PUBLISHABLE SUMMARY

Harmful algal blooms (HAB) have important, deleterious, economic, environmental and human health effects worldwide. The most prevalent of these HAB species in the Gulf of Mexico is the toxic dinoflagellate, *Karenia brevis*. Brevetoxins produced by *Karenia brevis* can have severe negative effects, causing fish, marine mammal and marine bird kills and Neurotoxic Shellfish Poisoning (NSP), via consumption of contaminated shellfish, and respiratory distress via exposure to aerosolized toxins, in humans. *Karenia brevis* is also known to produce multiple compounds other than brevetoxin, that are allelopathic and hemolytic. Together with brevetoxins, these compounds can affect filter-feeders such as bivalves.

Commercially and ecologically important shellfish, including suspension filter-feeding bivalves, are negatively affected by *K. brevis*, yet effects of this alga on particular shellfish species remains poorly understood. Although *Karenia brevis* is already known to cause lethal and sub-lethal effects in some shellfish species, only very few studies investigated cellular and molecular responses in bivalves upon HAB exposure. Exposure of bivalves to HABs may induce an inflammatory response involving hemocytes and oxidative activity. The need to understand cellular and molecular mechanisms during the response of bivalves to HABs appears necessary. The objectives of this project were to characterise the cellular inflammatory responses and oxidative activities of bivalves to HAB exposure, and the potential involvement of hemocytes in transportation of toxin.

Exposure of two bivalve species (*C. virginica* and *M. mercenaria*) to *K. brevis* resulted in alteration of hemocyte parameters, potentially affecting immune capacities of animals and their capacity to deal with a potential secondary stress (environmental modification, pollution or infection). Intense inflammatory responses were observed in several tissues (gills, mantle, connective tissue, digestive gland) along with an increase of circulating hemocytes. Only a mild oxidative stress at cellular or tissue level (light increase of lipid peroxidation in oysters) was detected. Moreover, results were often inconsistent between species as well as maturation stages.

Observed results on hemocytes led to further explore cell physiology of this cell type, thought to be involved in bivalve homeostasis. Indeed, although ROS production in bivalve molluscs is mostly studied for its defence involvement, ROS may also be involved in cellular and tissue homeostasis. Results show that ROS in unstimulated hemocytes do not originate from cytoplasmic NADPH-oxidase, nitric oxide synthase or myeloperoxidase, but from mitochondria. In contrast to mammalian cells, incubation of hemocytes with rotenone (complex I inhibitor) had no effect on ROS production. Incubation with antimycin A (complex III inhibitor) resulted in a dose-dependent ROS production decrease while an over-production is usually reported in vertebrates. In hemocytes of *C. gigas*, the production of ROS seems similarly dependent on both $\Delta\psi_m$ and ΔpH. These findings point out differences between mammalian models and bivalve cells.

Direct *in vitro* exposure of hemocytes to *K. brevis* cells or purified toxin (brevetoxin) did not induce the same pattern of response reported above, with no effect observed on hemocyte parameters. It was hypothesized that in vivo effects of brevetoxin on hemocytes may be indirect, potentially affecting other cells/tissues which, in turn, may alter hemocyte biology and role in homeostatis. We proposed an effect of brevetoxin on digestive cells prior alteration of hemocyte parameters by demonstrating an effect of brevetoxin on the biology of these cells. Altered digestive cells then affected hemocyte parameters without the need of cell-to-cell contact, suggesting secretion of soluble signals in surrounding medium.

Exposure of adult bivalves to *K. brevis* resulted in alteration of gonadic development and reproduction success, potentially altering maintenance and/or growth of local bivalve populations. These results led us to perform complementary *in vitro* and *in vivo* experiments. Direct exposure of gametes and embryos to *K. brevis* cells resulted in reduction of fertilization success and increase in abnormalities and death of embryos. The effects of exposing embryos and gametes to *K. brevis* on survival and growth were dose-dependent and time-dependent. For both clams and oysters, 500 cells.mL^{-1} was enough to result in significant effects. Exposures of adults to *K. brevis* also resulted in delayed ripeness and a reduction of viability and DNA content of isolated sperms. Results of these *in vivo* and *in vitro* suggest that exposure to *K. brevis* during bivalve gametogenesis or during early life stages could affect recruitment and stability of bivalve populations as blooms and spawning periods overlap.

The accomplished research provided fundamental insight into the implication of hemocytes and digestive cells in the fate of algal toxin as well as the inflammatory and oxidative responses of bivalves upon HAB exposures. The present project allowed us determining some key cellular events. Obtained results provided a solid basis to further investigate how the physiological state of bivalves, such as maturation stage, reproduction, stress status can influence toxin accumulation and detoxification from tissues of commercially-important bivalves. This project thus provided useful information for shellfish aquaculture, restoration and management in regions impacted by HABs. This research also contributed to improve shellfish safety, by providing information on bivalve toxification and detoxification processes, which can benefit worldwide consumer health protection.