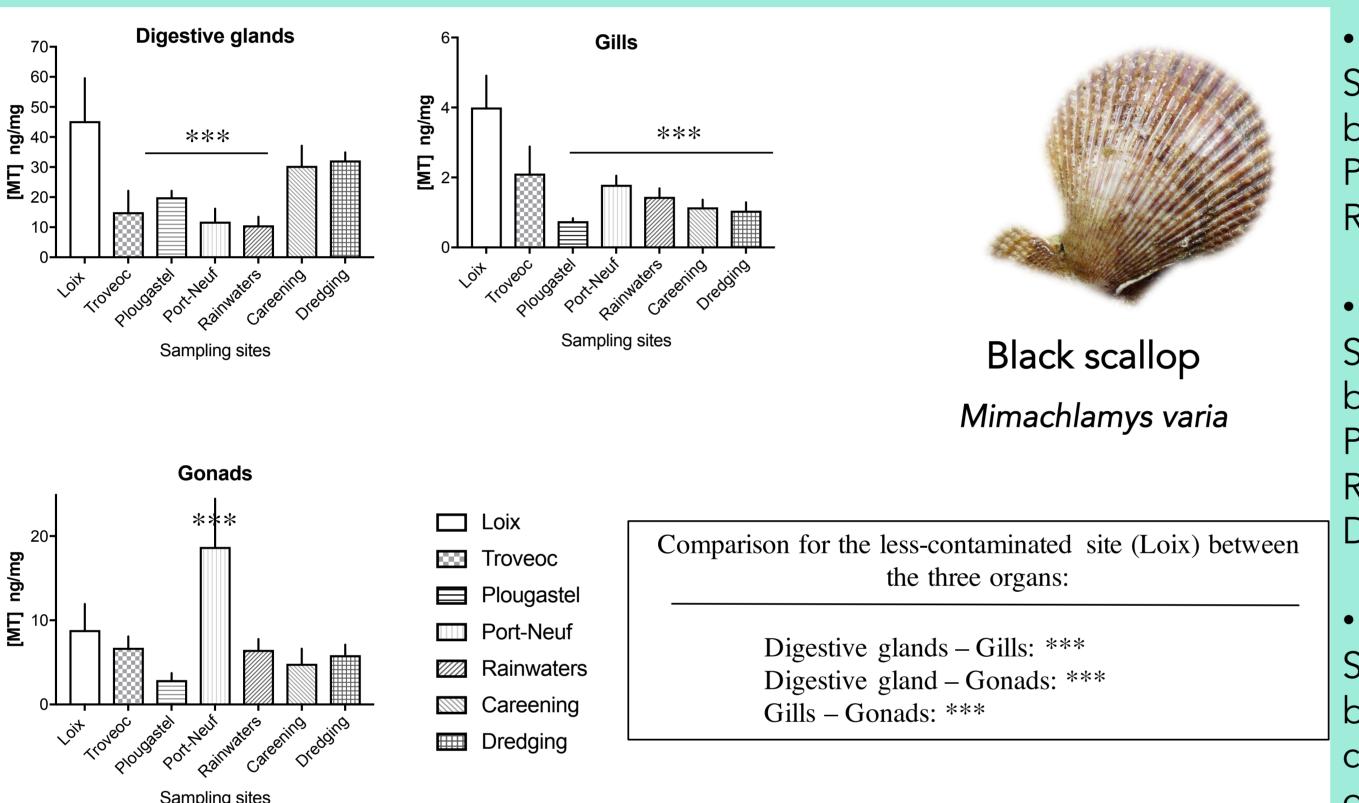
#### Introduction & Context

Human activities development of the Atlantic coast (France) lead to chronic pollution of the environment by a mixture of organic type (pesticides, hydrocarbons, phytosanitary) and inorganic (metals) contaminants. These last years, an environmental regulation with Marine Strategy Framework Directive (MSFD, 2008/56/EC) and OSPAR commission for example, have been developed for the preservation of coastal environments, giving rise to studies of aquatic biomonitoring. The use of biomarkers allows observing the impact of pollutants on coastal species like the marine bivalve Mimachlamys varia. Various biomarkers considered as defence biomarkers have been considered in this research to study oxidative stress by Superoxide Dismutase (SOD), detoxication of organic compounds by Glutathione-S Transferase (GST), lipidic peroxidation with Malondialdehyde (MDA), immune processes with Laccase and Metallothioneins (MTs) involved in uptake, storage, and excretion of metals.

### Study model & Sampling strategy

Black scallop Mimachlamys varia is a filter and sedentary mollusk. Specimen were collected in March 2016 in 13 sites (see map opposite) contrasted by their level of pollution for a distance exceeding 500 Km in the French Atlantic coast (open area) & in harbour (semi-open area). Metallothioneins (MTs), SOD, GST, MDA, laccase which are biomarkers assays were measured to compare responses in several tissues (gills, digestive glands, gonads) of organic and inorganic pollutants effects.

## In situ Metallothionein concentration modulations in three organs



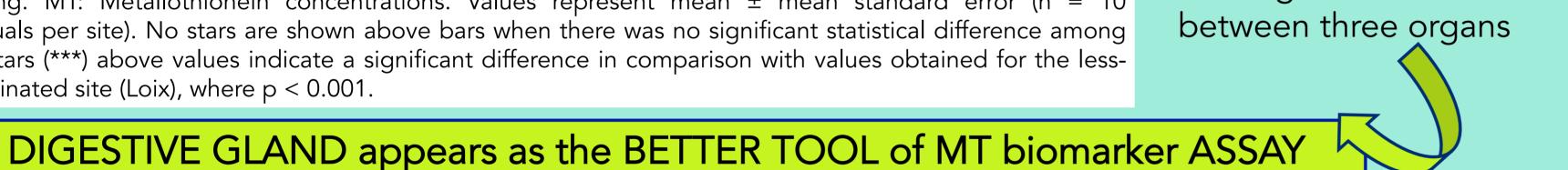
Legend: Biomarkers assessed in the final fraction of Mimachlamys varia digestive glands, gills and gonads (see Material and Methods) for March 2016 from Loix, Troveoc, Plougastel, Port-Neuf, Rainwaters, Careening, Dredging. MT: Metallothionein concentrations. Values represent mean  $\pm$  mean standard error (n = 10 individuals per site). No stars are shown above bars when there was no significant statistical difference among sites. Stars (\*\*\*) above values indicate a significant difference in comparison with values obtained for the lesscontaminated site (Loix), where p < 0.001.

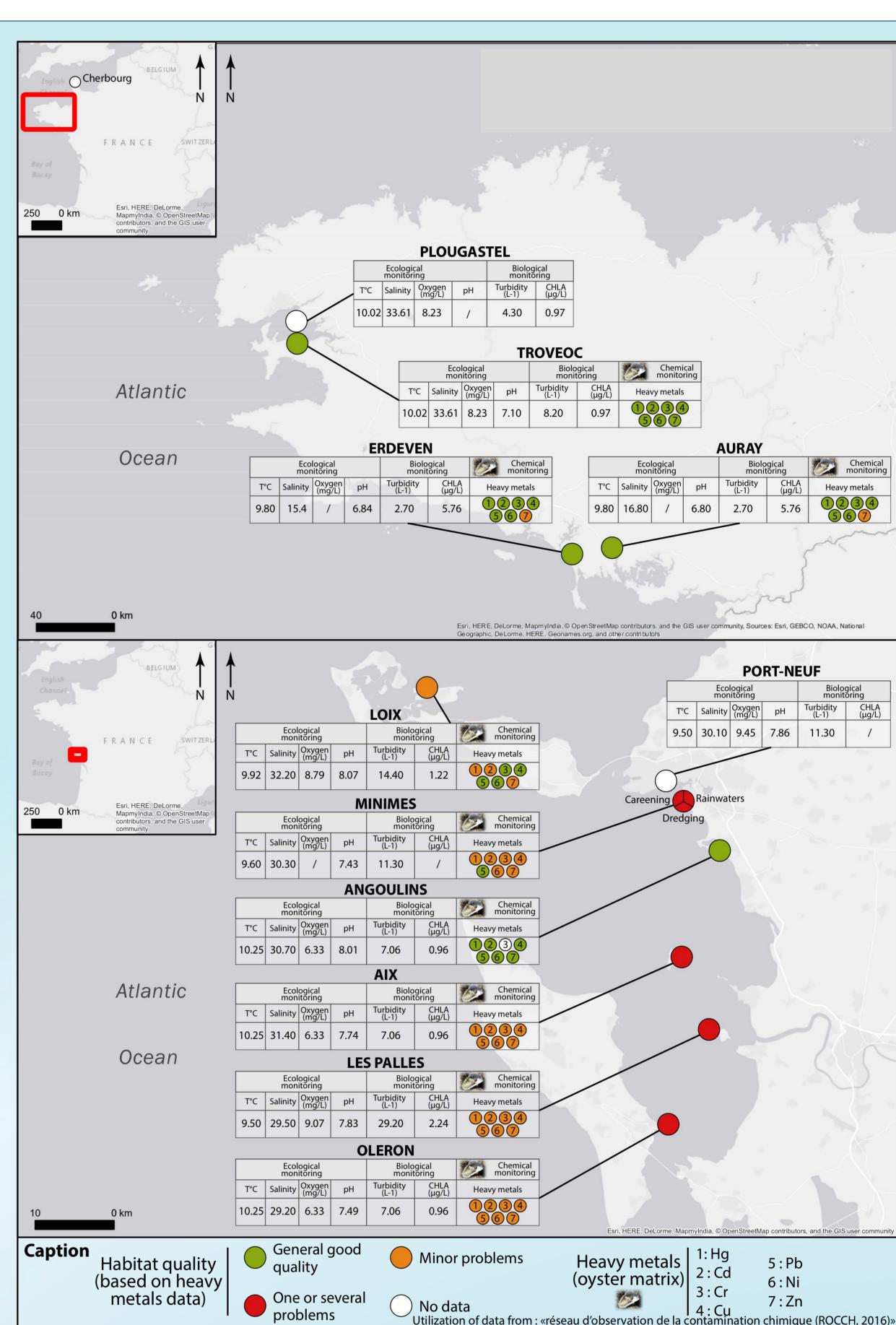
 For digestive gland Significant differences between Loix and Troveoc, Plougastel, Port-Neuf and Rainwaters

 For gills Significant differences between Loix and Plougastel, Port-Neuf, Rainwaters, Careening and Dredging

For gonads Significant differences between Port-Neuf (higher concentration) and all the other sites

Real significant difference between three organs





## In situ Multi-Biomarkers Approach

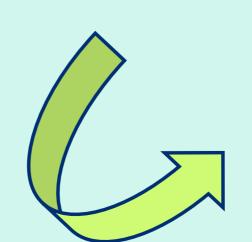
**Table**: Biomarker responses in digestive glands and gills in *Mimachlamys varia* with less-contaminated site Loix (SOD, GST, MDA, Laccase) and study sites Trovéoc, Plougastel, Fairing area, Outlet rainwater, Angoulins, Les Palles, Port-Neuf, Oléron, Aix Isle, Outlet dredging, Erdeven and Auray. Data are expressed with mean  $\pm$  SD (n = 10/site, 3 replicates per individu).

Biomarkers		Tissue	Loix	Plougastel	Trovéoc	Careening	Outlet rainwaters	Angoulins	Les Palles	Port-Neuf	Oléron	Aix Isle	Outlet dredging	Erdeven	Auray
Defence	Superoxide dismutase (U.min <sup>-1</sup> .mg prot <sup>-1</sup> )	Digestive gland	25.5 ± 2.5 a	68.8 ± 7 ** a	226 ± 19 ***	223 ± 17 ***	245.3 ± 14 *** a	281 ± 24 ***	194.2 ± 12 *** a	126.0 ± 10 *** a	87.4 ± 11 ***	111.4 ± 8 ***	21.5 ± 2.3 a	39.2 ± 3.5 a	53.2 ± 4.2 a
		Gills	126 ± 6	172.8 ± 15	54.8 ± 5.1 ***	68.5 ± 7 ***	85.2 ± 6.9 **	137 ± 12	129.2 ± 19		77.7 ± 11 ***	137.0 ± 11	172.1 ± 25 **	100.3 ± 8.2	119.4 ± 12
	Glutathion S-transferase (mU.min <sup>-1</sup> .mg prot <sup>-1</sup> )	Digestive gland	1622 ± 145 a	2127 ± 143 **	1907.4 ± 88 *	1736.4 ± 127	1036.9 ± 156 *** a	206.5 ± 58 ***	324.5 ± 80 *** a	842.9 ± 191 *** a	1103 ± 159 *	544 ± 85 ***	763.6 ± 120 *** a	1818.3 ± 193	2493.4 ± 176
		Gills	403 ± 67	312 ± 57	165 ± 33	342 ± 27	218 ± 21	182 ± 27**	138 ± 29*	384 ± 16	208 ± 30	173 ± 39	103 ± 19 *	$260 \pm 39$	NA
Damage	Malondialdehyde (µM.mg prot <sup>-1</sup> )	Digestive gland	$3.3 \pm 0.2$	6.9 ± 0.6 *** a	7.9 ± 0.8 *** a	$4.3 \pm 0.5$	$3.5 \pm 0.4$	3.3 ± 1	4.1 ± 0.6 a	$3.9 \pm 0.4$	$2.2 \pm 0.2$	$2.7 \pm 0.3$	$3.0 \pm 0.4$	$4.5 \pm 0.6$	3.6 ± 0.3 a
		Gills	$4.7 \pm 1.6$	$3.5 \pm 0.3$	$2.7 \pm 0.3$	$2.8 \pm 0.4$	$3.7 \pm 0.6$	$5.5 \pm 0.5$	$6.6 \pm 1.4$	$3.1 \pm 0.3$	$3.6 \pm 0.9$	$3.3 \pm 1.2$	$3.7 \pm 0.5$	$3.7 \pm 0.4$	7.5 ± 1.9 *
l	_	Digestive gland	$4.6 \pm 0.7$	$4.0 \pm 0.5$ a	$6.0 \pm 0.5$	$6.3 \pm 0.8$	$6.3 \pm 0.7$	8.5 ± 1 ***	8.2 ± 0.6 ***	$5.9 \pm 0.5$	7.7 ± 0.8 ***	7.1 ± 0.6 ***	$4.9 \pm 0.4$	$4.0 \pm 0.4$	2.9 ± 0.3 a
Immuno- modulation	Laccase (U.min <sup>-1</sup> .mg prot <sup>-1</sup> )	Gills	5.2 ± 0.5	7.6 ± 0.5 *	7.7 ± 0.9 *	5.8 ± 1.2	7.0 ± 1	8.4 ± 0.9 ***	9.1 ± 0.9 ***	$5.8 \pm 0.9$	6.7 ± 1.2	5.9 ± 2.7	$4.5 \pm 0.4$	$3.9 \pm 0.6$	6.1 ± 0.9

significant differences between Loix (less-contaminated site) and other sites: \*p < 0.001; \*\*\*p < 0.001, significant differences between digestive glands and gills: a < 0.01, significant differences between digestive glands and gills: b < 0.05

#### Results:

- Specific activities related pollutants
- depend on the sampling sites and organs



- A predominant signal:
   In Digestive glands: MTs, GST & SOD, MDA
   In Both organs (Digestive glands & Gills): Laccase

#### References: • Breitwieser, M., Viricel, A., Churlaud, C., Guillot, B., Martin, E., Stenger, P-L., Huet, V., Fontanaud, A., Thomas-Guyon, H., 2017. First data on three bivalve species exposed to an intra-harbour polymetallic contamination (La Rochelle, France). Comp. Bioch. and

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