Biochemical biomarkers and histopathology in juvenile *Solea senegalensis* for early warning assessment of marine ecosystem health

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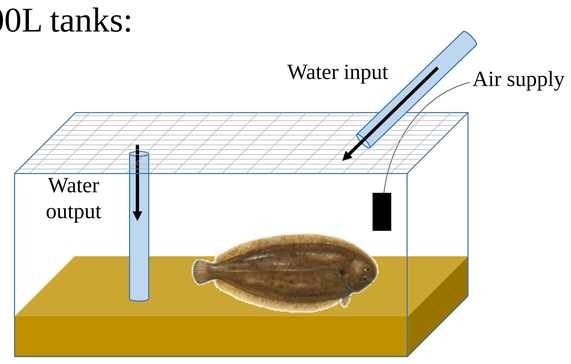
Introduction

Human originated contaminants can appear diluted in estuarine and marine waters or accumulate in sediments. In this context, the use of benthic species for the assessment of biological effects of marine pollution is crucial for marine environment monitoring. In the Bay of Biscay, the common flatfish *Solea* sp., is candidate to be recognized as sentinel species in pollution monitoring programmes. The present study uses juvenile *Solea* sp. (23.24± 1.22cm standard length) exposed to contamination conditions to better understand toxicity processes involved based on biochemical biomarkers and histopathology. *Solea senegalensis* were exposed to three different experimental set ups: (a) contaminated sediments (Sed1; Sed2; Sed3); waterborne metal (Cd) and (c) waterborne organic pollutant (Benzo(α)pyrene). A battery of biochemical biomarkers was analysed in samples of liver and brain: Catalase (CAT), glutathione S-transferase (GST) and acetylcholinesterase (AChE).

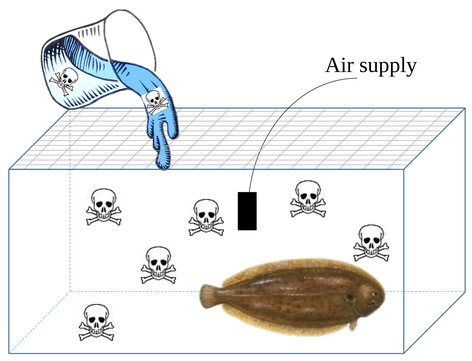
Material and Methods

Experimental setup

After acclimatation, farmed juvenile *Solea senegalensis* were exposed to different treatments in 500L tanks:

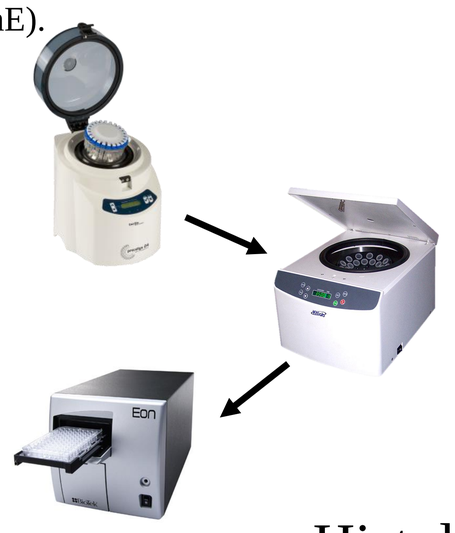


Sediment exposure
Sed1: sampled from a near prestine area (Plentzia)
Sed2: Mix of Sed1 and Sed2
Sed3: sampled from a polluted harbour (Pasaia)



Waterborne Cd exposure
(1µg/l; 10µg/l; 1000µg/l)

Waterborne BαP exposure
(0.1µg/l; 1µg/l; 100µg/l disolved in 0.005%DMSO)



Biochemical analyses

Sample homogeneisation
in 0.1M K-phosphate buffer (pH 7.4) at 4°C, 6500 x 15sec

Centrifugation
at 4°C, 12,000 *g* for 30min.

Biochemical measurement
AChE (in brain); CAT and GST (in liver)

Histological approaches

Liver samples were fixed in 4% neutral buffered formaldehyde; dehydrated in a graded series of ethanol, cleared and embedded in paraffin. 5µm sections were stained with haematoxylin-eosin and analysed under a light microscope.

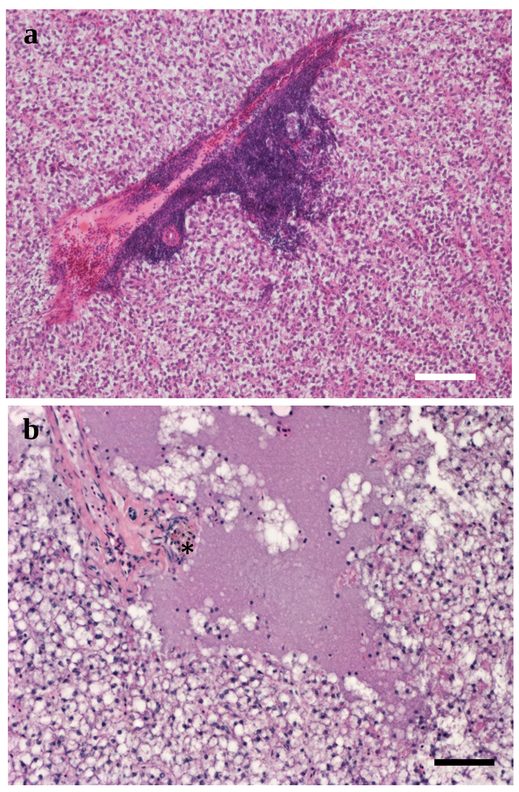


Fig. 1 Hepatic sections of *S. senegalensis* stained with haematoxylin and eosin. (a) Hepatic tissue showing important lymphocytic infiltration surrounding a blood vessel. (b) Severe case of hepatic necrosis and melanomacrophage centres (*). (c) Hydropic vacuolation of biliary epitelial cells. White scale bar: 100µm; black scale bar: 50µm.

Results

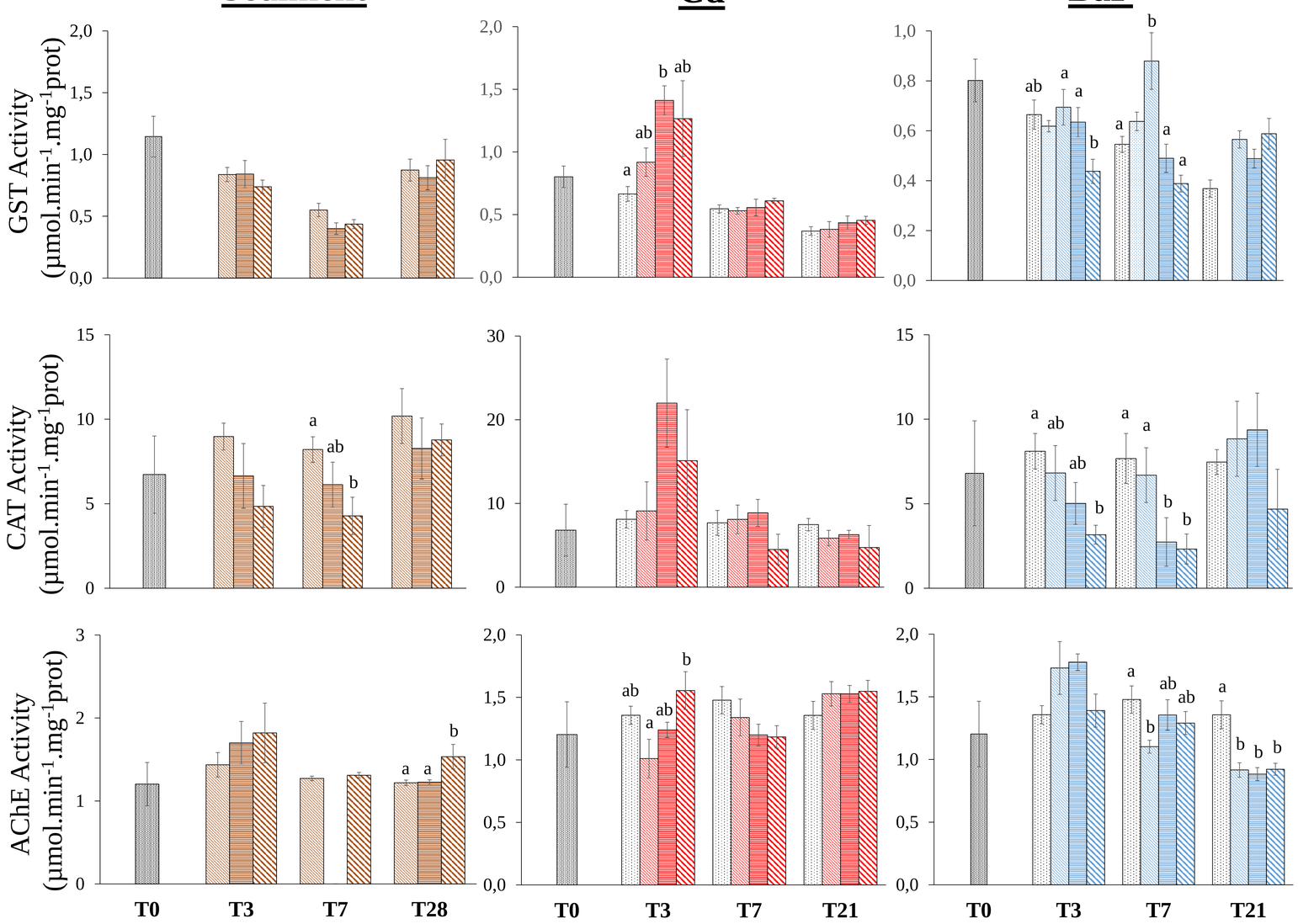


Fig. 2 Enzymatic activities measured in liver and brain of juvenile *S. senegalensis* exposed to different sediments (left); different concentrations of Cd (centre) and BaP (right) for different exposure times. n=6; intervals indicate standard error; letters indicate significant differences between experimental groups of a same sampling time (α=0.05)

Table 1. Prevalence (%) of hepatic alterations identified in juvenile *S. senegalensis* for each laboratory experiment. n=12; MMCs: Melanomacrophage centres; HV: Hydropic vacuolation; CPF: Concentric Periductal Fibrosis. Bold prevalence indicates significant difference with groups of the same experiment determined by the Z-score test (α=0.05).

	Sediment										**Cd**												**BaP**											
	T0	T3			T7			T28			T0	T3				T7				T21				T0	T3				T7				T21			
		Sed1	Sed2	Sed3	Sed1	Sed2	Sed3	Sed1	Sed2	Sed3		Control	Low	Mid	High	Control	Low	Mid	High	Control	Low	Mid	High		Control	Low	Mid	High	Control	Low	Mid	High	Control	Low	Mid	High
Hyperaemia	25	0	8.3	16.7	8.3	8.3	16.7	8.3	16.7	**33.3**	0	25	7.7	0	7.7	7.7	0	0	**38.5**	0	11.1	0	21.4	0	15.4	23.1	7.7	15.4	0	0	15.4	23.1	0	0	66.7	21.4
MMCs	0	0	41.7	33.3	16.7	8.3	8.3	33.3	**50**	25	25	15.4	15.4	30.8	15.4	69.2	53.9	69.2	46.2	55.6	66.7	66.7	78.6	25	61.5	53.9	61.5	61.5	61.5	76.9	61.5	76.9	44.4	57.1	**33.3**	64.3
Lymphocytic infiltration	8.3	0	8.3	0	0	0	0	0	0	8.3	20	23.1	8.3	0	23.1	0	0	7.7	61.5	0	0	**66.7**	53.9	25	15.4	30.8	30.8	15.4	0	15.4	53.9	7.7	11.1	14.3	66.7	7.1
Necrosis	41.7	**0**	41.7	83.3	50	16.7	83.3	50	33.3	83.3	43.8	46.2	69.6	38.5	84.6	76.9	69.2	84.6	84.6	44.4	66.7	33.3	64.3	**43.8**	69.2	61.5	76.9	84.6	61.5	76.9	76.9	61.5	55.6	57.1	66.7	85.7
HV of epithelial cell of bile ducts	0	0	0	0	8.3	8.3	0	8.3	8.3	8.3	0	7.7	0	0	7.7	0	7.7	7.7	0	11.1	11.1	66.7	**0**	0	7.7	0	7.7	15.4	0	**38.5**	0	0	0	0	0	7.1
CPF of bile ducts	8.3	0	0	33.3	16.7	8.3	0	33.3	33.3	25	12.5	23.1	30.8	30.8	38.5	53.9	46.2	38.5	38.5	33.3	55.6	**100**	42.9	12.5	30.8	30.8	16.7	69.2	38.5	69.2	53.9	38.5	66.7	28.6	66.7	50

Conclusions

Biochemical biomarkers measured in *S. senegalensis* permitted to differentiate degree of biological responses to contaminants after short-term exposure. Exposure to contaminated sediments and waterborne toxicants led to reduction of catalase and glutathione S-transferase activities observable after three days. Induction of acetylcholinesterase activity tended to occur from seven days of exposure. In comparison. histopathological responses were detected after long-term exposures showing higher prevalence of liver alterations such as hyperaemia, melanomacrophage centres and necrosis. The present laboratory experiments helped characterizing the impact of pollution in sole at different levels of biological organization and different time scales. This work supports the potential of *Solea* sp. as sentinel species for the assessment of general health status of the marine biota in the context of biomonitoring programmes.

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