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An *in-situ* study of the impacts of urban wastewater on the immune and reproductive systems of the freshwater mussel *Elliptio complanata*

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ABSTRACT

The goal of this study was to examine the disruptive effects of municipal effluents on the immune and reproductive systems of freshwater mussels. For 30 days, caged mussels were immersed in the Rivière des Mille Îles (Quebec, Canada), 150 m both upstream and downstream from two urban wastewater treatment plants: station F (Fabreville) and station A (Auteuil), which serve the city of Laval. Station F is 12 km upstream from station A. The immune and reproductive statuses of the mussels were thereafter determined. Though the weight/shell length ratio was not affected, the effluent induced mortality up to 60% at downstream sites. Total hemocyte counts increased, and phagocytosis and lysozyme activities were induced at station F, whereas these responses were suppressed at station A. Heterotrophic bacteria levels in mussels were negatively correlated with phagocytosis, showing the importance of this process in defending against infection. Inflammation biomarkers such as nitric oxide and cyclooxygenase activity were the same for all sites but were positively correlated with phagocytosis activity. The production of vitellogenin (Vtg)-like proteins was significantly induced at the site downstream from station A and was strongly associated with phagocytosis. This was further supported through analysis of covariance, of Vtg responses against phagocytosis, revealing that Vtg was no longer induced at the sites upstream and downstream from station A. The data support the contention that Vtg was involved, in part at least, in the immune system in mussels. Both Vtg and immune status are impacted by urban effluents and should be considered when using the Vtg biomarker to search for the presence of (xeno)estrogens in contaminated environments.

1. Introduction

Most domestic and some industrial wastewaters are processed by municipal wastewater treatment plants (OECD, 2004). Over the last 20 years, the release of contaminants harmful to freshwater ecosystems has substantially decreased. Despite these improvements in wastewater treatment, effluents are still considered an important source of pollution for both freshwater and marine ecosystems. In North America, the decline of some freshwater mollusc populations was partly attributed to the degradation of water quality by municipal effluents (Lydeard et al., 2004). Chemicals such as heavy metals, polyaromatic hydrocarbons (PAH), endocrine disruptors (e.g., nonylphenol, ethynyl-estradiol) and pharmaceuticals are potentially toxic to aquatic organisms, especially to their immune and reproductive systems (Sonnenschein and Soto, 1998; Fournier et al., 2000). Despite this situation, relatively few studies have been carried out to evaluate the influence of effluents on the immune and reproductive systems of freshwater mussels. Freshwater bivalves are benthic filter-feeding animals and thus are directly exposed to contaminants from either dissolved or particulate compartments. Moreover, these organisms are sessile, dwell in the sediment–water interface and live for relatively long periods (up to 30 years in some species), which makes them relevant sentinel species for ecotoxicological studies.

The immune system of bivalves is based on the phagocytosis of pathogens (Roch, 1999). The secretion of various humoral factors (agglutinins, cytokines etc.) and other cellular processes such as natural killer (NK)-like cell and lysozyme activity have also been described (Hubert et al., 1997; Malagoli and Ottaviani, 2005). The humoral defences use the production of reactive oxygen species (ROS), agglutinins (lectins), antimicrobial peptides and lysozymes (Canesi et al., 2002a). Lysozymes are a component of the non-specific immune response against infectious bacteria in the bivalves (Mydlarz et al., 2006) and are secreted in plasma by hemocytes after pathogen recognition or a physiological stress (Pipe, 1990; Carballal et al., 1997; Hong et al., 2006; Monari et al., 2007). Nitric oxide (NO) plays an important role in immunity against bacteria and microparasites (Smith et al., 2000; Tafalla et al., 2003; Villamil et al., 2007). NO is produced by NO-synthases in hemocytes during phagocytosis and reacts with hydrogen peroxide to form peroxynitrite, a highly potent bactericide (Fang, 1997; Gourdon et al., 2001). Moreover, as it does in

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vertebrates, NO may act as an immunomodulator and mediates the effects of estrogens and opioids on immunity and inflammation (Galloway and Depledge, 2001; Stefano et al., 2003). Cyclooxygenase (COX) is involved in the first step of arachidonic acid oxidation leading to the production of prostaglandins, which are readily induced during inflammatory reactions in many tissues of the mussel. COX is also involved in the signaling pathways leading to hemocyte bactericidal activity (Canesi et al., 2002b).

Recent studies have shown that endocrine disruption in bivalves occurred after exposure to individual estrogenic compounds and urban effluents (Quinn et al., 2004; Ortiz-Zarragoitia and Cajaraville, 2006). Production of the egg-yolk protein vitellogenin (Vtg) seems to be relatively sensitive to estrogen exposure (Gagné et al., 2001; Aarab et al., 2006; Matozzo et al., 2008). Interestingly, Vtg has recently been thought to be involved in several immune processes in fish, such as opsonization and the phagocytosis of pathogenic bacteria (Li et al., 2008b). Furthermore, this protein is overproduced in males during bacterial infection and shows antibacterial and hemagglutinating properties (Shi et al., 2006). Moreover, recent studies showed that estradiol-17β can modulate immune functions of hemocytes via active estrogen receptors (Canesi et al., 2004, 2006; Gauthier-Clerc et al., 2006). These findings suggest possible interactions between the immune and endocrine systems in aquatic organisms in that the egg-yolk protein Vtg might contribute to immunocompetence.

The objective of this study was to examine the effects of municipal wastewaters on both the immune system and gonad status (reproduction) in caged freshwater mussels in a river subjected to input from two urban effluent discharge points. The immune system was characterized at both the cellular and humoral levels: phagocytosis, NK-like cytotoxic activity, COX activity, NO and lysozyme secretion. In parallel, gametogenic activity was studied in terms of gonad maturity, gonad size (gonado-somatic index) and levels of Vtg-like proteins. An attempt was made to highlight the relationships between the immune response and gonad integrity in experimentally caged mussels exposed *in-situ* to municipal effluent dispersal plumes.

2. Materials and methods

2.1. Mussel handling and exposure experiment

Wild freshwater *Elliptio complanata* mussels were collected in June 2007 in the Rivière Richelieu (Quebec, Canada), which is not subjected to any direct sources of pollution. The animals were then maintained for 3 months in aquariums at 15 °C, with a 16 h-light/8 h-dark cycle. They were fed daily with concentrates of phytoplankton (Phytoplex®) and laboratory-cultured *Pseudokirchneriella subcapitata* algae. At the time of collection, mussels were in ripe and spawning stage. After three months, the gonads were mostly at the post-spawning and undeterminate stage. The mussels were then placed in experimental cages according to a standardized methodology (ASTM, 2001). Briefly, 15 mussels (7.04 ± 8 cm shell length; 24 ± 5 g total weight) were placed in each of 5 cylindrical nets, which were attached to a PVC frame (1 m^2 surface area) covered by a rough net cage for protection from predators.

The exposure experiment took place in the Rivière des Mille Îles, located in the Montréal area (Quebec, Canada). The Rivière des Mille Îles receives treated wastewater from the two treatment plants for the city of Laval, with a population of about 345 000. The city releases its treated municipal wastewaters at two discharge points in the Rivière des Milles Isles: station F (Fabreville) and station A (Auteuil) (Fig. 1). For 30 days in October 2007, when water temperatures were approximately 16–20 °C, two cages were deployed at each study site (station F and station A): one cage at upstream and the other cage was placed downstream the

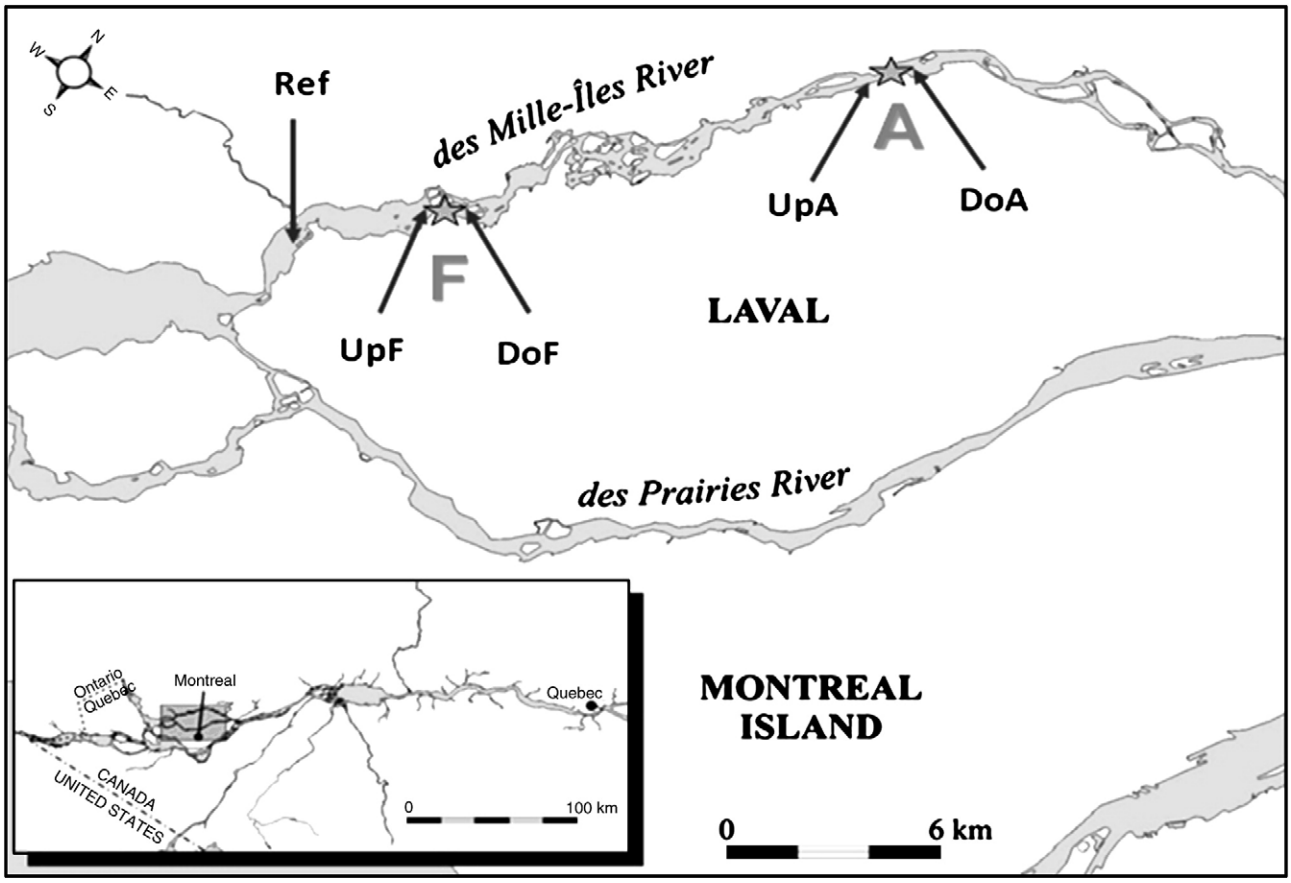

Fig. 1. Map of the study area, showing the wastewater treatment plant point of release F (Fabreville) and A (Auteuil) (stars) and the exposure sites where mussel were immersed in cages (arrows).