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Water Quality Assessment and Objectives for Bamfield Inlet

TECHNICAL REPORT

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EXECUTIVE SUMMARY

This document presents a summary of the ambient water quality of Bamfield and Grappler Inlets, situated near the community of Bamfield, located on the west coast of Vancouver Island, British Columbia (BC). The water quality assessment of the inlets form the basis for the recommended water quality objectives designed to protect existing and future water uses.

Situated in the traditional territory of the Huu-ay-aht First Nation and adjacent to the Pacific Rim National Park Reserve, Bamfield offers unparalleled access to a wide array of environments including unique coastal, marine and rainforest habitats. During the summer months, the population of Bamfield increases substantially. There has been a growing concern over the health of the inlet and potential impacts on human health related to elevated bacteriological contamination. There is currently no community sewage collection or treatment facility within the area.

A preliminary water quality assessment was conducted in 2001/2002, which highlighted elevated fecal coliforms during the summer months exceeding both shellfish consumption and recreational use provincial water quality guidelines. Therefore, a three-year monitoring program was established between 2005 and 2008, focusing on the summer months, to confirm and identify sources of the bacteriological contamination. Sources of contamination were identified using new, innovative monitoring tools, such as caffeine analysis, nitrogen isotope signatures, and microbial source tracking (MST). These relatively new techniques provided a weight-of-evidence approach to demonstrate human contributions to fecal contamination in the study area.

The results of this study indicate that exceedances of the BC Ministry of Environment (MOE) water quality guidelines for shellfish and recreation for both fecal coliforms and enterococci are occurring and tend to be clustered within the inner Bamfield inlet area. As freshwater inputs are minimal, bacteriological contamination is likely coming from anthropogenic sources within the inlets. Furthermore, the MST, caffeine and nitrogen isotope analytical tools, while having limitations, were also a valuable means to characterize the sewage inputs. While MST analysis was limited to those results with

fecal coliforms above 40 CFU/100ml, the majority of the results did confirm the presence of human contamination, thus signifying a strong correlation between elevated microbiological concentrations and human waste.

In order to maintain and protect the water quality in Bamfield and Grappler inlets, short-term and long-term ambient water quality objectives were set for both fecal coliforms and enterococci. The short-term objectives are based on primary and secondary recreation uses, while the long-term objectives are proposed for future shellfish harvesting.

Future monitoring recommendations include attainment monitoring at all 18 Bamfield marine sites and the two freshwater sites, every 3-5 years, depending on available resources and what land use activities, including upgrades to sewage discharges, are underway within the inlet. This monitoring should be conducted on a weekly basis for five weeks within a 30-day period between August and September. Samples should be analyzed for fecal coliforms and *enterococci* in marine waters and fecal coliforms and *E. coli* in freshwater. It is also recommended that of the new techniques used in this study, MST be considered for use in future monitoring work.

Management options to protect human health and overall water quality of Bamfield and Grappler Inlets should include posting a warning in Bamfield Inlet, by the local health authority, advising the public of the potential health risk and the local government should consider a community sewage collection/treatment system process, through liquid waste management planning.

Water Quality Objectives for Bamfield and Grappler Inlets.

Time Period	Variable	Objective Value
Short-term (5-10 years)	Fecal Coliform	≤ 200 CFU/100 mL (geometric mean based on a minimum of five weekly samples collected over a 30-day period)
	Enterococci	≤ 20 CFU/100 mL (geometric mean based on a minimum of five weekly samples collected over a 30-day period)
Long-term (>10 years)	Fecal Coliform	≤ 14 CFU/100 mL (median based on a minimum of five weekly samples collected over a 30-day period)
	Enterococci	≤ 4 CFU/100 mL (median based on a minimum of five weekly samples collected over a 30-day period)

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1.0 INTRODUCTION

1.1 BACKGROUND

The village of Bamfield is one of many eco-tourism centers on the west coast of Vancouver Island. Not only is Bamfield one of the start/end points for the West Coast Trail, which hosts approximately 10,000 hikers per year, it is a layover spot for kayakers and canoeists in Barkley Sound, and a port for the Lady Rose, which brings day passengers from Port Alberni to Ucluelet to Bamfield (and return). Fishermen flock to the area during the fishing season (primarily the summer months) staying in the many resorts and B&B's located within the inlet. The beauty and ruggedness of the area supports many nature cruises, whale watching tours and campers who come to enjoy the scenery and laid back lifestyle. Bamfield is also situated within the Huu-ay-aht First Nation traditional territory.

Bamfield was originally the site of the Pacific Cable Board (PCB) Cable Station, which served as the eastern terminus of the trans-Pacific telegraph cable from 1901 – 1959. In 1968, the National Research Council asked the five western Canadian universities to establish a marine biology station on the Pacific Coast and in 1969 the former cable station property was purchased. The Bamfield Marine Sciences Center (BMSC) has provided world-class facilities to a community of leading research biologists, ecologists, and oceanographers. The BMSC is located at the confluence of Grappler and Bamfield inlets and is owned by the non-profit Western Canadian Universities Marine Sciences Society, whose members are the University of Alberta, University of British Columbia, University of Calgary, University of Victoria, and Simon Fraser University. For the majority of the year, Bamfield has a population of approximately 300 permanent residents. During the summer tourist season, this number can increase to more than 2,000 people. There is currently no community sewage collection or treatment facility within the area. Resorts and individual households may have primary treatment, and the effluent is discharged directly into the inlet in the intertidal zone. With the increase in activity during the summer months there has been a growing concern over the health of the inlet, and potential impacts on human health related to elevated bacteriological contamination.

A stringent standard for shellfish growing water is necessary due to the filter feeding mechanism of bivalve shellfish than can concentrate bacteria. A sanitary closure to shellfish harvesting in Bamfield and Grappler Inlets is currently in place (Fisheries and Oceans Canada, 2009). Maintenance of the microbiological quality and safety of waterbodies for recreational use and shellfish harvesting is essential to prevent risks to human health and economic losses due to closures.

In 2001/2002 a preliminary assessment of Bamfield and Grappler Inlets was conducted in a partnership between the Ministry of Water, Land and Air Protection (now Ministry of Environment (MOE)), Environment Canada, local area shellfish growers, BMSC, Ministry of Health, and the Alberni Clayoquot Regional District (ACRD). Sampling was conducted at 12 locations within the inlet and consisted of monthly grab samples for fecal coliform analyses collected from October 2001 to September 2002. Within Bamfield Inlet, the study found elevated fecal coliform levels during the summer months exceeding both shellfish consumption and recreational use guidelines. Further monitoring was recommended to focus on the summer period, to fulfill the five weekly samples in 30 days requirement under the British Columbia water quality guidelines, and to increase the number of sampling locations within the inlet.

In 2005, a three-year sampling program was established to address the recommendations from the preliminary study and to identify the source of the bacteriological contamination. Sources of contamination were identified using new, innovative monitoring tools, such as caffeine analysis, nitrogen isotope signatures, and bacterial source tracking. These relatively new techniques provide a weight-of-evidence approach to demonstrate human contributions to fecal contamination in the study area. This report provides a summary of the three-year (2005-2007) assessment and proposes water quality objectives for Bamfield and Grappler Inlets.

1.2 WATER QUALITY OBJECTIVES

Water quality objectives are prepared for specific bodies of fresh, estuarine, and coastal marine surface waters of British Columbia as part of the Ministry of Environment's mandate to manage water quality. Objectives are prepared only for those waterbodies and water quality characteristics that may be affected by human activity now or in the future.

Water quality objectives are based on scientific guidelines that are safe limits of the physical, chemical, or biological characteristics of water, biota (plant and animal life) or sediment, which protects water use. Objectives are established in British Columbia for waterbodies on a site-specific basis. They are derived from the guidelines by considering local water quality, water uses, water movement, and waste discharges.

Water quality objectives are set to protect the most sensitive designated water use at a specific location. For marine waters, designated uses include:

- shellfish harvesting and human health;
- aquatic life and wildlife;
- recreation and aesthetics.

By protecting the most sensitive water use, all designated uses for a given waterbody are also protected.

Water quality objectives have no legal standing at this time and are not directly enforced. However, they do provide policy direction for resource managers for the protection of water uses in specific waterbodies. Objectives guide the evaluation of water quality, the issuing of permits, licences and orders, and the management of fisheries and the province's land base. They also provide a reference against which the state of water quality in a particular water body can be checked, and help to determine whether basin-wide water quality studies should be initiated. Water quality objectives are also a standard for assessing the Ministry's performance in protecting water uses.

Water quality objectives are established to protect all uses that may take place in a waterbody. Monitoring is undertaken to determine if all the designated water uses are being protected. The monitoring usually takes place at a critical time, when the water quality objective may not be met, that is generally determined as part of the water quality objective setting exercise. It is assumed that if all designated water uses are protected at the critical time, then they will also be protected at other times when the threat is less. For practical reasons, the monitoring usually takes place during a five-week period, which allows the specialists to measure the worst, as well as the average condition in the water. For some waterbodies, the monitoring period and frequency may vary, depending upon the nature of the problem, severity of threats to designated water uses, and the way the objectives are expressed (i.e. mean and/or maximum values).

2.0 SITE DESCRIPTION

Bamfield is located in the heart of the Pacific Rim National Park on the West Coast of Vancouver Island, 89 km from Port Alberni and 77 km from Lake Cowichan. Bamfield Inlet is a protected inlet located on the south shore of Barkley Sound, and flows into Trevor Channel (Figure 1). Grappler Inlet is located to the east and adjacent to Bamfield Inlet. The area is within the traditional territories of the Huu-ay-aht First Nation, sections of Crown and private residential land, and portions of the Pacific Rim National Park Reserve. The village of Bamfield is transected by the inlet (approximately 180 m across) making boats the common mode of transportation between the east and west sides. The “west side”, also referred to as Mills Peninsula, is home to the Canadian Coast Guard station and is distinguished by the historic boardwalk connecting homes, docks and businesses running along its side. Along with the Bamfield Marine Sciences Center, the community school, Centennial Park, the boat launch and a pub reside on the “east side”. The “east side” is accessible via a gravel road from Port Alberni or Lake Cowichan.

2.1 CLIMATE

The regional climate can be characterized by mild temperature and heavy winter rains. The mean annual rainfall is 270 cm with the heaviest rains occurring from November to February (Environment Canada, 2007). Snowfalls are rare. The driest months are July and August. Winds are moderately strong and blow predominantly from the southeast or southwest, and occasionally from the north. Winter storms can be expected from late October to mid-March.

2.2 OCEANOGRAPHY

The surface water temperature ranges from 8-17 °C with a thermocline between 4-6 m (BMSC, 2007). Below the thermocline, temperatures range from 7-10 °C. The surface water salinity ranges from 13-32 ppt with a shallow halocline in the top few metres. Below 10 m the salinity is 31-32 ppt. The surface water oxygen concentration averages 6-7 mg/L, dropping off to 4-5 mg/L near bottom. Water clarity varies throughout the year with reduced visibility during the plankton blooms of the summer months.

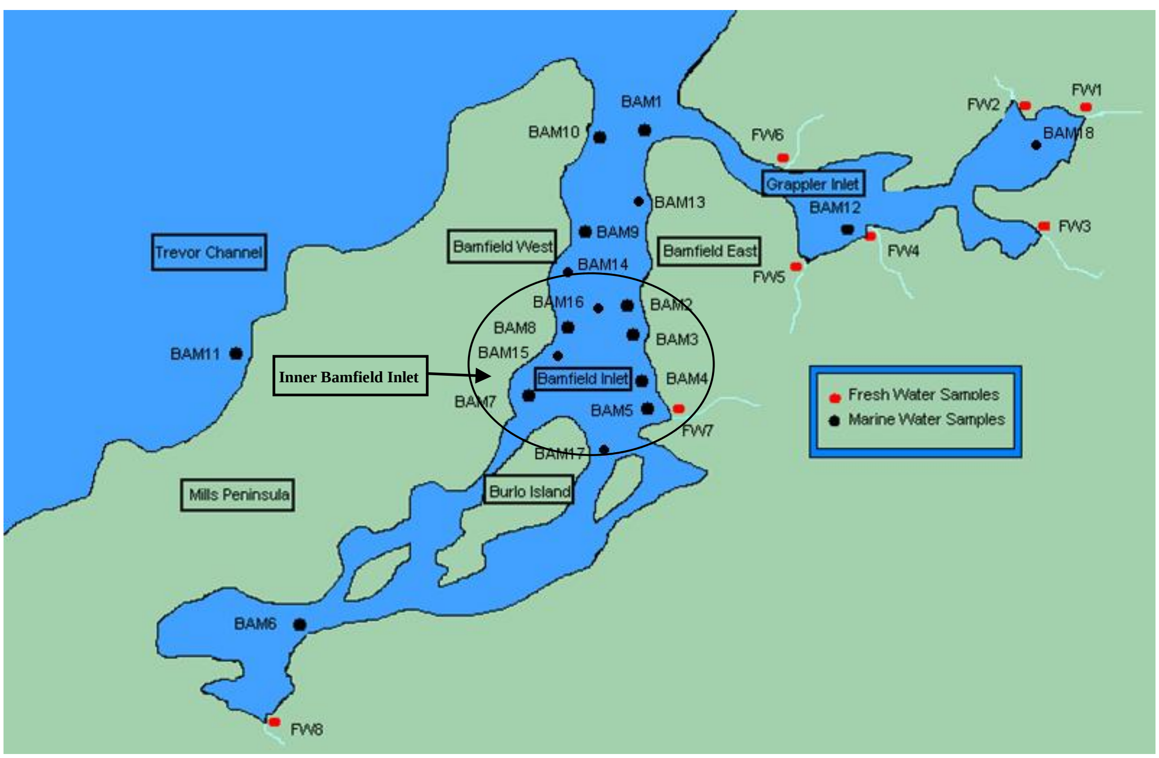

Figure 1. Bamfield and Grappler Inlets and their associated sampling locations.

Tides in the Barkley Sound area are the mixed semi-diurnal type with two unequal cycles per day. The lowest tides occur during the evenings in winter, and the mornings in summer. The maximum predicted amplitude is 3.9 m. Information on flushing rates and circulation patterns of the inlets are currently not available.

Bamfield Inlet consists of an inner and outer basin, separated by a few small islands (Burlo and Rance Islands). The inner basin is shallow with an average depth of 3 m and maximum depth of 9 m. The head of the inlet is comprised of a large mud flat area, which has two intermittent streams flowing into it. There is a shallow shelf, approximately 1 m in depth, which separates the two basins. The shelf, along with the seasonal instream flow to the inner basin, likely reduces flushing of the inner inlet. The mid-basin zone (or start of the outer basin), where most of the residential area is located, has an average depth of 10 m. There is another small intermittent stream, which flows into the east side of the inlet, just north of Rance Island. Moving out towards the mouth of the inlet, the inlet becomes deeper with an average depth of 20 m and a maximum depth of 45 m at the entrance.

Grappler Inlet is comprised of two basins separated by a shallow channel. The entrance to the inlet is narrow, with an average depth of 7 m. This opens up into Port Desire, the first basin, with an average depth of 5 m. Another narrow and shallow channel then connects Port Desire to the inner basin. This channel is passable only during moderate to high tides. The inner basin is open and shallow, with an average depth of 2 m. Sugsaw Creek flows into this inner basin, and flows all year round.

Predominant features of the shoreline in both Bamfield and Grappler inlets are rocky headlands, islands, reefs, caves, tidepools and surge channels. A wide range of soft sediments is found intertidally, including sand, mud and gravel. This is especially apparent in the inner basins of both inlets. Subtidally, the deeper areas are filled with silt while the shallower areas are overlain with gravel, sand or shell.

3.0 WATER USES

3.1 RECREATION

Bamfield and Grappler inlets provide significant recreational values for both foreshore residents and visitors. It's an important part of the community's identity, and very important socio-economically. Boating, including canoe and kayaking, are very popular activities within the inlet, both as a means of transport between the west and east sides of the community and for recreational purposes. Swimming occurs during the summer months from the many docks located along the foreshore. Brady's Beach, located on the west side of Mills Peninsula, is a popular location among locals and tourists.

3.2 FISHERIES

Fishing is the primary attraction to the area. While most fishing activities are conducted out in Barkley Sound, there are locals who fish from the docks as well as at the entrance to Bamfield Inlet. Species of interest include salmon, ling cod, rockfish, and halibut. Numerous shellfish species are found in both inlets however, the area is currently closed for harvesting. Seals, sea otters, and the occasional whale have been sighted within the inlets.

Students from the BMSC conduct research activities within the intertidal areas of both Bamfield and Grappler Inlets, looking at the flora and fauna found in this marine setting. Some scuba diving occurs around the area of the BMSC. However, the majority of the research projects are conducted in Barkley Sound, away from any significant human influences.

3.3 WILDLIFE

The foreshore areas of Bamfield and Grappler Inlets provide habitat to a variety of species typical of the west coast of Vancouver Island, including blacktail deer, black bear, cougar, wolf, eagles, hawks, owls, seagulls, and numerous other species of small birds and mammals.

3.4 DESIGNATED WATER USES

Designated water uses are those water uses that are designated for protection in a watershed or waterbody. Water quality objectives are designed for the substances or conditions of concern in a watershed so that their attainment will protect all designated uses. Currently for Bamfield and Grappler Inlets, the greatest risk is to human health when spending time in or on the water, swimming, boating or fishing. In addition to recreational use, the water should also be protected for aquatic life and wildlife. Furthermore, while the inlet and surrounding area is closed for shellfish harvesting, it is a goal of local residents and first nations to re-open all or parts of the area for future shellfish harvesting. In this regard, shellfish harvesting and consumption by humans is the most sensitive designated use for the future.

4.0 INFLUENCES ON WATER QUALITY

The primary concern with regards to potential impacts on water quality in Bamfield and Grappler inlets are associated with anthropogenic activities, specifically the discharge of human sewage throughout the inlets.

4.1 PERMITTED DISCHARGES

Currently there is no community sewage collection and treatment system in place at Bamfield, however there are 12 authorized commercial wastewater treatment facilities, including the BMSC. There are at least four un-authorized commercial operations. The majority of single family dwellings discharge either to the inlet directly or to septic tanks, if space is available.

Even those residents that do not directly discharge to either inlet may potentially cause fecal contamination if their septic fields are failing, not properly located, or not maintained. The many vessels that dock in the Bamfield Inlet are another source of human bacterial contamination. Vessels traveling to the area are not allowed to directly discharge to the marine environment while in port. However there are no pump out stations provided in the inlet, and control of these non-permitted discharges is limited.

4.2 WILDLIFE

Wildlife can influence water quality through fecal contamination, resulting in elevated levels of microbiological indicators (e.g. fecal coliforms, enterococci, and *Escherichia coli*) in water. Fecal contamination of water by animals is generally considered to be less of a concern to human health than contamination by humans because there is less risk of inter-specific transfer of pathogens.

Bamfield and Grappler Inlets contain significant old-growth wildlife habitat and provide a home for a wide variety of warm-blooded species (Section 3). In addition, the inlets are home to many marine mammals such as seals, otters, and mink. Therefore, a risk of fecal contamination from natural wildlife populations within the inlets does exist.

5.0 STUDY DETAILS AND METHODS

To better understand the sources of contamination in Bamfield and Grappler Inlets, this study focused on bacteriological indicators, such as fecal coliforms and enterococci, while also using caffeine levels, microbial source tracking (MST) techniques, and nitrogen isotope levels found in mussels to determine if the source of contamination was of human origin. To complete the study, MOE partnered with the community of Bamfield, ACRD, Huu-ay-aht First Nation, and the BMSC to set goals, provide an opportunity for public input, and assist with field work.

5.1 SAMPLING SITES AND SCHEDULE

Sampling was conducted between 2005 and 2007 during the peak summer months (July – August) at 18 sample sites throughout Bamfield and Grappler Inlet (Figure 1). This includes one control site (BAM11), Brady's Beach, located on the west side of Mills Peninsula, away from any direct influence from either inlet. The control site is not without its limitations, as it is subject to a greater degree of flushing, however it was the best site within reasonable distance to Bamfield. Table 1 provides a list of each sample site with a detailed description. Two freshwater sites were also established, both in Bamfield Inlet, to address any potential upslope contamination. Marine and freshwater samples were both collected on the same day.

Table 1. Marine (BAM 1-18) and freshwater (BAM FW7 and 8) sample sites in Bamfield Inlet.

EMS ID	Site Name	Site ID	Detailed Site Description
E259797	Bamfield 1 - Biological Station	BAM1	At Bamfield Marine Station outfall
E259798	Bamfield 2 - Kingfisher Marina	BAM2	Off of Kingfisher Marina, blue roof
E259799	Bamfield 3- Red Cross Station	BAM3	Out from storm drain just south of station
E259800	Bamfield 4 - Hawkeye Marina	BAM4	In front of Hawkeye Marina
E259801	Bamfield 5 - Federal Dock	BAM5	Just south of loading area
E259813	Bamfield 6 - Head Bamfield Inlet	BAM6	At head of inlet, on east side
E259802	Bamfield 7 - McKay Bay Lodge	BAM7	At McKay Bay Lodge, north of dock
E259803	Bamfield 8 - Mills Landing Cottages	BAM8	In front of cottages
E259804	Bamfield 9 - Spore Residence	BAM9	Spore residence, blue house
E259805	Bamfield 10 - General Store	BAM10	Dock in front of general store
E259806	Bamfield 11 - Brady's Beach (reference)	BAM11	North end of swimming area
E259807	Bamfield 12 - SeaBeam Resort	BAM12	Dock in front of resort - Grappler's Inlet
E259808	Bamfield 13 - Greenhouses	BAM13	Adjacent to greenhouses near BMS
E259809	Bamfield 14 - Community Hall	BAM14	House across from Community hall
E259810	Bamfield 15 - Imperial Eagle Lodge	BAM15	Dock in front of Imperial Eagle Lodge
E259811	Bamfield 16 - Buoy Y54	BAM16	Mid-inlet next to red buoy Y54
E259812	Bamfield 17 - Burlo and Rance Islands	BAM17	Mid-inlet between Burlo and Rance islands
E259814	Bamfield 18 - Head of Grappler Inlet	BAM18	Head of Grappler inlet, out from dock
E259877	Bamfield Freshwater 7	BAM FW7	East side, south of BAM5 and federal dock
E259878	Bamfield Freshwater 8	BAM FW8	Far end of Bamfield Inlet, south BAM6

In 2005, samples were collected on July 12, 19, 26, August 2, 10 and 16. In order to link sampling events with peak population times (primarily driven by fishing and tourism); sampling in subsequent years was not initiated until later in August and carried out through to early September. Thus, in 2006 sampling was conducted on August 10, 15, 22, 29 and September 6. Similarly for 2007, sampling was conducted on August 8, 14, 21, 28 and September 4. Sampling was generally done in the morning (between 08:00 and 10:00) to ensure transport of samples on the Lady Rose to Port Alberni. During the study period, most samples were collected on a rising tide, just after the low, and on a few occasions on a falling tide just before the low, as the end of discharge pipes were more visible. There were two occasions during 2007 when samples were collected during high tides, just as they were beginning to drop (August 21 and September 4). See Appendix 1 for more tide information.

5.2 MICROBIOLOGICAL INDICATORS

The microbiological quality of marine waters used for recreating and harvesting of seafood is imperative, as contamination of these systems can result in high risks to human health, as well as significant economic losses due to closure of beaches and shellfish harvesting areas (Scott *et al*., 2002). Water contaminated with human feces is generally regarded as a greater risk to human health, as the water is more likely to contain human-specific enteric pathogens, including *Salmonella enterica*, *Shigella* spp., Hepatitis A virus, and Norwalk-group viruses. The direct measurement and monitoring of pathogens in water, however, is difficult due to their low numbers, intermittent and generally unpredictable occurrence, and specific growth requirements (Krewski *et al*., 2004; Ishii and Sadowsky, 2008). To assess health risks, resource managers commonly measure fecal indicator bacteria levels (Field and Samadpour, 2007; Ishii and Sadowsky, 2008), whose presence is used to indicate the fecal contamination of water. The most commonly used indicator organisms for assessing the microbiological quality of water are the total coliforms, fecal coliforms (a subgroup of the total coliforms more appropriately termed thermotolerant coliforms as they can grow at elevated temperatures), *Escherichia coli*, a thermotolerant coliform considered to be specifically of fecal origin (Edberg *et al*., 2000), and enterococcus, a subgroup of the fecal streptococci, normally found in the gastrointestical tract of warm-blooded animals (Yates, 2007).

Fecal coliforms have been used extensively for many years as indicators for determining the sanitary quality of surface, recreational, and shellfish growing waters. However, research in recent years has shown there are many differences between the coliforms and the pathogenic microorganisms they are a surrogate for, which limits the use of coliforms as an indicator of fecal contamination (Scott *et al*., 2002). Therefore, additional microbes such as *E. coli* and enterococci have been suggested for use as alternative indicators (Griffin *et al*., 2001). *E. coli* is principally associated with fecal contamination (Kloot *et al*., 2006) while, enterococci are considered especially reliable as indicators of health risk in marine environments (Cabelli, 1983). It should be noted that Environment Canada still base their shellfish harvesting designations on fecal coliform measurements.

Thus, in this study, both fecal coliforms and enterococci were measured in the marine environment and *E. coli* in the two freshwater streams. Surface (0.5 m) grab samples for bacteriological analysis were collected on a weekly basis for five consecutive weeks from mid-July to mid-August at all sites. However, during 2005, six samples were collected over a 34 day period. It was decided to use all 6 sample dates in the statistical calculations for guideline comparisons as it made the data set more robust. Samples were collected according to Resource Inventory Standards Committee (RISC) standards (RISC, 1994) using a sampling pole (marine sites only) to minimize contamination. Samples were shipped on ice via the Lady Rose to Port Alberni, where the marine samples were then transported to North Island Labs in Courtenay, BC and the freshwater samples were sent via courier to Cantest Labs in Burnaby. When duplicate samples were collected, the results were incorporated into the 30-day average, thus some averages may be based on six samples in 30 days, rather than five.

5.3 MICROBIAL SOURCE TRACKING

While elevated levels of fecal indicator bacteria can indicate a potential risk to human health and provide evidence of fecal pollution, they cannot identify the source of contamination. When this is unknown, the actual risk to human health is uncertain and it is not always clear where to direct management efforts. Over the last decade, researchers have developed a range of microbial source tracking (MST) tools that can be used to distinguish human-sourced fecal contamination from that of animals (Ahmed *et al*., 2010). Some current methods include ribotyping, pulse-field gel electrophoresis, denaturing-gradient gel electrophoresis, repetitive DNA sequences (Rep-PCR), host-specific 16S rDNA genetic markers (*Bacteroides*), and antibiotic resistance analysis (Scott *et al.*, 2002; Meays *et al*., 2004). Each method appears to have distinct advantages and disadvantages and currently there is no standard method that has been adopted for source tracking (Meays *et al*., 2004). Determining which method or combination of methods to use for any given situation will depend on a number of factors including: the goal of the project; the level of detail required (broad scale results -human /non-human versus detailed results – human, livestock species, wildlife species);

availability of resources; time constraints; and access to a lab with expertise to analyze the samples.

The goal of this study was to determine if fecal contamination was occurring in Bamfield and Grappler inlets and, if so, to determine if the source was of human origin. Therefore, the MST technique used in this study combined the measurements of fecal indicator bacteria (i.e. fecal coliform) with the detection of human specific markers for *Bacteroides*. The *Bacteroides* technique can be used to discriminate and identify the source of contamination if it is human, ruminant (including deer), horse, pig, or dog. When interpreting the *Bacteroides* results, a score of 1 denotes a positive result, while a score of 0.5 denotes a potential positive result. This MST technique provides a presence/absence result and cannot quantify the amount of contamination from each source identified. Other considerations for selecting this method included the limited resources available for the study and the ability to use the Pacific Environmental Services Centre (PESC) laboratory in North Vancouver who specialize in this technique.

Samples for *Bacteroides* analyses were collected in a 1 L sterilized plastic bottle on a weekly basis, in conjunction with the bacteriological samples, for five consecutive weeks at all marine sites. These samples were shipped to North Island Labs, where they were filtered and prepared for further analysis. Only samples with fecal coliform counts of 40 colony forming units (CFU)/100 mL or higher had the additional *Bacateroides* analysis performed. The selected filtered samples were subsequently shipped in a cooler on ice to PESC and analyzed following the protocols developed by Bernhard and Field (2000).

5.4 CAFFEINE ANALYSIS

As mentioned above, there are certain shortcomings with relying solely on indicator bacteria to assess risks associated with pathogenic microorganisms in water. Methods for MST in aquatic environments have also been developed to distinguish animal from human sources, however, expense, reproducibility, and standardization have also been problems for these approaches (Scott *et al*., 2002). Recently, caffeine has been examined as a potential chemical marker for surveillance of human fecal input into source water (Peeler *et al*., 2006). As an active ingredient in many beverages, pharmaceuticals, and food products, and only being partially metabolized in the human body, caffeine has been found in many different aquatic ecosystems (Buerge *et al*., 2003). The stability of caffeine in the aquatic environment, and its source-specificity make caffeine an ideal surrogate of human-derived fecal pollution of source water (Weigel et al., 2002). In many instances, there appears to be an association between elevated caffeine concentrations and population densities.

Water samples for caffeine analysis were collected during the first and last week of the five week sample period for all three years, with the exception of 2007 when the samples were collected on the first and fourth week. Samples were collected using 1 L plastic bottles at just below the surface (0.5 m). The samples were stored on ice and subsequently frozen upon return to the MOE office in Nanaimo. Caffeine samples for each summer were sent as a batch to the University of Victoria Water Quality Lab for caffeine extraction following standard methodologies (Sergei Verenitch, Senior Lab Manager UVIC, pers. comm.). It should be noted that minimum detection limits changed during each consecutive sampling year as the methods for caffeine analyses improved. Minimum detection limits were 10 ng/L for 2005, 4 ng/L for 2006 and 2 ng/L for 2007. On the occasion when duplicate samples were collected, the average of the two numbers was calculated and used for data analysis.

As caffeine samples were collected twice per year from each site during the sampling period, the two values were averaged to produce an annual result for site-to-site and year-to-year comparison. The exception to this was in 2005, when caffeine samples were only collected at 7 locations on the first date (July 12, 2005), and at all sites on the second sampling date (August 10, 2005), therefore averages were only calculated for the 7 sites and the remaining sites are individual results.

5.5 NITROGEN ISOTOPE ANALYSIS

Traditional methods for monitoring sewage dispersal around sewage outfalls often use spot sampling of physiochemical tracers, such as nutrients and salinity, or bacteriological concentrations. Recent work indicates that stable isotopic signatures of the receiving environment biota may indicate the dispersal of sewage nitrogen (McKinney *et al*., 2001;

Rogers, 2003). Often the marine environment can be nitrogen-limited, and algae will assimilate nitrogen additions from alternative sources, such as sewage effluent (Lyngby and Mortensen, 1994). Human sewage is characterized by an enrichment of ^{15}N in the discharge, which can be easily distinguished from pure marine un-impacted sites. Normal oceanic $\delta^{15}N$ levels are approximately 6.7‰ (Gartner *et al*. 2002) whereas sewage effluent generally has observed $\delta^{15}N$ levels of 10-20‰. Mussels have been shown to be effective biomonitors, able to accumulate and concentrate a variety of toxins, resulting in bioaccumulations and biomagnifications (Rogers, 1999). Several studies have confirmed that benthic organisms and filter feeders from sewage-impacted areas have isotopic signatures distinct from those collected at reference sites (Tucker *et al*., 1999; Rogers, 2003).

Analysis of nitrogen isotopic signatures involved the collection of Pacific blue mussels (*Mytilus trossulus*) once during each study year (August 9, 2005, September 6, 2006 and August 27, 2007) from sixteen sites within Bamfield Inlet. Mussels ranged in size from 30 mm (BAM 1) to 103 mm (BAM 11) with an average size of approximately 56 mm. There was one exception where a mussel collected from BAM 1 was 160 mm in length, and this individual was processed separately. It was thought that this mussel may, in fact, be a California mussel (*M. californicus*) due to its size. The mussels were collected during low tide from docks and pilings adjacent to the water sample sites. The adductor muscle was removed from each mussel and placed in a tissue cup. Multiple mussels (7-12 mussels per site) from the same area were pooled to ensure minimum sample wet weight of 20-30 milligrams. These samples were sent to the UVIC lab where they were freeze-dried, homogenized by grinding and analyzed for $\delta^{15}N$ using thermal combustion elemental analyzer coupled with isotope ratio mass spectometer. Replicate samples were collected at randomly selected sites during each sample year. Replicate samples consisted of multiple mussels per site similar to the original sample. The replicate results were used as individual data points to both increase the data set and to illustrate consistency between samples collected at one station.

5.6 QUALITY ASSURANCE / QUALITY CONTROL

Quality assurance and quality control was verified by collecting duplicate samples. Duplicate microbiological and caffeine samples were collected by filling two sample bottles in as close to the same time period as possible (one right after the other) at a monitoring location. For duplicate mussel samples, the mussels at a location were collected and randomly split into two groups to be processed for nitrogen isotope analysis. The UVIC lab also conducted duplicate nitrogen isotope analysis on occasion, whereby they split a sample at the lab, to assess analytical technique. Finally duplicate MST samples were only conducted if duplicate fecal coliform data was collected for a particular site and both results were greater than 40 CFU/100 mL. The percent difference was then calculated between the laboratory duplicate results reported for the various samples and techniques used in this study. The maximum acceptable percentage difference between duplicate samples is 25% (RISC, 1997). However, this interpretation only holds true if the results are at least 10 times the detectable limits for a given parameter, as the accuracy of a result close to the detectable limit shows more variability than results well above detectable limits. As well, some parameters (notably bacteriological indicators) are not homogeneous throughout the water column and therefore we expect to see a higher degree of variability between replicate samples.

6.0 WATER QUALITY ASSESSMENT

6.1 QUALITY ASSURANCE / QUALITY CONTROL RESULTS

Fifty-six sets of duplicate samples were collected for microbiological parameters during the sampling program (Appendix II). Relative percent mean differences were not calculated as 68% of the results were less than 10 times the detection limits (<1 CFU/100 ml), and therefore the guidelines for interpreting acceptability do not apply. There were a few exceptions when the results for duplicate samples had high variability thus relative percent differences could be calculated. For example on August 10, 2006 at BAM 5, fecal coliform results were 170 CFU/100 mL and >1,600 CFU/100 mL, however enterococci results for the same date and location were similar, 108 CFU/100 mL and

126 CFU/100 mL (relative percent difference of 15.4%). In these instances contamination may have occurred during collection/analysis, but it is more likely the result of environmental conditions that were highly variable, as both these analyses (fecal coliform and enterococci) came from the same bottle. This suggests that there are many non-fecal coliforms that contribute to the fecal coliform results.

Duplicate samples were collected for nitrogen isotope analysis (10 samples) and caffeine (5 samples) (Appendix II). Nitrogen isotope analysis duplicate results were all within acceptable limits with relative percent differences ranging from 0 % to 9.9%. Detection limits for caffeine varied per sample year, <10 ng/L for 2005, <4 ng/L for 2006, and <2 ng/L for 2007, due to analytical improvements. The caffeine results were either just at or slightly below the acceptable 10 times detection limits level. However, relative percent differences for caffeine duplicate samples were calculated and ranged from 10.2% to 28.9%. Three of the five duplicate samples fell within the maximum acceptable percentage difference (25%) for duplicate samples. The exceptions were in 2006 at BAM 15 with relative percent difference of 28.9%, and 2007 at BAM 4 with relative percent difference of 28%.

The duplicate results for MST analysis are more descriptive than numerical (*i.e.* presence/absence). However human, ruminant, and pig were positively identified in the 4 duplicate sets of samples. It should be noted that the level of confidence in a positive pig result is not as high as any of the other organisms because pig primers for one of the markers have been noted to cross-prime with ruminant animals (PESC, 2006).

6.2 MICROBIOLOGICAL INDICATORS

The British Columbia (BC) MOE water quality guidelines (Table 2) were used to assess water quality in Bamfield and Grappler Inlets based on the designated use of the water (*i.e.*, shellfish harvesting, and primary and secondary contact recreation). Primary contact refers to direct contact with water over most of the body's surface, to the point of complete submergence, or where there is substantial risk of ingestion or intimate contact with eyes, ears, nose, mouth, or groin, such as swimming and scuba diving. Secondary contact refers to an activity where a person would have very limited direct contact with the water, usually only the feet and hands, and little risk of complete immersion, such as boating, kayaking, canoeing, and fishing. These water quality guidelines are set at levels intended to prevent health problems in healthy adults. Children, seniors and domestic animals may be more susceptible to illness. As small pieces of fecal matter in a sample can skew the overall results for a particular site, the medians (for aquatic life-shellfish) and geometric means (for recreation) are used to determine if the water quality guideline is exceeded, as extreme values would have less effect on the data.

Table 2. The BC MOE water quality guidelines for microbiological indicators (CFU/100 mL) (Warrington, 1988). Medians and geometric means are calculated from at least five weekly samples in a 30-day period and 90th percentiles require 10 samples in 30 days.

Water use	*E. coli* (freshwater only)	Enterococci	Fecal coliforms
Aquatic life • Shellfish harvesting	Less than or equal to 14 CFU/100 mL (median)	Less than or equal to 4 CFU/100 mL (median)	Less than or equal to 14 CFU/100 mL (median)
Aquatic life • Shellfish harvesting	Less than or equal to 43 CFU/100 mL (90th percentile)	Less than or equal to 11 CFU/100 mL (90th percentile)	Less than or equal to 43 CFU/100 mL (90th percentile)
Recreation • Primary contact	Less than or equal to 77 CFU/100 mL (geometric mean)	Less than or equal to 20 CFU/100 mL (geometric mean)	Less than or equal to 200 CFU/100 mL (geometric mean)
Recreation • Secondary contact • Crustacean harvesting	Less than or equal to 385 CFU/100 mL (geometric mean)	Less than or equal to 100 CFU/100 mL (geometric mean)	None applicable

6.2.1 Fecal coliforms

Individual values for fecal coliforms ranged from below detection limits (<1 CFU/100 mL) to a maximum of 1,600 CFU/100 mL. Figure 2 illustrates the fecal coliform geometric means calculated for all sites for each year. The geometric mean for all sites ranged from 2 CFU/100 mL at BAM 11 (2007) to 144 CFU/100 mL at BAM 4 (2006). BAM 11 (control site) was consistently lower than all other sample locations, and met the water quality guidelines for shellfish harvesting and primary recreation (there is no fecal coliforms guideline for secondary recreation) on all occasions. While fecal coliform geometric means were elevated in the inner Bamfield Inlet (*i.e.*, area around the vicinity of Federal dock extending over to the fisheries dock; see Figure 1) all sites met the fecal coliform primary recreation guideline of 200 CFU/100ml for each of the 3 summers sampled. However, the 2006 results were generally higher than either 2005 or 2007 data.

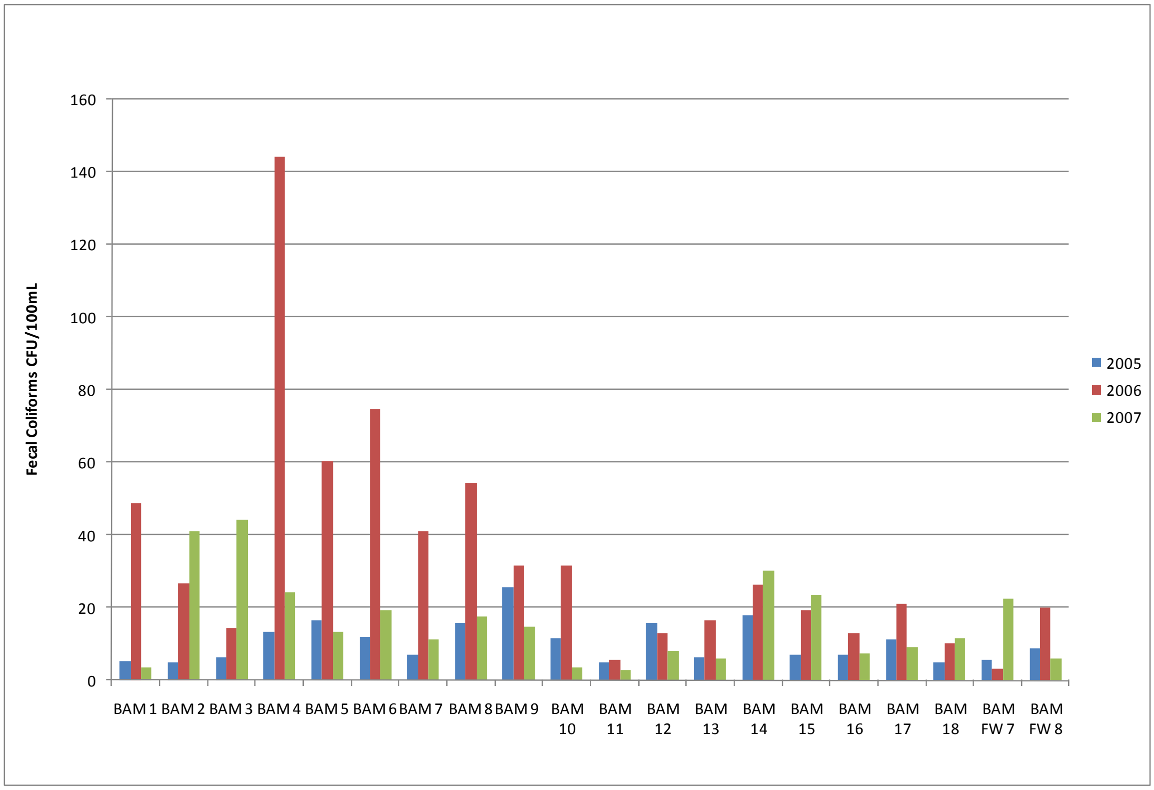

Figure 2. Fecal coliform geometric mean results for 2005-2007.

When comparing the fecal coliform median values for all sites against the shellfish harvesting (aquatic life) guideline ($\leq$14 CFU/100 ml) there were some exceedances in all three years. The fecal coliform median values ranged from 2 CFU/100 mL at BAM 11 (2007) to 89 CFU/100 mL at BAM 4 (2006) (Figure 3). There were 4 exceedances in 2005, 14 in 2006 and 6 in 2007. These exceedances were more prevalent in the inner Bamfield Inlet area.

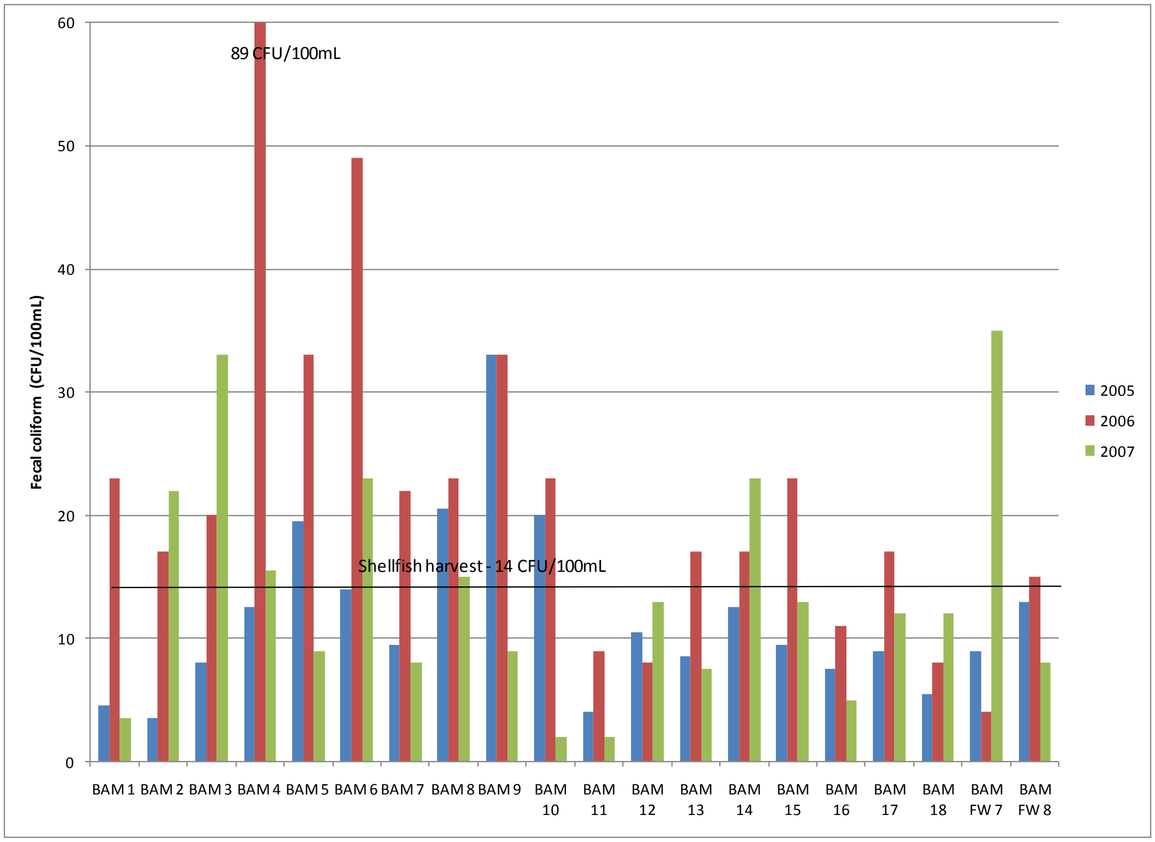

Figure 3. Fecal coliform median values (CFU/100 mL) for 2005-2007.

Fecal coliforms were also measured at two freshwater locations flowing into Bamfield Inlet, with individual values ranging from <1 CFU/100 mL at FW7 (2006) to a maximum of 103 CFU/100 mL at FW7 (2007). The fecal coliform geometric means for these two sites were low ranging from 3 CFU/100 mL at FW7 (2006) to 20 CFU/100 mL at FW8 (2006) (Figure 2). These values were well below the primary recreation guideline of 200 CFU/100 ml.

As bivalves are filter feeders and concentrate pathogens, the concentration of coliforms in the meat on a per 100 g basis can be expected to be 10 to 100 times the concentration in 100 mL of the water in which they grow (Warrington, 1988). For this reason, the quality of growing waters must be very high. Currently, there are no water quality shellfish harvesting guidelines for fresh water environments. While shellfish harvesting is not occurring in the freshwater environments, the shellfish harvesting guidelines were applied to the two freshwater inputs as they are potential sources of fecal contamination into Bamfield Inlet. The shellfish harvesting fecal coliform median guideline value of ≤ 14 CFU/100 mL was exceeded twice during the sampling period (FW7- 35 CFU/100 mL median value in 2007 and FW8 – 15 CFU/100 mL median value in 2006) (Figure 3). This illustrates the potential for upland sources to contribute to fecal contamination in the marine environment.

6.2.2 Enterococci

Individual values ranged from below detectable limits (<0.5 CFU/100 mL) to a maximum of 682 CFU/100 mL. Figure 4 illustrates the enterococci geometric means calculated for all sites for each of the three years, which ranged from 1 CFU/100 mL at BAM 11 (2006) to 144 CFU/100 mL at BAM 4 (2006). The primary recreation guideline (20 CFU/100 mL) for enterococci was exceeded 6 times throughout the sampling period: 4 sites (BAM 4, 5, 6, and 8) in 2006; and 2 sites (BAM 5 and 8) in 2007. The secondary recreation guideline (100 CFU/mL) was only exceeded on one occasion at BAM 4 in 2006. These exceedances were also largely clustered within the inner Bamfield inlet area, with the exception of BAM 6 at the head of Bamfield Inlet.

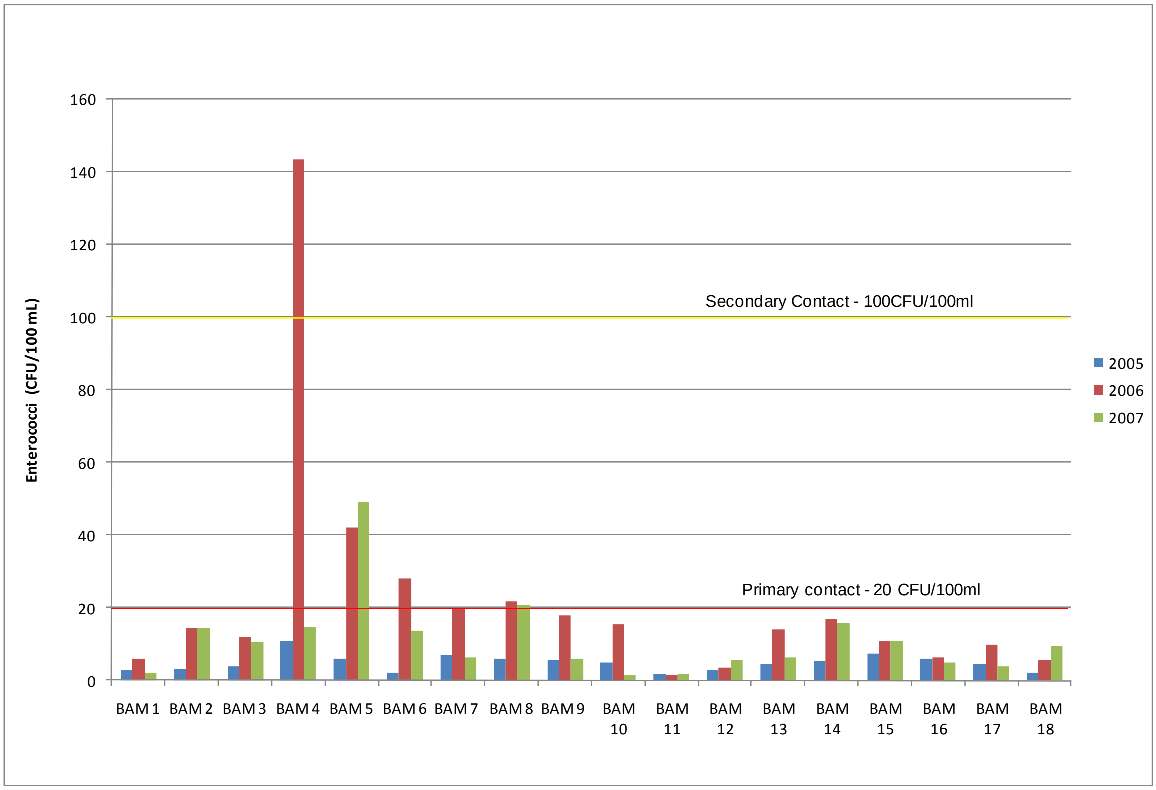

Figure 4. Enterococci geometric means (CFU/100 mL) for 2005-2007.

There were numerous exceedances of the shellfish harvesting guideline for enterococci (≤4 CFU/100 mL) at most sites in all three sample years (Figure 5). The enterococci median values ranged from 1 CFU/100 mL (BAM 18 in 2005 and BAM 1, 10 and 11 in 2007) to 142 CFU/100 mL at BAM 4 (2006). Of the 18 sites sampled, there were 11 exceedances in 2005, 17 in 2006 and 14 in 2007. These exceedances occurred throughout much of the sample area.

Enterococci concentrations were consistently low at BAM 11, the control site, where influence from human sewage would be negligible.

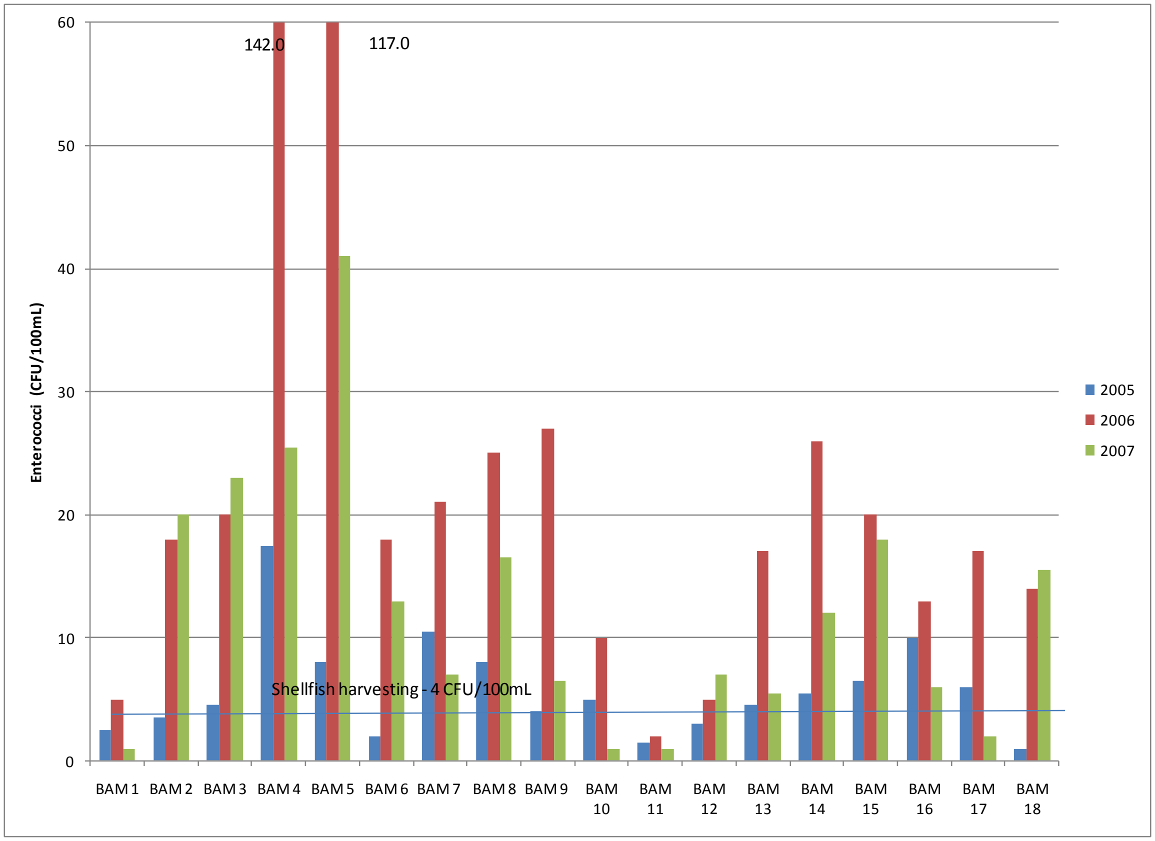

Figure 5. Enterococci median values (CFU/100 mL) for 2005-2007.

6.2.3 *E. coli*

E. coli concentrations were only measured in the two freshwater sites, and ranged from below detectable limits (< 1 CFU/100 mL) to 102 CFU/100 mL (FW7-2007). The geometric means for these two sites ranged from 2 CFU/100 mL (FW7- 2006) to 15 CFU/100 mL (FW8-2006), well below both the primary and secondary recreation guidelines for *E. coli*. Median values were also low and ranged from 2 CFU/100 mL at site FW7 (2006) to 11 CFU/100 mL at site FW8 (2006), well below the shellfish harvesting guideline of ≤14 CFU/100 mL. Overall, the range of values for both fecal coliforms and *E. coli* were similar, however, fecal coliform concentrations generally tended to be slightly higher than *E. coli* concentrations, likely due to the contribution of coliforms from non-fecal sources.

6.2.4 Proposed Water Quality Objectives

Microbiological data from this study showed that Bamfield Inlet, and to a lesser extent Grappler Inlet, is clearly subject to bacteriological contamination, particularly in the areas adjacent to the community of Bamfield. The control site, BAM 11, illustrates that natural or background concentrations of bacterial contamination are very low. The outer Bamfield sites are likely subject to flushing as well as compared to the inner basin. It is also apparent that microbial input to Bamfield Inlet from the freshwater sites is minimal. This suggests that the bacteriological contamination is coming from anthropogenic sources within the inlets.

During the summer (the peak tourist season at Bamfield), bacteriological levels in this area can exceed BC Water Quality Guideline levels for primary and secondary recreation. This poses a health risk to swimmers and at times, boaters or kayakers. In addition, shellfish harvesting, while closed in the inlet and surrounding area, is a designated water use that local shellfish harvesters and residents would like to see re-established. Therefore, a short term and a long-term water quality objective are proposed for both fecal coliforms and enterococci. The short-term objectives are based on primary and secondary recreation uses, while the long-term objectives are proposed for future shellfish harvesting. While fecal coliforms do have their limitations as indicators (see Section 5.2), they were chosen in addition to enterococci, as they are more relevant to the Environment Canada shellfish regulations. Objectives established for shellfish harvesting may only be applicable to portions of the inlets as the area is potentially opened for harvesting through options such as conditional management plans, seasonal openings or depuration. However, any future shellfish harvesting would be dependent on the success of measures taken to reduce bacteriological contamination. ***Thus, the proposed short-term (5-10 years) water quality objective is that the geometric mean of a minimum of five weekly samples collected within a 30-day period must not exceed 20 CFU/100 mL for enterococci and must not exceed 200 CFU/100 mL for fecal coliforms at all sites within Bamfield and Grappler Inlets. The proposed long-term (>10 years) water quality objective is that the median of a minimum of five weekly samples collected within a 30-day period must not exceed 4 CFU/100 mL for enterococci and must not***

exceed 14 CFU/100 mL for fecal coliforms at all sites within Bamfield and Grappler Inlets. The 90th percentiles were not used as part of the water quality objectives development, as they are based on 10 samples collected in 30 days, which is not convenient or cost effective for the Bamfield area.

6.3 MICROBIAL SOURCE TRACKING

Over the three-year study period, *Bacteroides* analyses were conducted on 12 of the 15 sample dates (Table 3). In 2005, two sample dates (July 19 and August 10), with a total of 10 samples, were analyzed. All 5 sample dates in 2006 had sufficient fecal coliform concentrations to conduct the *Bacteroides* analysis, with a total of 31 samples analyzed. Similarly in 2007, all five sample dates had at least one site with sufficient concentrations, for a total of 17 samples. In all samples submitted, human markers were identified. In addition to identifying human fecal matter, the MST analysis also identified ruminants (i.e. deer), pig, and dogs as sources of contamination. There were also two dates when horse was identified, both in 2005 (July 19, and August 10). The level of confidence in a positive pig result is not as high as any of the other organisms because pig primers for one of the markers have been noted to cross-prime with ruminant animal (PESC, 2006). The likelihood of pig fecal material present in Bamfield Inlet is nil, therefore these results are more likely representative of ruminant animals (*i.e*. deer). There have been horses present on occasion in the Bamfield area.

Figure 6 illustrates MST results for human only contamination broken down by site. The MST results confirm the presence of human sewage contamination throughout Bamfield Inlet. The results are consistent with the elevated bacteriological levels found in the inner harbor (BAM 4, 5, 6, 9 and 14) along with the presence of caffeine and elevated nitrogen isotope levels (see Sections 6.3 and 6.4). Of particular interest, Bam 11, the control site, did not have any human sources identified through the MST analysis.

Table 3. Summary of *Bacteroides* results for Bamfield and Grappler Inlets (2005-2007). Note: 1.0 = confirmed presence of human markers and 0.5 = potential presence of human markers.

	July 19/05		August 10/05			August 10/06				August 15/06			August 22/06				August 29/06			Sept 6/06			Aug 8/07		Aug 14/07		August 21/07				August 28/07				Sept 4/07		
Station Name	Human	Horse	Human	Ruminant	Horse	Human	Ruminant	Pig	Dog	Human	Ruminant	Pig	Human	Ruminant	Pig	Dog	Human	Ruminant	Pig	Human	Ruminant	Pig	Human	Ruminant	Human	Ruminant	Human	Ruminant	Pig	Dog	Human	Ruminant	Pig	Dog	Human	Ruminant	Pig
BAMFIELD 1						1.0	1.0										1.0	1.0	1.0																		
BAMFIELD 2						0.5	1.0													1.0	0.5	1.0					1.0	1.0							1.0	1.0	
BAMFIELD 3						1.0	1.0																				1.0	1.0			1.0	1.0	1.0				
BAMFIELD 4		1.0				0.5	0.5			1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.5	1.0	0.5	0.5					1.0	1.0			1.0	1.0		1.0			
BAMFIELD 5			1.0	1.0		1.0	1.0						1.0	1.0	1.0												1.0	1.0			1.0	1.0					
BAMFIELD 6	0.5					1.0	0.5	0.5		1.0	0.5	1.0					1.0	1.0	0.5																0.5	0.5	1.0
BAMFIELD 7						0.5	0.5													1.0	1.0	0.5					1.0	1.0		1.0							
BAMFIELD 8			1.0	1.0		0.5	0.5						1.0	1.0	1.0												1.0	1.0									
BAMFIELD 9			1.0	0.5		1.0	0.5										1.0	1.0	1.0																1.0	1.0	
BAMFIELD 10						0.5	0.5										1.0	1.0	0.5																		
BAMFIELD 11																																					
BAMFIELD 12	1.0																			1.0	0.5	0.5															
BAMFIELD 13						0.5	0.5																														
BAMFIELD 14	1.0		1.0	1.0		1.0	0.5		1.0				1.0	1.0	1.0	1.0							1.0	1.0													
BAMFIELD 15						1.0	0.5	0.5	1.0																		1.0	1.0									
BAMFIELD 16	1.0					1.0	1.0	0.5	1.0																		1.0	1.0									
BAMFIELD 17			1.0	0.5	0.5															1.0	0.5	1.0															
BAMFIELD 18						0.5	0.5																		0.5	1.0									0.5	1.0	1.0

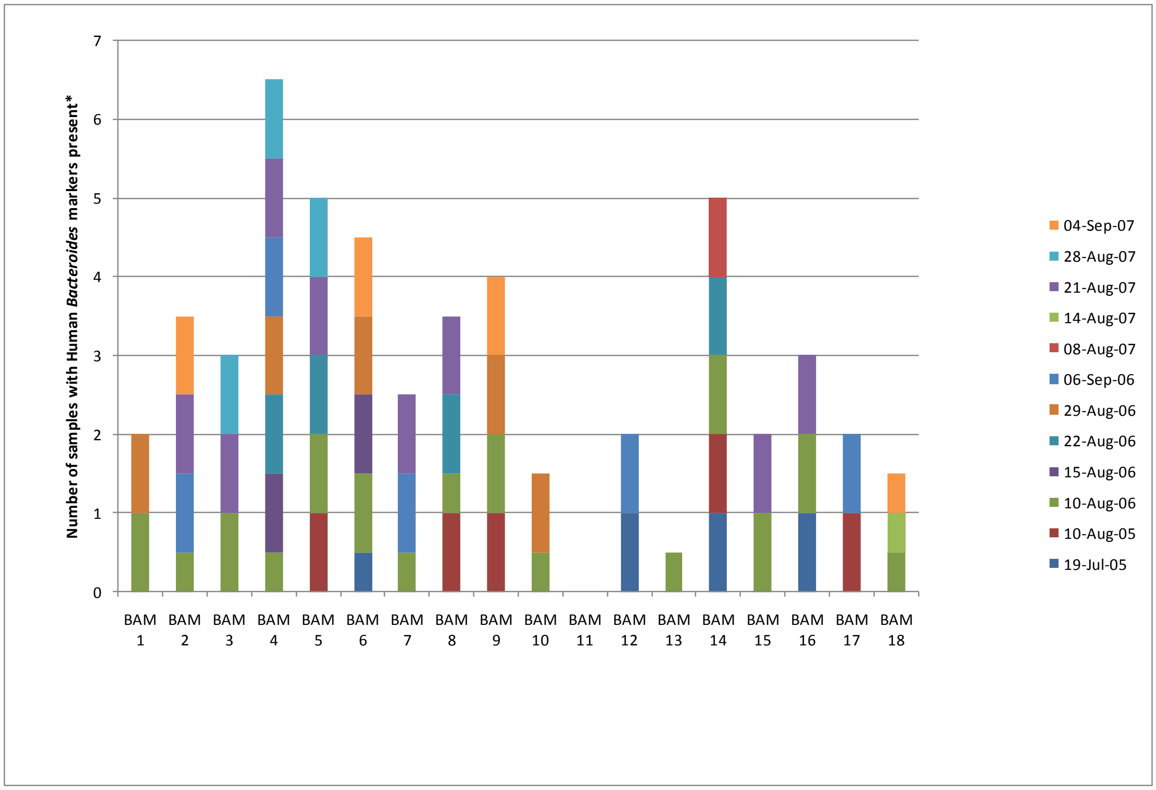

Figure 6. Number of samples per site that contain confirmed *Bacteroides* human markers. *Samples which confirm human sources = 1.0, while samples which are potentially human = 0.5.

6.4 CAFFEINE

Caffeine was detected in each of the 18 sample sites, including the reference site (BAM 11) on one occasion in 2007 (Figure 7). Observing caffeine at the reference site is not unusual and can be attributed to many things such as the natural occurrence of caffeine in the environment from plant species (Peeler *et al*., 2006) and the presence of swimmers or boaters at Brady Beach (BAM 11).

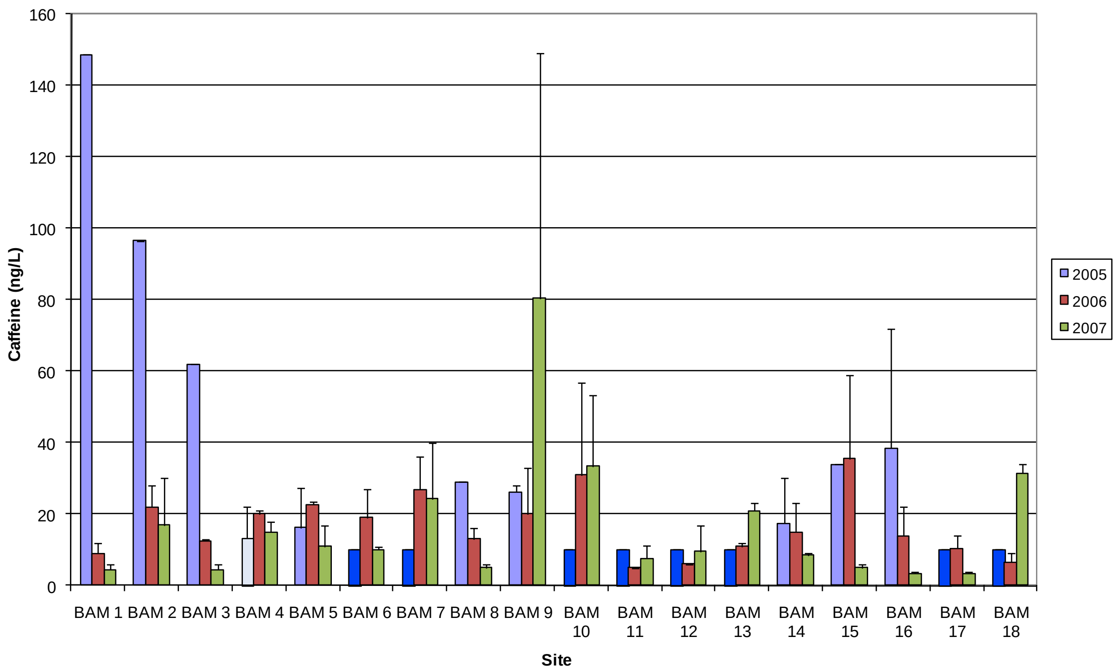

Figure 7.Yearly average caffeine concentrations (ng/L) at Bamfield marine sites 2005-2007. The striped values for 2005 indicate values at minimum detection limits (MDLs). MDLs varied for each year (2005 MDL = 10 ng/L; 2006 MDL = 4 ng/L; 2007 MDL = 2 ng/L). The error bars represent standard deviation when averages were calcualted.

Caffeine concentrations varied depending on the sampling location ranging from 2 ng/L to 149 ng/L. Year-to-year variability was observed at BAM 1, 2, and 3, where the 2005 caffeine concentrations were relatively higher than the 2006 and 2007 results. The higher values at the entrance to Bamfield Inlet (BAM 1) in 2005 may be in response to tide levels and flushing of the inlet. The caffeine samples collected on August 10, 2005 were obtained during a falling tide, when water from the inlet was moving out towards Trevor Channel. In general, most samples were collected during the low slack tide. Overall, there are tendencies for the higher caffeine values to be located at sites within the inner Bamfield Inlet area, particularly on the west side of the inlet (BAM 7, 8, 9, 15 and 16). Thus, it appears the caffeine results in general, are supportive of the microbiological data and the *Bacteroides* results which all illustrate the presence of human sewage in Bamfield inlet, primarily the inner area.

6.5 NITROGEN ISOTOPES

The $\delta^{15}N$ isotopic signature results obtained from the mussels collected in the inlet range from 7.4‰ in 2007 (BAM 11-reference) to a maximum of 11.1‰ (BAM 18-head of Grappler Inlet) in 2006 (Figure 8). The highest value in all three sample years comes from either the head of Bamfield Inlet or Grappler Inlet. The head of Bamfield Inlet (BAM 6) also tended to have some of the overall higher fecal coliform and enterococci results, as well as relatively high human contamination confirmed through the MST analysis. The heads of both inlets have very few homes associated with those areas as compared to the mouth or outer portion of both inlets. However, these areas are relatively shallow, sheltered and poorly flushed. The bottom substrates at the heads of both inlets are comprised primarily of fine organic sediments. Therefore, it may be reasonable to assume that the fine particles associated with the human sewage inputs into the Inlets, move with the tides settling out in the head of the Inlets. This would suggest that mussels collected from these areas would tend to have higher $\delta^{15}N$ isotopic signatures. However the difference between reference (BAM 11) $\delta^{15}N$ isotopic signature results and these sites with little development is only 2-3‰. This difference could also be attributed to other factors such as nitrogen cycling (i.e. uptake of N^{14} versus N^{15}) in shallow versus deep water sites.

While the 2005 and 2006 $\delta^{15}N$ results were very similar at all sites, there was an overall decrease in $\delta^{15}N$ of about 1‰ in the 2007 results. There were no obvious changes to sewage input or sources of nutrients to the inlets between all three years. Of importance to note, is that in 2007 the head of Bamfield inlet (BAM 6) still had the highest $\delta^{15}N$ value which is consistent with 2005 and 2006 results. Again the differences between years could also be attributed to the variability of the $\delta^{15}N$ level in primary producers related to the source of nitrogen and the fractionation of nitrogen that occurs during uptake and metabolism (McKinney *et al*., 2001).

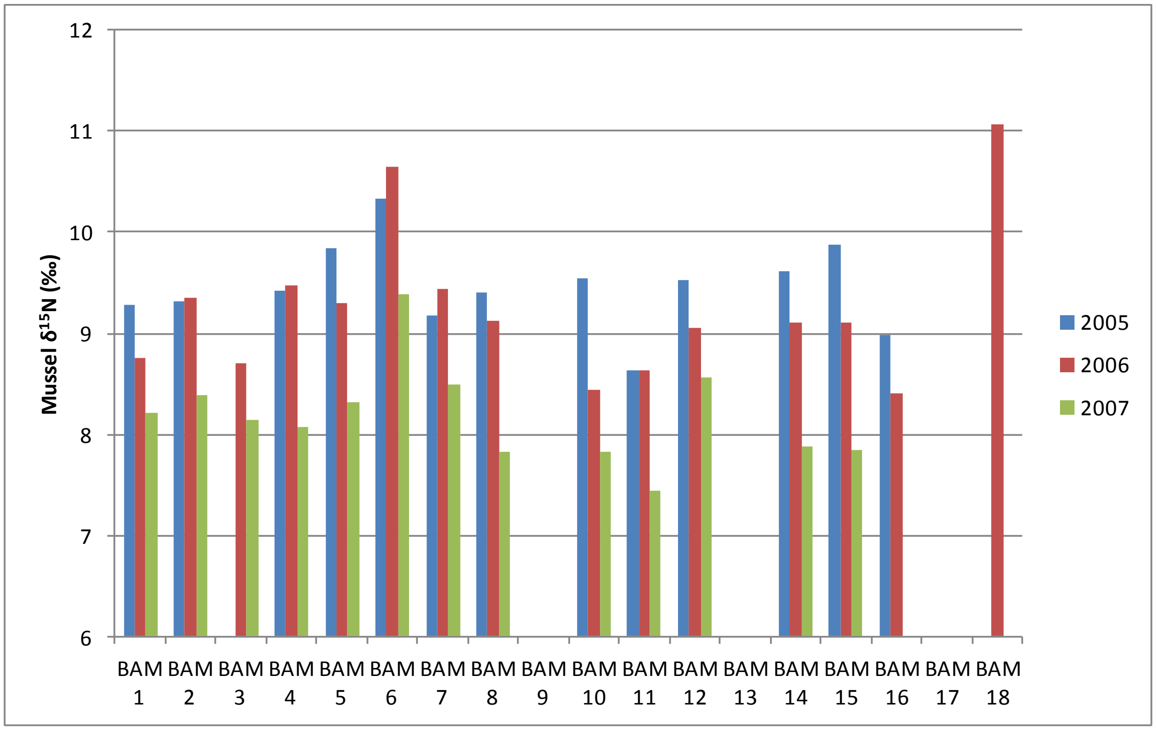

Figure 8. Annual average $\delta^{15}N$ (‰) of mussels found in Bamfield Inlet (2005-2007).

In general, the $\delta^{15}N$ data indicates a potential enrichment of up to 3‰ in the head waters of Bamfield and Grappler Inlet sites relative to the reference location at Brady's Beach. There is even less difference in nitrogen isotope levels between reference and the more impacted sites (inner Bamfield inlet). There were a few occasions when the $\delta^{15}N$ results at a particular site were slightly lower than those observed at the reference site (BAM 10 and 16 in 2006; BAM 16 in 2007). BAM 16 is a mid channel buoy marker and likely to have more flushing than sites located closer to shore where deposition is more likely to occur. However, there has not been a lot of research looking at the use of nitrogen isotopes in the marine environment. Most of the research has involved large waste water effluent discharges and substantial land use or anthropogenic type activities within the test area, which supported differences observed in $\delta^{15}N$ levels. With regards to Bamfield inlet, the human sewages sources are relatively small but numerous, making the nitrogen isotope analysis more difficult. Overall, more work is required in this area, such as seasonal differences, sediment deposition and nitrogen uptake, before some definitive conclusions can be made from the nitrogen isotope results.

7.0 WATER QUALITY OBJECTIVES

The results from this assessment suggest that human sewage is the main contributor to microbiological contamination in both inlets. Short-term and long-term water quality objectives are proposed for both fecal coliforms and enterococci. The short-term objectives are based on primary and secondary recreation uses, while long-term objectives are proposed for future shellfish harvesting. Objectives established for shellfish harvesting may only be applicable to portions of the inlets, as the area that could be opened for harvesting may be limited to options such as conditional management plans, seasonal openings, or depuration. However, any future shellfish harvesting would be dependent on the success of measures taken to reduce bacteriological contamination. The objectives are summarized in Table 4.

Table 4. Summary of proposed water quality objectives for Bamfield and Grappler Inlets.

Time Period	Variable	Objective Value
Short-term (5-10 years)	Fecal Coliform	≤ 200 CFU/100 mL (geometric mean based on a minimum of five weekly samples collected over a 30-day period)
	Enterococci	≤ 20 CFU/100 mL (geometric mean based on a minimum of five weekly samples collected over a 30-day period)
Long-term (>10years)	Fecal Coliform	≤ 14 CFU/100 mL (median based on a minimum of five weekly samples collected over a 30-day period)
	Enterococci	≤ 4 CFU/100 mL (median based on a minimum of five weekly samples collected over a 30-day period)

Designated water uses: recreation, aquatic life – shellfish harvesting and wildlife

While the fecal coliform and enterococci results have highlighted a potential threat to human health, the supporting data add further weight in confirming that the source of this contamination is human. While the MST, caffeine and $\delta^{15}N$ analytical tools are relatively new techniques requiring further refinement as stand alone tools, they are valuable in confirming contamination due to human sewage. Furthermore the sampling occurs in a dynamic environment where tides, currents, weather, and sources of contamination are constantly changing. Each of the tools used responds differently in the environment, due to varying stabilities and biological interactions, in some cases making correlations difficult. For example, fecal coliforms are relatively short lived in marine waters, while caffeine is relatively stable and $\delta^{15}N$ can be affected by the nitrogen cycle process. Regardless, *Bacteroides* analysis, caffeine, and, to a lesser degree, $\delta^{15}N$ confirm the presence and influence of human sewage throughout Bamfield and Grappler Inlets. They are a valuable means to characterize the sewage inputs and should be considered in future monitoring work.

The water quality objectives recommended here take into account the impacts from past land use and should be periodically reviewed and revised to reflect any future improvements in water quality.

8.0 MONITORING RECOMMENDATIONS

The recommended water quality monitoring program for Bamfield and Grappler inlets is summarized in Table 5. The attainment monitoring should be conducted every 3-5 years based on staff and funding availability, and whether activities, such as land use or upgrades to sewage discharges, are underway within the inlet. In order to capture the periods where water quality concerns are most likely to occur (*i.e.*, highest population use of the area and lack of dilution) we recommend that a minimum of five weekly samples be collected within a 30-day period between late July and early September. Samples should be collected at all 18 marine sites as well as the 2 freshwater sites. Samples should be analyzed for microbiological indicators; marine (fecal coliforms and *enterococci*) and freshwater (fecal coliforms and *E. coli*).

It is also recommended that the new techniques used in this study be considered in any future monitoring work. Of particular interest would be the *Bacteroides* analysis if funds were limited, as this technique provided the strongest agreement with the microbiological results and thus, the presence of human contamination.

Table 5. Proposed schedule for future water quality monitoring in Bamfield and Grappler Inlets.

Sample Sites	Frequency and timing	Parameters to be measured
Bamfield and Grappler Inlets (18 sites) and 2 freshwater sites in Bamfield Inlet	August – September: five weekly samples in a 30-day period	fecal coliforms (both marine and freshwater), enterococci (marine only) and *E. coli* (freshwater only)

9.0 MANAGEMENT OPTIONS

The most significant influence on bacteriological contamination in Bamfield Inlet is the various untreated and partially treated sewage discharges to the intertidal and foreshore area. With the exception of the Bamfield Marine Station, there is no sewage collection and treatment system for the Huu-ay-aht First Nation and the community of Bamfield found within the Alberni-Clayoquot Regional District. Under these conditions, the Vancouver Island Health Authority in partnership with MOE, posted a warning in Bamfield Inlet in July 2010, advising the public of the potential health risk in the Inlet due to the presence of human sewage.

The following sections briefly discuss management options to protect human health and overall water quality of Bamfield and Grappler Inlets.

9.1 OFFICIAL COMMUNITY PLANS

There is a clear link between land-use planning required of local governments in the *Local Government Act* and waste management plans in the *Environmental Management Act* (*EMA*). An official community plan (OCP) is a statement of objectives and policies regarding future land-use patterns in incorporated municipalities or in designated areas of regional districts. They are approved by the Ministry of Community, Sport and Cultural Development (MCSCD). The OCP provides a clear statement to the public and the province about local government's growth management objectives and provides the rationale for subsequent land-use regulations. An OCP was developed for the Bamfield community in 2000 and it is currently under review. Where OCPs are in place, the local government planning statement (bylaw) typically forms the basis of waste management plans. However in Bamfield the current OCP does not address liquid waste management and the protection of human health.

9.2 LIQUID WASTE MANAGEMENT PLANS (LWMP)

The *Environmental Management Act (EMA)* allows municipalities and regional districts to develop LWMPs for approval by the Minister of Environment. The LWMP supports the OCP by minimizing the adverse environmental impacts of the OCP and ensuring that development is consistent with Ministry of Environment waste management objectives. In other words, the LWMP is a strategy to reduce and minimize pollution and plan for the future. It applies to existing wastewater treatment facilities with primary treatment or no treatment, and can be implemented in stages, taking into account the assimilative capacity of the receiving environment, the ability to finance the upgraded sewage facilities, and public input to the waste management planning process.

Wastes to be addressed in the Liquid Waste Management Plan should include, but are not necessarily limited to:

- municipal sewage;
- urban storm water runoff;
- combined sewer overflows;
- septic tank pumpage;
- pump station overflows;
- sewage treatment plant sludge;
- industrial or commercial wastes discharged to municipal sewers;
- septic tanks and other sewage disposal systems not connected to the community sewer system; and
- any other effluent specified by a manager.

Estimates of waste quantity and quality should be based on long-term growth projections. For waste management planning areas where official land use plans are not in place or where it is deemed the background information of a land use plan is inadequate, growth projections must be developed.

9.3 RECOMMENDATIONS

While the current OCP review process is an opportunity for the community to develop a strategy to deal with waste management concerns, it is recommended that the LWMP process be implemented for the Bamfield area to address local concerns and priorities. The opportunity exists for partnerships to be established between the Huu-ay-aht First Nation, the Bamfield Marine Station, Alberni-Clayoquot Regional District and the community of Bamfield to provide a cost effective sewage collection system for all partners. This would protect both human and environmental health in the Bamfield area while maintaining future economic opportunity for the community. It is worth noting that the Ministry of Community, Sport and Cultural Development favours funding of municipal capital works that have undergone a LWMP process.

Barring the LWMP process, the partners may seek to register the treatment and discharge of effluent under *EMA's*, Municipal Sewage Regulation. This process could solely focus on direct discharges to the marine environment. This approach could be a starting position for addressing point source discharges with attention to non-point discharges occurring at a later phase associated with community development.

10.0 LITERATURE CITED

Ahmed, W., R. Yusuf, I. Hasan, A. Goonetilleke, and T. Gardner. 2010. Quantitative PCR assay of sewage-associated *Bacteroides* markers to assess sewage pollution in an urban lake in Dhaka, Bangladesh. Can. J. Microbial. 56: 838-845.

Berhard, A.E. and Field, K.G. 2000. A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Precotella* genes encoding 16S rRNA. Applied and Environ. Microbiol. 66(4): 1587-1594.

Brettar, I. and M.G. Höfle. 1992. Influence of ecosystematic factors on survival of *Escherichia coli* after large-scale release into lake water mesocosms. Applied and Environmental Microbiology 58(7): 2201 – 2210.

British Columbia Ministry of Environment (BC MOE) 1994. Environmental Lab Manual for the Analysis of Water, Wastewater, Sediment and Biological Materials, Province of BC.

Buerge, I.J., T. Poiger, M.D. Muller, and H.R. Buser. 2003. Caffeine, an anthropogenic marker for wastewater contamination of surface waters. Environ. Sci. Technol. 37:691-700.

Cabelli, V.J. 1983. Health effects for marine recreation waters. USEPA 600/1-80-031. Health Effects Research Laboratory, Research Triangle Park, N.C.

Demarchi, D.A. 1996. An Introduction to the Ecoregions of British Columbia. Ministry of Environment, Lands and Parks, Victoria. B.C. Available online at: http://www.env.gov.bc.ca/ecology/ecoregions/intro.html

Edberg, S.C., E.W. Rice, R.J. Karlin, and M.J. Allen. 2000. *Escherichia coli*: the best biological drinking water indicator for public health protection. J. Appl. Microbiol., 88:106S-116S.

Field, K.G. and M. Samadpour. 2007. Fecal source tracking, the indicator paradigm, and managing water quality. Water Res., 41:3517-3538.

Fisheries and Oceans Canada, 2009. http://www.pac.dfo-mpo.gc.ca/ops/fm/shellfish/biotoxins/closures/sanitary_e.htm

Gartner, A., P. Lavery, and A.J. Smit. 2002. Use of δ15N signatures of different functional forms of macroalgae and filter-feeders to reveal temporal and spatial patterns in sewage dispersal. Mar Ecol Prog Ser 235: 63–7

Griffin, D.W., E.K. Lipp, M.R. McLaughlin, and J.B. Rose. 2001. Marine recreation and public health microbiology: quest for the ideal indicator. BioScience 51: 817-825.

Howell, J.M., M.S. Coyne and P.L. Cornelius. 1996. Effect of sediment particle size and temperature on fecal bacteria morality rates and the fecal coliform/fecal streptococci ratio. J. Environ. Qual. 25: 1216 – 1220.

Ishii, S. and M.J. Sadowsky. 2008. *Escherichia coli* in the environment: Implications for water quality and human health. Microbes Environ., 23(**2**): 101-108.

Jawson, M.D., L.F. Elliott, K.E. Saxton, and D.H. Fortier. 1982. The effect of cattle grazing on indicator bacteria in runoff from a Pacific Northwest watershed. J. Environ. Qual. 11: 621 - 627.

Kloot, R.W., B. Radakovich, X. Huang, and D. Brantley. 2006. A comparison of bacterial indicators and methods in rural surface water. Environ. Monior. Assess. 121: 275-287.

Krewski, D., J. Balbus, D. Butler-Jones, C.N. Haas, J. Isaac-Renton, K.J. Roberts, and M. Sinclair. 2004. Managing microbiological risks of drinking water. J. Toxicol. Environ. Health Part A, 67:1591-1617.

Lyngby, J. and M. Mortensen. 1994. Assessment of nutrient availability and limitation using macroalgae. J Aquat. Ecosyst. Health 3: 27-34.

McKinney, R.A., W.G. Nelson, M.A. Charpentier, and C. Wigand. 2001. Ribbed mussel nitrogen isotope signatures reflect nitrogen sources in coastal salt marshes. Ecol, Applications 11(1):203-214.

Meays, C.L., K. Broersma, R. Nordin, and A. Mazumder. 2004. Source tracking fecal bacteria in water: a critical review of current methods. J. Environ. Man. 73: 71-79.

MINFILE 2005. Ministry of Energy and Mines Mineral Inventory. Available online at: http://www.em.gov.bc.ca/mining/geolsurv/minfile/

Peeler, K.A., S.P. Opsahl, and J.P. Chanton. 2006. Tracking anthropogenic inputs using caffeine, indicator bacteria, and nutrients in rural freshwater and urban marine systems. Environ. Sci. Technol. 40: 7616-7622.

PESC (Pacific Environmental Science Centre), 2006. Bacterial Source Tracking results submitted to MOE.

RISC (Resource Inventory Standards Committee). 1994. Ambient Freshwater and Effluent Sampling Manual. Available online at: http://ilmbwww.gov.bc.ca/risc/pubs/aquatic/ambient/index.htm

RISC. 1997. Guidelines for Interpreting Water Quality Data. Available online at: http://www.ilmb.gov.bc.ca/risc/pubs/aquatic/interp/index.htm

Rogers, K.M. 1999. Effects of sewage contamination on macroalgae and shellfish at Moa Point, New Zealand using stable carbon and nitrogen isotopes. NZ J Marine Freshwater Res. 33:181-188.

Rogers, K.M. 2003. Stable carbon and nitrogen isotope signatures indicate recovery of marine biota from sewage pollution at Moa Point, New Zealand. Mar. Pollul. Bulletin 46:821-827.

Scott, T.M., Rose, J.B., Jenkins, T.M., Farrah, S.R., and Lukasik, J. 2002. Microbial cource tracking: current methodology and future directions. Appl. Environ. Microbiol. 68(12): 5796-5803.

Stephenson, G.R. and R.C. Rychert. 1982. Bottom sediment: a reservoir of *Escherichia coli* in rangeland streams. J. Range Manage. 35: 119-123.

Tiedemann, A.R., D.A. Higgins, T.M. Quigley, H.R. Sanderson and D.B. Marx. 1987. Responses of fecal coliform in streamwater to four grazing strategies. J. Range Manage. 40(4): 322 – 329.

Tucker, J., N. Sheats, A.E. Giblin, C.S. Hopkinson and J.P. Montoya. 1999. Using stable isotopes to trace sewage-derived material through Boston Harbour and Massachusetts Bay. Mar. Environ. Res. 48: 353-375.

Warrington, P.D. 1988. Water quality criteria for microbiological indicators, technical document. British Columbia Ministry of Environment. Victoria, BC.

Yates, M.V. 2007. Classical indicators in the 21st century – far and beyond the coliform. Water Environ. Res., 79 (3):279-286.

Appendix I

Table 1. Tide information for Bamfield and Grappler Inlets

Sample Date	Tide Info (time and depth)	
July 12, 2005	High 04:21 PDT - 9.47ft	Low 10:52 PDT - 3.26 ft
July 19, 2005	Low 05:42 PDT - 1.40 ft	High 12:19 PDT- 9.76 ft
July 26, 2005	High 04:45 PDT - 10.32 ft	Low 11:01 PDT - 2.56 ft
August 2, 2005	Low 06:05 PDT - 2.39 ft	High 12:40 PDT - 9.26 ft
August 10, 2005	High 04:02 PDT - 9.53 ft	Low 10:08 PDT - 3.58 ft
August 16, 2005	Low 04:30 PDT - 2.17ft	High 11:10 PDT - 9.14 ft
August 10, 2006	Low 08:01 PDT - 0.46 ft	High 14:23 PDT - 11.33 ft
August 15, 2006	High 05:32 PDT - 9.15 ft	Low 11:26 PDT - 4.51 ft
August 22, 2006	Low 06:51 PDT - 1.95 ft	High 13:10 PDT - 9.81 ft
August 29, 2006	Low 09:59 PDT - 4.63 ft	High 16:17 PDT - 10.48 ft
September 6, 2006	Low 06:11 PDT - 1.16 ft	High 12:33 PDT - 10.76 ft
August 8, 2007	Low 03:37 PDT - 2.47 ft	High 10:16 PDT - 8.68 ft
August 14, 2007	Low 08:19 PDT - 1.61 ft	High 14:34 PDT - 10.61 ft
August 21, 2007	High 07:30 PDT - 7.65 ft	Low 12:09 PDT - 6.47 ft
August 28, 2007	Low 07:14 PDT - 1.25 ft	High 13:33 PDT - 11.09ft
September 4, 2007	High 07:14 PDT - 8.47 ft	Low 12:14 PDT - 6.04 ft

Appendix II.

Table 1. Duplicate microbiological samples collected during the study period.

		Enterococci CFU/100mL		Fecal Coliforms CFU/100mL	
Sample Date	Location	Sample #1	Sample #2	Sample #1	Sample #2
12-Jul-05	Bam 1	4	1	<2	n/a
	Bam 2	22	19	17	n/a
19-Jul-05	Bam 4	21	21	95	49
	Bam 5	11	6	33	23
26-Jul-05	Bam 7	15	2	2	4
	Bam 8	<1	2	5	2
02-Aug-05	Bam 10	2	1	2	2
	Bam 11	2	<1	<2	2
10-Aug-05	Bam 13	3	2	14	23
10-Aug-06	Bam 5	108	126	170	>1600
	Bam 15	31	21	170	33
15-Aug-06	Bam 4	229	510	49	130
	Bam 8	19	71	33	13
22-Aug-06	Bam 3	6	14	33	7
	Bam 14	18	12	220	79
29-Aug-06	Bam 1	21	28	350	920
	Bam 9	1	13	7	79
06-Sep-06	Bam 9	8	5	17	33
	Bam 16	12	14	11	23
08-Aug-07	Bam 7	11	2	8	8
	Bam 11	2	1	7	5
14-Aug-07	Bam1	<1	6	8	5
	Bam 18	3	16	4	49
21-Aug-07	Bam 9	1	3	11	7
	Bam 13	4	1	8	2
28-Aug-07	Bam 4	39	12	95	17
	Bam 10	1	1	<2	2
04-Sep-07	Bam 5	9	13	5	5
	Bam 8	149	12	7	>1600

n/a- duplicate did not get done due to lack of A-1 media

Table 2. Duplicate results for nitrogen isotope and caffeine samples.

Date	Sample Location	Result	Mean	% difference
$\delta^{15}N$ isotopic signature results		‰	‰	
09-Aug-05	BAM 1A	9.13	9.28	3.23
	BAM 1B	9.43		
	BAM 4A	9.33	9.34	0.21
	BAM 4AR	9.35		
	BAM 4B	9.57		
	BAM 7	9.17	9.17	0.52
	BAM 7R	9.17		
	BAM 10	9.58	9.55	0.00
	BAM 10R	9.53		
	BAM 11A	8.79	8.64	4.75
	BAM 11B	8.74		
	BAM 11C	8.38		
06-Sep-06	BAM 2A	9.31	9.35	8.66
	BAM 2B	9.77		
	BAM 2BR	8.96		
	BAM 3	8.63	8.70	1.61
	BAM 3R	8.77		
	BAM 18A	11.00	11.07	1.17
	BAM 18B	11.13		
27-Aug-07	BAM 1	8.21	8.21	0.00
	BAM 1R	8.21		
	BAM 11A	7.44	7.82	9.72
	BAM 11B	8.20		

Caffeine results		ng/L	ng/L	% difference
2005 - no duplicates				
10-Aug-06	BAM 5A	32.00	34.20	13.10
	BAM 5B	36.50		
	BAM 15A	59.00	68.95	28.90
	BAM 15B	78.90		
08-Aug-07	BAM 7A	9.20	8.60	13.90
	BAM 7B	8.00		
28-Aug-07	BAM 4A	19.90	17.50	28.00
	BAM 4B	15.00		
	BAM 10A	14.40	13.70	10.20
	BAM 10B	13.00		