# Microbial Pollution: A Key Factor to Consider in the Management of Municipal Wastewater Effluents from the "Smallest" of Sewage Outfalls

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## ABSTRACT

Payne, J.F., Fancey, L.L., Park, L., Andrews, C.A., Cole, L. French, B. and Thoms, T. 2004. Microbial Pollution: A Key Factor to Consider in the Management of Municipal Wastewater Effluents from the "Smallest" of Sewage Outfalls. Can. Tech. Rep. Fish. Aquat. Sci. 2538: v + 22 p.

High volume municipal effluents may come under stringent regulations in the near future while small sewage outfalls dot the Newfoundland and Labrador coastline. It has been established that sewage outfalls receiving effluents from populations as small as 50 people or less, have the potential to contaminate near shore intertidal and sub-tidal sediments to a considerable degree with various bacteria including *Clostridium sp.*, and coliforms including *Escherichia coli*. Studies were carried out at a number of sites including Harbour Grace, Harbour Breton and Carbonear Bay. Sediments from some small outfalls contained levels of *Clostridium sp.* comparable to levels reported from a site in the United States receiving sewage from a population numbering in the hundreds of thousands. Studies in Harbour Grace and Carbonear Bay also indicated that sediments in deeper waters can act as reservoirs for high levels of *Clostridium sp.* loading. Microbes (bacteria and viruses) in sewage may pose risks to the health of fish and marine mammals as well as human consumers of fish products. These results raise questions about the potential for cumulative impacts of clusters of small sewage outfalls versus single larger outfalls such as those in St. John's Harbour and Halifax Harbour that are presently receiving priority regulatory attention. Also with respect to potential risks to human health and the environment, microbial and viral contamination, along with such parameters as organism disease and pathology, might be more important to monitor than traditional "end-of-pipe" water quality parameters.

## RÉSUMÉ

Payne, J.F., Fancey, L.L., Park, L., Andrews, C.A., Cole, L. French, B. and Thoms, T. 2004. Microbial Pollution: A Key Factor to Consider in the Management of Municipal Wastewater Effluents from the "Smallest" of Sewage Outfalls. Can. Tech. Rep. Fish. Aquat. Sci. 2538: v + 22 p.

Les effluent municipaux à grand volume pourraient bientôt faire l'objet de règlements stricts, mais pas les nombreux petits émissaires d'évacuation d'eaux usées situés le long des côtes de Terre-Neuve et Labrador. Il a été établi que les émissaires qui reçoivent les effluents de collectivités comptant aussi peu que 50 habitants, ou même moins, peuvent causer une importante contamination des sédiments intertidaux et infralittoraux par diverses bactéries, comme *Clostridium sp.*, *Escherichia coli* et divers coliformes (p. ex. coliformes fécaux). Des études ont été menées à ce sujet à de nombreux endroits, y compris Harbour Grace et Harbour Breton ainsi que la baie Carbonear. Les sédiments à proximité de certains petits émissaires contiennent une concentration de *Clostridium sp.* semblable à celle observée dans les sédiments des milieux qui reçoivent les eaux usées d'une collectivité étatsunienne de plusieurs centaines de milliers d'habitants. Les études à Harbour Grace et dans la baie Carbonear ont également montré que les sédiments en eaux plus profondes peuvent constituer des réservoirs à charge élevée en *Clostridium sp.* Les micro-organismes (bactéries et virus) présents dans les eaux usées peuvent présenter des risques pour la santé des poissons, des mammifères marins et des consommateurs de produits de la pêche. À la lumière de ces résultats, des questions sont soulevées à propos des effets cumulatifs possibles d'un ensemble de petits émissaires par rapport à l'impact d'un seul grand émissaire, comme ceux dans les ports de St. John's et d'Halifax. Des études ont également été entreprises sur la santé des poissons qui vivent à proximité d'émissaires. Aussi en considèrant les risques potentielles concernant la santé humaine et l'environment, il serait plus important d'évaluer les paramètres pathologiques et maladies d'organismes, que d'évaluer les paramètres de la qualité d'eau à la fin des 'conduites d'eau'

# INTRODUCTION

Sewage contamination is linked to problems of eutrophication, water borne pathogens and toxic substances (Wu 1999). Eutrophication may cause changes in species composition and function, while waterborne pathogens derived from sewage can constitute a human health hazard either directly from contaminated waters or indirectly from contaminated seafood. Regarding toxic substances, acute as well as a variety of chronic effects have been documented in fish and invertebrates in association with sewage contamination (Payne et al. 1996; Khan and Payne 1997; Schmidt et al. 1999 and Woodworth et al. 1999). Even sewage free of "industrial" effluents such as in St. John's Harbor in Newfoundland and Labrador, has had noted effects on the health of resident fish (French et al. 2000). Thus it is understandable that sewage contamination has become a priority issue for the Department of Fisheries and Oceans and Canada's National Programme of Action (NPA) for the Protection of the Marine Environment from Land Based Activities (Federal/Provincial/Territorial Advisory Committee on Canada's National Programme of Action for the Protection of the Marine Environment from Land-based Activities 2000). This was reaffirmed in the *Montreal Declaration on the Protection of the Marine Environment from Land-based Activities* which identified both sewage and nutrient loading as priority issues.

Investigation into the impacts of sewage on the marine environment is particularly important in Newfoundland and Labrador, where many communities discharge untreated sewage directly into the coastal marine environment near fish and fish habitat, commercial fishing grounds, or recreational areas. It is essential to understand the spatial extent and degree of impact of municipal sewage effluent on marine ecosystem health in terms of the *Oceans Act* as well as under the fish habitat provisions of the *Fisheries Act*. Assessment of sewage impacts in Newfoundland and Labrador inshore waters serves to test the ability of natural habitats to support commercial, aboriginal and recreational fisheries, especially in coastal regions where domestic municipal sewage is discharged. The information gathered in such assessments will permit ecosystem–based planning to assist with regulatory or policy decisions which are necessary to protect the near-shore marine environments and to ensure sustainable development and management of Canada's marine waters.

This pilot study investigates the potential for broad-scale contamination of near-shore marine sediments by the discharges of municipal sewage in several small communities in Newfoundland and Labrador. A microbial index, the enumeration of sporulative anaerobes (*Clostridium* sp. spores) in sediments, was evaluated as an environmental quality parameter to define zones of cumulative impacts by sewage effluents. Unlike fecal coliforms, which die off relatively quickly over time in seawater, *Clostridium* spores found in human wastes remain viable for years, and measurement of their levels in sediments has potential as a tool for defining zones of impact from sewage discharge. (O'Reilly et al. 1995).

## METHODS AND MATERIALS

Sediment samples were taken for microbiological analyses from four contaminated areas and five reference areas in Newfoundland and Labrador, Canada (Fig. 1). Samples of bottom sediment were taken from shallow near-shore sites at two contaminated areas, Harbour Grace and Carbonear Harbour, on September 12, 2001 and September 13, 2001 respectively. Sediment samples were also collected from onshore sites contaminated with 13 municipal and 111 individual (single dwelling) domestic sewage outfalls at Harbour Breton, September 19, 2001; other onshore sites were sampled at Victoria and Salmon Cove on November 21, 2001. Sample details are given in Appendix 1. Reference sites were selected in areas where there were neither sewage outfalls nor resident populations nearby (Fig. 2). Reference samples were collected from Chance Cove and Bellevue Beach on October 7, 2001; Chance Cove (southern shore) and Long Beach on October 9, 2001; and Gooseberry Cove, October 10, 2001.

Samples were collected from shallow near-shore areas at Harbour Grace Harbour and Carbonear Harbour in line transects using an Eckman grab deployed from a 7.5 m open boat. The Eckman grab sampler was lowered by rope manually and triggered closed at the bottom. When retrieved onboard, the grab was immediately placed in a shallow plastic tray that had been rinsed with 70% ethanol. Immediately upon retrieval, the grab was opened from the top, and a sub-sample was aseptically removed from the top of the sediment in the grab using a sterile plastic scoop. The sediment was placed inside a labelled, sterile Whirlpak bag that was held upright on ice until analysed.

At Harbour Breton, Victoria and Salmon Cove, sediment samples were taken by hand from the beach at the intertidal area. Beach sediment was sampled at low tide (Harbour Breton) or on the receding tide (Salmon Cove). A long-handled shovel was rinsed with 70% ethanol and used to scoop an initial sediment sample from near the end of the outfall pipe. The sediment was then sub-sampled aseptically using a sterile plastic scoop, and the sediment was placed inside a labelled, sterile Whirlpak bag that was held upright on ice until analysed. Alternatively, if easily accessible, sediments were scooped directly from the beach outfall area or sampling site using a sterile plastic spoon. Replicate field samples were collected from several sites by scooping two sediment samples from the same area and storing each in separate Whirlpak bags. Field replicates were analysed as separate samples. Reference samples were collected similarly.

Samples were analysed by Jacques Whitford Ltd., St John's, NL within several days of collection. Total and fecal coliforms and *E.coli* were enumerated using the Most Probable Number (MPN) method (Department of Fisheries and Oceans 1988). Sporulative anaerobes were enumerated using a modification of the Food and Drug Administration (FDA 1998) method for *Clostridium sp.* Detailed microbiological methodology for enumeration of sporulative anaerobes is given in Appendix 2. Laboratory replicates and internal reference standards were analysed along with collected sediment samples to monitor quality control.

## RESULTS

Total and fecal coliforms, *E. coli* and sporulative anaerobe counts from reference sites are given in Table 1. Reference sites showed relatively low microbiological contamination with Chance Cove (Southern Shore) indicated to be the most pristine. Of the five reference sites, only one, Chance Cove, Trinity Bay, showed any sporulative anaerobe activity yielding only 10 CFU g wet weight$^{-1}$ or less.

Results for contaminated sites, (Table 2) in Harbour Grace, Carbonear, Harbour Breton and Victoria/Salmon Cove were consistent with contamination from various levels of raw domestic sewage. At Harbour Grace, sediment samples HG-01 to HG–09 were taken in a transect along the middle of the entire harbour basin with HG–01 furthest towards shore and HG-09 furthest seaward (Fig. 3). Sporulative anaerobe counts indicated a consistent level of contamination ranging from 3800 to 7300 CFU g wet weight$^{-1}$. Samples taken at four outfall sites (HGOTF#1 to HGOTF#4) had sporulative anaerobe counts ranging from 90 to 9600 CFU g wet weight$^{-1}$. The sediment sample from site HGOTF #4 gave the highest coliform and *E.coli* counts indicating recent sewage input. Visual inspection of the sample at the time of collection revealed obvious undissolved tissue paper and raw fecal material.

In Carbonear Harbour (Fig. 4), sediment samples were taken along two transects, one parallel to one side of the harbour (CRB #1 to CRB #5), and another parallel to the opposite side of the harbour (CRB #6 to CRB #9). Samples were taken at two outfall sites (CRBOTF #1 and CRBOTF #2). The total coliform counts were highest near the outfall sites (CRBOTF #1, CRBOTF #2 and CRB #5) where the sporulative anaerobe counts were lowest. Conversely, for the transect samples, the sporulative anaerobe counts ranged from 580 to 3300 CFU g wet weight $^{-1}$ whereas coliform counts were low to negative.

Sediment samples were taken at outfall sites in Harbour Breton (Fig. 5). Sporulative anaerobe counts ranged from 1100 to 67000 CFU g wet weight $^{-1}$. Fecal coliform and *E. coli* counts indicated recent sewage contamination. One sample (V#1), taken from a freshwater pond (Fig. 6) that had been contaminated in recent months with overflow from a nearby sewage lagoon in Victoria, indicated coliform contamination and gave a sporulative anaerobe count (5000 CFU g wet weight $^{-1}$) that was within the range found in Harbour Grace. Samples V#2 to V#8, taken from the beach area in Salmon Cove, had relatively lower counts for all indicators.

## DISCUSSION

Marine sewage disposal has been the method of choice in Newfoundland and Labrador for many rural and urban communities. Cull (2000) has found, in a comparison of levels of organic waste from four major sources in Newfoundland and Labrador, that sewage is the second largest contributor of organic wastes next to offal from fish plant wastes. In Newfoundland and Labrador communities, sewage effluent is discharged,

mostly untreated, directly into the near-shore marine environment via open effluent outfall pipes that often empty into the intertidal area. Wave and tidal action then washes the effluent away from the immediate area of disposal and redistributes it. The potential effects of sewage on the marine environment have not always been considered particularly harmful. In the case of smaller communities where the most cost-effective means is marine sewage disposal, it is often assumed that the vast ocean is able to assimilate, or otherwise degrade, destroy or mitigate any potentially harmful effects.

Although microbiological techniques have been most commonly applied, fecal steroid biomarkers such as coprostanol have also been used to indicate sewage loading (e.g. Parrish et al. 2000; Edwards et al. 1998). Hudson et al. (2001), examined sediments in Trinity Bay, Newfoundland and Labrador for 5β-stanols such as coprostanol and were unable to detect them in offshore sediments and found only low levels in certain inshore sediments. These authors state that this finding suggests "raw sewage discharges in rural Newfoundland and Labrador are efficiently degraded or dispersed or that inputs are highly localised".

Traditional microbiology has used indicator organisms such as coliforms, fecal coliforms and, specifically, *E. coli,* to indicate recent sewage contamination. The marine environment is ultimately hostile to sewage pathogens, and they die off with time. Labile microorganisms, although useful in the short term, are not good indicators for delineation of long-term spatial and temporal impacts of sewage input. The stresses of the marine environment dictate that if a microbe is to be a useful marker to delineate the extent of long-term distribution of marine sewage contamination, it must be resilient to prolonged environmental exposure and therefore persist over time in the sea. Klein and Houston proposed the use of *Clostridium perfringens* as such an indicator in 1899 (cited in Bisson and Cabelli 1979). Although not necessarily as good a sign of recent sewage pollution as some others, *Clostridium perfringens* has somewhat of an advantage in detecting long-term sewage input because it produces resistant spores that can survive the adverse conditions presented by the marine environment. Moreover, anaerobic spores may remain viable for decades or possibly even centuries. (Duncanson et al. 1986 as cited by O'Reilly et al. 1995). The relative difficulty of culture of such sporulative anaerobes has generally been a deterrent to its routine use. The enumeration of environmentally resistant sporulative anaerobes in environmental samples, however, may give a more reliable indication of sewage input into the marine environment regardless of the stresses put upon the test organism. This is particularly important in situations where detection and delineation of long-term sewage contamination or impact is more relevant or informative than recent input. Bisson and Cabelli (1979 and 1980) suggested the use of *Clostridium perfringens* as an indicator in such circumstances. Hill et al. (1993), examined benthic distribution of sewage sludge using *Clostridium perfringens* as an indicator using the mCP method and found that *C. perfringens* counts were highest in the top 1 cm of sediment and exceeded 9000 CFU g dry weight $^{-1}$ of sediment. *Clostridium perfringens* is associated with fecal wastes and causes wound infections and gas gangrene (Emerson and Cabelli 1982).

In this pilot study, we investigated the suitability of sporulative anaerobe enumeration (in comparison to total coliform, fecal coliform and *E. coli* counts) as a signature of sewage contamination in benthic sediments. Viable spores were counted, using a pour plate technique as described in Appendix 2 (FDA 1998) to provide an indication of the distribution of raw sewage from several rural Newfoundland and Labrador communities.

As a first step, refining the delineation of the zone of impact of sewage contamination would provide a basis on which to build an assessment of the potential for this anthropogenic input to effect eutrophication or impact marine benthic communities. It would also aid in identifying the scope of fish health impacts or contamination of seafood products. The action of wave and current in the marine environment may serve to transport sewage inputs away from the point source of entry, leading to potential impacts in areas away from the source. Therefore, the assessment of potential impacts in the marine environment must also include evaluation of the degree of sewage contamination of nearby offshore areas.

In the reference sites sampled, the low numbers of clostridia correctly indicated a pristine environment. Hill et al. (1993) found that *C. perfringens* counts in North Sea sediments were found to be low (4 CFU g wet weight of sediment$^{-1}$). In near-shore sampling sites, such as Harbour Grace and Carbonear Harbours, sediments yielded consistently high levels of spore formers even when coliform and *E.coli* indicators provided negative results. The sites of Harbour Grace and Carbonear Harbour were chosen as representative effluent -receiving marine waters, with surrounding small towns that directly discharge untreated domestic sewage. Samples were collected from near-shore sites in these harbours to assess the spatial extent of sewage contamination. Results indicate that harbour sediments were contaminated with sewage microorganisms, not only directly near outfall sites, but also in areas within a 1 to 2 km radius. The level of sporulative anaerobes found in Harbour Grace and Carbonear indicate persistent sewage contamination at a level of magnitude comparable to areas with very large populations. (O'Reilly et al. 1995). Overall, although *E. coli* and total coliforms die-off rather quickly, *Clostridium sp.* can provide a sensitive and reliable signature of the spread of sewage pollution, including from very small outfalls.

Salmon Cove was chosen as a sampling site to evaluate the area for potential sewage contamination as this site was closed to recreational use due to high coliform counts in the summer of 2001.

All of the sediment samples obtained from shore sites at Harbour Breton indicated that *Clostridium* levels were in a range higher than the other sites sampled and were in fact comparable to levels reported in the United States, that receive effluent from populations in the hundreds of thousands to millions. (O'Reilly et al. 1995). Evidence of such high contamination is surprising considering that Harbour Breton is a small community on the southern coast of insular Newfoundland with a population of approximately 2290. In this community, domestic sewage effluent is disposed of raw into the marine environment, and flows directly into the harbour of the community

through outfalls from either individual homes or from groups of up to approximately 100 dwellings. In contrast to Harbour Grace and Carbonear near-shore sites, sediment samples were taken onshore in Harbour Breton, directly from outfall areas on the beach at low tide. For the sites sampled in Harbour Breton, this study has established that even sewage outfall sites receiving effluents from populations as small as 50 people or less have the potential to contaminate near-shore sediments to a considerable degree with various bacteria, including *Clostridium* and coliforms.

The present report focuses on microbial studies at outfall sites but it would also be of interest to investigate for effects on the health of fish in association with sewage contamination. French et al. (2000) previously noted a variety of effects in winter flounder (*Pleuronectes americanus)* collected from St. John's Harbor, which receives a high volume of sewage, but no major industrial effluents per se. The observations on fin rot and altered hematology in flounder in this study may be primarily linked to microbial pollution. Pilot studies are also presently being carried out on mussels and some histopathogical lesions have been observed (unpublished data). Overall it is also worth noting that with respect to potential risks to human health and the environment, microbial and viral contamination, along with such parameters as organism disease and pathology, might be more important that traditional "end-of-pipe" water quality parameters.

## CONCLUSIONS

High volume municipal effluents may come under stringent regulations in the near future while small sewage outfalls dot the Newfoundland and Labrador coastline. It has been established that sewage outfalls receiving effluents from populations as small as 50 people or less, have the potential to contaminate near shore intertidal and sub-tidal sediments to a considerable degree with various bacteria including *Clostridium*, total coliforms, fecal coliforms and *E. coli.* Microbes (bacteria and viruses) in sewage may pose risks to the health of fish and marine mammals as well as human consumers of fish products. Considering microbes as well as other aspects of sewage pollution such as eutrophication, or impacts on fish health, these results raise questions about the potential for cumulative impacts of clusters of small sewage outfalls versus single larger outfalls such as those in St. John's Harbour and Halifax Harbour which are receiving priority regulatory attention.

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Table 1. Microbiological results –Reference sites.

| Sample Number | Total Coliform MPNg $^{-1a}$ | Fecal Coliform MPNg$^{-1a}$ | *E. coli* MPNg$^{-1a}$ | Anaerobes Sporulative CFUg$^{-1a}$ |
|---|---|---|---|---|
| Chance Cove, Trinity Bay (A)[b] | 2.3 | <0.3 | Negative | 10 |
| Chance Cove, Trinity Bay (B)[b] | 0.4 | <0.3 | Negative | 5 |
| Bellevue Beach (A) | 0.4 | <0.3 | Negative | <10 |
| Bellevue Beach (B) | 0.4 | <0.3 | Negative | <10 |
| Chance Cove, Southern Shore (A) | <0.3 | Negative | Negative | <10 |
| Chance Cove, Southern Shore (B) | <0.3 | Negative | Negative | <10 |
| Long Beach (A) | 0.4 | <0.3 | Negative | <10 |
| Long Beach (B) | <0.3 | Negative | Negative | <10 |
| Internal lab control | <0.022 MPN mL$^{-1}$ | Negative | Negative | <1 CFU mL$^{-1}$ |
| Gooseberry Cove (A) | <0.3 | Negative | Negative | <10 |
| Gooseberry Cove (B) | 15 | 15 | 15 | <10 |
| Internal lab control | <0.022 MPN mL$^{-1}$ | Negative | Negative | <1 CFU mL$^{-1}$ |

[a] wet weight
[b] A and B are field replicates

Table 2. Microbiological results – Contaminated sites.

| Sample Number | Total Coliform MPN $g^{-1a}$ | Fecal Coliform MPN $g^{-1a}$ | *E. coli* MPN $g^{-1a}$ | Anaerobes Sporulative CFU $g^{-1a}$ |
|---|---|---|---|---|
| **Harbour Grace** | | | | |
| HG-01 | <0.3 | Negative | Negative | 4800 |
| HG-02 | <0.3 | Negative | Negative | 3800 |
| HG-03 | <0.3 | Negative | Negative | 5600 |
| HG-04 | <0.3 | Negative | Negative | 5600 |
| HG-05 | <0.3 | Negative | Negative | 6600 |
| HG-06 | 4 | 4 | <3 | 6900 |
| HG-06 Lab Replicate | 0.9 | <0.3 | Negative | 4900 |
| HG-07 | 0.4 | <0.3 | Negative | 7300 |
| HG-08 | 0.4 | <0.3 | Negative | 5200 |
| HG-09 | <0.3 | Negative | Negative | 6300 |
| Internal Lab Control | <0.022 | Negative | Negative | <1 CFU $mL^{-1}$ |
| HGOTF#1 | 46 | 0.4 | 0.4 | 9600 |
| HGOTF#2 | 2.3 | 2.3 | 2.3 | 1100 |
| HGOTF#3 | <0.3 | Negative | Negative | 90 |
| HGOTF#4 | 110 | 46 | 9.3 | 3900 |
| | | | | |
| **Carbonear** | | | | |
| CRB #1 | <0.3 | Negative | Negative | 2100 |
| CRB #2 | <0.3 | Negative | Negative | 650 |
| CRB #3 | <0.3 | Negative | Negative | 1600 |
| CRB #4 | 0.4 | <0.3 | Negative | 3300 |
| CRB #5 | 24 | 24 | 24 | 580 |
| CRB #6 | 1.5 | <0.3 | Negative | 2500 |
| CRB #7 | 0.4 | <0.3 | Negative | 2900 |
| CRB #8 | 0.7 | <0.3 | Negative | 2900 |
| CRB #8 Lab Replicate | <0.3 | Negative | Negative | 2800 |
| CRB #9 | <0.3 | Negative | Negative | 2400 |
| CRB OTF #1 Breakwater | 7.5 | <0.3 | Negative | 170 |
| CRB OTF #2 Fraize | 46 | 0.4 | 0.4 | 110 |
| Internal Lab Control | <0.022 | Negative | Negative | <1 CFU $mL^{-1}$ |
| Internal Lab Control replicate | <0.022 | Negative | Negative | <1 CFU $mL^{-1}$ |

Table 2. (Cont'd.)

| Sample Number | Total Coliform MPN $g^{-1a}$ | Fecal Coliform MPN $g^{-1a}$ | *E. coli* MPN $g^{-1a}$ | Anaerobes Sporulative CFU $g^{-1a}$ |
|---|---|---|---|---|
| **Harbour Breton** | | | | |
| HB #1A[b] | 24 | 1.5 | 1.5 | 12000 |
| HB #1B | 15 | 4.3 | 1.5 | 23000 |
| HB #2A | 24 | 24 | 24 | 16000 |
| HB #2B | 46 | 9.3 | 4.3 | 8900 |
| HB #3A | 9.3 | 9.3 | 4.3 | 17000 |
| HB #3B | 110 | 15 | 15 | 27000 |
| HB #3B Lab Replicate | 46 | 24 | 24 | 31000 |
| HB #4A | 110 | 110 | 3.8 | 62000 |
| HB #4B | 46 | 9.3 | 9.3 | 67000 |
| HB #5A | 9.3 | 0.9 | 0.4 | 1100 |
| HB #5B | 46 | 0.9 | 0.4 | 2000 |
| HB #6A | 110 | 0.7 | 0.7 | 2500 |
| HB #6B | 110 | 9.3 | 9.3 | 3900 |
| HB #7A | 460 | 240 | 24 | 10000 |
| HB #7B | 460 | 240 | 240 | 21000 |
| Internal Lab Control | <0.022 | Negative | Negative | <1 CFU $mL^{-1}$ |
| HB #8A | 210 | 210 | 210 | 2700 |
| HB #8B | 1100 | 1100 | 1100 | 6100 |
| HB #8B Lab Replicate | 75 | 75 | 75 | 6300 |
| HB #9A | 1100 | 9 | 3 | 18000 |
| HB #9B | 460 | 9 | 4 | 16000 |
| HB #10A | 150 | 150 | 150 | 36000 |
| HB #10B | 1100 | 39 | 39 | 33000 |
| HB #11A | 1100 | 240 | 240 | 23000 |
| HB #11B | 1100 | 460 | 460 | 15000 |
| HB #12A | 93 | 93 | 43 | 2100 |
| HB #12B | 1100 | 460 | 460 | 3400 |
| HB #13A | 460 | 460 | 460 | 9100 |
| HB #13B | 1100 | 1100 | 44 | 2500 |

Table 2. (Cont'd.)

| Sample Number | Total Coliform MPN $g^{-1a}$ | Fecal Coliform MPN $g^{-1a}$ | E. coli MPN $g^{-1a}$ | Anaerobes Sporulative CFU $g^{-1a}$ |
|---|---|---|---|---|
| **Victoria /Salmon Cove** | | | | |
| V #1 | 46 | 1.9 | 1.5 | 5000 |
| V #2 | <0.3 | Negative | Negative | <10 |
| V #3 | 2.3 | 0.9 | 0.9 | <10 |
| V #3 Lab Replicate | 2.3 | 2.3 | 2.3 | <10 |
| V #4 | 0.4 | 0.4 | 0.4 | <10 |
| V #5 | <0.3 | Negative | Negative | 10 |
| V #6 | 0.9 | 0.4 | 0.4 | 30 |
| V #6 Lab Replicate | <0.3 | Negative | Negative | 35 |
| V #7 | 9.3 | 9.3 | 9.3 | <10 |
| V #8 | 9.3 | 1.5 | 1.5 | <10 |
| Internal Lab Control | <0.022 | Negative | Negative | <1 CFU $mL^{-1}$ |

[a]wet weight
[b]A and B are field replicates

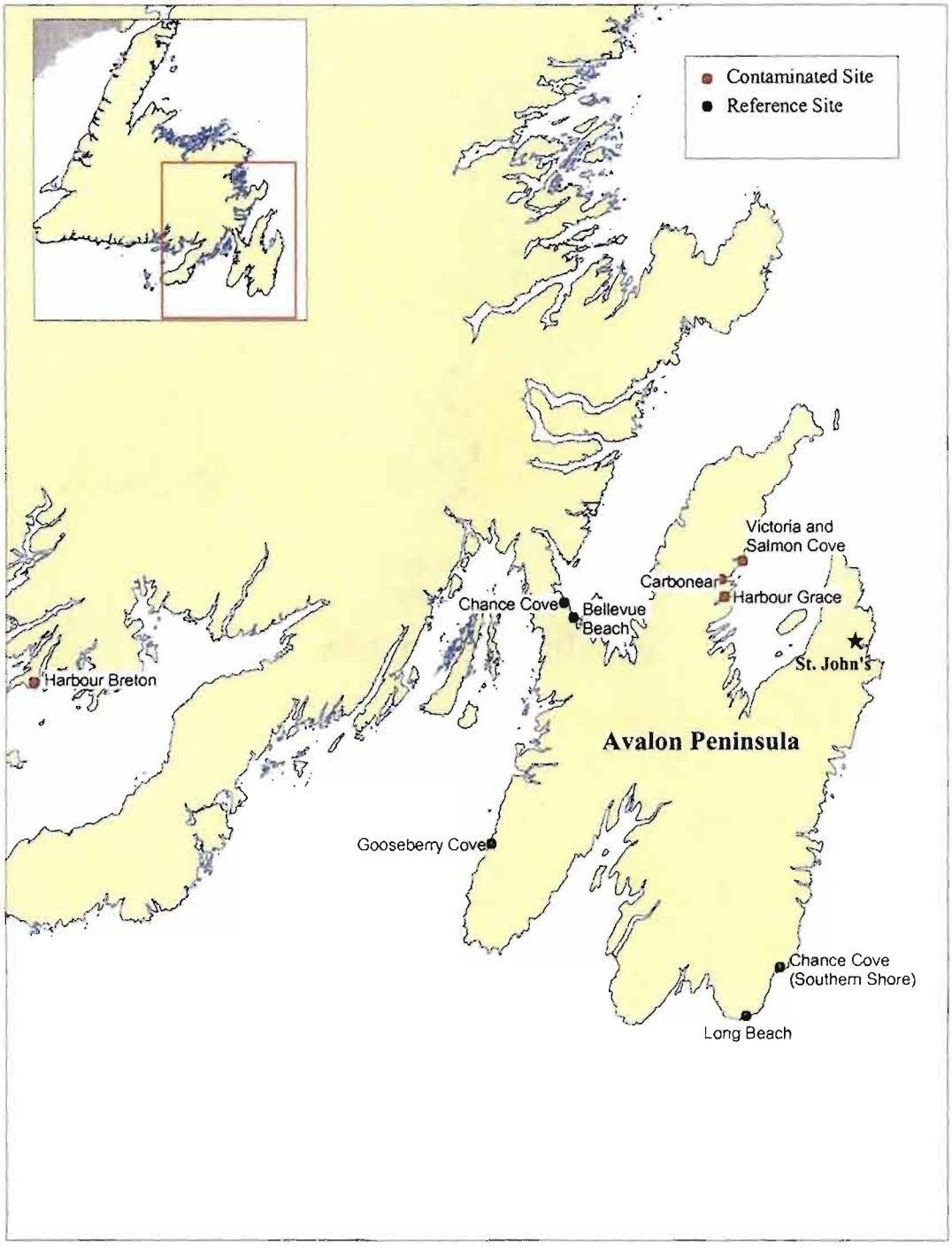


Figure 1. Study sampling sites (Contaminated and Reference).

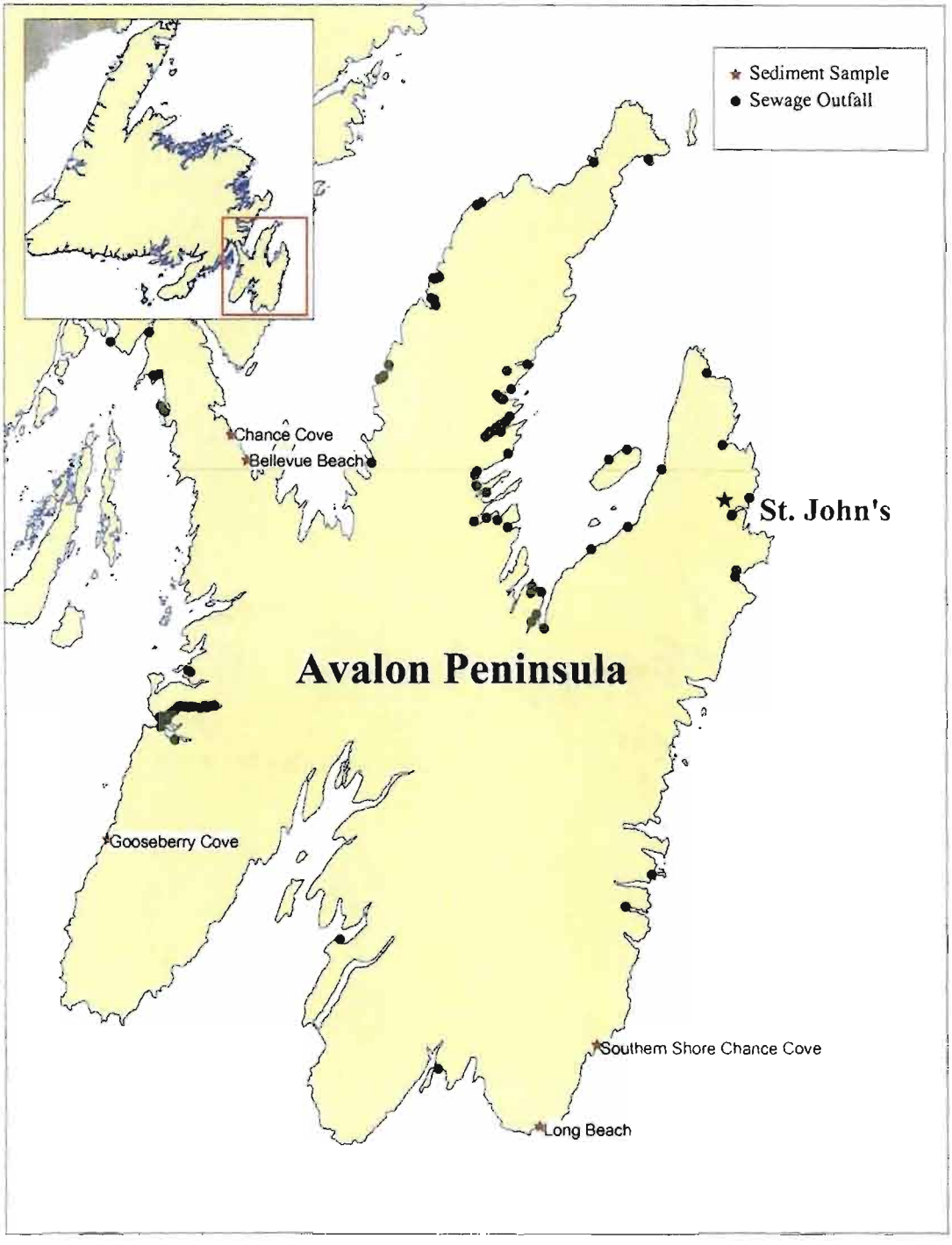


Figure 2. Reference sites (Shown Isolated from Sewage Outfalls).

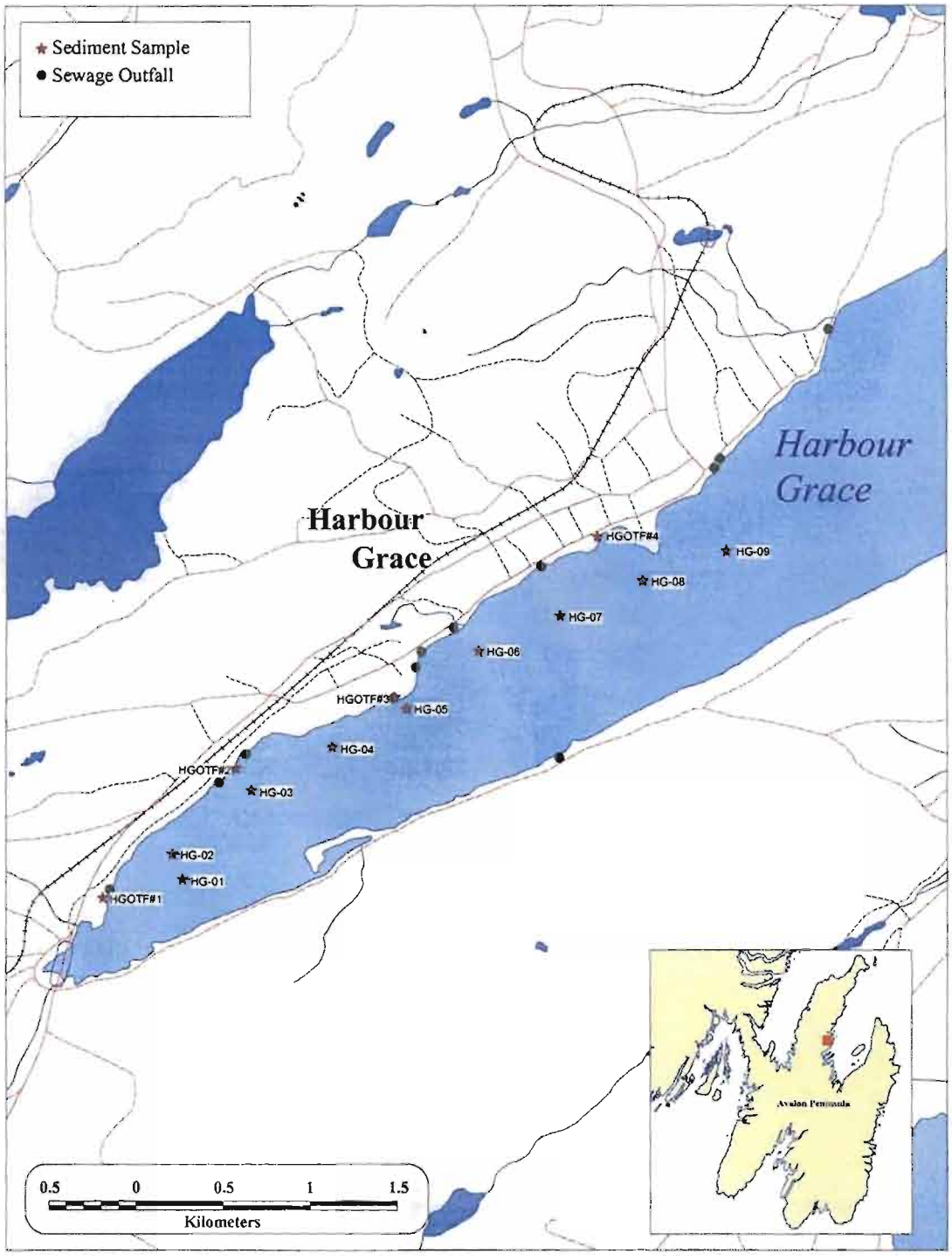


Figure 3. Sampling sites at Harbour Grace.

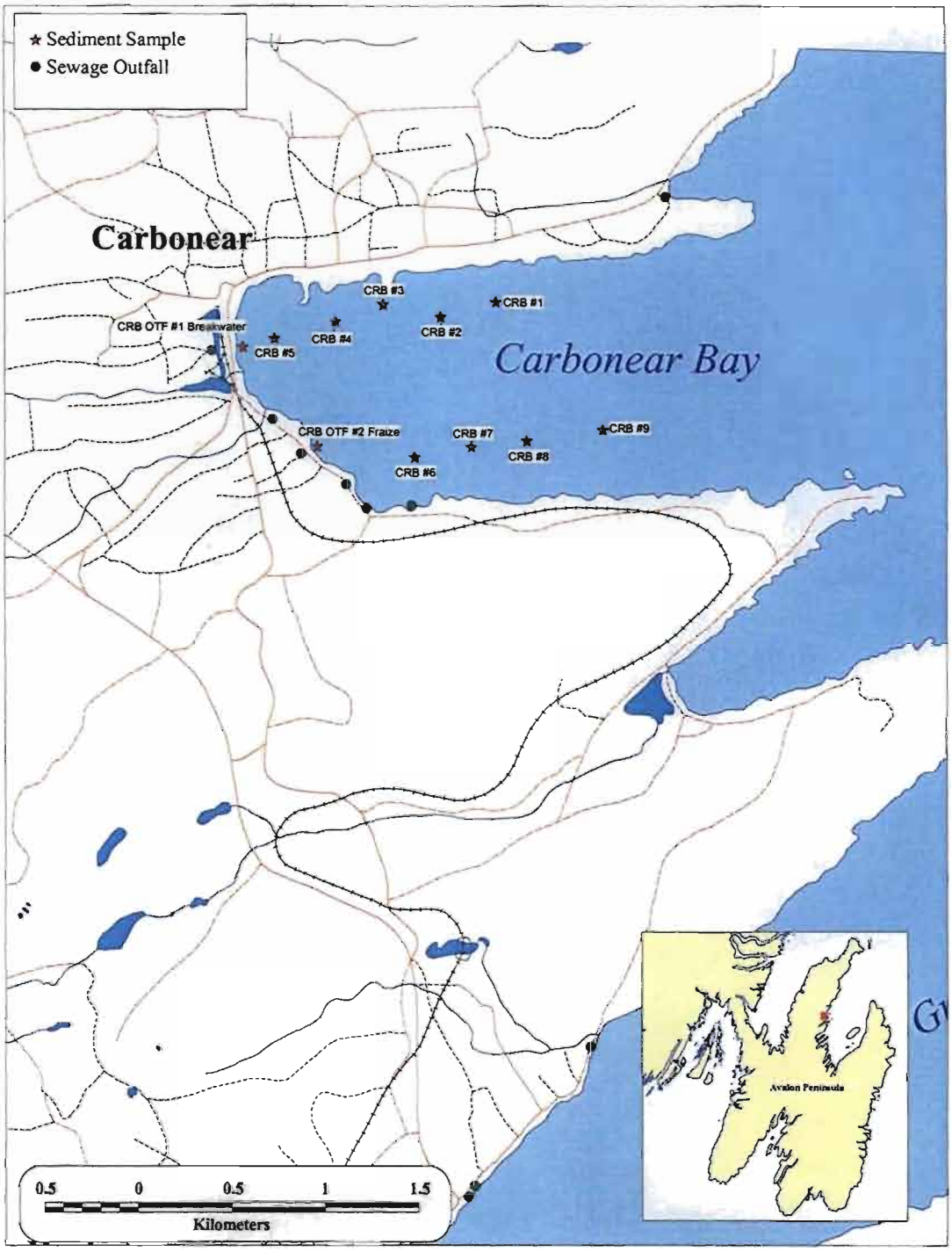


Figure 4. Sampling sites at Carbonear.

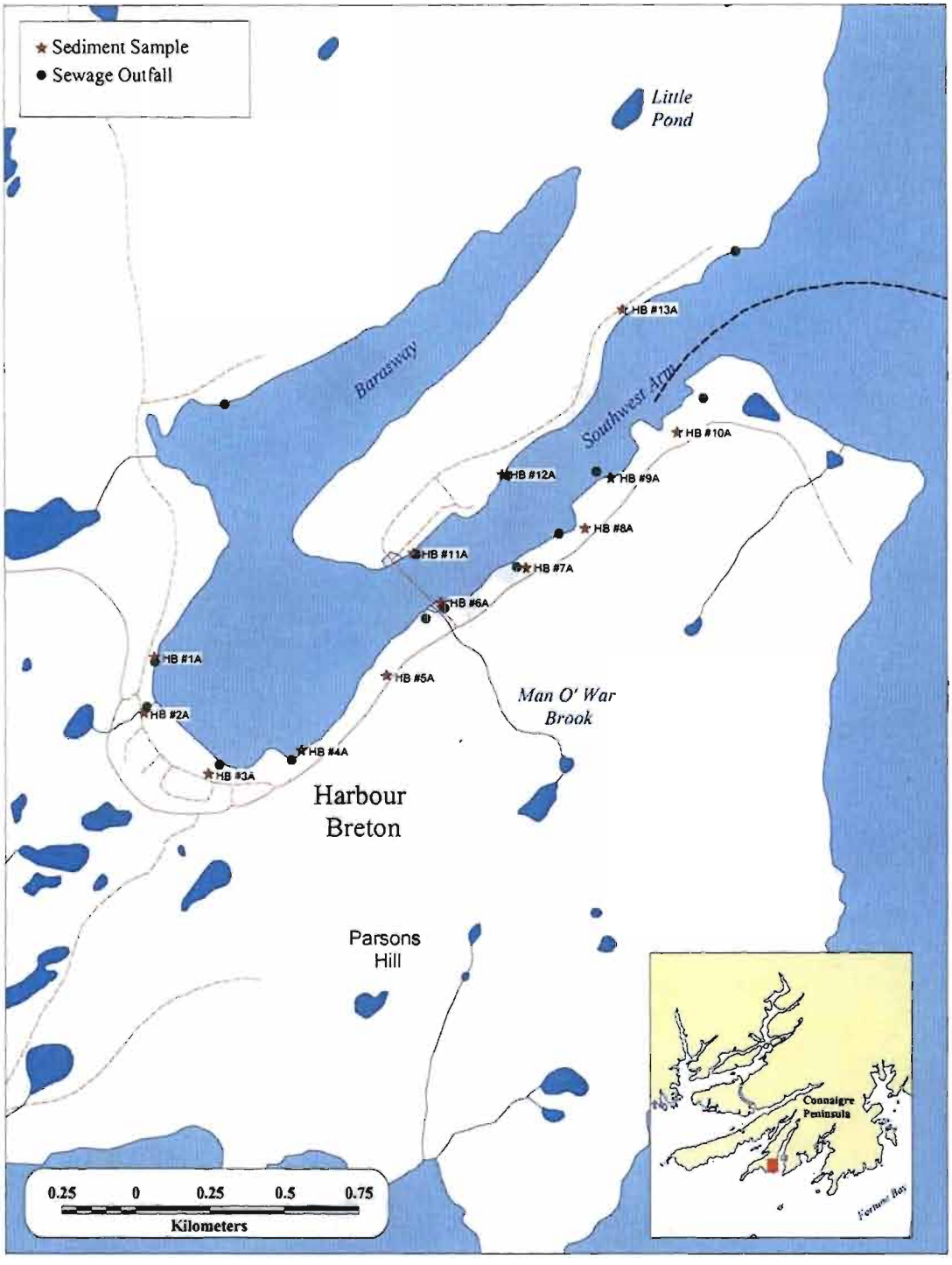


Figure 5. Sampling sites at Harbour Breton.

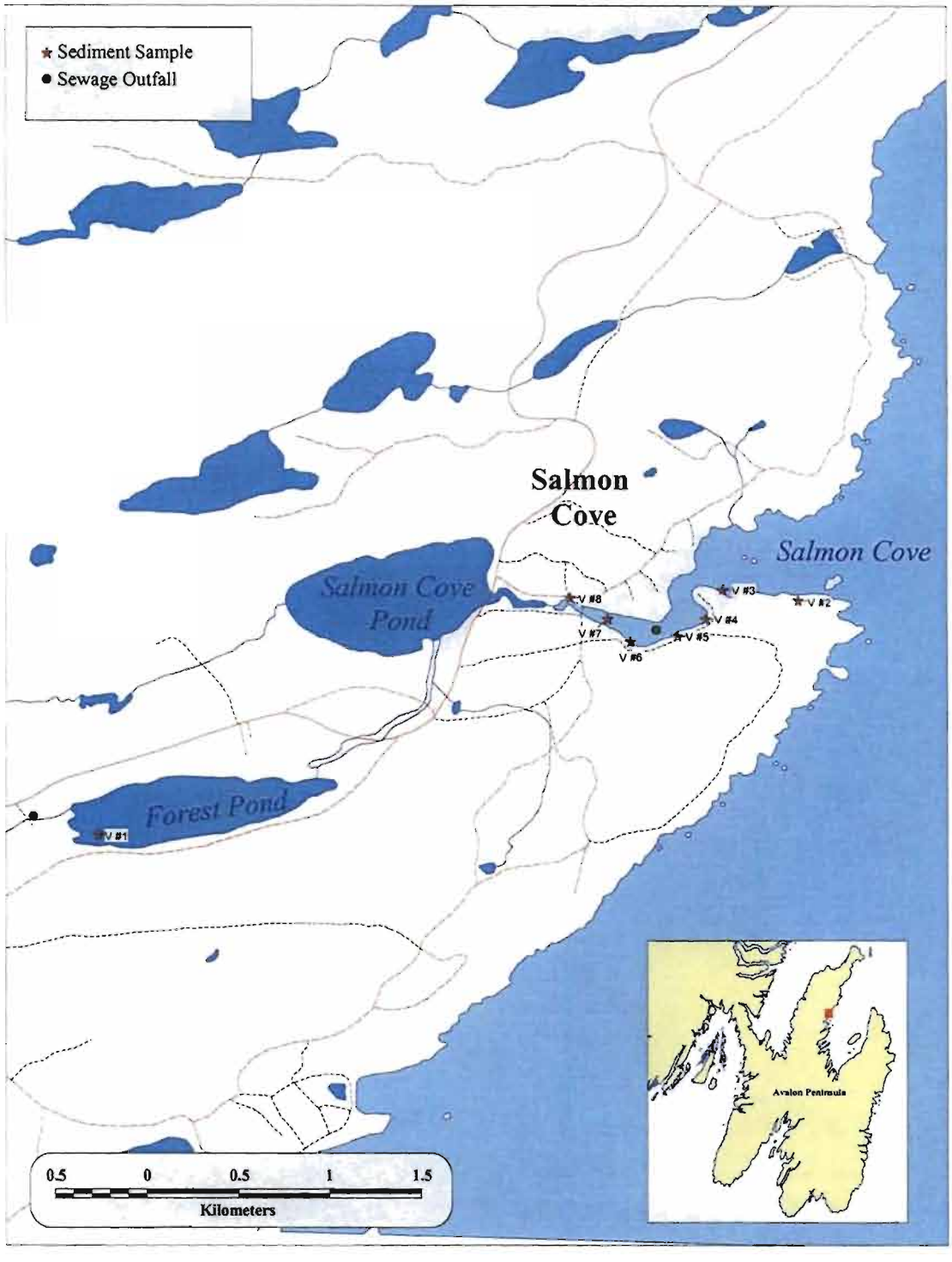


Figure 6. Sampling sites at Salmon Cove and Victoria.

Appendix 1. Sampling details.

| Sample Number | Sample Type | Latitude | Longitude | Date of Collection in 2001 |
|---|---|---|---|---|
| **Reference Sites** | | | | |
| Chance Cove (A)* | sediment from beach | 47°40.530'N | 053°49.143'W | October 7 |
| Chance Cove (B) | field replicate of (A) | 47°40.530'N | 053° 49.143'W | October 7 |
| Bellevue Beach (A) | sediment from beach | 47°38.294'N | 053°47.034'W | October 7 |
| Bellevue Beach (B) | field replicate of (A) | 47°38.294'N | 053°47.034'W | October 7 |
| Southern Shore Chance Cove (A) | sediment from beach | 46°45.730'N | 053°00.689'W | October 9 |
| Southern Shore Chance Cove (B) | field replicate of (A) | 46°45.730'N | 053°00.689'W | October 9 |
| Long Beach (A) | sediment from beach | 46°38.388'N | 053°08.285'W | October 9 |
| Long Beach (B) | field replicate of (A) | 46°38.388'N | 053°08.285'W | October 9 |
| Gooseberry Cove (A) | sediment from beach | 47°04.278'N | 054°05.674'W | October 10 |
| Gooseberry Cove (B) | field replicate of (A) | 47°04.278'N | 054°05.674'W | October 10 |
| | | | | |
| **Contaminated Sites** | | | | |
| **Harbour Grace** | | | | |
| HG-01 | bottom sediment-grab sample | 47°40.370'N | 053°14.986'W | September 12 |
| HG-02 | bottom sediment-grab sample | 47°40.446'N | 053°15.036'W | September 12 |
| HG-03 | bottom sediment-grab sample | 47°40.648'N | 053°14.682'W | September 12 |
| HG-04 | bottom sediment-grab sample | 47°40.789'N | 053°14.319'W | September 12 |
| HG-05 | bottom sediment-grab sample | 47°40.914'N | 053°13.980'W | September 12 |
| HG-06 | bottom sediment-grab sample | 47°41.096'N | 053° 13.659'W | September 12 |
| HG-06 Lab. Replicate | bottom sediment-grab sample | 47°41.096'N | 053°13.659'W | September 12 |
| HG-07 | bottom sediment-grab sample | 47°41.211'N | 053°13.287'W | September 12 |
| HG-08 | bottom sediment-grab sample | 47°41.325'N | 053°12.913'W | September 12 |
| HG-09 | bottom sediment-grab sample | 47°41.422'N | 053°12.535'W | September 12 |
| HGOTF#1 | bottom sediment-grab sample | 47°40.308'N | 053°15.349'W | September 12 |
| HGOTF#2 | bottom sediment-grab sample | 47°40.715'N | 053°14.756'W | September 12 |
| HGOTF#3 | bottom sediment-grab sample | 47°40.948'N | 053°14.037'W | September 12 |
| HGOTF#4 | bottom sediment-grab sample | 47°41.456'N | 053°13.126W | September 12 |

| Sample Number | Sample Type | Latitude | Longitude | Date of Collection in 2001 |
|---|---|---|---|---|
| | | | | |
| **Carbonear** | | | | |
| CRB #1 | bottom sediment-grab sample | 47°44.252'N | 053°12.588'W | September 13 |
| CRB #2 | bottom sediment-grab sample | 47°44.204'N | 053°12.822'W | September 13 |
| CRB #3 | bottom sediment-grab sample | 47°44.236'N | 053°13.069'W | September 13 |
| CRB #4 | bottom sediment-grab sample | 47°44.182'N | 053° 13.272'W | September 13 |
| CRB #5 | bottom sediment-grab sample | 47°44.129'N | 053° 13.532'W | September 13 |
| CRB #6 | bottom sediment-grab sample | 47°43.800'N | 053°12.916'W | September 13 |
| CRB #7 | bottom sediment-grab sample | 47°43.834'N | 053°12.675'W | September 13 |
| CRB #8 | bottom sediment-grab sample | 47°43.856'N | 053°12.439'W | September 13 |
| CRB #8 Lab Replicate | bottom sediment-grab sample | 47°43.856'N | 053°12.439'W | September 13 |
| CRB #9 | bottom sediment-grab sample | 47°43.894'N | 053°12.115'W | September 13 |
| CRB OTF #1 Breakwater | bottom sediment-grab sample | 47°44.099'N | 053°13.817'W | September 13 |
| CRB OTF #2 Fraize | bottom sediment-grab sample | 47°43.825'N | 053°13.449'W | September 13 |
| **Harbour Breton** | | | | |
| HB #1A* | sediment from beach | 47°28.44'N | 055°49.63'W | September 19 |
| HB #1B | sediment from beach | 47°28.44'N | 055°49.63'W | September 19 |
| HB #2A | sediment from beach | 47°28.34'N | 055°49.66'W | September 19 |
| HB #2B | sediment from beach | 47°28.34'N | 055°49.66'W | September 19 |
| HB #3A | sediment from beach | 47°28.23'N | 055°49.49'W | September 19 |
| HB #3B | sediment from beach | 47°28.23'N | 055°49.49'W | September 19 |
| HB #3B Lab Replicate | sediment from beach | 47°28.23'N | 055°49.49'W | September 19 |
| HB #4A | sediment from beach | 47°28.27'N | 055°49.24'W | September 19 |
| HB #4B | sediment from beach | 47°28.27'N | 055°49.24'W | September 19 |
| HB #5A | sediment from beach | 47°28.40'N | 055°49.01'W | September 19 |
| HB #5B | sediment from beach | 47°28.40'N | 055°49.01'W | September 19 |
| HB #6A | sediment from beach | 47°28.53'N | 055°48.86'W | September 19 |
| HB #6B | sediment from beach | 47°28.53'N | 055°48.86'W | September 19 |
| HB #7A | sediment from beach | 47°28.59'N | 055°48.63'W | September 19 |
| HB #7B | sediment from beach | 47°28.59'N | 055°48.63'W | September 19 |
| HB #8A | sediment from beach | 47°28.66'N | 055°48.47'W | September 19 |
| HB #8B | sediment from beach | 47°28.66'N | 055°48.47'W | September 19 |
| HB #8B Lab Replicate | sediment from beach | 47°28.66'N | 055°48.47'W | September 19 |

| Sample Number | Sample Type | Latitude | Longitude | Date of Collection in 2001 |
|---|---|---|---|---|
| HB #9A | sediment from beach | 47°28.75'N | 055°48.40'W | September 19 |
| HB #9B | sediment from beach | 47°28.75'N | 055°48.40'W | September 19 |
| HB #10A | sediment from beach | 47°28.83'N | 055°48.22'W | September 19 |
| HB #10B | sediment from beach | 47°28.83'N | 055°48.22'W | September 19 |
| HB #11A | sediment from beach | 47°28.62'N | 055°48.93'W | September 19 |
| HB #11B | sediment from beach | 47°28.62'N | 055°48.93'W | September 19 |
| HB #12A | sediment from beach | 47°28.76'N | 055°48.69'W | September 19 |
| HB #12B | sediment from beach | 47°28.76'N | 055°48.69'W | September 19 |
| HB #13A | sediment from beach | 47°29.05'N | 055°48.36'W | September 19 |
| HB #13B | sediment from beach | 47°29.05'N | 055°48.36'W | September 19 |
| **Victoria/Salmon Cove** | | | | |
| V #1 | sediment from beach | 47°46.159'N | 053°12.163'W | November 21 |
| V #2 | sediment from beach | 47°47.000'N | 053°09.483'W | November 21 |
| V #3 | sediment from beach | 47°47.127'N | 053°09.536'W | November 21 |
| V #3 Lab Replicate | sediment from beach | 47°47.127'N | 053°09.536'W | November 21 |
| V #4 | sediment from beach | 47°47.084'N | 053°09.595'W | November 21 |
| V #5 | sediment from beach | 47°47.032'N | 053°09.601'W | November 21 |
| V #6 | sediment from beach | 47°46.964'N | 053°09.659'W | November 21 |
| V #6 Lab Replicate | sediment from beach | 47°46.964'N | 053°09.659'W | November 21 |
| V #7 | sediment from beach | 47°46.837'N | 053°09.954'W | November 21 |
| V #8 | sediment from beach | 47°46.896'N | 053°10.122'W | November 21 |

Appendix 2. Enumeration Method for Mesophilic Sporulative Anaerobes (specifically *Clostridium sp)*

Sediment samples were collected aseptically, stored in sterile containers and were kept on ice (1-4°C) for no more than 48 hours before analysis. A 1:10 dilution of the sample was prepared by aseptically adding 25 ± 0.2 g of sediment into 225 mL of 0.1% peptone water. A homogeneous solution was prepared by hand shaking. Other serial dilutions were prepared in peptone broth as necessary using this initial dilution. Sediment sample dilutions were heat treated for 10 minutes at 75°C to activate spores. For each dilution of a sample, 1 mL was aseptically transferred into separate, duplicate, appropriately labelled Petri dishes. BDH Meat Liver agar (12-15 mL, cooled to 43-45 °C) was added to each plate. Samples and agar were mixed immediately. Agar was allowed to solidify for 15-20 minutes, then plates were inverted and incubated under anaerobic conditions (anaerobic jar containing an activator pack and anaerobic indicator strip) at 35 ± 2C, for 18-24 hours. Meat liver agar contains sulfite which when reduced to $H_2S$ by *Clostridium* species causes a blackening of the medium due to the presence of iron salt. Plates containing 20-200 colonies were selected and the number of colonies present recorded with the dilution factor. (Jacques Whitford Environment Limited, modification of FDA 1998, protocol).