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Environment and immunomodulation: effects of the soluble fraction of Norwegian heavy fuel on the immune system of Pacific oyster, *Crassostrea gigas*.

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Introduction: Estuaries and coastal areas are often subjected to much pollution, including pesticides and polycyclic aromatic hydrocarbons (PAHs) produced by anthropic activities. Bay of Marennes-Oléron is an estuarine coastal zone in which there is a high level of aquacultural production and this has adverse effects in aquatic organisms. In fact, PAHs, which are widely spread throughout aquatic ecosystems, have a low polarity and so there is a clear tendency to bioaccumulate in marine organisms (1). The harmful effects of such contaminants on the organism's immune system, leading to a decreased resistance to pathogens, is a relatively new area of research and it still poorly understood.

The aim of the present work was to detect the impact in vivo of PAHs on the immune system of the Pacific oyster, *Crassostrea gigas*.

Materials and Methods:

Pacific oysters:

One hundred and fifty Pacific oysters, in sea water at 11°C in two tanks.

PAHs:

The soluble fraction of heavy fuel of Norwegian type with the 16 most important PAHs of the European pollutant list and the US EPA.

Contamination:

Two groups of 125 oysters in two tanks (1200L).

One contaminated tank, the soluble fraction of heavy fuel at environmental concentration of 760 $ng.l^{-1}$ (± 120$ng.l^{-1}$) in seawater, five days in a closed circuit.

One control tank, in non-contaminated seawater with a closed circuit.

At the end of the contamination, contaminated and control oysters were transferred to two new 1200L tanks supplied with non-contaminated seawater with 0,3 $m^3.h^{-1}$, for one month.

Haemolymph sampling:

At day 0 (transfer day), 3, 14 and 30 post contamination, 15 oysters per group and per day

Analyses:

Flow cytometry: haemocyte viability, forward and side scatter profiles, phagocytosis and mitochondrial activity.

Spectrophotometry: Specific phenoloxidase-like activity (specific PO-like activity) and lysozyme concentration.

Statistical analyses: The normality was verified with an Anderson-Darling test. A F test to normal data was applied to analyse the differences between contaminated and control oysters. P values lower than 0.05 were used to identify significant differences.

Results - Discussion:

Exposure to heavy fuel doesn't modify the percentage of haemocyte populations, mortality and total mitochondrial activity in the Pacific oysters (data not shown).

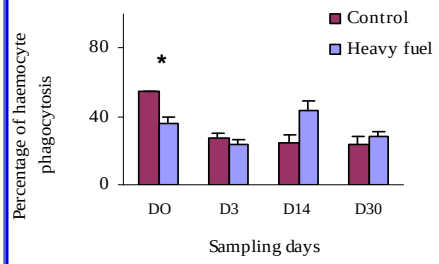

Fig 1: *Percentage of haemocyte phagocytosis during the kinetics studies for control Pacific oysters (■) and contaminated Pacific oysters (■), with * = statistical difference for p≤ 0,05 and n=5 samples.*

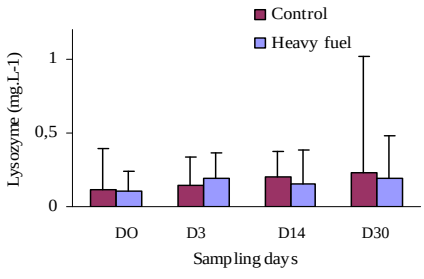

Fig 2: *Lysozyme concentration during the kinetics studies for control Pacific oysters (■) and contaminated Pacific oysters (■), with * = statistical difference for p≤0,05 and n=9 samples.*

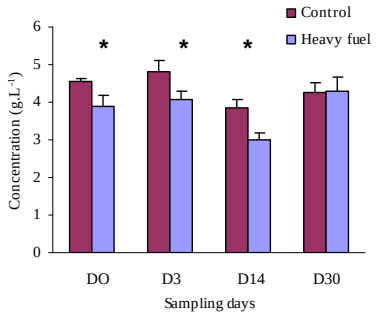

Fig 3: *Specific PO-like activity during the kinetics studies for control Pacific oysters (■) and contaminated Pacific oysters (■), with * = statistical difference for p≤0,05 and n=9 samples.*

A significant reduction in haemocyte phagocytosis of contaminated Pacific oysters was observed at the transfer day (D0) by flow cytometric (fig 1). Phagocytosis would appear to be an immune biomarker which is modulated by the environmental concentration of PAHs (2, 3).

An insignificant modulation of the lysozyme concentration of contaminated Pacific oysters was observed by spectrophotometry at all the kinetics studies (fig 2), but the protocol has still to be optimized.

A significant decrease in specific PO-like activity was observed by spectrophotometry up to day 14 of decontamination, in contaminated Pacific oysters (fig 3). PAHs produced a decrease in phagocytosis (2), which could induce a variation in calcium flow (4). Furthermore, the proPO-PO activation system was calcium dependant (5). There should therefore be a relationship between the diminution of phagocytosis and specific PO-like activity after environmental contamination by PAHs.

Conclusion:

This study demonstrates the early response of humoral factors after an environmental contamination by PAHs in vivo. Finally, **humoral factors would appear to be bioindicators of environmental pollution by PAHs.**

References

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