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Environment and immunomodulation: effects of the soluble fraction of Norwegian heavy fuel on the immune system of Sea bass, *Dicentrarchus labrax*.

Anne Bado-Nilles[1], Claire Quentel[2], Stéphane Le Floch[3], Jacqueline Arzel[3], Michel Auffret[4], Béatrice Gagnaire[5], Tristan Renault[5], & Hélène Thomas-Guyon[1]

[1]LBEM-CNRS Université de La Rochelle, France, [2] AFSSA site de Brest, France, [3]CEDRE, Brest, France, [4]LEMAR-CNRS, Université de Bretagne Occidentale, France, [5]LGP IFREMER La Tremblade, France

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 sea bass, *Dicentrarchus labrax.*

Materials and Methods:

Fish:

Three hundred and forty four sea bass, 144g (± 32), in sea water at 11°C in two tanks.

PAHs:

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

Contamination:

Two groups of 172 fish in two tanks (1200L).

One contaminated tank, the soluble fraction of heavy fuel at the 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 closed circuit.

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

Blood sampling :

At day 0 (transfer day), 1, 3, 9, 14 and 30 post contamination, ten fish per group and per day.

Analyses

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

Spectrophotometric analysis: alternative complement pathway activity (ACH50) and lysozyme concentration.

Statistic 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 fishes. P values lower than 0.05 were used to identify significant differences.

Results - Discussion:

Exposure to heavy fuel doesn't modify the percentage of leucocyte populations, phagocytosis or total mitochondrial activity in the sea bass (data not shown).

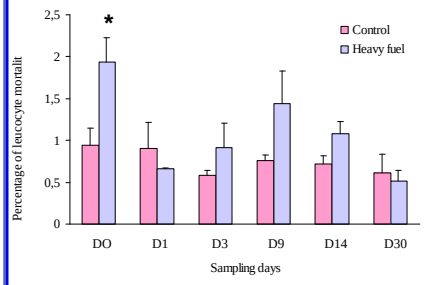

***Fig 1**: Percentage of leucocyte mortality during the kinetics studies for control sea bass ( ) and contaminated sea bass ( ), with * = statistical difference for p≤ 0,05 and n≥5 samples.*

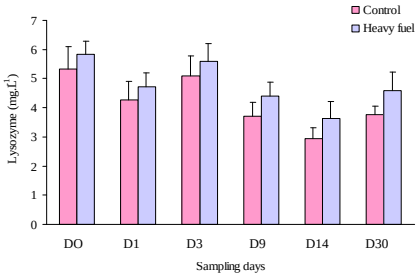

***Fig 2:** Lysozyme concentration during the kinetics studies for control sea bass ( ) and contaminated sea bass ( ), with * = statistical difference for p≤0,05 and n=10 samples.*

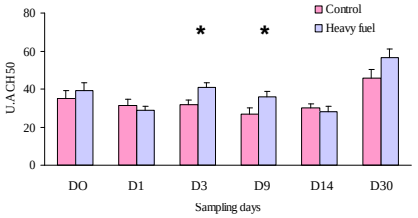

***Fig 3 :** Alternative complement pathway activity during the kinetics studies for control sea bass ( ) and contaminated sea bass ( ), with * = statistical difference for p≤0,05 and n=10 samples.*

A significant reduction in leucocyte viability of contaminated sea bass, observed at the transfer day (D0) by flow cytometry (fig 1), suggests that the PAHs are immunotoxic at the environmental concentration. A "physiological stress", produced by PAHs, can therefore induce a modulation of the organisms' immune system. In fact, damaged cells can be replaced rapidly during the decontamination period, ostensibly leading to a diminution of cellular mortality.

A insignificant increase in lysozyme concentration in contaminated sea bass was observed at all the kinetics studies by spectrophotometry (fig 2).

From day three to day nine of decontamination, a significant increase in ACP50 was observed by spectrophotometry in contaminated sea bass (fig 3). Results suggest that a stress induced by experimental conditions would appear to hide ACH50 activity from transfer day (DO) to day one.

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

(1) Walker *et al.*, 2001. Principles of ecotoxicology, 2nd Edition, Taylors & Francis : 8-9
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