Spatial and temporal faecal coliform variations in surface water, sediments and cultivated oyster's meat, *Crassostrea virginica*, from Richibucto estuary, New Brunswick.

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RÉSUMÉ

Au Canada, la classification sanitaire des zones côtières détermine des zones ouvertes, conditionnelles, fermées ou prohibées à la récolte ou l'aquaculture des mollusques. Cette classification est principalement basée sur les concentrations de coliformes fécaux dans l'eau et la chair des mollusques, en utilisant *Escherichia coli* comme indicateur. L'objectif général de cette étude était de suivre les variations spatiales et saisonnières de la contamination fécale dans une zone ouverte, une zone conditionnelle et une zone fermée de l'estuaire de la Richibouctou, N. B., Canada. La concentration d'*E. coli* a été mesurée dans quatre compartiments, soient l'eau de surface, les sédiments et des huîtres américaines (*Crassostrea virginica*) cultivées en suspension et sur le fond, à cinq stations d'échantillonnage, de juillet 2003 à août 2004. Les paramètres physico-chimiques (température, salinité et concentration de seston) étaient similaires d'une station à l'autre.

La première partie de cette étude, qui s'est concentrée sur la zone conditionnelle, visait à évaluer divers indicateurs de la salubrité des huîtres. Aucune différence significative entre la concentration de coliformes fécaux dans les huîtres cultivées en suspension et celles cultivées sur le fond n'était détectable. La concentration de coliformes fécaux était significativement plus faible dans l'eau que dans les sédiments ou dans les huîtres cultivées en suspension et sur le fond. Le facteur temps avait un effet significatif sur la concentration d'*E. coli* dans tous les compartiments. Même si les concentrations d'*E. coli* dans l'eau étaient positivement corrélées avec celles dans les huîtres et celles dans les sédiment, dans 17 à 22% des cas les limites de contamination n'étaient pas respectées à la fois dans l'eau et dans les huîtres (14 NPP/100 mL et 230 NPP/100 g respectivement). Les concentrations de coliformes fécaux dans l'eau et dans la chair des huîtres ne furent simultanément au-dessus de leurs limites qu'à deux reprises durant cette étude.

La deuxième partie de l'étude visait à tester la fiabilité du système de classification des zones côtières actuellement en vigueur au Canada, en suivant la contamination par les coliformes fécaux dans trois zones (ouverte, conditionnelle et fermée). Nos résultats démontrent que les échantillons d'eau provenant des trois zones respectaient les caractéristiques d'une zone ouverte, telles qu'établies par le Programme canadien de Contrôle de Salubrité des Mollusques. Cependant, 10, 22 et 24% des échantillons d'huîtres provenant respectivement de la zone ouverte, de la zone conditionnelle et de la zone fermée ont dépassé la limite de fermeture de 230NPP de colonies d'*E. coli* /100 g de chair d'huîtres.

ABSTRACT

In Canada, the sanitary classification of coastal zones distinguishes opened, conditional, closed and prohibited areas for shellfish harvesting. The classification is based on levels of faecal coliform (*Escherichia coli*) concentration in water and shellfish meat. The general objective of the study was to determine if there are differences in faecal coliform counts in relation to seasonal or spatial distribution patterns in an opened, conditional and closed shellfish growing zone located in the Richibucto estuary, N. B., Canada. *E. coli* concentrations in surface water, sediments, suspended and bottom cultured American oysters (*Crassostrea virginica*) were determined from July 2003 to August 2004.

The first part of this study, regarding the conditional zone, faecal coliform concentration levels in water were significantly lower then in sediments and suspended and bottom cultured oysters. The seasonal variations (sampling dates) had a significant influence on faecal coliform concentration in components. Faecal coliform concentrations in water were positively strongly correlated with those recorded from oysters and sediments. Moreover, in 17 and 22% of readings, thresholds for potential closure were exceeded for water (14 MPN/100 mL) and for oysters meat (230 MPN/100 g) respectively. On two occasions, the concentration of faecal coliforms in water and oysters simultaneously exceeded the maximum permissible levels for harvesting. Inconsistencies in our results bring into question whether the concentration of *E. coli* in water is the most suitable indicator for defining oyster contamination. The current findings are meant to contribute to an improved understanding of bacterial contamination for shellfish harvesting and hopefully lead to improved management strategies of coastal areas.

Results from the second part of the study, concerning coastal zones classification, indicated that surface water from all three classified zones monitored in the Richibucto estuary (New Brunswick, Canada), according to CSSP water quality standards, respect the characteristics of an opened zone. 10, 22 and 24% of oyster's meat samples from an open, conditional and closed zone respectively exceeded the threshold for closure criteria of 230 MPN of *E. coli* colonies /100 g

INTRODUCTION

The US National Shellfish Sanitation Program (NSSP) was developed in 1924 following an outbreak of typhoid fever in USA resulting from the ingestion of contaminated shellfish (Burkhardt III *et al.* 2000). Twenty-four years later, the Canadian Shellfish Sanitation Program (CSSP) was created to ensure the safety of Canadian shellfish products. In Canada, coastal shellfish harvesting areas are classified into three distinct categories: open, conditional or closed to shellfish aquaculture activities. Classification is primarily based on faecal coliform concentration levels in surface waters and shellfish meat. The organism most widely used as an indicator, which originates from the intestinal tract of warm-blooded animals, is the bacterium *Escherichia coli* (Youn-Joo, 2002; Pérez *et al.* 2001; Watkins *et al.* 1988). The presence of *E. coli* in the aquatic environment is considered as a useful indicator of contamination related to raw sewage and of potentially harmful micro-organisms to human health (Cools *et al.* 2001; Solic *et al.* 1999).

In Atlantic Canada, the shellfish industry is rapidly expanding (MacRae *et al.* 2005). Not unlike other species, bivalves harvested in large numbers become susceptible to certain diseases caused by parasites, viruses and bacteria. Because bivalves are filter feeders they have the potential to concentrate contaminants from water during active feeding (Mugg Pietros and Rice 2003; Ward *et al.* 2003; Solic *et al.* 1999). This can present a health risk to consumers, especially in those instances where they are eaten raw or undercooked as is the case for oysters (Barillé *et al.* 1997; Murphee and Tamplin, 1991; Gerba *et al.* 1980). Findings by Davies *et al.* (1995) demonstrated that marine sediments provide a favourable environment for the survival of *E. coli* bacteria. The capacity of sediment to act as reservoirs has been previously described from freshwater beaches where *E. coli* concentrations were found to be significantly higher in sand cores than in the water column (Wheeler Alm *et al.* 2003). Additional work confirmed that sediment agitation attributed to recreational activities and storm surges result in the re-suspension of faecal coliform in the water column (Crabill *et al.* 1999). Temperature affects both bacterial survival times in water and sediments (Wilson *et al.* 2000, Youn-

Joo 2002, Trousselier *et al*. 2004) as well as filtration rate of oysters (Loosanoff, 1958). Therefore, both are subject to variation with seasonal changes of temperature.

The Canadian Shellfish Sanitation Program (CSSP) is jointly administered by the Department of Fisheries and Oceans (DFO), the Canadian Food Inspection Agency (CFIA) and Environment Canada (EC). In practice, water samples are analysed by Environment Canada while shellfish samples are processed by Canadian Food Inspection Agency. To approve an area for shellfish harvest, the *E. coli* content in water samples must not exceed an average MPN (Most Probable Number) of coliform bacteria colonies of 14/100 mL out of a minimum of 15 samples, and no more than 10% of the samples may exceed 43 MPN/100 mL (Health Products and Food Branch. Ottawa. 2002). Environment Canada also applies a geometric mean and the P90 (90^{th} percentile) for all samples in assessing the classification of coastal zones. Oyster meat samples must not exceed 230 MPN/100 g. Generally, shellfish stock bacterial standards are used for the evaluation of depuration effectiveness and for the verification of data from previously opened areas which are closed under a specific management plan. Even though it has been pin-pointed as a reservoir of faecal coliforms, sediment is not used for purposes of coastal zones classification (Davies *et al.* 1995; Crabill *et al.* 1999; Wheeler Alm *et al.* 2003).

Coastal and shoreline development, wastewater collection and treatment facilities, faulty septic tanks, food processing plants, urban runoff, bird feces, disposal of human wastes from boats, agriculture activities, and bathers all contribute to faecal contamination of recreational waters (Benedict and Neumann, 2004; Noble *et al.* 2004; Trousselier *et al.* 2004; Crowther *et al.* 2001). Goyal *et al.* (1979), Hussong *et al.* (1981) and Larkin and Hunt (1982) agree that based on a lack of significant correlations in faecal contamination between shellfish meat and water, faecal coliform concentration in water may not be the most appropriate indicator of shellfish safety.

The primary objective of the first part of this study was to investigate the effectiveness of using water contamination as an indicator of shellfish salubrity. More specifically, the concentrations of *E. coli* in surface water, sediments and in suspended and bottom cultured American oysters (*Crassostrea virginica* Gmelin) were monitored in a conditional zone of the Richibucto estuary, New-Brunswick, Canada. To our knowledge, this is the first study that simultaneously monitors levels of *E. coli* in water, sediments and oysters.

The aim of the second part of this study was to test the consistency, and therefore the reliability of the current classification system employed in Canada. Faecal coliform concentrations were monitored in surface water, sediments and cultured American oysters (*Crassostrea virginica* Gmelin) from an open, a conditional and a closed zone of the Richibucto estuary.

MATERIALS AND METHODS

Experimental design

Five sampling stations were installed in the Richibucto estuary (Figure 1), New Brunswick (46° 70 '20" N, 64° 85' 50" W). One (numbered 1 on the chart) was located in an open zone near Indian Island. Three sampling stations (numbered 2, 3 and 4 respectively) were located in the Aldouane River in a zone classified as conditional for shellfish growing activities during this study. The last sampling station (numbered 5) was located in Mooney's Creek, a closed zone situated near summer cottages and within four hundred meters from a wastewater treatment plant.

Oysters ranging between 55 and 65 mm in shell length were obtained from a nearby commercial lease located in an open zone. Oysters were cultured in suspension and on the bottom in floating and non-floating Vexar® bags respectively. Non-floating bags lacked lateral floaters to eliminate buoyancy. Each bag (Figure 2) housed 225 oysters, a density commonly used by industry. Each station was comprised of four floating and four non-floating bags tied to a back line in alternate fashion and firmly anchored to the bottom by two 20 kilogram cement blocks (Figure 3). Water depth at each sampling station varied between three meters and a half meter, at high and low tides respectively. According to Doré and Lees's study (1995) on mussels (*Mytilus edulis*), the majority of *E. coli* cells are digested instead of being excreted as pseudofeces. As a result, the risk of bottom cultured oysters being contaminated by feces and pseudofeces from oysters cultured in suspension, is minimal.

Experimental units were deployed on July 7th 2003 and samples of water, sediment and oysters were collected at low tide between July 14th 2003 and August 30th 2004. While samples were collected on a weekly basis between July and September 2003, sampling frequency was reduced to once a month for October and November in 2003 and for April in 2004. On February 11th, May 5th and May 12th 2004 only water and sediment samples were recorded. Ice condition prevented sampling during December 2003 and January 2004 and regular weekly sampling resumed as of May 2004.

On November 25th 2003, several days prior to ice formation, floating bags were sunk to the bottom in order to protect oysters from being crushed by ice. In order to simulate harvesting practices by oyster growers, only floating bags were sampled during the winter months. Storms and severe winter conditions in 2003 contributed to damaged equipment (ropes and bags). In spring 2004, experimental units were reconditioned and new oysters of comparable size, origin and density as for the preceding year were introduced into the bags. All animals were acclimated one week prior to sampling program.

Sampling and microbiological tests

All sampling was performed by boat during the ice-free seasons and by foot in winter. Surface water was collected approximately 20 cm beneath the surface (standard depth from Environment Canada sampling protocols) using a sterile 250 mL plastic bottle. Because faecal coliforms in sediment are only found in the first 10 to 15 mm of bottom substrate, approximately 200 g of surface sediment were collected at this depth on each experimental station with a Ponar© grab and were subsequently stored in a sterile glass container in preparation for microbiological analysis. Oysters were sampled at each station from one floating and one non-floating bag randomly selected. An oyster sample consisted of approximately twelve oysters or 100 g of wet meat.

All microbiological processing of water and shellfish were based on protocols outlined by the Health Products and Food Branch (2002). Samples were kept in a cooler at temperatures below 10ºC and were never in direct contact with ice. Surface water, sediments and oysters samples were analysed using the LTB/EC multiple fermentation tubes method. Units determined by this method are the Most Probable Number (MPN) of faecal coliform colonies / 100 mL of water, 100 g of sediment or 100 g of shellfish meat. Samples were diluted with Peptone water (0.5%) before inoculation in fermentation tubes. Peptone water contains minerals, thereby minimizing bacterial mortality due to osmosis during the dilution process. The lowest value detectable with the LTB/EC microbiological method is <2 MPN/100 mL for water and <20 MPN/100 g for sediment and oyster contamination. For purposes of statistical analysis and to ensure valid numerical values, 50% of the limit of detection was used as the lowest possible value (Allard J. personal communication). Therefore, 1 MPN/100 mL for water and 10 MPN/100 g for sediments and oysters samples were used as minimal detectable thresholds for statistical validations.

Physico-chemical factors

Water temperatures (ºC) and salinity (ppt) were measured at the surface and near the bottom using an electronic probe (YSI Model 85). Total suspended particulate matter (mg/L) was likewise determined at both depths following the protocol described by Aminot and Chaussepied (1983) for seston quantification.

Quantitative analysis

Indicators of shellfish salubrity

For this part of the study, the concentration of faecal coliforms was studied in four components (water, sediments, suspended and bottom cultured oysters) and three replicates (sampling stations) in the conditional zone. All sampling was conducted on 31 different sampling dates.

Data were neither normally distributed nor homoscedastic, and no simple numeric transformation could contribute to its modification. Therefore, non-parametric statistics were used. A repeated measures generalized linear mixed model using iteratively reweighed likelihoods was used to fit the model (Wolfinger and O'Connell 1993). These statistical analyses were performed using SAS (SAS Institute 1999). The effect of components (water, sediments, suspended and bottom cultured oysters), sampling stations and sampling days (temporal factor) on faecal coliform concentrations was analysed. Spearman tests were used to evaluate correlation coefficients between the faecal content of each component and physico-chemical parameters (Zar, 1999).

Testing of criteria for coastal zone classification

For this part of the study, the concentration of faecal coliforms in water, sediment and oysters was compared among the three zones (open, conditional and closed). As no significant difference between the concentration of faecal coliforms in bottom- and suspension- cultured oysters had been detected in the conditional zone, both culture methods were combined for statistical analyses.

Because the zone factor was not replicated, a pseudoreplication conflict was apparent. Therefore, simple statistics (geometric mean, median, 90^{th} percentile and percentages) and quadrants squatter plots were used to demonstrate our results. Scatter plots, based on epidemiology risk and odds ratio analyses, are divided in four quadrants. A "positive" quadrant, showing an open zone ideal characteristics, a "negative" quadrant representing a closed zone characteristics and two quadrants that combines positive and negatives results.

RESULTS

Indicators of shellfish salubrity

Because of a shallow water column, surface and bottom water temperature, as well as salinity, were not significantly different (Figure 4). Water temperature varied from -1.2ºC during winter to 22.1ºC in summer. Water salinity fluctuated from 18 to 27.5 ppt. Total suspended matter in water ranged between 1.1 mg/L to 11 mg/L.

The temporal factor (sampling days) had a strong significant effect on faecal coliform concentration in surface water, sediments and oysters, whether it was tested using a linear ($F = 7.64$, $p = 0.006$, $n = 303$), a square ($F = 7.77$, $p = 0.006$, $n = 303$), a cubic ($F = 7.54$, $p = 0.006$, $n = 303$) or a quadratic ($F = 7.11$, $p = 0.008$, $n = 303$) model.

Therefore, further analyses were conducted with the effect of time statistically kept constant. No significant effect attributable to sampling stations was detected (F = 0.33, p = 0.716, n = 303) (Figure 5). There was a significant difference in faecal contamination between treatments (F = 2.94, p = 0.034, n = 303). Differences of least squares means demonstrated that faecal contamination in water was significantly lower than that in sediment, suspended and bottom cultured oysters (t = -2.81, p = 0.005, n = 303; t = -2.46, p = 0.014, n = 303; t = -2.76, p = 0.006, n = 303 respectively) (Figure 6). No significant differences in faecal coliform counts (t = -0.94, p = 0.3471, n = 303) was observed between suspended and bottom cultured oyster. As a result, suspended and bottom cultivated oysters were treated together and named “oysters” for purposes of Spearman’s correlation tests.

Spearman correlation tests (Table 1) showed a positive significant correlation between faecal coliform concentration for all three components (Figures 7, 8, 9). Likewise, water temperature displayed a positive correlation with faecal coliform contamination for the three compartments. Total suspended particulate matter displayed a significant positive correlation with faecal coliform contamination of only water and oysters.

During the study, mean faecal coliform content in water (Figure 7) and oysters (Figure 9) occasionally exceeded the maximum permissible threshold defining opened and conditionally opened.For water, 5 of 30 readings (17%) exceeded 14 MPN of faecal coliform colonies. Moreover, 6 of 27 results (22%) recorded for oysters exceeded 230 MPN of faecal coliform colonies. On only two occasions did faecal coliform contents simultaneously exceed the maximum permissible contamination levels in water and oysters and that would normally result in closure of a harvestable zone.

Testing of criteria for coastal zone classification

In the open zone, water quality satisfied currently used classification standards, as the median, the 90th percentile (P90) and the geometric mean were under the threshold for closure of 14 MPN /100 mL and no water sample exceeded 43 MPN/100 mL (Table 2 and Figure 10). The median, the geometric mean as well as the P90 value for the concentration of *E. coli* in oysters were inferior to the limit of 230 MPN/100 g. Even if these statistical values for oyster's faecal contamination are under the limit of 230MPN/100 g, 10.34% of oyster's meat samples exceeded this contamination threshold in the open zone (Figure 10). Similar results as oysters were obtained with sediments samples with a median of 19.90MPN/100 g, a geometric mean of 36.22MPN/100 g and a P90 of 120 MPN/100 g (Table 2).

In relation to the conditional zone, water quality remained within the established classification standards, as the median and the geometric mean were under the threshold for closure of 14 MPN/100 mL and no water sample exceeded 43 MPN/100 mL (Table 2 and Figure 11). Nevertheless, the 90th percentile for water faecal contamination reached 17 MPN/100 mL. The median and geometric mean of oyster's faecal content were inferior then 230 MPN/100 g. However, the P90 value was of 453 MPN/100 g and 22.22% of oyster's samples from the conditional zone exceed the critical value of 230 MPN/100 g (Table 2 and Figure 11). Following a similar contamination pattern as oysters, sediments faecal coliform concentrations were represented by a median, geometric mean and a P90 of 19.90, 43.71 and 707 MPN/100 g respectively (Table2).

From the closed zone, water contamination was represented by a median and geometric mean under the maximum permissible limit for closure of 14 MPN/100 mL. Nonetheless, the geometric mean and P90 were of 4.86 and 17 MPN/100 mL (Table 2). Moreover, only 3.33% of water samples from this zone exceeded the closure threshold of 43 MPN/100 mL (Figure 12). Oyster meat samples from this zone had a median and geometric mean faecal contents of 30.00 and 55.38 MPN/100 g respectively. As well as a P90 of 360 MPN/100 g, 24% of all oyster's samples retrieved from this closed zone exceeded the permissive threshold of 230 MPN/100 g (Table 2 and Figure 12). Comparable results as oysters were recorded from sediments that had a faecal coliform content median of 19.9 MPN/100g and a geometric mean of 43.71 MPN/100 g, as well as a 90th percentile of contamination that reached 330 MPN/100 g (Table 2).

DISCUSSION

Indicators of shellfish salubrity

The primary purpose of this study was to determine whether faecal coliform levels in water represent the most effective indicator of shellfish contamination. Our findings reveal that there is a positive correlation between *E. coli* concentration in water and oysters, and that both are subject to temporal fluctuations. Despite this observation, our findings also demonstrate that maximum contamination levels of water (14 MPN/100 mL) and oysters (230 MPN/100 g), levels that have been retained to justify the closure of a harvestable zone, are not always achieved in a similar manner over a fixed period of time. In fact, our findings convincingly demonstrate that synchrony between the maximum permissible contamination levels of water and oysters for a zone to be classified as closed, occurred on only two occasions.

Tides and currents can alter the contamination profile of both water and oysters, resulting in readings that might vary significantly, even over very short periods of time. Such findings are consistent with those of Bordalo (2003) who previously reported small-scale temporal variations in water contamination by faecal coliform demonstrating for example that water quality improved dramatically during high tides. Given that our study was based on sampling at low tide, it would be interesting to determine how findings might compare if a similar study was to be undertaken at high tide.

Our study revealed that faecal coliform in sediment can reach levels as high as those found in oysters. Furthermore, we observed that *E. coli* in sediment display temporal variations as was the case for water and oysters. Cools *et al.* (2001) showed that *E. coli* could survive for periods of up to 65 - 80 days in sand particles. Furthermore, Wheeler Alm *et al.* (2003), Baudart *et al.* (2000) and Obiri-Danso and Jones (2000) indicated that weather and strong winds can contribute significantly to an increase of bacterial concentrations in surface waters via resuspension of sediments. In addition, Benedict and Neumann (2004), Noble *et al.* (2004), Trousselier *et al.* (2004) and Crowther *et al.* (2001) demonstrated that *E. coli* concentrations in water can rise significantly during and shortly after major rainfall events due to increased agricultural and urban runoff. Given that our findings suggest that sediments may act as an important reservoir, and taking into consideration the observations described by the authors cited above, is it conceivable that perhaps the sediment may turn out to be a more useful indicator of oyster contamination than water.

Water temperature had a significant influence on faecal coliform concentration as shown by its positive correlation in relation to *E. coli* concentrations in the three components studied. The concentration of *E. coli* in water and oysters tended to be high from the beginning of June to the end of October at which time water temperature ranged between 10 and 23ºC. This also corresponded to a time during which oysters are known to be most actively feeding according to Loosanoff (1958). Temperature is likewise an important factor in regulating faecal coliform activity and our results support Husong *et al.* (1981) who proposed that seasonal variations of coliform concentration in oysters should be considered when assessing the potential impact of bacterial infestation in a growing area.

Testing of criteria for coastal zone classification

According to the quality standards for water only, our sampling stations from the three coastal zones monitored throughout this study (opened, conditional and closed) could have been opened to shellfish aquaculture and harvesting. In contrast, 10, 22 and 24% of the oysters samples collected at the same three zones exceeded the contamination limit of 230 MPN of E. coli/ 100 g. Our study shows that there are important fluctuations in faecal coliform levels in oyster's meat as the 90th percentile is significantly higher than the median or the geometric mean for each of the three zones. Our results support the conclusion of Goyal *et al.* (1979), Hussong *et al.* (1981) and Larkin and Hunt (1982) that faecal contamination in water may not be the most appropriate indicator of shellfish safety as, in their studies, there was no significant correlation between faecal contamination in shellfish meat and water.

Because agencies do not sample as late into the fall season as we did for purposes of this study, it is possible that our findings underestimate the amplitude of the contamination levels. For example, in the same region, water was monitored by Environment Canada only from late May to the end of September. Therefore, low counts obtained by our study during late fall and winter may result in biasing interpretation due to the impact of a "diluting factor" in terms of the proportion (%) of results that were over the maximum permissible limit of faecal coliform concentration resulting in obligatory closure.

A variety of sources of faecal bacteria, including biotic and abiotic factors, can contribute to microbial loading into an estuary. In our study area, the closed zone is bordered by cottages and waste-water treatment plant. It is highly likely that these represent the major sources of contamination. In contrast, the open and conditional zones are more likely contaminated by non-point sources such as fishing and pleasure boats that unload their domestic wastes when entering or exiting the port.

Coastal pasturelands are considered as an important non-point source of faecal coliform and pesticides due to runoff during periods of heavy rain (Benedict and Neumann, 2004; Noble *et al.* 2004; Trousselier *et al.* 2004; Crowther *et al.* 2001). Throughout the sampling period, birds including ring-billed gulls (*Larus delawarensis*), double-crested cormorants (*Phalacrocorax auritus*) and common terns (*Sterna hirundo*), were often seen near or on the floating bags and their faecal matter was spotted directly on the floating structures in all three zones. Birds are an important concern for shellfish harvesters who believe that they may have a critical impact on organic and inorganic loading and faecal contamination in the Richibucto estuary.

Researchers are trying to develop a faecal coliform profile based on DNA sequencing of cells to determine the major sources (birds, humans, cattle, etc.) of potential microbial infections for a given region (Stoeckel *et al.* 2004). This method should accelerate the identification process of faecal coliform sources by concerned agencies and consequently help optimise costal zones management.

CONCLUSION

In conclusion, a possible approach in determining contamination levels would be to perform direct tests on oyster meats prior to placing the product on the market. Finally, since our findings demonstrated that sediments contain high faecal coliform levels, and knowing that they have the capacity to act as major reservoirs, it would be useful to monitor environmental conditions to better understand how and which forces come together to favor sediment re-suspension and its possible impact on faecal coliform accumulation in oysters.

Our work raises the question whether the current standards of *E. coli* levels in water, represent the most appropriate criteria in determining the state of microbial contamination of oysters. We believe that consideration should be given to revised management strategies based on an improved understanding of shellfish contamination patterns

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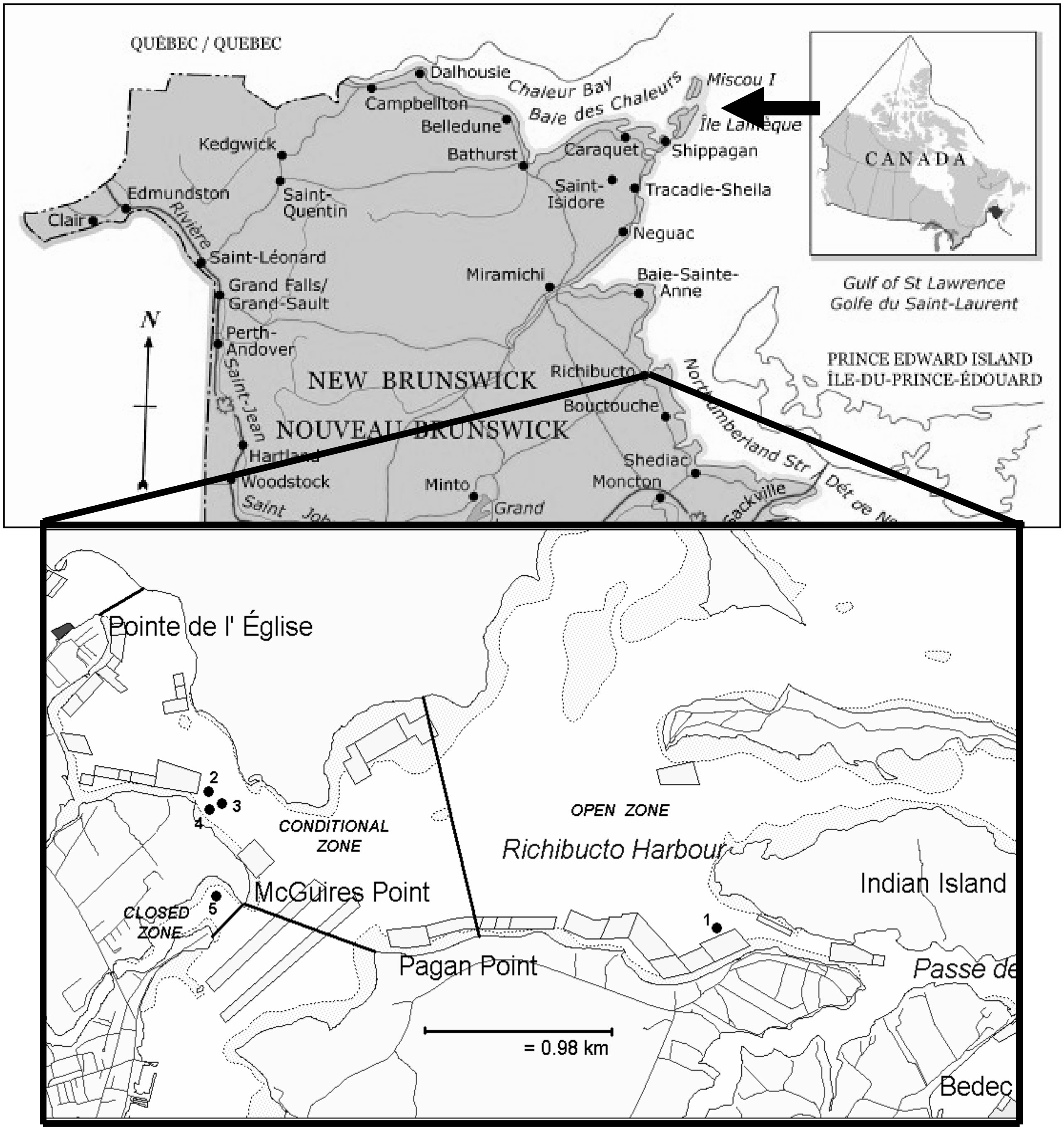

Figure 1: Location of the five sampling stations (●) at the three classified zones (open, conditional and closed) of the Richibucto estuary.

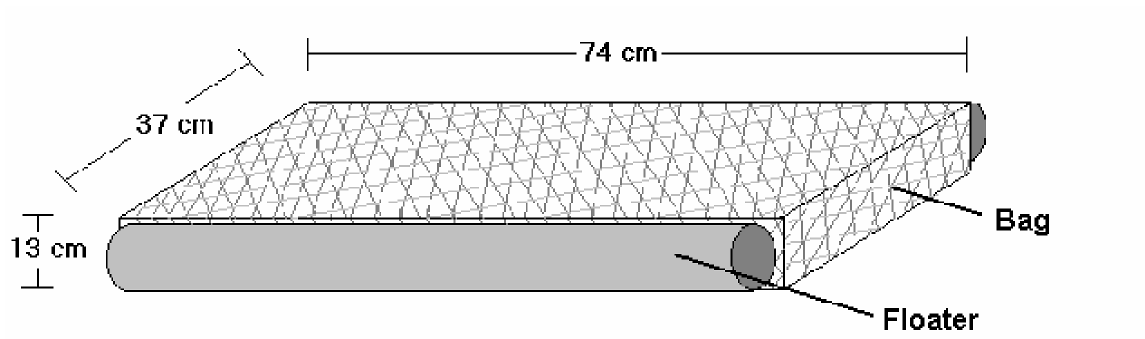

Figure 2: Diagram of a floating bag.

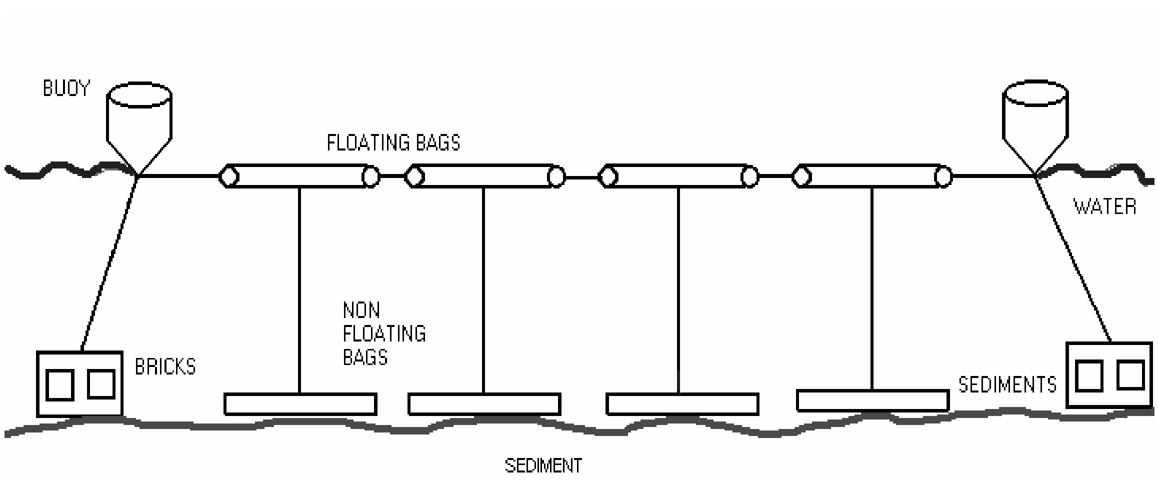

Figure 3: Side view of the experimental design for one sampling station.

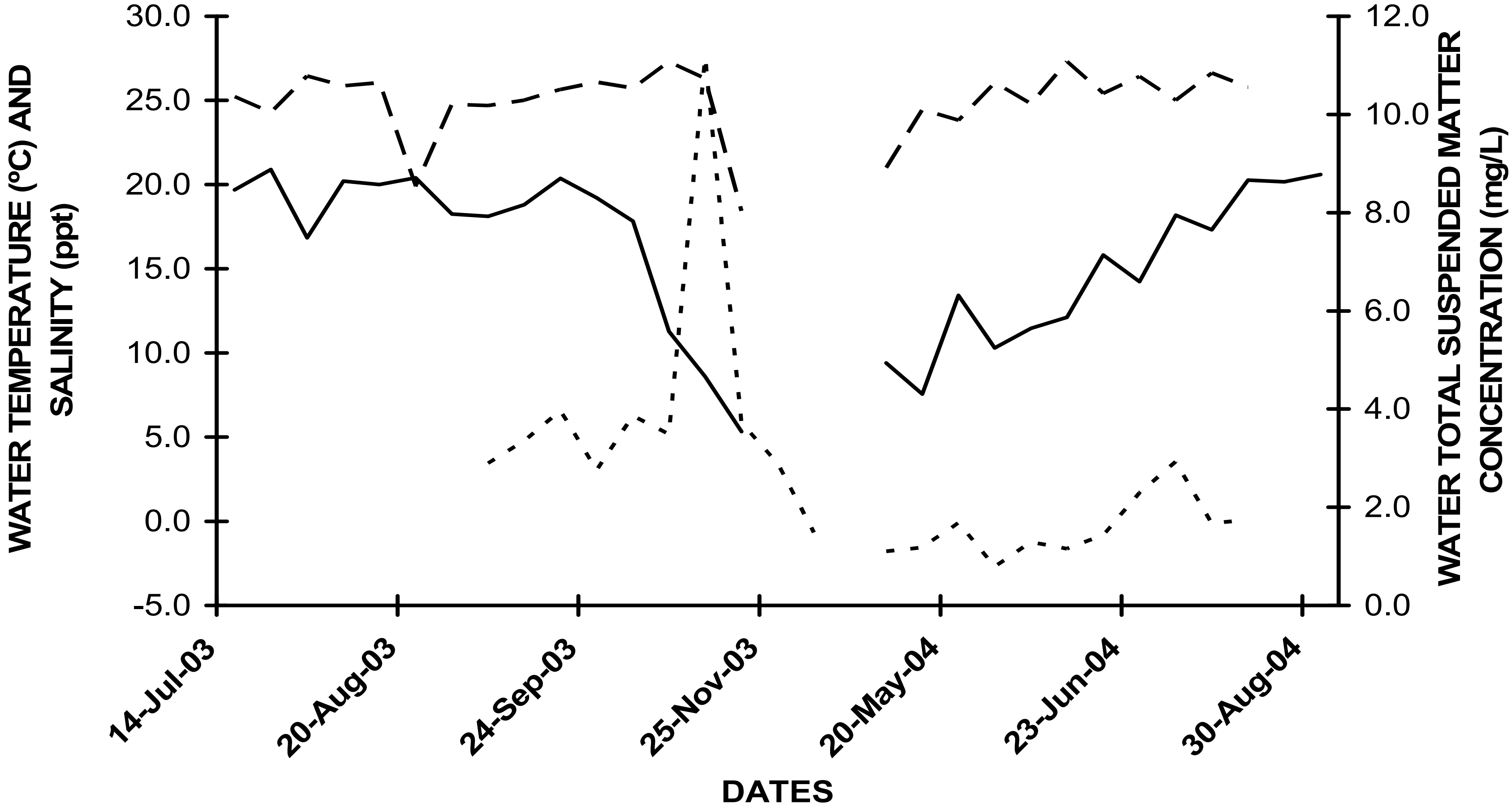

Figure 4: Surface water mean temperature (——), salinity (– – –) and total suspended matter (------) from the Richibucto estuary conditional zone.

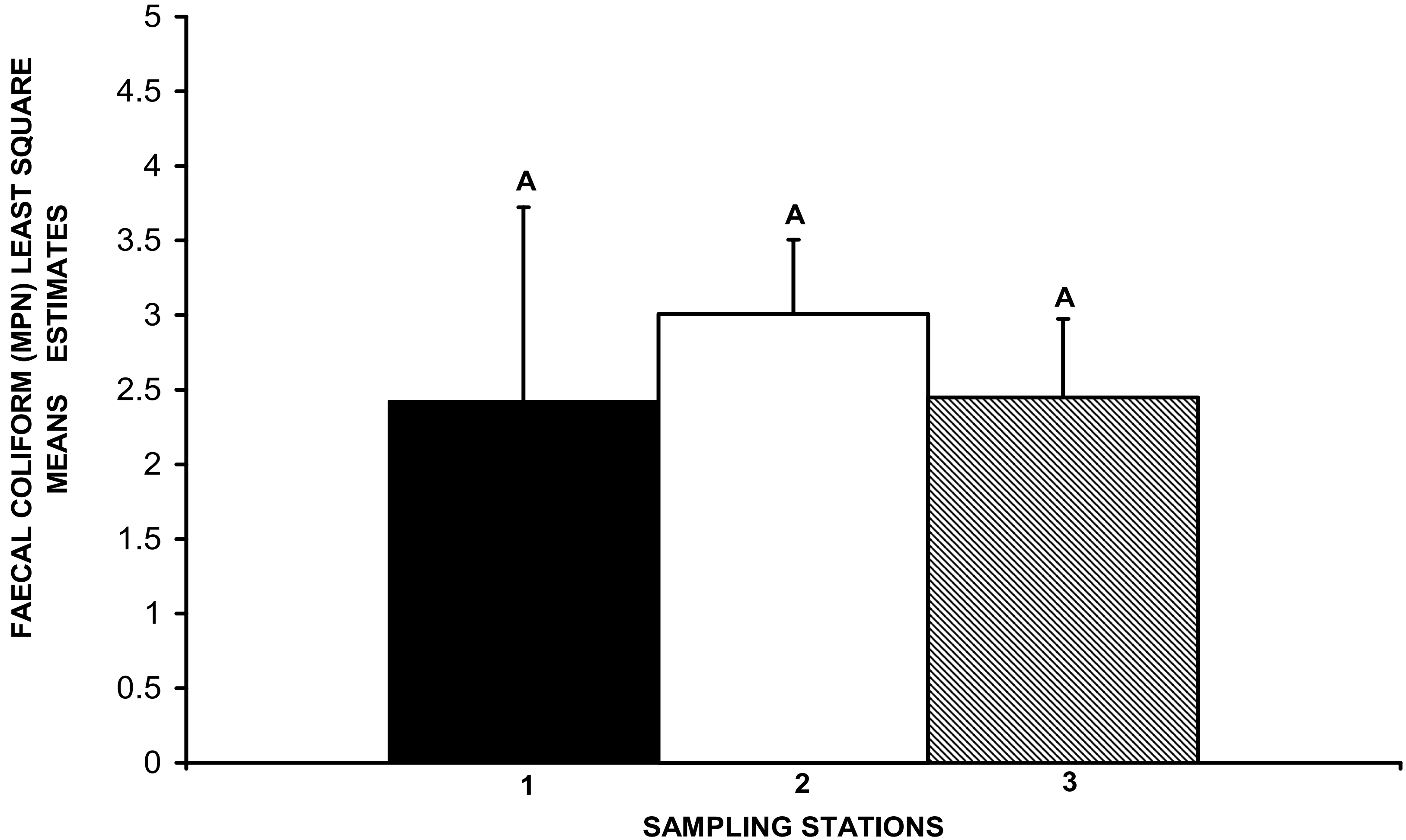

Figure 5: Faecal coliform (MPN) least square means estimates with standard errors, for all four treatments combined, at each sampling station of the conditional zone.

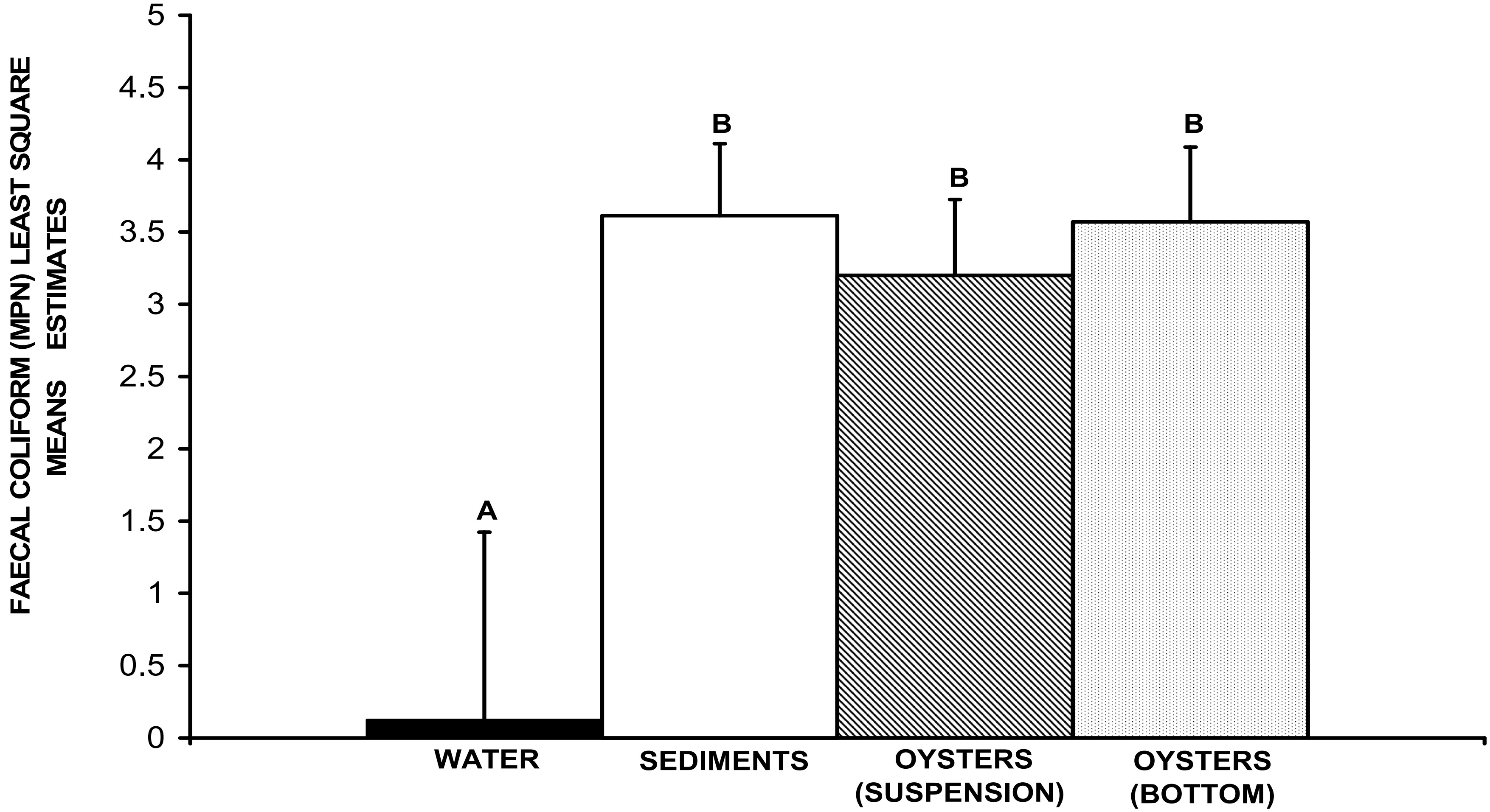

Figure 6: Faecal coliform (MPN) least square means estimates with standard errors, for all three stations combined, of each treatment of the conditional zone.

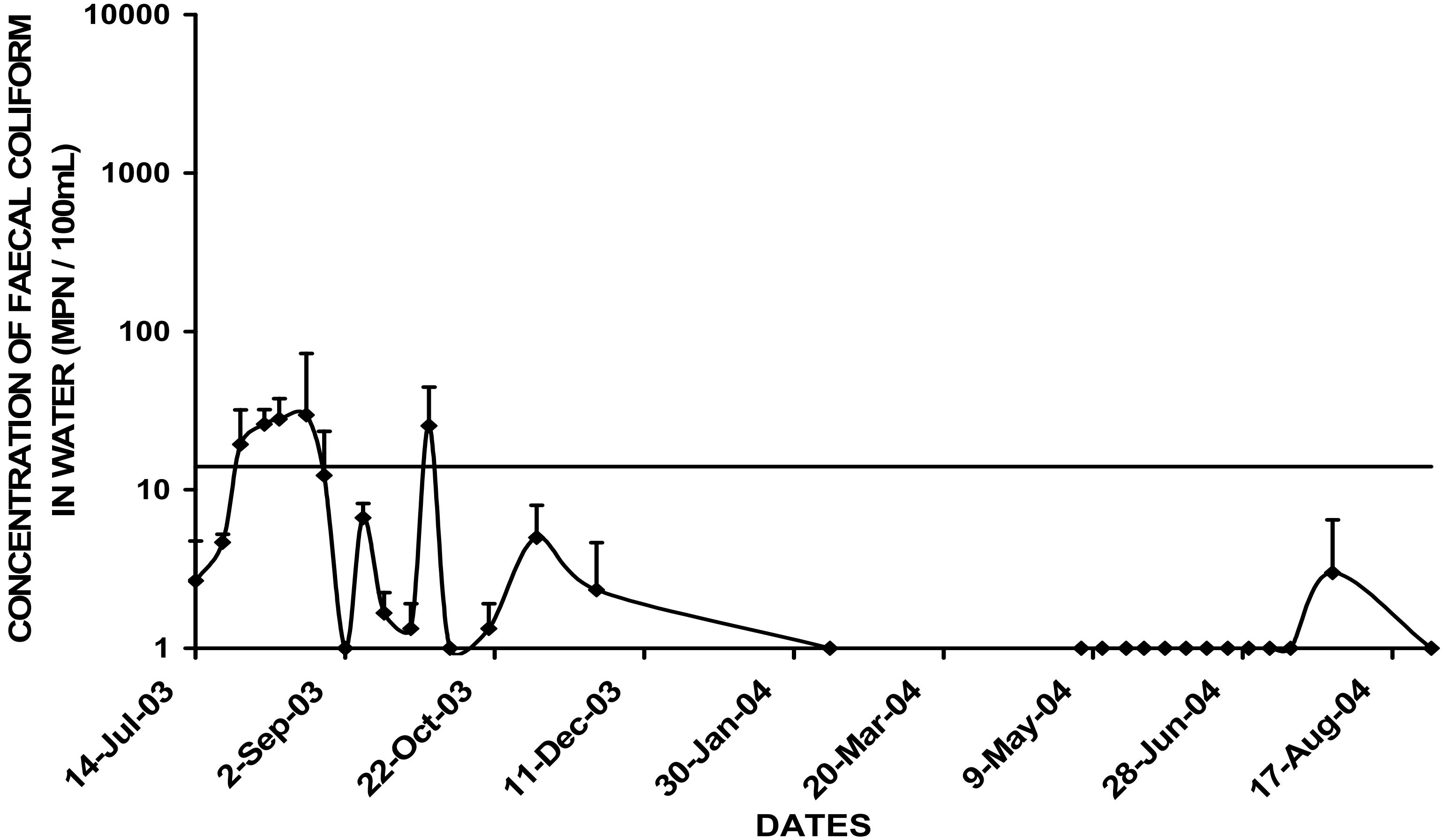

Figure 7: Water mean faecal coliform (MPN) content in function of sampling dates for the conditional zone. Water faecal contamination threshold for potential closure of 14 MPN of faecal coliform colonies is indicated with a horizontal line.

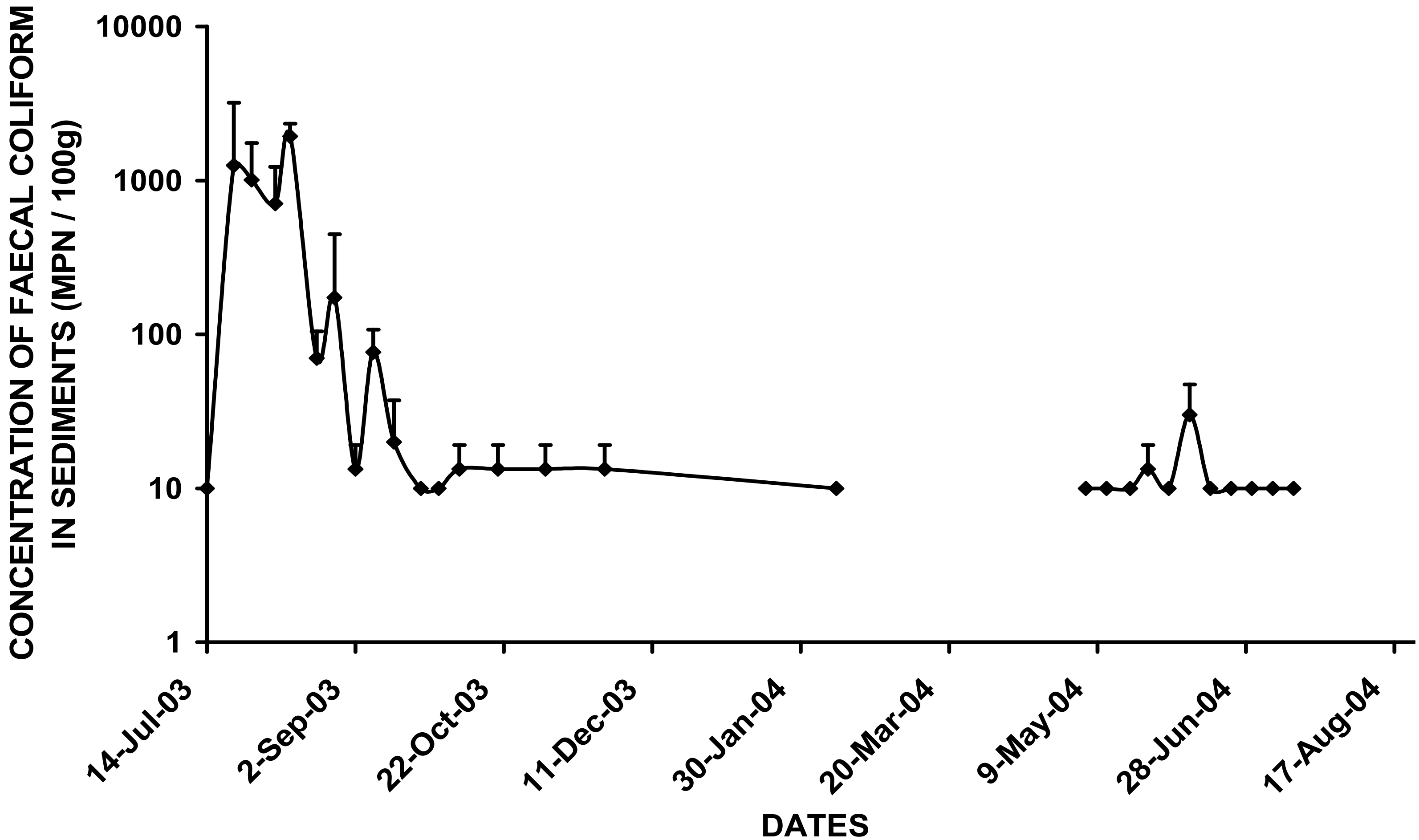

Figure 8: Sediments mean faecal coliform (MPN) content in function of sampling dates for the conditional zone.

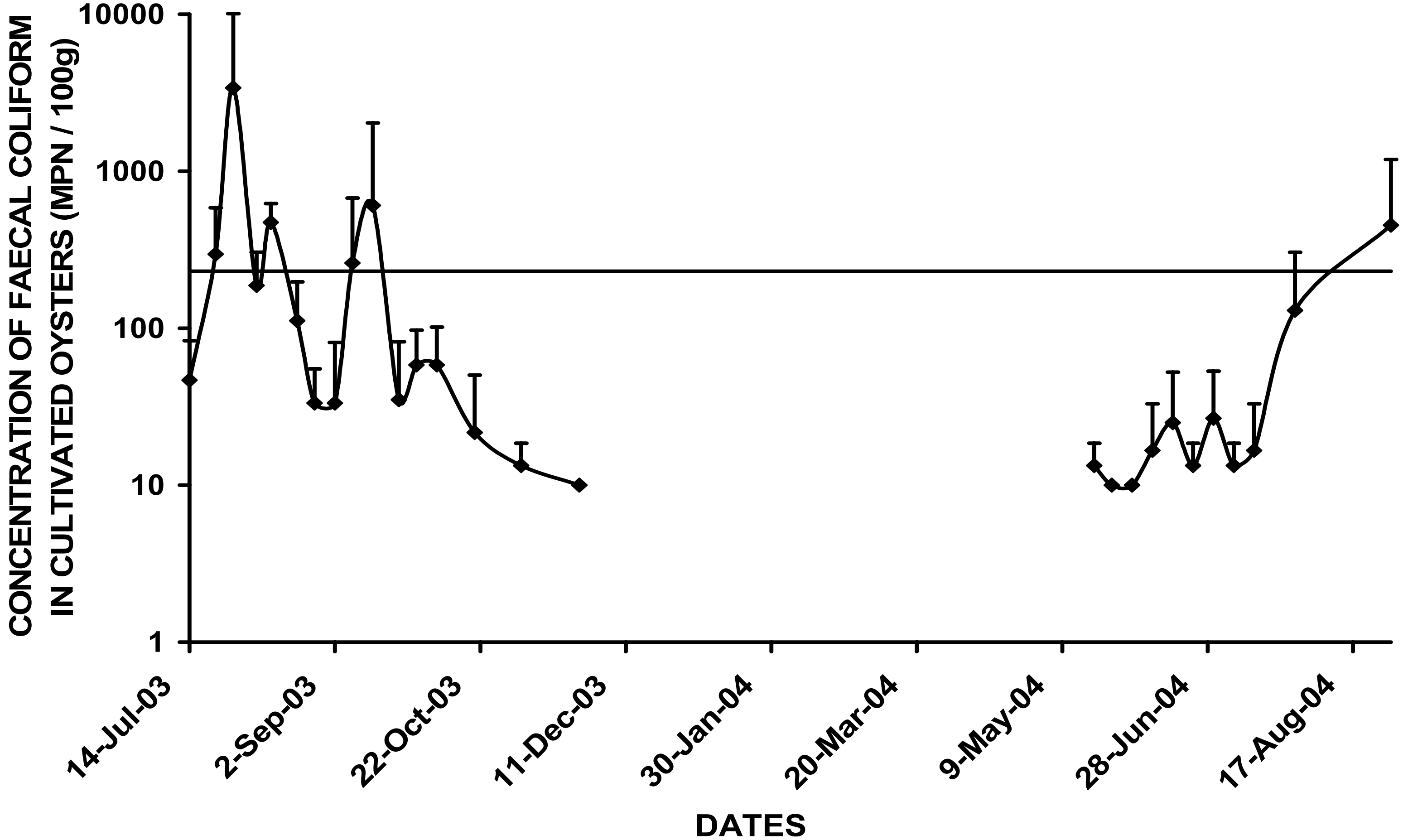

Figure 9: Oysters mean faecal coliform (MPN) content in function of sampling dates for the conditional zone. Oyster's faecal contamination threshold for potential closure of 230 MPN of faecal coliform colonies is indicated by the horizontal line.

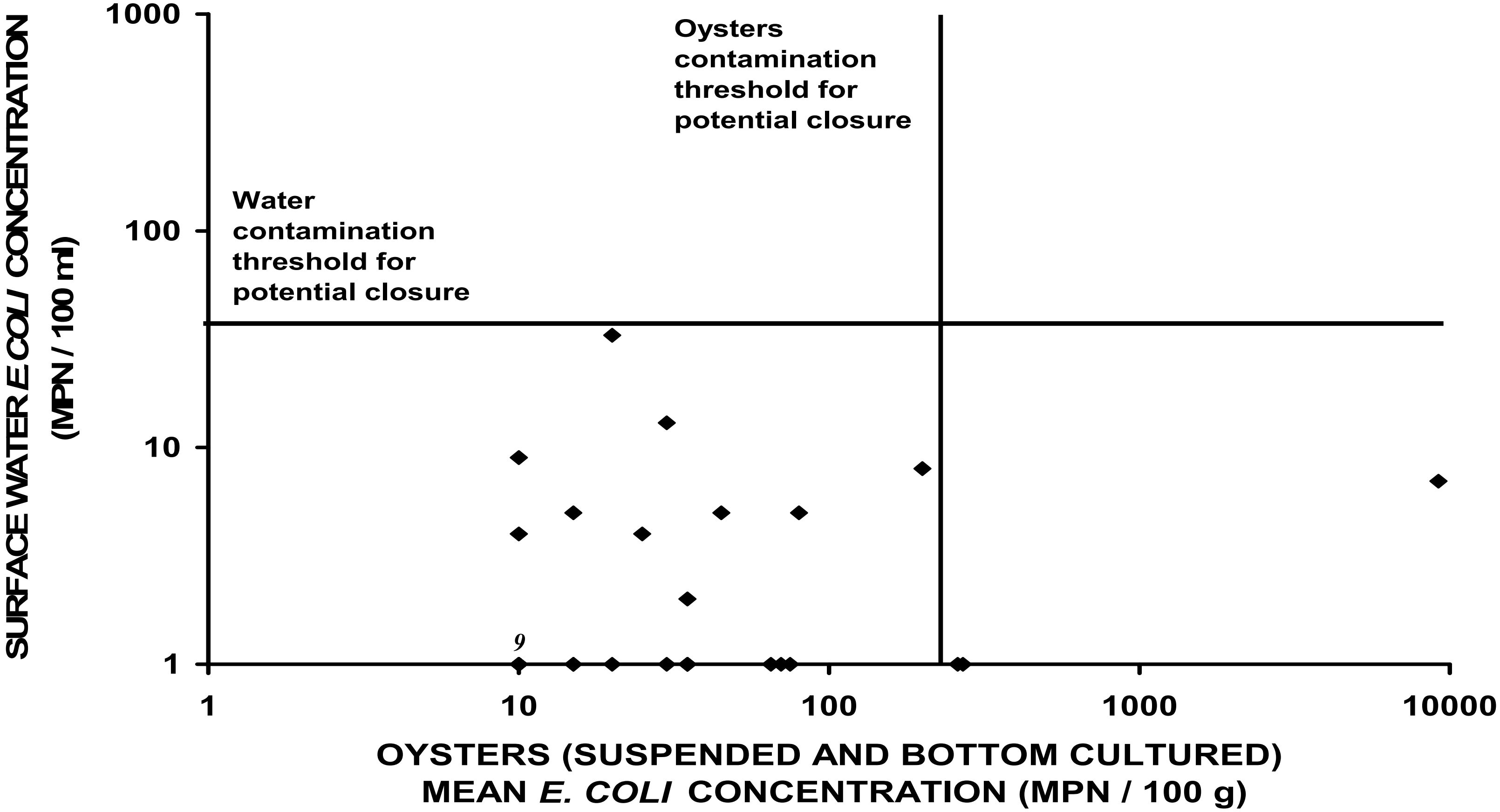

Figure 10: Quadrants scatter plot, with contamination thresholds for potential closure, comparing the concentration of *E. coli* in water (MPN / 100ml) and oysters (MPN / 100g) for the open zone, N=29.

NOTE: The number 9 represents the number of data superimposed for this particular result.

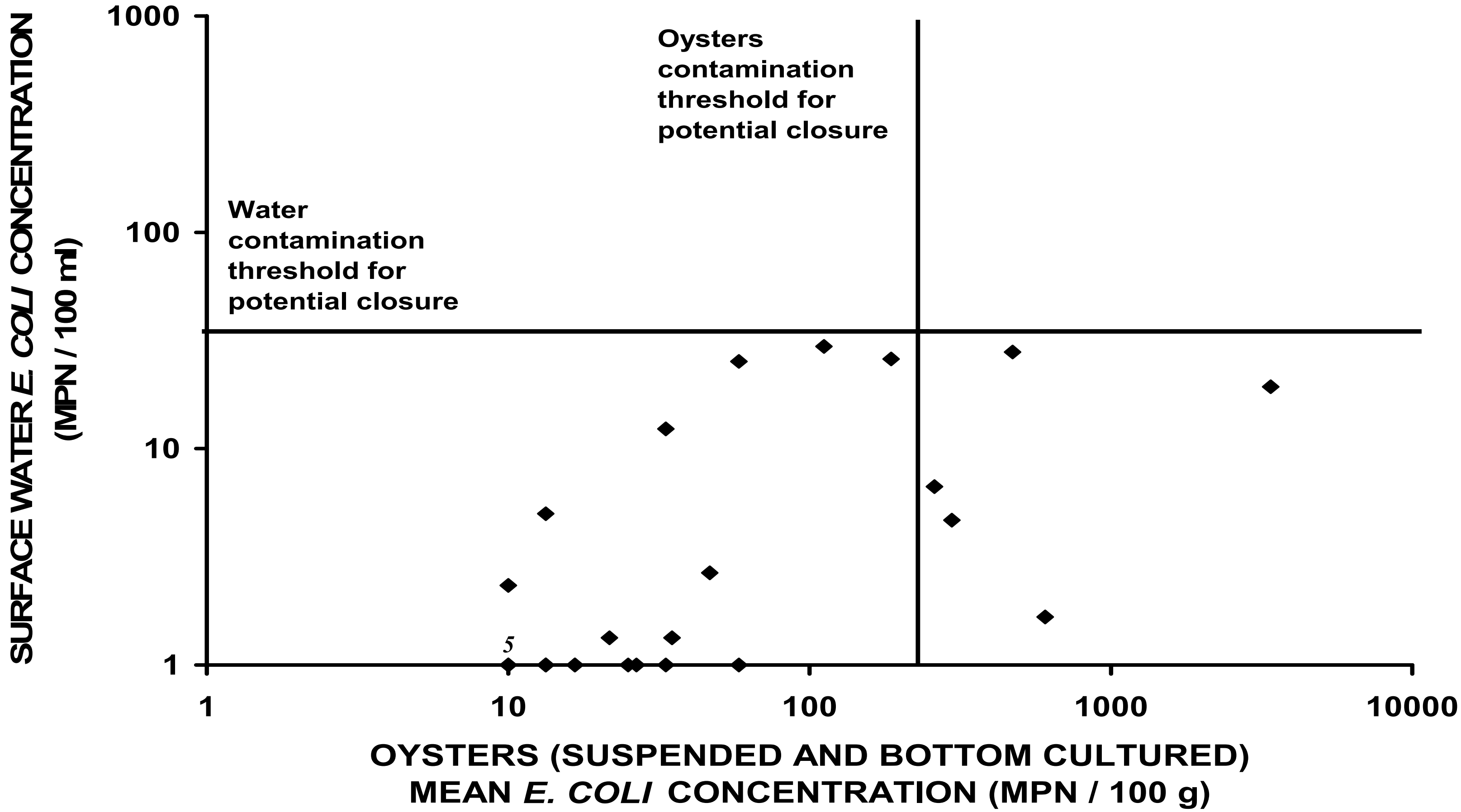

Figure 11: Quadrants scatter plot, contamination thresholds for potential closure, comparing the concentration of *E.coli* in water (MPN / 100ml) and oysters (MPN / 100g) for the conditional zone, N=25.

NOTE: The number 5 represents the number of data superimposed for this particular result.

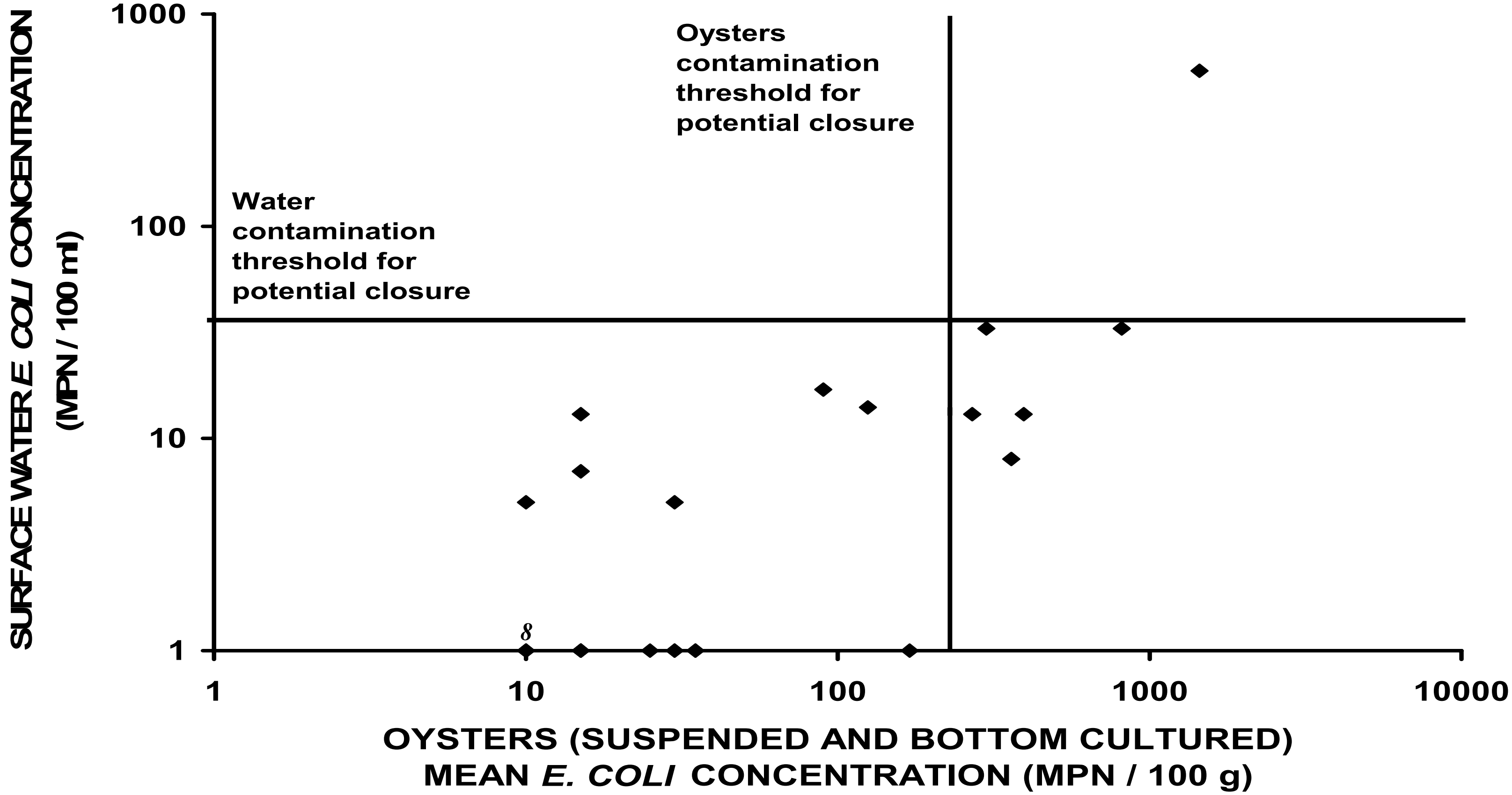

Figure 12: Quadrants scatter plot, contamination thresholds for potential closure, comparing the concentration of *E. coli* in water (MPN / 100ml) and oysters (MPN / 100g) for the closed zone, N=25.

NOTE: The number 8 represents the number of data superimposed for this particular result.

Table 1: Spearman's correlation coefficients (2-tailed) between compartments (water, sediments and oysters) and physico-chemical parameters (water temperature, salinity and total suspended matter), from the conditional zone.

		SEDIMENTS (MPN/100g)	OYSTERS (MPN/100g)	WATER SALINITY (ppt)	WATER TEMPERATURE (ºC)	TOTAL SUSPENDED MATTER (mg/L)
WATER	r_s	0.692	0.588		0.336	0.567
(MPN/100ml)	p	0.000	0.000	NS	0.001	0.000
	N	78	81		81	57
SEDIMENTS	r_s		0.606		0.369	
(MPN/100g)	p		0.000	NS	0.001	NS
	N		75		75	
OYSTERS	r_s				0.506	0.472
(MPN/100g)	p			NS	0.000	0.000
	N				78	54

*** NS = non-significant*

Table 2 : Water, sediment and cultivated oysters faecal coliform contamination median, geometric mean, 90th percentile and percentages of their closure threshold (43 MPN/100mL for water and 230 MPN/100g for oysters meat).

Zones	Compartments	N	Median	Geometric mean	% water samples > 43 MPN / 100ml	% oysters samples > 230 MPN / 100g	P90 MPN
Open	**Water**	31	1.90	2.94	0		8
	Oysters	29	30.00	42.59		10.34	200
	Sediments	29	19.90	36.22			120
Conditional	**Water**	30	1.90	3.56	0		17
	Oysters	27	33.33	58.70		22.22	453
	Sediments	28	19.90	39.22			707
Closed	**Water**	30	1.90	4.86	3.33		17
	Oysters	25	30.00	55.38		24.00	360
	Sediments	29	19.90	43.71			330

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