Modulation of immune parameters by chemical environmental pressures in wild populations of European bullhead, Cottus sp. from Vesle Basin (Champagne, France)

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1. Introduction

Champagne is one of the French regions where pesticide uses are particularly high either for agriculture or viticulture. A large number of these chemical substances present in the aquatic environment are able to disturb homeostasis and physiological adaptations of organisms by modulating one or more biological functions. The natural immune functions, which are particularly important in ectotherm vertebrate as fish, are potential target of numerous xenobiotics. In fact, many cellular responses as phagocytosis, cellular mortality and cellular subpopulations distributions are used as immunotoxicity biomarkers in fish. In the present study, these three immunological biomarkers were developed to evaluate immune status of European bullhead, *Cottus sp.*, a sedentary fish, in the presence of various agricultural pressures in the Vesle River system.

2. Materials and methods

Five different sites on the Vesle basin were selected due to their various environmental anthropogenic pressures. All the chosen sites were in possession of wild populations of European bullheads (Figure 1). These sites were located on two rivers, the Vesle (Bouy, Prunay and Muizon) and the Ardre (Serzy and Courtagnon). Situated in a forested sector, Courtagnon was used as a reference site with no direct environmental inputs of chemicals. Serzy station was localized within an intensive viticulture area. Bouy site was highly influenced by intensive cereal farming. Prunay station was impacted by agricultural and viticultural pressures. Finally, Muizon site was chosen due to their position in the downstream of Reims City (Champagne, France).

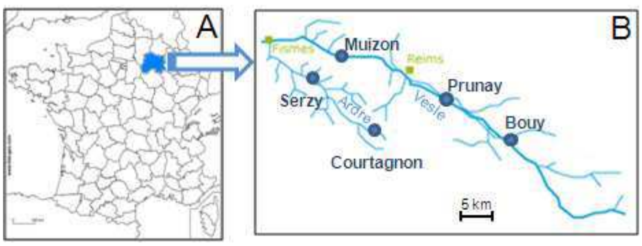

Figure 1: Localization of sampling sites on Vesle river system, with A = localization of sites in France and B = localization of the selected sampling sites in Vesle basin.

During spring, summer and autumn 2010, 20 adult European bullheads were caught by electrofishing in each selected sites. Spleens were interesting due to sampling facilities and to their immune function. After spleen tissue disruption, leucocytes were isolated using density gradient centrifugation and cell suspensions were adjusted to 10^6 cells.mL^{-1}. Leucocyte subpopulation compositions, cell mortality and phagocytosis activity were analysed with a Cyan™ ADP flow cytometer connected to hypercyt®intellicyt (Beckmann Coulter).

3. Results and discussion

3.1. Morphological and seasonal variations

Fish biometric characteristics (length and weight) seem to have no impact on selected immune parameters.

The seasonal variation induced modifications of selected immune biomarkers without discrepancy between studied sites. In summer, fish had normal leucocyte population distributions with roughly 80 % of lymphocytes and 20 % of granulocytes-monocytes. In spring and autumn, lymphocyte proportions were reduced whereas those of granulocytes-monocytes increased. For cellular mortalities, a peack was detected in spring and the bottom value in summer. Phagocytosis activity was significantly higher in fish caught in autumn (Figure 2).

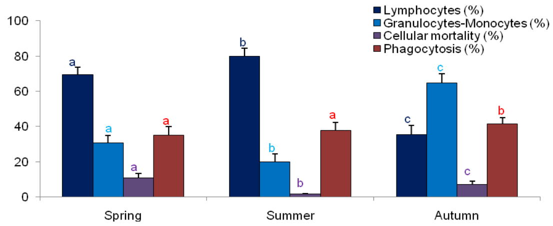

Figure 2: Seasonal variations of immune-related biomarkers for European bullhead. Values correspond to mean of all sites (n = 100) ± ET. For each biomarker, the same letter indicated no statistical difference (p ≤ 0.05).

3.2. Impact of various environmental pressures

Independently of seasonal variations, environmental characteristics of each site seem to disturb selected immune markers (Figure 3). The alteration of splenic leucocyte distributions in Bouy and Prunay sites compared to other stations were rather identical between seasons. The highest cell mortalities were observed in sites situated in areas with intensive agriculture or viticulture (Bouy, Prunay and Serzy). Moreover, phagocytosis activities were significantly reduced in fish caught in the three same sites, excepted in summer for Prunay and in autumn for Bouy. We can notice here that bullhead sampling in autumn at Bouy station suffered from numerous visible pathologies.

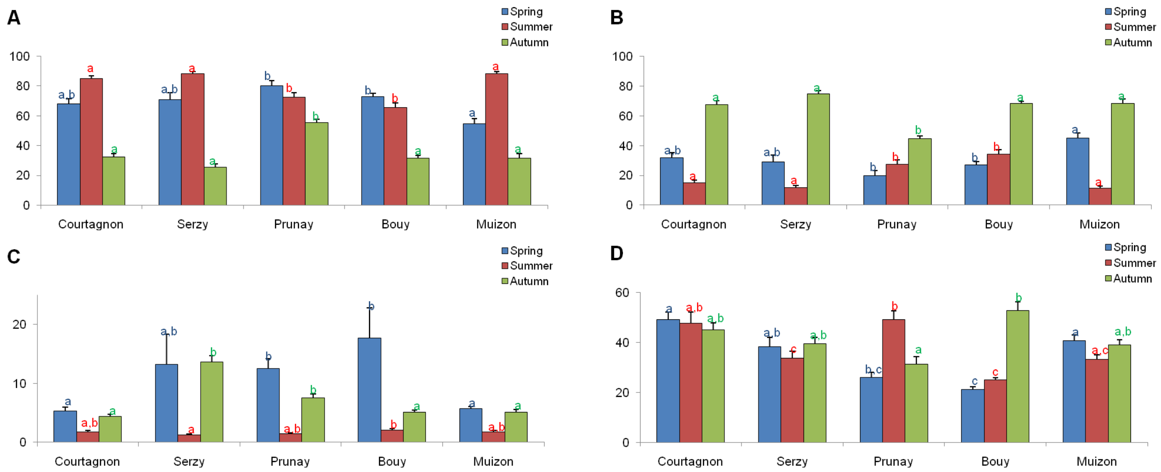

Figure 3: Biomarker responses for European bullhead captured at the different sampling sites and season with A = lymphocytes (%), B = granulocytes-monocytes (%), C = leucocyte mortality (%) and D = phagocytosis (%). Values correspond to mean (n = 20) ± ET. For each season, sites annoted with different letters are statistically significant (p ≤ 0.05).

4. Conclusions

These first results may indicate possible immunotoxicological impacts on bullhead from Vesle basin, of seasonal variations and agri-viticultural practices, with more impact of cereal farming influence than of wine-growing area. Hence, further ambitious studies with multi-annual development, have to complete the present data in order to improve the knowledge of pesticides induced immunomodulations. Moreover, data are needed to better characterize the effects of biotic and abiotic confounding factors on these biomarker base levels and to define their natural variability ranges for assessment of wild fish health.

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