Toxicity testing of sediment collected in the vicinity of effluent discharges from seafood processing plants in the Maritimes.

Benoit A Lalonde[1], Paula Jackman[2], Ken Doe[2], Christine Garron[1], Jamie Aubé[2]

[1] Environment Canada, Water Science and Technology Directorate, 45 Alderney Dr, Dartmouth, Nova Scotia, Canada, B2Y 2N6, Benoit.lalonde@ec.gc.ca

[2] Environment Canada, Science and Technology Branch, Environmental Science Centre, PO Box 23005, Moncton, New Brunswick, Canada, E1A 3E9

There are over 1100 fish processing plants in Canada and the majority of them discharge untreated effluents directly to marine or estuarine receiving environments. The purpose of this study was to evaluate chemical and toxicological characteristics of sediments near fish processing plant effluent discharges to assess potential impacts of seafood processing effluents on receiving environments. Eighteen sediment samples were collected near effluent discharges of six seafood processing plant outfalls in New Brunswick, Canada in the winter of 2006. Ammonia levels ranged from <0.2 to 3480 µg/g, sulphide levels ranged from <0.4 to 6970 µg/g and redox ranged from -255 to 443 mV. Only one sample had a Microtox™ Solid-Phase Test IC_{50} value below 1000 mg/kg while three samples caused greater than 30 % reduction to amphipod survival. Redox, sulphide and ammonia concentrations were all found to be significantly related to both *Eohaustorius estuarius* survival and Microtox™ (*Vibrio fischeri*) IC_{50}. An increase in sulphide (R^2=0.584; 0.750) and ammonia (R^2=0.478; 0.552) and a decrease in redox

(R^2=0.485; 0.651) were associated with an increase in toxicity to *E. estuarius* and Microtox™, respectively. The highest toxicity to both test organisms, and the most contaminated sediments based on physical/chemical characteristics measured, was observed in samples from Blacks Harbour.

Introduction

The seafood processing industry is of national economic importance in Canada with 75% of Canada's 1100 facilities located in the Atlantic Provinces. The majority of seafood processing plants discharge their effluent directly to marine and estuarine receiving environments without municipal wastewater treatment.

Fish plants in Canada are subject to the Fish Processing Operation Liquid Effluent Guidelines under the Canada Fisheries Act which recommend: screening the effluents for solids removal, well-designed outfall discharging below low tide and the recovery of certain high strength wastes associated with fish meal processing (Environment Canada 1975). In addition, a few provinces (such as NB and BC) have enacted performance-based standards for parameters such as BOD, TSS and oil and grease as part of the provincial permitting process. These targets are often dependant on the size of the seafood processing plants rather than on environmental risks.
Historically, the characterisation of fish plant effluent has focussed mainly on a few conventional parameters such as biological oxygen demand (BOD), total suspended solids (TSS), nitrogen and oil and grease (AMEC 2003, NovaTec 1994). Those studies

indicate that, in general, fish processing effluents contain high levels of BOD, TSS, nitrogen and oil and grease. Four studies (Novatec 1994, Wells and Schneider 1975, Wasave and Kulakarni 2004 and Lalonde *et al*. 2007) have investigated the toxicity of fish processing effluent, and those studies indicate that the effluent is toxic to a variety of aquatic organisms. However, Lalonde *et al*. (2007) also showed that almost a quarter of the effluent tested were not toxic.

There are only a few studies on the effects of seafood processing effluent on receiving environments. The focus of these studies is either on the determination of physico-chemical characteristics of sediments located at the outfall from seafood processing plants (Dale and Dawson 1975, Dominator Marine Services 2001) or on the differences of fish or benthic invertebrate populations at the outlet of estuaries receiving effluent from seafood processing plants, in comparison with non-disturbed estuaries (Dale and Dawson 1975, Thériault 2006). Studies on the potential toxicity of sediment in the receiving environment of seafood processing plant effluent and the potential zone of impact of those effluents have not previously been published. The purpose of this study was to assess both chemical and toxicological characteristics of sediments to evaluate potential impacts of seafood processing effluent to receiving environments.

Methods

Sediment samples were collected from receiving environments of six seafood processing plants in March 2006 (Figure 1). Plants were located in Blacks Harbour, Lamèque, Tracadie, Caraquet, Côte Sainte Anne (Ste Anne) and Cap-Pelé and they processed respectively, Atlantic herring, northern shrimp, snow crab, herring roe, lobster and lobster (raw). The 4L sediment samples were collected by scuba divers at the effluent outfall of the plants (0m) as well as at 10m and 100m distance from the outfalls (along the prevailing current direction) for a total of 18 samples. The only plant in operation at the time of the sediment collection was in Blacks Harbour.

The sediment samples were thoroughly homogenized and subsampled for analysis of sulphide, redox potential (Eh), and ammonia by specific ion electrodes (Jackman and Doe 2006). In addition to the analysis of the sediment samples, the level of ammonia was measured in the overlying water at the start and end of the amphipod survival toxicity test. Two sediments were analysed in duplicate for quality control purposes. A calculation was performed to determine the amount of unionized ammonia based on the test temperature, salinity, and pH in the water samples (Bower and Bidwell 1978). Particle size was determined by Maxxam Analytics Inc based on Walton (1978). Sediment samples were acid digested (H_2SO_4/HNO_3/$KMNO_4$) prior to mercury concentration determination by cold vapor atomic absorbtion spectrometry. The amphipod (*E. estuarius*) test procedure was conducted in accordance with the method described by Environment Canada (1998a) and the Microtox™ (*V. fischeri)* solid phase assay was conducted according to Environment Canada (2002).

Results

Ammonia concentrations ranged from <0.2 to 3480 µg/g, sulphide concentrations ranged from <0.4 to 6970 µg/g and redox ranged from -255 to 443mV (Figure 2). Samples collected at Blacks Harbour (0, 10 and 100m) and Tracadie (0m) had elevated levels of sulphide and ammonia compared to background concentrations found in unimpacted sites in New Brunswick and were described as strongly anaerobic (Jackman and Doe 2006). All samples obtained in Cap-Pelé and Ste Anne (except at 100m) had low levels of ammonia and sulphide and high redox readings. Mercury concentrations in all samples were below the detection limit of 0.06 mg/kg.

Pairwise correlations coefficients and associated Bonferonni probabilities were calculated between ammonia, sulphide and redox values. All three comparisons showed statistically significant relationships with correlation coefficients higher than 0.8 ($p<0.05$). The correlation coefficients were negative for the relationships between redox and ammonia and/or redox and sulphide and the correlation coefficient was positive between sulphide and ammonia.

Figure 2 depicts the high variability in concentrations of ammonia, sulphide and redox in receiving environments at different plants and distances to outfall. Sulphide concentrations decreased with increasing distance from the outfall except at Ste Anne and Cap-Pelé. Ammonia concentrations at Ste Anne also increased with increasing distance from the effluent outfall. Redox values at Ste Anne also decreased with distance, indicating either potential influence from another source or currents which caused greater deposition at 10m and 100m compared with the location of the outfall.

Only at Lamèque did we find the expected increase in redox values with an increase in distance from the effluent outfall. Cap-Pelé samples had the lowest sulphide and ammonia concentrations and highest redox levels of all sites sampled while Black's Harbour samples had the highest sulphide and ammonia concentrations, and the lowest redox values (Figure 2).

Ammonia concentrations in the overlying water at the end of the 10 day toxicity test using the amphipods were higher than the levels measured at the start of the test for all samples, except the Cap-Pelé samples which remained under the detection limit (<0.01mg/L). The highest concentrations and increases in concentrations before and after the ten day toxicity tests were measured in the three samples from Blacks Harbour as well as for the 0m sample from Tracadie.

Sediments from the three sampling stations (distances) in Blacks Harbour and Tracadie had a wide range of mean grain size while the four other locations had similar mean grain size at all three sampling stations (distances). An increase in mean grain size as a function of increasing distance from the outfall was detected for the Tracadie location only. Pearson correlation matrixes with associated Bonferonni probabilities were calculated between mean grain size and each physical and chemical parameter as well as each toxicity result and no statistically significant relationships were detected.

One-way analyses of variances (ANOVAs) were run to describe the relationship between either distance (3 levels) or locations (6 levels) and concentrations of sulphide, ammonia and redox values. Results from the ANOVAs clearly demonstrate that sulphide, ammonia and, redox were significantly different at different locations (p values ranged from <0.001 to 0.07) however no statistically significant differences were detected with distance (p values ranged from 0.877 to 0.952).

Amphipod % survival ranged from 0 to 100% (Figure 3). In Canada, Ocean Disposal Guidelines state that a test will be deemed as "fail" (toxic) if the mean 10 day survival is more than 30 percent lower than that in the control sediment and if this difference is statistically significant (Environment Canada 1998b). Of the eighteen samples collected, 15 were not considered toxic to the amphipod using Ocean Disposal Guidelines (Jackman and Doe 2006). All three samples which were considered toxic were collected in Black's Harbour (Figure 3). There were no amphipod survivors in all three samples (with five replicates per sample) from Blacks Harbour. At all other locations, all samples decreased in toxicity from 0m to 10m and by 100m all of them increased in toxicity again (Figure 3).

Microtox™ IC_{50} ranged from 659 to > 157 000 mg/L (Figure 3). In Canada, Ocean Disposal Guidelines state that a test will be deemed as "fail" (toxic) if the concentration of sample that is estimated to cause 50% inhibition of light production by the bacteria after 10 minutes of exposure is less than 1000 mg/L (Environment Canada 1998b). Of the eighteen samples collected, only one was considered toxic according to Ocean Disposal Guidelines while another sample was very close to the trigger concentration of 1000 mg/L. Both of those samples were collected in Blacks Harbour (Figure 3).

Sediment samples collected in Lamèque and Caraquet decreased in toxicity to Microtox™ with increasing distance from the outfalls of those seafood processing plants (Figure 3). All samples from Cap-Pelé had IC_{50} larger than 140 000 mg/L while the lowest IC_{50} (most toxic) in samples from Ste Anne was obtained at 100 m distance of the outfall (Figure 3).

One-way ANOVAs were run to describe the relationship between either distance (3 levels) or locations (6 levels) and toxicity results. Results clearly demonstrated that location ($p=0.000$-0.004) but not distance ($p=0.727$-0.761) from the outfall had a statistically significant effect on toxicity on amphipods and Microtox™ respectively.

Simple regressions between Microtox IC_{50} or amphipod (%) survival and the physical and chemical parameters yielded six statistically significant relationships (Table 1). Regression coefficients for the independent variables were negative for ammonia and sulphide, indicating an increase in toxicity with increasing ammonia or sulphide concentrations. Regression coefficients for redox were positive (Table 1), indicating a decrease in mortality with increasing redox values. For both toxicity tests, the regressions using sulphide as the independent variable had the highest R^2 value while regressions with ammonia for independent variable regressions had the lowest R^2 (Table 1). This demonstrated that sulphide concentrations explained the highest proportion of the variability of the dataset and also that there were other factors which contributed to the toxicity since a proportion of the variability of the dataset remained unaccounted for.

Simple regression between amphipod survival and ammonia concentration in water overlying the test sediment at the end of the toxicity test was also statistically significant ($p<0.001$) with a $R^2=0.557$. This indicates that unionized ammonia concentration in water overlying the test sediment was a significant contributor in explaining the variability in amphipod survival but there were other unknown factors involved. The regression coefficients and R^2 were very similar to those of the regression between the amphipod toxicity test and the concentration of ammonia in the sediment (Table 1).

Discussion

Fish plant effluents are usually characterized by high BOD, nutrients, suspended solids and oil and grease (AMEC 2003, NovaTec 1994). In general, environments which receive high amounts of nutrients (such as phosphorous and nitrogen) from effluents may experience an increase in the rate of primary productivity and this may contribute to local eutrophication. Eutrophication has been shown to cause major changes in species composition, structure and function of marine communities over large areas (Cloern 2001). Furthermore, when the sediment's capacity to assimilate organic inputs is exceeded and the sediment becomes anoxic, the sediment biogeochemistry will be altered towards a system dominated by anaerobic forms of metabolism and toxic degradation products (hydrogen sulphide and ammonia) which can be released into the environment and affect aquatic ecosystem health (Gowen *et al*. 1988; Holmer and Kristensen 1992). High levels of ammonia or sulphide in sediment have shown adverse effects in laboratory tests and a poorly developed benthic community in the field (Ankley *et al.* 1990). However, both ammonia and sulphide are non-persistent chemicals which can be readily transformed into non toxic nitrate and sulphate when in contact with aerated seawater (Stronkhorst *et al.* 2003). Modig and Olafasson (2001) state that as long as oxygen in the overlying water of the sediment is kept high, amphipods seem to avoid the high concentrations of sulphide in sediment by creating microhabitats (bioturbate the sediment) where sulphide is rapidly oxidized. They hypothesize that the amphipods can detect these areas where sulphide has been oxidized which leads to an aggregation in this area.

The six fish processing plants sampled in this study have been found to discharge effluents high in nutrients (Morry *et al.* 2006) which could be expected to contribute to eutrophication of the receiving environment over the long term. In fact, results indicated that sediment in the vicinity of most plants (except for the plant in Cap-Pelé) exhibited higher concentration of sulphide than are found in two other bays of New Brunswick (Bastien-Daigle *et al.* 2007). In the Bastien-Daigle *et al.* (2007) study, the authors measured average concentration of sulphide in two bays of New Brunswick as 159 to 314 μm and these bays were not subjected to fish farms nutrient input. In comparison, our study sites (except Cap-Pelé) had concentrations ranging from 552 to 20 800 uM (μg/g converted to μM). In comparison, bays located in the southwest region of New Brunswick and which are subjected to fish farms organic enrichment have been shown to have sulphide concentrations in the range of 4240 to 21600 μm (Wildish *et al*. 2001). The concentrations measured in some of our sample sites are very similar to those levels detected in the Wildish *et al.* (2001) indicating hypoxic and anoxic conditions of the sediments which were likely due to the fish plant effluent discharge.

In the Maritime Region benthic conditions below salmonid aquaculture sites using redox and sulphide performance-based standards (PBS) have been developed by Wildish *et al*. (2001). Using both parameters (sulphide and redox) of the PBS, 11 of the sediment samples are considered oxic, while additional 3 samples are considered only oxic in the redox parameter while the sulphide parameter designates them as hypoxic. One sample is considered hypoxic/anoxic while two samples are designated completely anoxic (Blacks Harbour 0 and 10m). The results from this study suggest that the performance based standards might be useful as a surrogate to sediment toxicity for fish plant discharges testing as only the three samples from Black's Harbour showed high toxicity to both amphipod and Microtox and all three were described as hypoxic/anoxic and anoxic by the PBS.

Some of the data indicated greater contamination at sites 10m or 100m from effluent discharges points then at 0m. Other sources of nutrient enrichment were sought in an effort to explain the higher levels of contamination, however no other significant discharges in the study areas were found. It is possible that the effluent solids could be deposited some distance from the source, due to tidal influence, current patterns, salinity or thermal variations. These factors have been considered very important in study designs for environmental effects monitoring of discharges to marine environments from mining and pulp and paper mills in Canada (Environment Canada 2005). Previous studies have also shown the importance of oceanographic characteristics (current, tidal amplitude, temperature, etc.) as important predictors of impacts to the receiving environment from effluents (Commons *et al.* 1996, Purnama and Kay 1999). Receiving environments which receive nutrient rich discharges and which are partially enclosed or have poor flushing rates have been shown to retain greater concentrations of nutrients than dynamic systems (Brodie 1995). This is probably the cause of the high amounts of sulphide and ammonia in Blacks Harbour which, although it has high tidal amplitude (over 7m registered in nearby Back Bay), has an outfall at the end of a narrow channel which might not have a high flushing rate. A breakwater in place in close proximity to the outfall in Ste Anne may have caused the effluent to settle at the 10 and 100m sampling locations. Mironova and Murajova (2007) found that the presence of a breakwater located close to the outlet of a sewage discharge caused settling of organic matter from the discharge.

The species processed or type of processing performed at the plants might also influence the degree of environmental degradation caused by fish processing effluent releases. Lalonde *et al.* (2007) showed significant differences in nutrient loads from plants processing different species or even processing the same species but with a different processing technique. The effect of species processed on nutrient loading and sediment eutrophication or toxicity could not be measured in this study since most plants processed different species. The effect of processing type on sediment toxicity is apparent in the differences in sediment toxicity measured at Cap-Pelé (raw lobster), compared with Ste Anne (cooked lobster). Sediments from Ste Anne were higher in toxicity, indicating that processing type could influence sediment quality. This is supported by evidence from Lalonde et al. (2007), which described significant differences in effluent quality from one processing stream to another. However other factors such as tides, current patterns and salinity and temperature variations between the two locations might also explain some of the differences detected in this study.

Of all the samples tested for toxicity, only samples from Blacks Harbour were considered toxic according to the Canadian Ocean Disposal Guidelines (Figure 3). However only Blacks Harbour was sampled while the plant was in operation. The other five processing plants are seasonal and were not processing at the time of the year when sampling occurred. Toxicity of sediments at those plants which are closed for the season could be expected to be higher during times of peak operation.

The simple regressions between physical and chemical parameters and sediment toxicity (Table 1) revealed weak but statistically significant relationships, suggesting that at least some of the toxicity is the result of nutrient loading in the receiving environment. However the moderate R^2 values suggest that there may be more factors that might have contributed to the toxicity such as chemical constituents not measured in this study. For example, persistent anthropogenic contaminants (PCBs, PBDEs, mercury, organochlorine pesticides) previously detected in the receiving environment of fish plants (Ernst *et al.* 1982, Lalonde *et al.* 2007, Carawan *et al.* 1979). Other chemicals, which are known to compose part of fish plant processing effluent, such as cleaners, degreasers, disinfectants and sanitizers. (Lalonde *et al.* 2007) could contribute to the toxicity as well. From the regression/ANOVA analysis conducted, sulphide appeared to be the strongest predictor of toxicity to *E. estuarius* and Microtox™. This relationship is supported by Moller *et al.* (1994) and Wang and Chapman (1999) who respectively reported significant relationships between Microtox™, invertebrates and sulphide levels in sediment.

The relationship between ammonia concentration in sediment and toxicity had the lowest R^2 but still was statistically significant in our study. In contrast, a study by Anderson *et al.* (1998) found no statistically significant relationships between survival of the amphipod *R. abronius* and concentrations of ammonia in sediment, although the authors concluded that mortality in samples with ammonia concentrations greater than 4 µg/g (3% of their samples) could have been due to ammonia. Thirteen of the eighteen sediment samples in our study had ammonia levels above 5 µg/g. Interestingly our results show some conflicting responses of amphipod survival as a function of ammonia concentrations since the samples taken at Black's Harbour at 100 m distance had levels of ammonia from 68.2 to 71.9 µg/g and 0% survival while the sample from Tracadie (at 0m) had levels of ammonia of 139 µg/g and an 80% amphipod survival rate. These kinds of results are reflected in the R^2 from the regression output which shows that a good proportion of the variability of amphipod survival is not explained by ammonia concentration. Jackman and Doe (2006) calculated an LC_{50} of 12.6 µg/g for ammonia-N in sediment for *E. estuarius*. These results are not fully corroborated by our dataset since there are a few samples which had concentrations of ammonia in sediment higher than the LC_{50} value calculated by Jackman and Doe (2006) but the survival rate for amphipods was well above 50% (Tracadie at 0m with an ammonia concentration of 139.9 µg/g and a survival rate of 80%).

Un-ionized ammonia concentration of the overlying water measured at the start and end of the ten day toxicity test was found to correlate negatively with amphipod survival. However only one sample in this study (from Blacks Harbour) exceeded published LC_{50} concentration for porewater unionized ammonia which had a range of 0.7-3.48 mg/L (Moore *et al*. 1997, Kohn *et al.* 1994). All others samples in this study had overlying water un-ionized ammonia concentrations which were less than 0.5 mg/L. Jackman and Doe (2006) calculated an LC_{50} of 2.2 mg/L for undissociated ammonia in overlying water, for *E. estuarius*. The maximum concentration for undissociated ammonia in this study was 2.52 mg/L in a sample from Blacks Harbour which also had 100% mortality to the amphipod. The two other samples from Blacks Harbour had undissociated ammonia concentrations at the end of the amphipod toxicity test of 0.4 mg/L as well as 100% mortality to the amphipod. These results again support the conclusion that there are other factors (such as sulphides) which contribute to the toxicity of sediments near fish plant effluent discharges. Carr *et al.* (1996) stated that if significant correlations exist between ammonia, sulphide, and bioassays endpoints, but the concentrations of these factors rarely equalled or exceeded the concentrations expected to cause toxicity according to established sediment quality guidelines, it would be reasonable to conclude that other factors are contributing to the toxicity.

This study has established the relationships between effluent discharge from fish processing plants and the resulting toxicity of sediment in the receiving environments at six New Brunswick locations. To enhance the dataset, a benthic invertebrate population and community survey should also take place at those locations to establish the impacts of those sediments to the organisms living in-situ. Furthermore, sediment toxicity testing should be expanded to survey other facilities which process different species as well as to ensure that sampling is carried out during peak processing season.

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Table 1 - Simple regressions between toxicity results and physical and chemical parameters.

Dependent	Independent	N	Coefficient	F ratio	p	R^2
Log_{10}(Microtox IC_{50})	Log(NH_3)	18	-0.551	19.710	0.000	0.552
Log_{10}(Microtox IC_{50})	Log(sulphide)	18	-0.580	48.110	0.000	0.750
Log_{10}(Microtox IC_{50})	redox	18	0.003	29.851	0.000	0.651
Arcsin(Amphipod%survival)	Log(NH_3)	18	-19.151	14.679	0.001	0.478
Arcsin(Amphipod%survival)	Log(sulphide)	18	-19.092	22.429	0.000	0.584
Arcsin(Amphipod%survival)	redox	18	0.024	15.046	0.001	0.485

Table 1 depicts the results from the regression analysis between the toxicity tests results as the dependent variable and the physical and chemical parameters of the sediment as independent variables.

Figure Captions

Figure 1. Map of New Brunswick (Canada) showing sediment sampling locations (●)

Figure 1 shows the locations of the sediment sampling stations in the vicinity of fish processing plants in New Brunswick, Canada.

Figure 2. Relationships between sediment sampling locations and distances as a function of ammonia, sulphide and redox.

Figure 2 depicts the concentrations of ammonia, sulphide and redox levels in sediments collected in six different locations and three different distances (0, 10 and 100m) of fish processing plants in New Brunswick, Canada.

Figure 3. Toxicity testing results as a function of distance from effluent discharge at 6 seafood processing plant locations

Figure 3 represents the toxicity tests results (on Microtox™ and amphipods) based on sediments collected in six different locations and three different distances (0, 10 and 100m) of fish processing plants in New Brunswick, Canada.

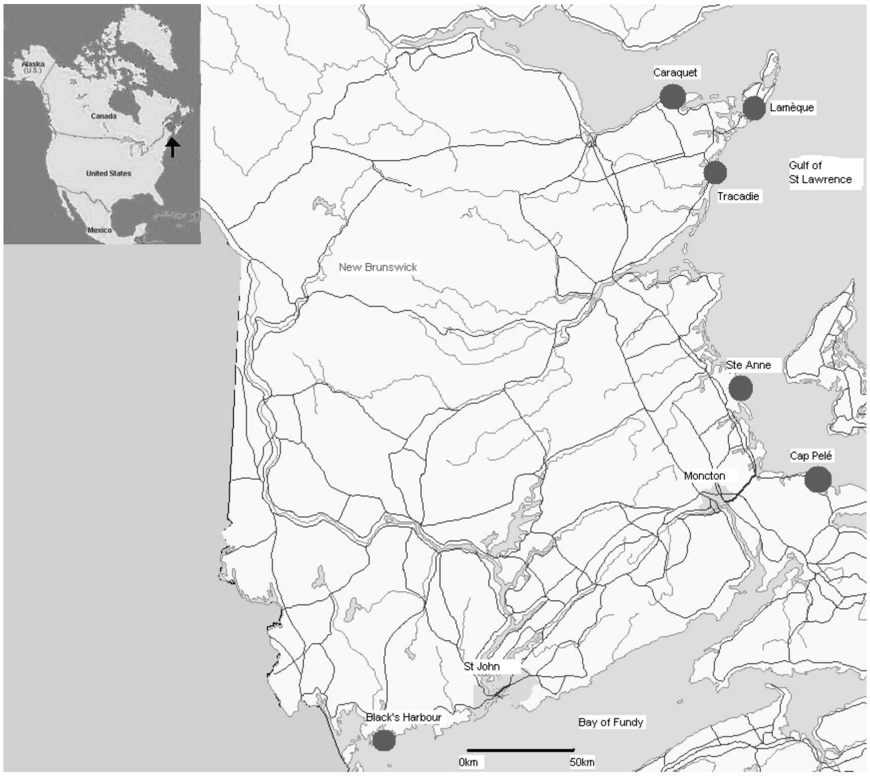

Figure 1. Map of New Brunswick (Canada) showing sediment sampling locations (●)

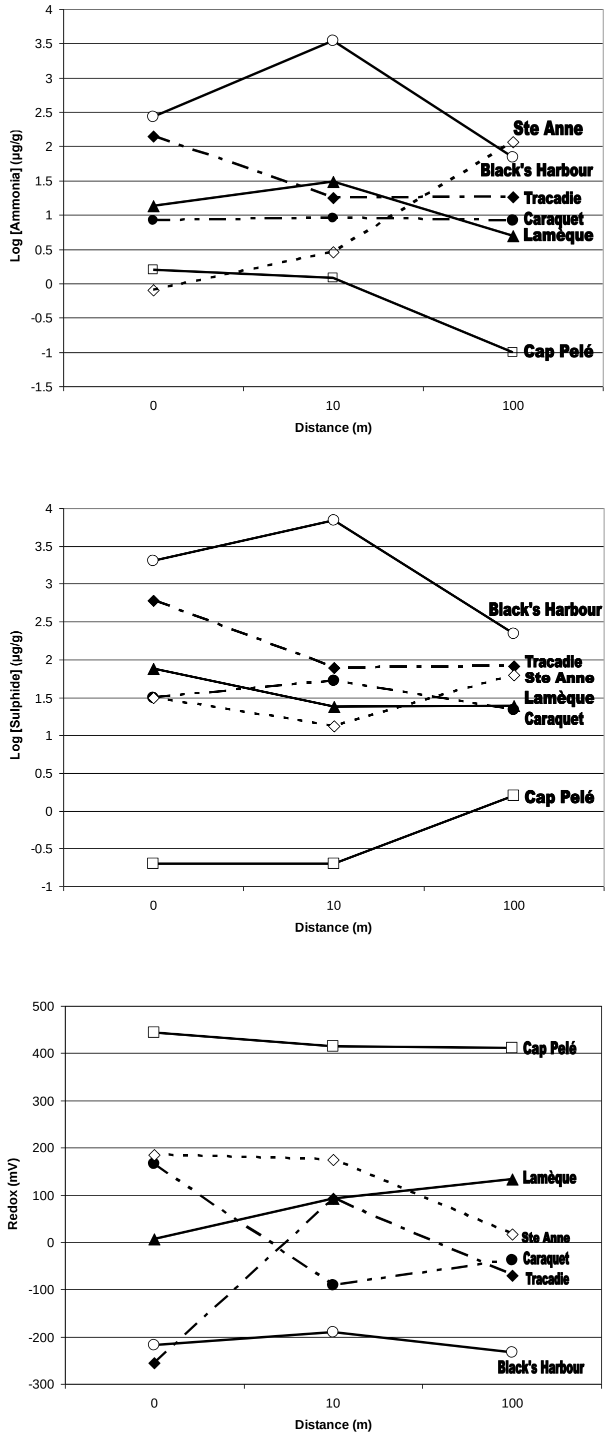

Figure 2 - Relationships between sediment sampling locations and distances as a function of ammonia, sulphide and redox.

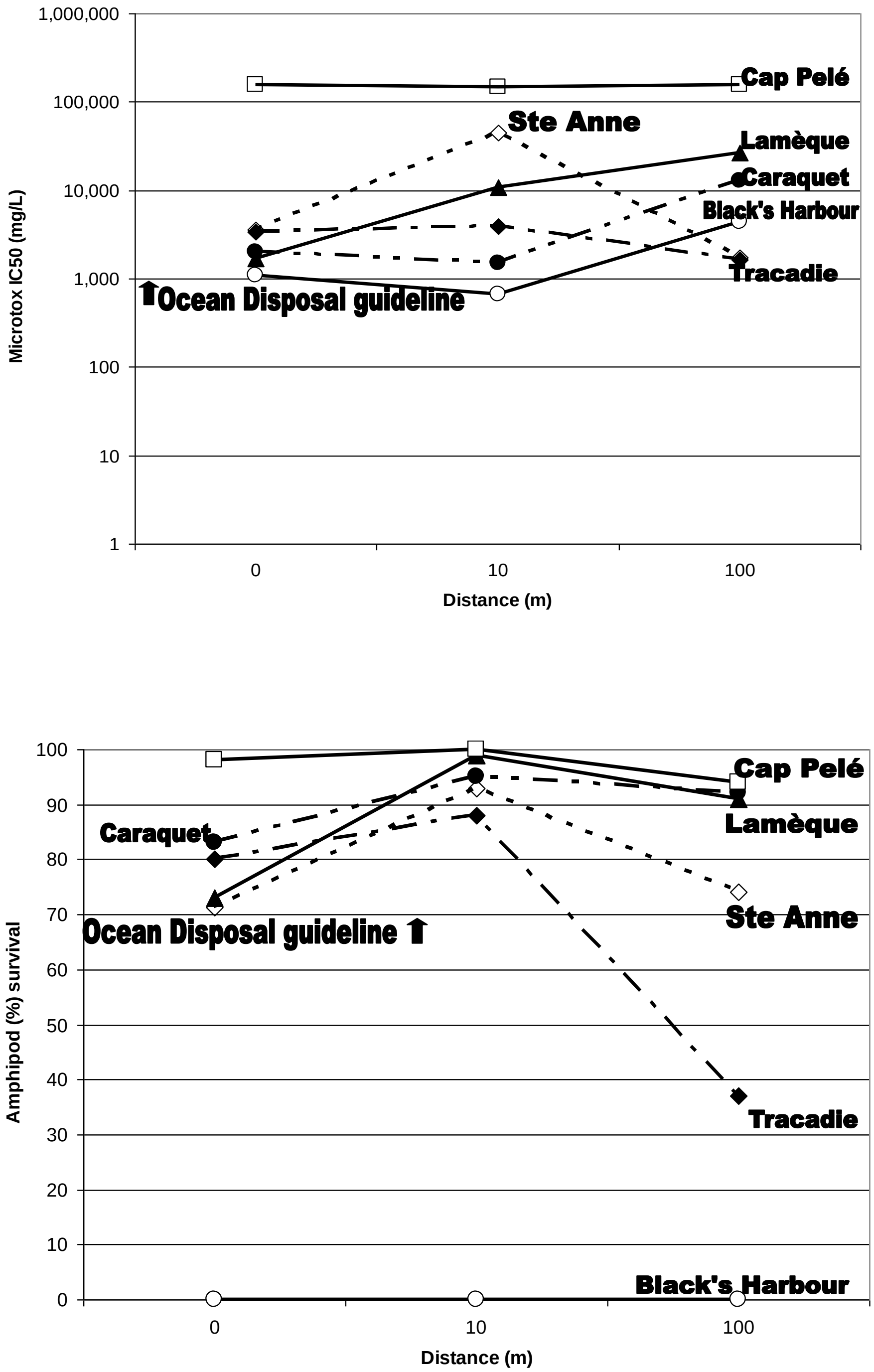

Figure 3 – Toxicity testing results as a function of distance from effluent discharge at 6 seafood processing plant locations