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Clostridium perfringens in Long Island Sound Sediments: An Urban Sedimentary Record

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ABSTRACT

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Clostridium perfringens is a conservative tracer and an indicator of sewage-derived pollution in the marine environment. The distribution of *Clostridium perfringens* spores was measured in sediments from Long Island Sound, USA, as part of a regional study designed to: (1) map the distribution of contaminated sediments; (2) determine transport and dispersal paths; (3) identify the locations of sediment and contaminant focusing; and (4) constrain predictive models. In 1996, sediment cores were collected at 58 stations, and surface sediments were collected at 219 locations throughout the Sound. Elevated concentrations of *Clostridium perfringens* in the sediments indicate that sewage pollution is present throughout Long Island Sound and has persisted for more than a century. Concentrations range from undetectable amounts to 15,000 spores/g dry sediment and are above background levels in the upper 30 cm at nearly all core locations. Sediment focusing strongly impacts the accumulation of *Clostridium perfringens* spores. Inventories in the cores range from 28 to 70,000 spores/cm^2, and elevated concentrations can extend to depths of 50 cm. The steep gradients in *Clostridium perfringens* profiles in muddier cores contrast with concentrations that are generally constant with depth in sandier cores. *Clostridium perfringens* concentrations rarely decrease in the uppermost sediment, unlike those reported for metal contaminants. Concentrations in surface sediments are highest in the western end of the Sound, very low in the eastern region, and intermediate in the central part. This pattern reflects winnowing and focusing of *Clostridium perfringens* spores and fine-grained sediment by the hydrodynamic regime; however, the proximity of sewage sources to the westernmost Sound locally enhances the *Clostridium perfringens* signals.

ADDITIONAL INDEX WORDS: *Bacteria, coastal zone management, contaminant, environment, fecal, metal, pollutants, pollution, population, quality, sewage, toxicity.*

INTRODUCTION

Long Island Sound Regional Study

The water and sediment quality of many coastal areas in the United States are impacted by discharges from urban centers, as well as by industrial and agricultural activities. Semi-enclosed marine areas, such as Long Island Sound, are particularly sensitive to anthropogenic activity (KOPPELMAN *et al.,* 1976; O'CONNER and EHLER, 1991; VESTAL *et al.,* 1995) because pollutants may be less efficiently removed, dispersed, or diluted than on open coasts. Long Island Sound is bordered by New York City, Huntington and Smithtown, NY, as well as the cities of Stamford, Bridgeport, Norwalk, New Haven, and New London, CT. These cities, and other population centers, are the source of direct input of pollutants in the form of sewage effluent, industrial discharge, dredge spoils, urban runoff, and atmospheric deposition (FARROW *et al.,* 1986; U.S. EPA, 1997; BREAULT and HARRIS, 1997). In addition, the Sound receives discharges from rivers, such as the Housatonic, Quinnipiac, Connecticut, and Thames, which drain extensive industrial and inland areas of Connecticut and Massachusetts (ZIMMERMAN *et al.,* 1996; HARRIS, 1997). The potential for adverse environmental effects (BRICKER *et al.,* 1992; U.S. EPA, 1994; LONG *et al.,* 1996), plus management concerns (*e.g.,* ROBERTSON *et al.,* 1991; VESTAL *et al.,* 1995) prompted the U.S. Geological Survey to undertake a multidisciplinary study (POPPE and POLLONI, 1998) to assess the influence of geologic processes on the ecosystem of the Sound. Concentrations of *Clostridium perfringens* spores in the sediments of Long Island Sound are used in this study as a tracer of sedimentary processes and as a proxy for other contaminants. This work is a component of the larger geochemical studies (BUCHHOLTZ TEN BRINK and MECRAY, 1998; BEUNING *et al.,* this volume; MECRAY and BUCHHOLTZ TEN BRINK, this volume; THOMAS *et al.,* this volume; VAREKAMP *et al.,* this volume). It contributes to the study's objectives of determining the regional distribution of contaminants in sediments of Long Island Sound, measuring contaminant inventories and rates of sediment mixing and accumulation, increasing our understanding of the processes by which pollutants migrate in the marine environment, and better predicting the fate of existing and future contaminants in Long Island Sound sediments.

Clostridium perfringens as a Tracer of Sewage Contamination

Clostridium perfringens, an enteric bacterium, is present in the intestinal tract of mammals. This bacteria, and its endospores, are excreted in human fecal material, pass through the sewage treatment process, and are discharged with effluent and sludge into the environment (NRC, 1993). The spores are inert in most temperate marine sediments (RIPPEY and WATKINS, 1992; DAVIES *et al.,* 1995) as both anoxia and elevated temperatures are necessary for significant growth. Consequently, the presence of *Clostridium perfringens* spores provides an excellent tracer and recorder of sewage input into an ecosystem (PARMENTER and BOTHNER, 1993; LUCENA *et al.,* 1996; WATKINS and BURKHARDT, 1996). The presence of *Clostridium perfringens* in sediments has been used as an indicator of sewage contamination in the sediments of Puget Sound (MATCHES *et al.,* 1974), Narragansett Bay (VALENTE *et al.,* 1992), New York Bight (CABELLI *et al.,* 1984; HILL *et al.,* 1993; SAWYER *et al.,* 1996), offshore of Los Angeles (SHANAVIS, 1998), Boston Harbor (PARMENTER and BOTHNER, 1993), and near Antarctica (EDWARDS *et al.,* 1998). In Long Island Sound, bottom waters are typically less than 10°C and submerged sediments do not reach temperatures favorable for growth, *e.g.,* greater than 35°C (K. Feldman, personal communication). The *Clostridium perfringens* concentrations measured in sediments from Long Island Sound are thus expected to provide an estimate of the location and magnitude of sewage contamination in the Sound. In addition, the concentrations of these spores are a valuable tracer for the magnitude and distribution of other urban contaminants in sediments because sewage discharge is often a significant source of pollutant metals (*e.g.,* silver (Ag), copper (Cu), zinc (Zn), mercury (Hg)) and other contaminants in coastal waters (FARROW *et al.,* 1986; U.S. EPA, 1997; BENOIT, *et al.,* 1999).

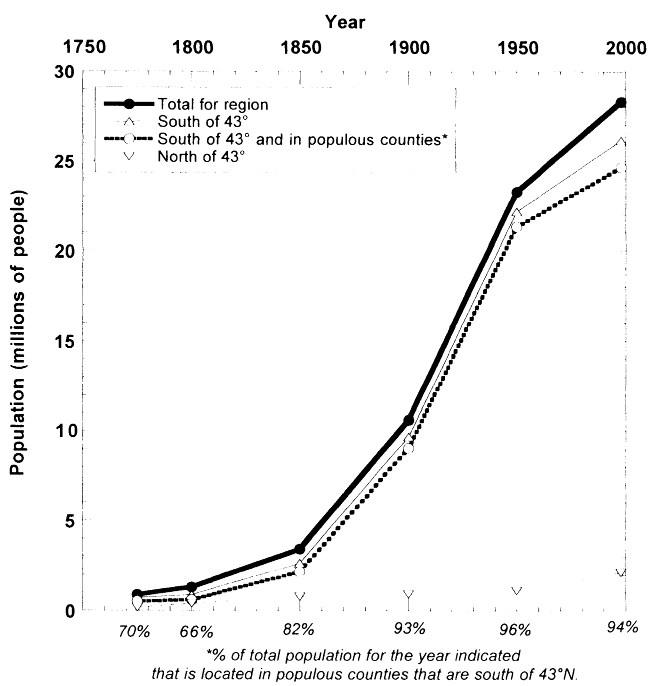


Figure 1. Integrated population growth for counties located in watersheds that drain to Long Island Sound (see Figure 2 for locations). The population that is nearest to Long Island Sound (south of 43°N) and dwelling in populous counties (those having >200,000 people in 1998) is more likely to provide a source of sewage contamination to the Sound than more distant or rural centers. Population data used to generate the plot is from the U.S. CENSUS BUREAU (1999) and ICPSR (1999).

Sources of *Clostridium perfringens* and Transport Processes

The input rates of various contaminant sources for Long Island Sound, and their relative importance, have changed over time due to population growth and increased industrial development. The rapid increase in population within the Long Island Sound watershed that occurred in the 1800 and 1900's (Figure 1) was also accompanied by increased urbanization, industrial activities, and establishment of municipal waste facilities. The greater density of water-water treatment plants (WWTP) around the western Sound (Figure 2) reflects the concentration of population in that area. FARROW *et al.* (1986) estimated that 85% of the sewage discharged to Long Island Sound was to the western basin, 11% to the central basin, and 4% to the eastern basin. Within the last 20 years, efforts to reduce environmental pollution have curtailed the input of some contaminants. Reductions have been made in the volume of discharged sewage solids, sewage metal loadings, and non-sewage sources of hydrocarbons and metals (NRC, 1993; U.S. EPA, 1997). Despite this, population density in the Long Island Sound region is expected to increase even more (CULLITON *et al.,* 1990), and continue to stress the marine environment. The patterns of *Clostridium perfringens* found in sediments provide both a record of past sewage contamination and a means to characterize the processes that transport contaminants.

The fate of *Clostridium perfringens* spores, or any contaminant that is introduced into coastal waters, is affected by a number of processes. The large-scale geologic processes of sedimentation, burial, resuspension, and winnowing affect the transport and accumulation of sediments, particulate matter, and contaminants associated with them (LERMAN, 1979; SANTSCHI *et al.*, 1990). The physical environment within Long Island Sound, which is created by the combination of bathymetry, current and wave action, and sediment sources, encompasses a wide spectrum of conditions and great spatial diversity in sedimentary environments (KNEBEL *et al.,* 1999; KNEBEL and POPPE, this volume). The current dynamics within the Sound also create a spectrum of sediment-transport rates (SIGNELL *et al.,* this volume) that cause physical processes at the sea floor to vary over time scales ranging from days to decades.

In this paper, we report the occurrence of *Clostridium perfringens* in sediments from cores and surface grab samples that were collected in 1996 throughout Long Island Sound. In addition, results from ongoing studies of contaminant metals, lithologic features, physical and chemical properties, and radiometric dating of the cores provide temporal constraints. These data: (1) yield a baseline for contaminant distribution; (2) provide estimates of the inventory of contaminated sediment; (3) outline transport and dispersal paths; (4) identify locations of sediment and contaminant focusing; and (5) pro-

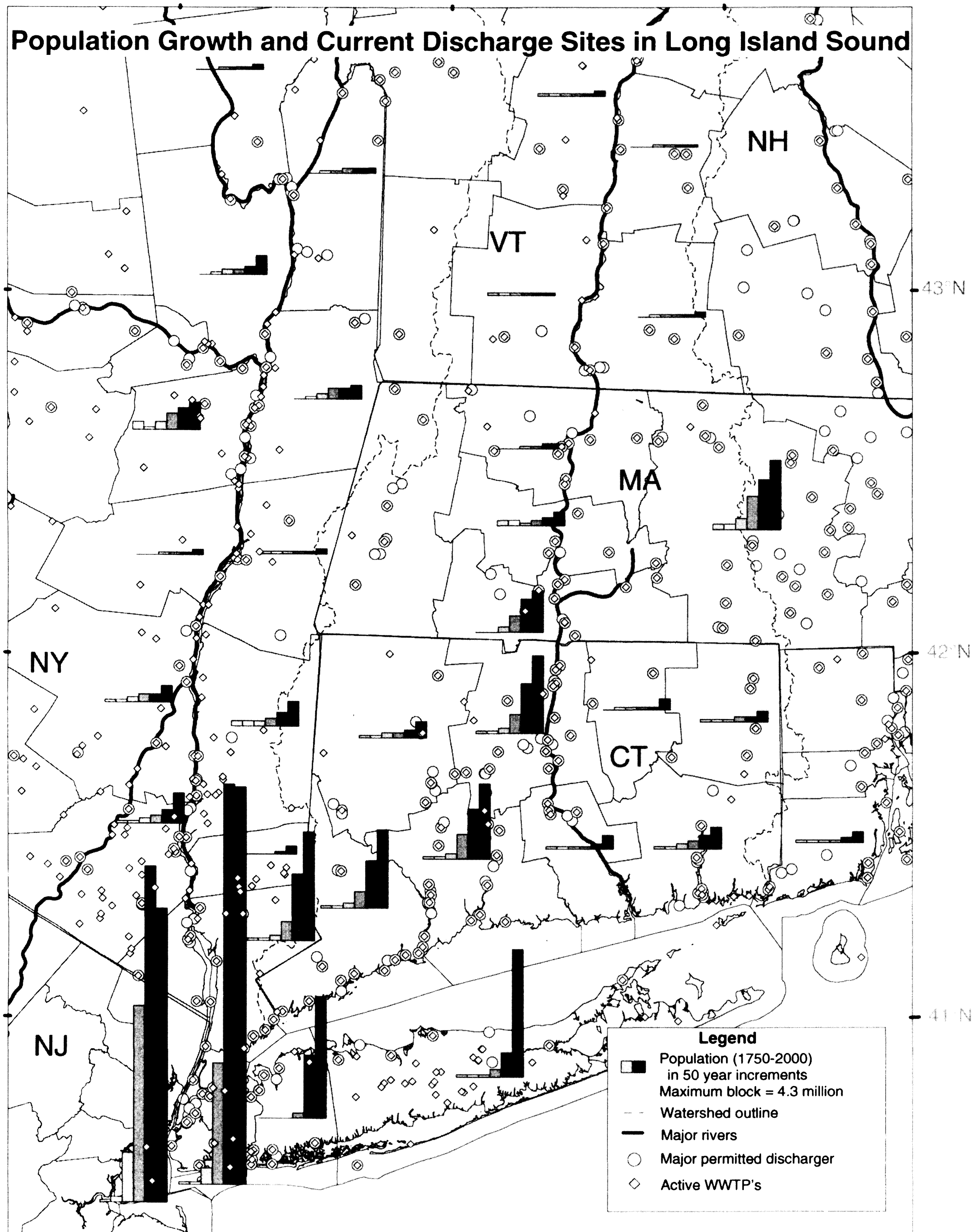


Figure 2. Population growth in the Long Island Sound region, by county from 1750 to 2000 A.D., shown with the location of permitted waste-dischargers in the region. Boundaries of counties, watersheds, and major rivers are also delineated for those watersheds that drain into Long Island Sound. Population data used to generate the plot is from the U.S. CENSUS BUREAU (1999) and ICPSR (1999). Locations of major permitted-dischargers and active sewage (WWTP) discharge sites are from the National Pollutant Discharge Elimination System (NPDES), which regulates direct discharges from municipal and industrial wastewater treatment facilities that discharge into the surface waters of the United States (U.S. EPA, 1997, 2000).

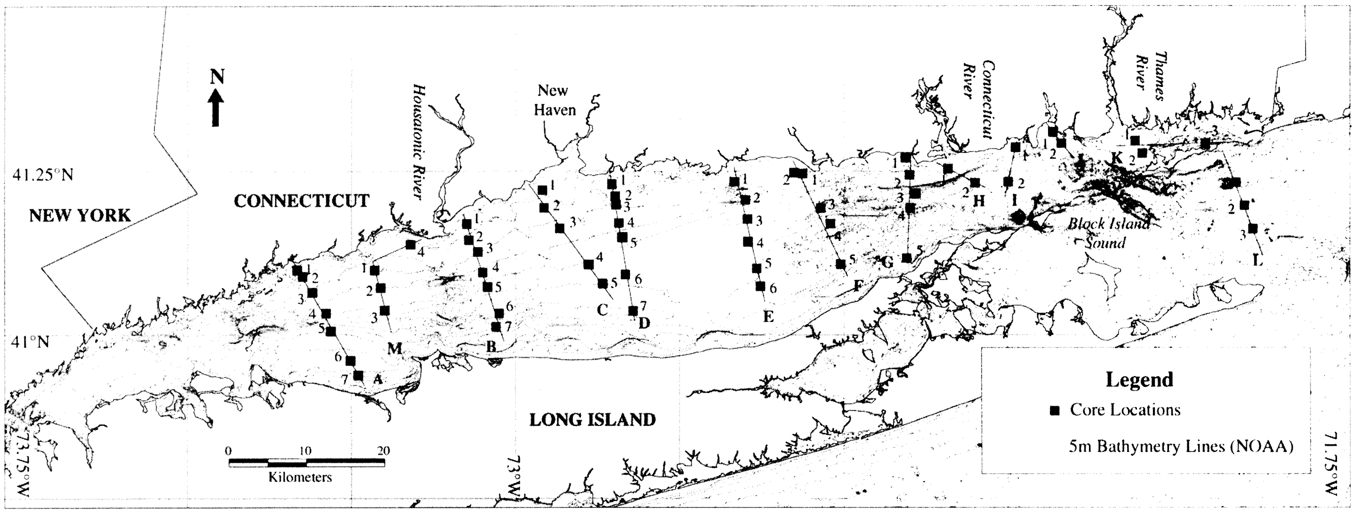


Figure 3. Locations where sediment cores were collected in June, 1996 in Long Island Sound on the R/V Seaward Explorer. Transects A through L are shown with the station numbers (*e.g.,* A1, A2, A3 . . .). Figure 5a shows the locations of grab samples that complemented the core distribution. Latitude and longitude of cores are in Table 1. Bathymetry is from NOAA (1999).

vide constraints for predictions of burial and remobilization rates.

METHODS

Study Area and Sample Collection

Sediment cores for geochemical analysis were collected in June 1996 at 58 stations along north-south transects (Figure 3; Table 1). Core sites were selected to include: (1) areas that are representative of particular sedimentary environments (KNEBEL *et al.,* 1998); (2) transects seaward of known contaminant sources; (3) regions where sidescan mosaics and biological community data were available (POPPE and POLLONI, 1998); and (4) sites offshore of land-based studies (VAREKAMP and SCHOLAND, 1996). In addition, 219 surficial samples were obtained that provided spatial coverage. These were collected along seismic track-lines in 1996, and in western Long Island Sound in 1997 (MECRAY and BUCHHOLTZ TEN BRINK, this volume).

Sediment cores were collected using the U.S. Geological Survey's hydrostatically-damped gravity corer (BOTHNER *et al.,* 1997), which collected 11-cm diameter cores up to 70 cm in length in clear, polycarbonate tubing. The corer was operated in both the piston and the piston-free modes. The piston mode provided slightly longer cores, whereas the piston-free mode provided slightly better sediment surfaces. Use of this corer minimized the disturbance of the sediment surface and made measurements of detailed gradients near the sediment-water interface possible. Between one and six replicate cores were collected at 29 of the stations. The overlying water collected in the corer was clear, and features on the surface (*e.g.,* fecal pellets) were distinct. A video camera was mounted on the frame of the corer and recorded the entry of the corer into the sediment. The video also allowed the core to be taken in a representative location, avoided placement of the sampling gear in dangerous spots, and recorded any leakage from the bottom of the core. On deck, the cores were capped, described, and stored vertically in a refrigerator (or archived frozen) for later sectioning and analysis. Overlying water was retained in the core barrel to reduce disturbance of the sediment interface, and salinity values were measured with a hand-held refractometer.

A Van-Veen grab sampler was used to collect the surface sediments. The grab sampler also had downward-looking video and still cameras attached in order to characterize the bottom and verify the integrity of the grab. After photographing and describing the sediment surface on deck, the overlying water was removed, and the upper 2-cm of sediment were collected with a Teflon-coated flat-bottom scoop. The sediment sample was placed in a pre-cleaned (5% HNO_3, distilled water, and methanol rinse) plastic container and homogenized. Aliquots were then taken for later analysis of *Clostridium perfringens* spores, grain size (POPPE *et al.,* 1998), and sediment chemistry (MECRAY and BUCHHOLTZ TEN BRINK, this volume).

Sample Analysis

On shore, X-radiographs were taken of all cores (in an upright position) to document fine-scale lithological features and provide insight for core sectioning. Immediately prior to sectioning, the overlying water was removed and profiles of bulk density and magnetic susceptibility were determined on each whole core with a multi-sensor core logger (MSCL; BOYCE, 1973). Cores were then vertically extruded and sectioned with titanium spatulas at 0.5-cm or 1.0-cm intervals, taking care to trim edges which may have been smeared during the process. Replicate cores were sectioned from stations A1, A3, B5, and C1. After three years of frozen storage at temperatures below $-10°$ C, replicate cores from stations M1,

Table 1. *List of core locations and Clostridium perfringens inventories.*

Station and Core name	Latitude (decimal degrees N)	Longitude (decimal degrees W)	Water depth (m)	Maximum depth (cm) of *enrichment**	*C. perfringens* inventory (spores/cm^2)
A1C1	41.098880	−73.331728	8.6	25	11419
A1C3	41.098693	−73.331730	9	28	14783
A1C5 (F)	41.098667	−73.331658	9.5	38	14112
A2C1	41.089117	−73.323840	11	36	19706
A3C1	41.064882	−73.309633	18	36	8844
A3C3	41.064503	−73.309038	18	36	8731
A4C1	41.033047	−73.287978	25	50	9449
A5C1	41.005263	−73.280750	28	60	16278
A6C3	40.960503	−73.251677	19	>25	>10523
A7C1	40.938217	−73.239193	15	30	7273
M4C1	41.137832	−73.160225	6.5	>25	>13968
M1C1	41.098640	−73.214553	12	>30	>2418
M2C2	41.072148	−73.205113	20	>40	>17060
M3C1	41.037455	−73.199165	28	>30	>12569
B1C2	41.168760	−73.074953	6.5	>40	>57590
B2C1	41.144432	−73.071685	12.5	>42	>37182
B3C2	41.126453	−73.057945	13	>35	>18339
B4C1	41.095192	−73.050622	22	>37	>9447
B5C3 (F)	41.073577	−73.042983	26	>20	>7812
B5C4	41.073433	−73.043463	26	>15	>11142
B5C5	41.073493	−73.043652	26	>53	>32907
B6C1	41.033012	−73.025110	37	55	9495
B7C1	41.012753	−73.030232	31	55	11329
C1C2	41.220560	−72.959663	9	>35	>21589
C1C3	41.220818	−72.959447	9	>30	>14877
C2C1	41.193568	−72.957062	13	34	11314
C3C1	41.162058	−72.933065	19	30	6156
C4C2	41.106932	−72.888073	28	>24	>17640
C5C1	41.078455	−72.867615	31	>20	>14364
D1C3	41.210333	−72.848500	9	36	17357
D1C4 (F)	41.229092	−72.853408	10	>32	>9889
D2C2	41.211270	−72.848292	12	>55	>10737
D3C2	41.198407	−72.847157	5	>50	>11293
D4C1	41.170468	−72.842900	18	>55	>33008
D5C3	41.147988	−72.836603	23	>36	>35275
D7C4	41.036952	−72.821153	36	>56	18176
E1C1	41.233570	−72.665713	13	35	3496
E2C1	41.205620	−72.649273	13	>35	>3234
E3C1	41.176575	−72.646057	31	48	9696
E4C1	41.141682	−72.645772	28	>44	>7902
E4C2 (F)	41.142208	−72.645150	28	>42	>6085
E5C2	41.101022	−72.632172	26	>24	>1866
E6C4	41.074383	−72.626448	23	>44	>3154
F1C5	41.246025	−72.562643	9	>50	>13320
F2C1	41.247608	−72.575877	9	40	8632
F3C1	41.193312	−72.535150	15	0	28
F4C1	41.169520	−72.520378	23	>16	>372
F5C1	41.107578	−72.504820	34	>50	>4917
G1C1	41.270000	−72.407167	5	38	65621
G2C1	41.243915	−72.400528	15	36	34092
G3C1 (F)	41.214573	−72.392198	46	46	5290
G3C2	41.215567	−72.391165	46	22	14078
G4C2	41.193355	−72.399345	44	0	158
G5C1	41.116830	−72.404773	20	>40	>2705
H1C1	41.253082	−72.341325	8	32	14661
H2C1	41.231733	−72.299888	38	34	3743
I1C1	41.286163	−72.238525	6	48	13716
I2C1	41.233370	−72.249853	37	26	4188
J1C2	41.309327	−72.181738	7	42	12843
J2C1	41.292085	−72.168177	10	38	13508
K1C1	41.296340	−72.055072	13	30	10670
K2C1	41.277075	−72.043988	15	40	10882
K3C3	41.291572	−71.946678	10	40	3346
L1C1	41.232865	−71.899238	40	10	849
L2C3	41.197338	−71.886482	33	18	1187
L3C1	41.1617967	−71.873250	35	14	553

(F) indicates cores that were stored frozen prior to analysis and were sectioned in 2 cm intervals, rather than 0.5 cm intervals.
* Maximum depth of enrichment is the depth below which *Clostridium perfringens* values are non-detectable.
Depths of core bottom (>) are given when background is not reached in the core and zero indicates background values throughout the core.

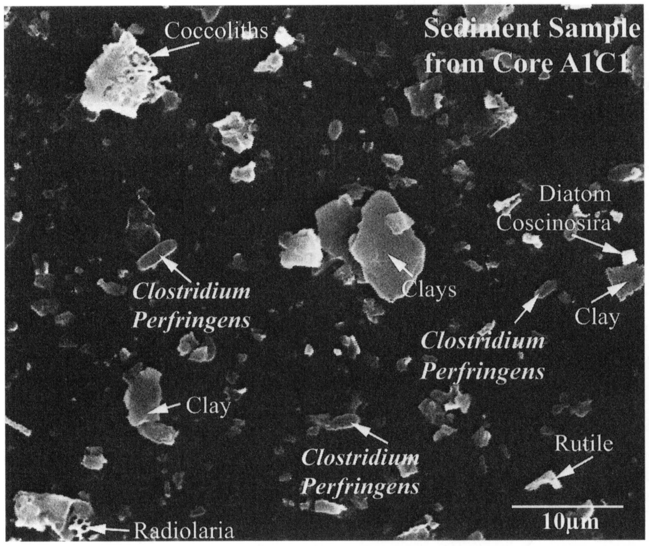


Figure 4. Scanning electron microscopy image of three *Clostridium perfringens* bacteria in a matrix of fine particles from the A1 sediment core (0–0.5 cm core depth).

B5, D1, E4, and G3 were sectioned in 2-cm intervals. All surfaces contacting the sediment (core barrels, sampling spatulas, aliquot containers) were rinsed with dilute acid (5% HNO_3), distilled water, and methanol and then air-dried in a laminar-flow hood. Each sediment section was homogenized, and an aliquot of wet sediment was removed for *Clostridium perfringens* analysis. Sectioning of all cores occurred at room temperature, and *Clostridium perfringens* aliquots were refrigerated immediately after slicing. The remaining wet samples were weighed, freeze-dried, water content was determined by weight loss, and the samples were archived for further analysis. Sediments from the grab samples (0–2 cm) were collected and prepared in a similar manner (BUCHHOLTZ TEN BRINK and MECRAY, 1998; MECRAY and BUCHHOLTZ TEN BRINK, this volume).

Concentrations of *Clostridium perfringens* spores (Figure 4) were determined by the membrane filtration and enumeration method of BISSON and CABELLI (1997) and EMERSON and CABELLI (1982), which is detailed in U.S. EPA (1995). Sample size was between 1 and 5 g of wet sediment, and each reported value is the average of two measurements. A portion of the sample was dried to determine the water content value used for conversion of measured data from units of spores per mass of wet sediment to reported values of spores per mass of dry sediment. Replicates were analyzed approximately every tenth sample, which resulted in a range of error from 2% to 30%, although most of the replicates had less than 15% error. The analytical blank for *Clostridium perfringens* was zero counts and the median detection limit for the method was 7 $\pm$ 8 spores/g dry sediment ($n = 156$), with detection limit values as low as 2 spores/g dry sediment and 97% of the detection limit values were <16 spores/g dry sediment. Values measured as non-detectable are considered to indicate uncontaminated, naturally-occurring background values.

Grain size was determined for the grab samples on wet sediments using standard laboratory methods (FOLK, 1974; POPPE *et al.*, 1985). The fine fraction of the sediment (% fines) was calculated as the silt-size fraction plus the clay-size fraction (*i.e.,* <63μm), in weight percent. Major and trace elements were determined by total digestion followed by Inductively Coupled Plasma Emission Spectrometry (MURRAY and LEINEN, 1996; MECRAY and BUCHHOLTZ TEN BRINK, this volume). X-radiographs and camera images used in interpreting the *Clostridium perfringens* distribution and transport were transferred from film to digital format. Data on sample locations, water depths, *Clostridium perfringens* concentrations and detection limits, water content, and sediment grain size were placed in an electronic database and are available from the U.S. Geological Survey.

Gamma-emitting radioisotopes, including ^{210}Pb and ^{137}Cs, were identified on a low-background Germanium well detector and used to quantify sediment accumulation and the depth of sediment mixing for cores (TUREKIAN *et al.,* 1980). Preliminary data are available for cores from stations A1, A7, B1, B5, C1, D1, D5, F1, and G1 (BUCHHOLTZ TEN BRINK, unpublished data). Sedimentation rates and age assignments were determined from: (1) the location of the ^{137}Cs peak attributed to bomb fallout, (RICHIE and MCHENRY, 1990); (2) the decay of excess ^{210}Pb under steady state conditions (APPLEBY and OLDFIELD, 1992; ROBBINS and HERSCHE, 1993); and (3) a numerical mixing and sedimentation model that generated a best-fit for profiles of both isotopes (SANTSCHI *et al.,* 1999, and references therein).

The MSCL analysis provided a continuous measure (0.5-cm intervals) of gamma-ray attenuation on the whole cores that was proportional to the sediment bulk density. This was compared to discrete measurements of water content on core aliquots to verify the accuracy of both methods and to estimate the variability in grain density. A grain density of 2.6 g/cm^2 was used to calculate wet- and dry- bulk density from measurements of MSCL attenuation and discrete water content analysis. Inventories of *Clostridium perfringens* in each core (spores/cm^2) were calculated by multiplying the *Clostridium perfringens* concentration (spores/g wet sediment) in each sediment interval by the corresponding wet bulk density (g/cm^3) and integrating over the length of the core. The calculated inventories are minimum values for cores that did not reach non-detectable, background values of *Clostridium perfringens* at depth.

RESULTS AND DISCUSSION

Sea-Floor Environment

Long Island Sound sediments are generally muddy in the west with a transition to coarser textures in the east (KNEBEL *et al.*, 1998; POPPE *et al.*, 1998, this volume). KNEBEL *et al.* (1998, 1999) and KNEBEL and POPPE (this volume) classified the sedimentary environments of the Sound into areas of fine-grained-deposition, sediment sorting and reworking, coarse-grained bedload transport (including sand waves and sand ribbons), and erosion or non-deposition. These environments closely follow the patterns of sediment transport predicted by wave and current modeling for the Sound (SIGNELL *et al.,* this volume). Sediments sampled in the Sound ranged from soft, black, noxious mud to well-sorted, coarse sand. Both the cores and the grabs exhibited lithologic properties that correspond to characteristics of the sedimentary environment in which they were located. The cores that were collected on transects A, M, B, C, D and K (Figure 3) were muddy and were located in areas of fine-grained deposition. They often had fine strata preserved in the core. The sea floor in these areas was covered with polychaete tubes and evidence of other infauna. Cores collected on transects E, F, G (except the most near-shore samples), H, I, J, and L were in areas where stronger current action had removed fine-grained sediment, or prevented its deposition. Bottom photographs showed rippled sands or gravelly deposits in these areas (BUCHHOLTZ TEN BRINK and MECRAY, 1998). The cores collected there were sandy throughout, frequently contained mollusk shells, and rarely had repetitive laminations in the x-radiographs.

Distribution of *Clostridium perfringens* in Surface Sediments

Concentrations of *Clostridium perfringens* that are elevated above a pre-anthropogenic background value were measured in surface sediments and core samples throughout Long Island Sound. The distribution of these elevated values indicates that the introduction of sewage-borne contaminants to the Sound is widespread and has occurred over a long period of time. *Clostridium perfringens* concentrations in the surficial interval (0–2 cm) range from non-detectable concentrations to 15,000 spores/g dry sediment (Figure 5a). The highest values are found in the western end of the Sound; very low, or non-detectable values, are found in the eastern region; and intermediate values are measured in the central and western basins. A triangulation-gridding technique was used to interpolate the *Clostridium perfringens* concentrations for the surface sediments of the entire Sound (Figure 5b). This distribution pattern is attributed to both sediment transport processes and proximity of the sediments to *Clostridium perfringens* sources.

Transport Processes Affecting *Clostridium perfringens*

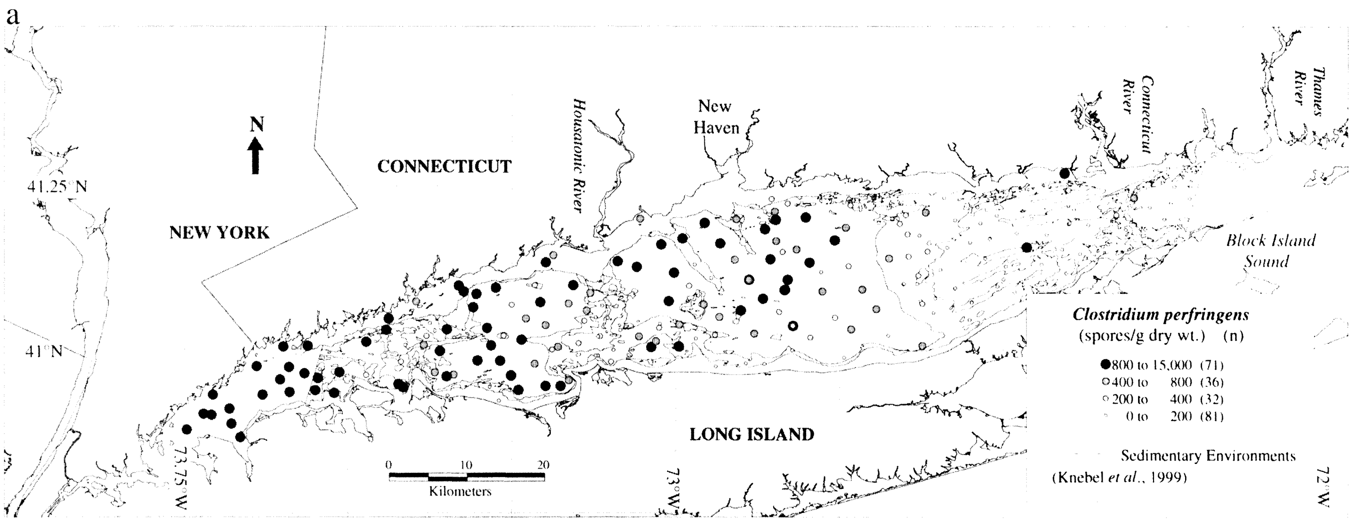
The *Clostridium perfringens* spores are elliptical, with a diameter of 1.5–2 μm, which places them in the clay-size fraction (Figure 4). Spores have a lower density than aluminosilicate sediments, and should be more easily transported and remobilized by the currents and waves in Long Island Sound (SIGNELL *et al.,* this volume) than clastic particles of similar size. Comparison of the *Clostridium perfringens* concentration in the sediments with the median grain size for each sample (Figure 6a) shows that the spores do not accumulate in sediments that have a long-term sandy character, such as that found in the dynamic eastern end of the Sound. The concentration range found in sands (non-detectable to 1600 spores/g dry sediment) is much less than the range found in fine sediment (10 to >5000 spores/g dry sediment). A decrease in spore concentrations with increasing grain size is observed for the sediments with median size larger than 15 μm (6 phi). The threshold and size-dependent removal are indicative of physical removal processes and suggest that spores do not interact (chemically or biologically) with the larger particles. WILLIAMS *et al.* (1997), using Osmium isotopes to trace sewage signals in surface sediments, also found that the concentrations of the sewage-specific tracer were greatest in fine-grained deposits.

The distribution of *Clostridium perfringens* spores in surface sediments (Figure 5) follow patterns that correlate with the bottom stress (see SIGNELL *et al.*, this volume), sediment texture (see POPPE *et al.,* this volume), and sedimentary environments (see KNEBEL and POPPE, this volume. The same pattern is observed for many particle-reactive metal contaminants (MECRAY and BUCHHOLTZ TEN BRINK, this volume). The qualitative correlation observed in the surficial maps is consistent with the positive correlation (log-log $R^2 = 0.68$) of *Clostridium perfringens* with the fraction of fine-grained sediments, and the higher *Clostridium perfringens* concentrations observed for depositional sites (Figure 6b) than for nearby non-depositional sites. The strong correlation between *Clostridium perfringens* concentrations and both the sedimentary environments and the sediment texture is a result of (1) the removal of the spores from areas of high bedload transport and (2) the greater capacity of the sediments in depositional areas to retain the *Clostridium perfringens* and other fine particles.

The eastern Sound and mid-basin shoal complexes are characterized as having sedimentary environments of coarse-grained bedload transport or erosion (KNEBEL and POPPE, this volume) and having currents that produce high bottom stress (SIGNELL *et al.,* this volume). These gravelly and sandy areas have very low concentrations (<200 spores/g dry sediment) of *Clostridium perfringens* (Figures 5 and 6b), although some patchiness results from pockets of finer material in the rougher topography.

As the Sound broadens from east to west, there is an area across the east-central Sound that contains environments of sediment sorting and coarse-grained bedload transport (KNEBEL and POPPE, this volume). *Clostridium perfringens* concentrations in this regime are <400 spores/g dry sediment (Figures 5 and 6b). Here, the sediments consist mostly of well-sorted, sand-sized particles that are predominately composed of silicate minerals. Resuspension and bedload transport in this region cause sediments to be repeatedly remobilized and allow efficient removal of the small *Clostridium perfringens* spores and other small particles by winnowing. The westward mean bottom flow (SIGNELL *et al.*, this volume) then focuses these fine-grained sediments and spores towards the central and western basins.

The central and western basins are characterized as predominantly depositional environments, in which the sediments consist of primarily silt and clay-sized particles. In these lower-energy areas, deposited sediment and *Clostridium perfringens* spores are less-readily remobilized by wave and current action, although they may experience mixing due to bioturbation. In the basin located south of New Haven, the *Clostridium perfringens* concentrations range from background levels to 3,000 spores/g dry sediment. The central and western basins are bounded, and separated, by non-depositional shoal complexes (Figure 3) on which erosion, sorting,



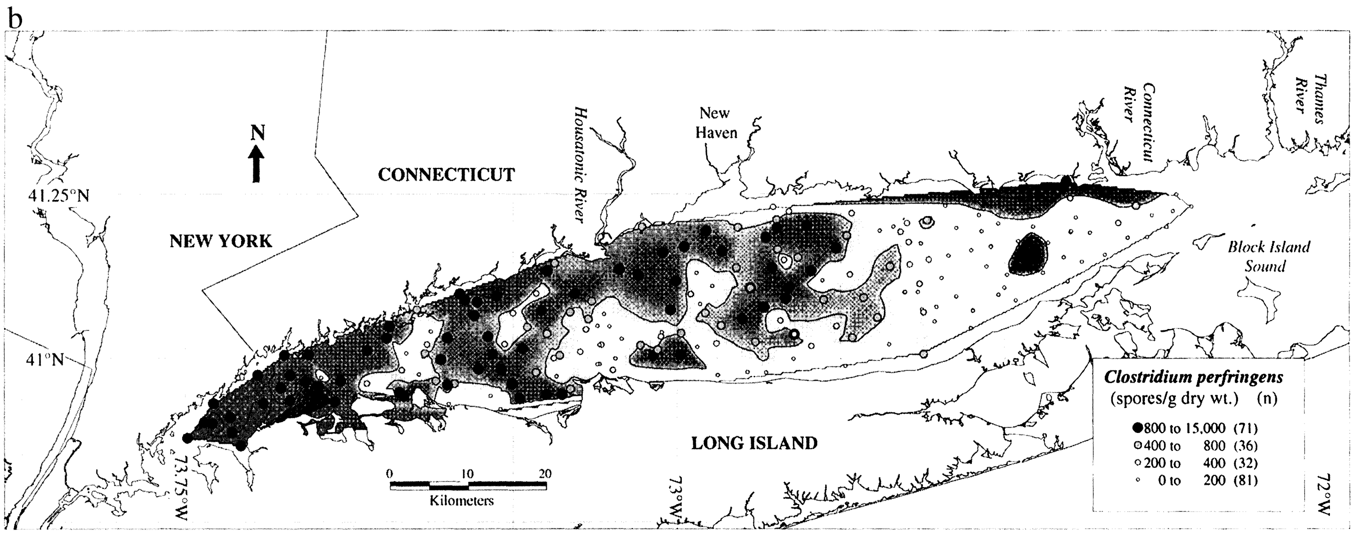


Figure 5a. Map of the location and concentration of *Clostridium perfringens* spores in surface sediment samples (0–2 cm). Higher concentrations are given as larger dots and darker colors. Lines delineate the sedimentary environments (KNEBEL *et al.*, 1999) of deposition, reworking, transport and erosion. Latitude and longitude of grab samples are in BUCHHOLTZ TEN BRINK and MECRAY (1998).

Figure 5b. Concentration map for *Clostridium perfringens* distribution in Long Island Sound sediments. Data was contoured from surface sediment measurements (Figure 5a) using the triangulation interpolation (TIN) technique.

and winnowing occurs and *Clostridium perfringens* concentrations (Figure 6b) are lower than in the nearby depositional sediments. Additionally, an axial depression, which extends across the central and western basins, experiences frequent periods of increased bottom stress that can winnow fine-grained materials and appears to correspond to slightly reduced values of *Clostridium perfringens.* Within the longitudinal bounds of the two central basins (Figure 6b), *Clostridium perfringens* values are concentrated between 200 and 2000 spores/g dry sediment in depositional areas (Figure 6b), whereas the concentrations in the other sedimentary environments range between 70 and 1000 spores/g dry sediment.

Clostridium perfringens concentrations at the westernmost end of the Sound (>1000 spores/g dry sediment) are uniformly greater than those located in similarly fine-grained sediments of the central and western basins. The numerous active WWTPs located in the westernmost Sound and western basin (Figure 2; U.S. EPA, 2000) that discharge into Long Island Sound may contribute to the elevated *Clostridium perfringens* values. The only locations in Long Island Sound that have higher values than the westernmost Sound are two sites that are anomalously located in the eastern Sound (where WWTPs are sparse) and may be affected by episodic input from the Connecticut River or local sources.

Source Proximity

Normalization of *Clostridium perfringens* concentrations to % fine-grained sediment (*i.e.,* % fines) corrects for dilution of

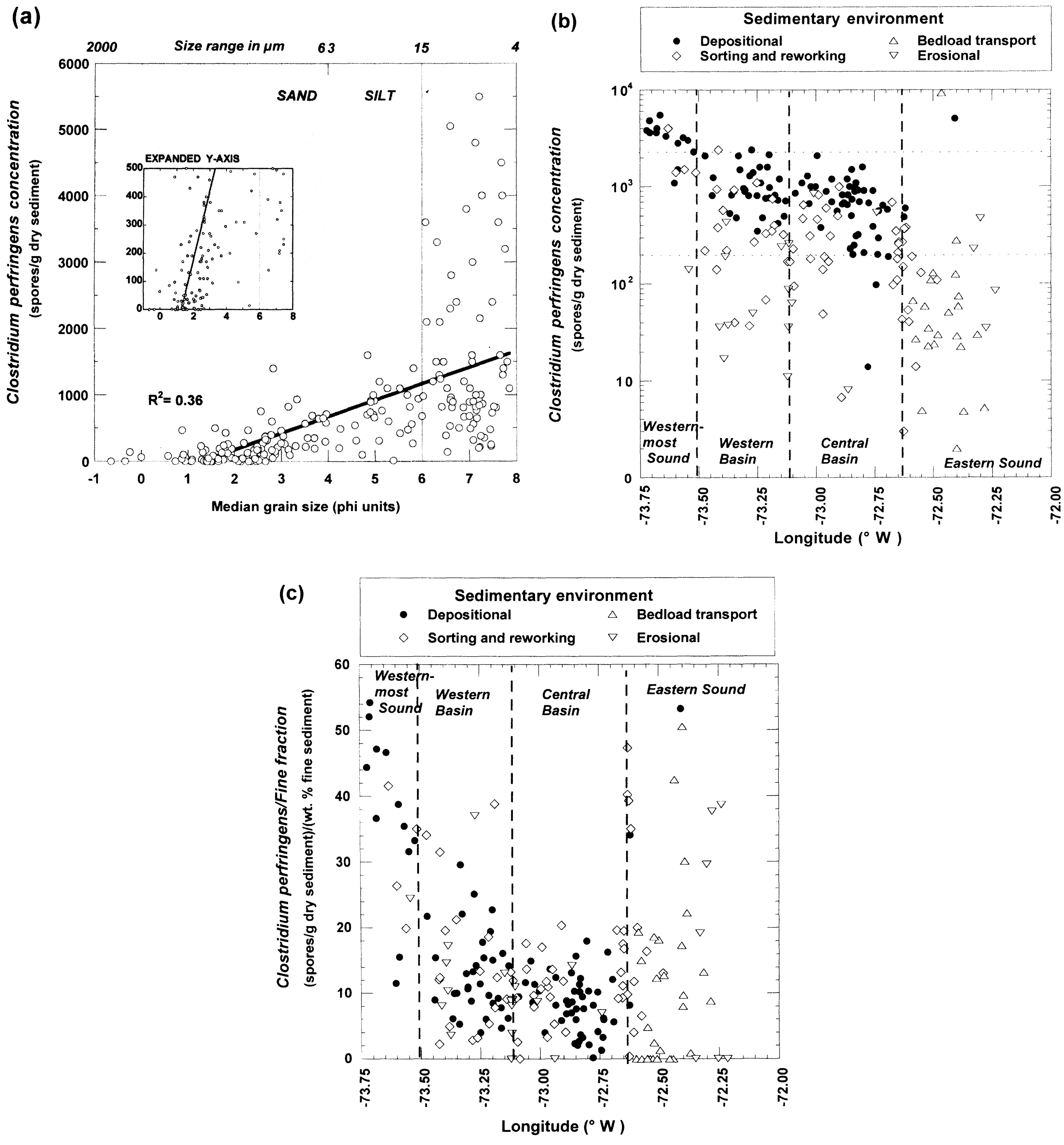


Figure 6a. *Clostridium perfringens* concentrations in Long Island Sound surface sediments plotted against the median grain size (method of moment statistics) for each sample. The lower axis shows size in phi units while shading delineates the range for sand and silt categories, with associated μm boundaries. The 15μm boundary is an upper limit for easily-remobilized fine silts. The inset is an expansion of the y-axis near the ordinate showing the regression line in the range where sediments are sandy. Figure 6b. *Clostridium perfringens* concentrations in Long Island Sound surface sediments as a function of longitude. Symbols indicate the sedimentary environment where each sample is located and dashed lines show basin boundaries. Dotted lines envelop the majority of the depositional samples in the western and central basins. Figure 6c. *Clostridium perfringens* concentrations in Long Island Sound surface sediments, normalized to the percent of fine-grained sediment for each surface sample and shown as a function of longitude. Symbols indicate the sedimentary environment where each sample is located and dashed lines show basin boundaries.

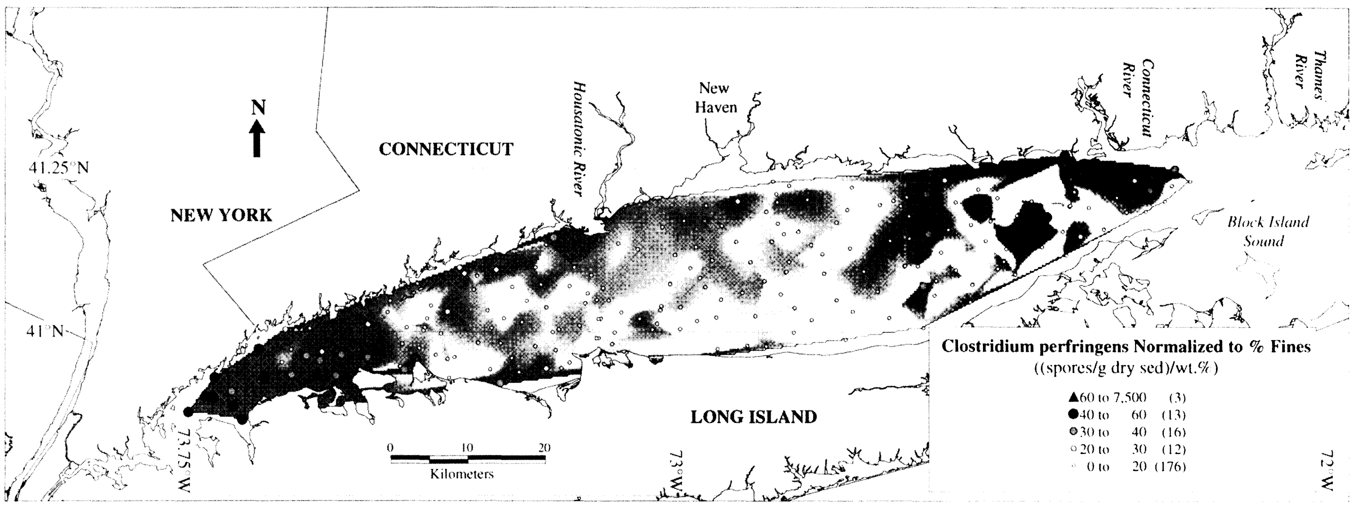


Figure 7. Map of *Clostridium perfringens* concentration normalized to the percent of fine-grained sediment for each surface sample. Individual samples are shown as points and shading gives interpolated values.

the spore-bearing, fine-grained fraction with variable amounts of coarse materials that have lower *Clostridium perfringens* concentrations. Trends in other factors that affect the *Clostridium perfringens* distribution, such as proximity to sources, can be more clearly observed in the normalized data. After normalization to % fines, the *Clostridium perfringens* concentrations in surface sediments range from background to 60 spores/g dry sediment/% fines (Figures 6c and 7).

There is a trend of significantly higher values for both depositional sediments and all sediments in the westernmost Sound ($n = 19$, average of 37 ± 13) over those in the western ($n = 64$, average of 13 ± 8) and central ($n = 71$, average of 9 ± 5) basins. In addition, values decrease eastward within the westernmost Sound ($R^2 = 0.40$). It is likely that the abundance, proximity and high discharge volume of WWTPs in the westernmost Sound and the East River (FARROW *et al.,* U.S. EPA, 2000) are responsible for this local signal.

Within the central and western basins, the normalized *Clostridium perfringens* values for surface sediments do not exhibit a longitudinal trend ($R^2 < 0.04$) (Figure 6c); however, values are slightly higher on the northern side of the Sound (Figure 7). The more uniform values in these two basins, relative to the westernmost and eastern Sound, may be a result of both more distant sources and the hydrodynamics, which can cause wide dispersal and homogenization of suspended source material.

The concentration of *Clostridium perfringens*/% fines in the eastern Sound has greater variance (mean of 17 ± 18) and patchiness than elsewhere in the Sound (Figures 6c and 7), perhaps due to the complex bathymetry and strong currents in the area. The influence of temporal or spatial variation in the source or transport of *Clostridium perfringens* persists, after normalization to the fine fraction reduces the effect of dilution. Potential sources of *Clostridium perfringens* for this region include the Connecticut River and the communities along the eastern shores, where WWTPs are sparse (Figure 2, U.S. EPA, 2000). There are too few depositional sites in the eastern Sound for comparison, but within the environment of coarse-grained bedload transport, there is a slight, statistically insignificant, trend of higher values towards the east. Consequently, it is difficult to determine from the surface samples in this study whether the Connecticut River inflow caused a local elevation in *Clostridium perfringens* contamination.

The accumulation of *Clostridium perfringens* in bulk sediments is primarily controlled by transport processes that affect the mobility of fine-grained sediments. A secondary factor, proximity to sewage sources, results in elevated contaminant concentrations in nearby sediments for the westernmost and northern Sound.

Distribution of *Clostridium perfringens* in Cores

Profiles of *Clostridium perfringens* in sediment cores (Figure 8) reveal spore inputs to the Sound that are widespread and have also impacted the Sound for more than a century. *Clostridium perfringens* profiles show concentrations above background levels from the core surface to depths of approximately 30 cm in cores that are from all regions of the Sound. The concentrations in modern and surficial muddy sediments, however, are more than 10 times greater than concentrations in sandy sediments. There are steep gradients in the *Clostridium perfringens* profiles of the muddier cores, which are located in the depositional regions. Several cores from depositional areas (*e.g.,* cores from stations A1, A2, A4, B6, B7, D1, D3, G1 in Figure 8) have *Clostridium perfringens* spore counts in deeper sections of the core ($\geq$ 40–50 cm) that are below the detection limit. Shorter cores (<40 cm) that were recovered from depositional areas do not reach background concentrations at depth (*i.e.,* cores from stations A6, M1, B3, B4, C4, C5, D2, D5). The low counts (<6 spores/g

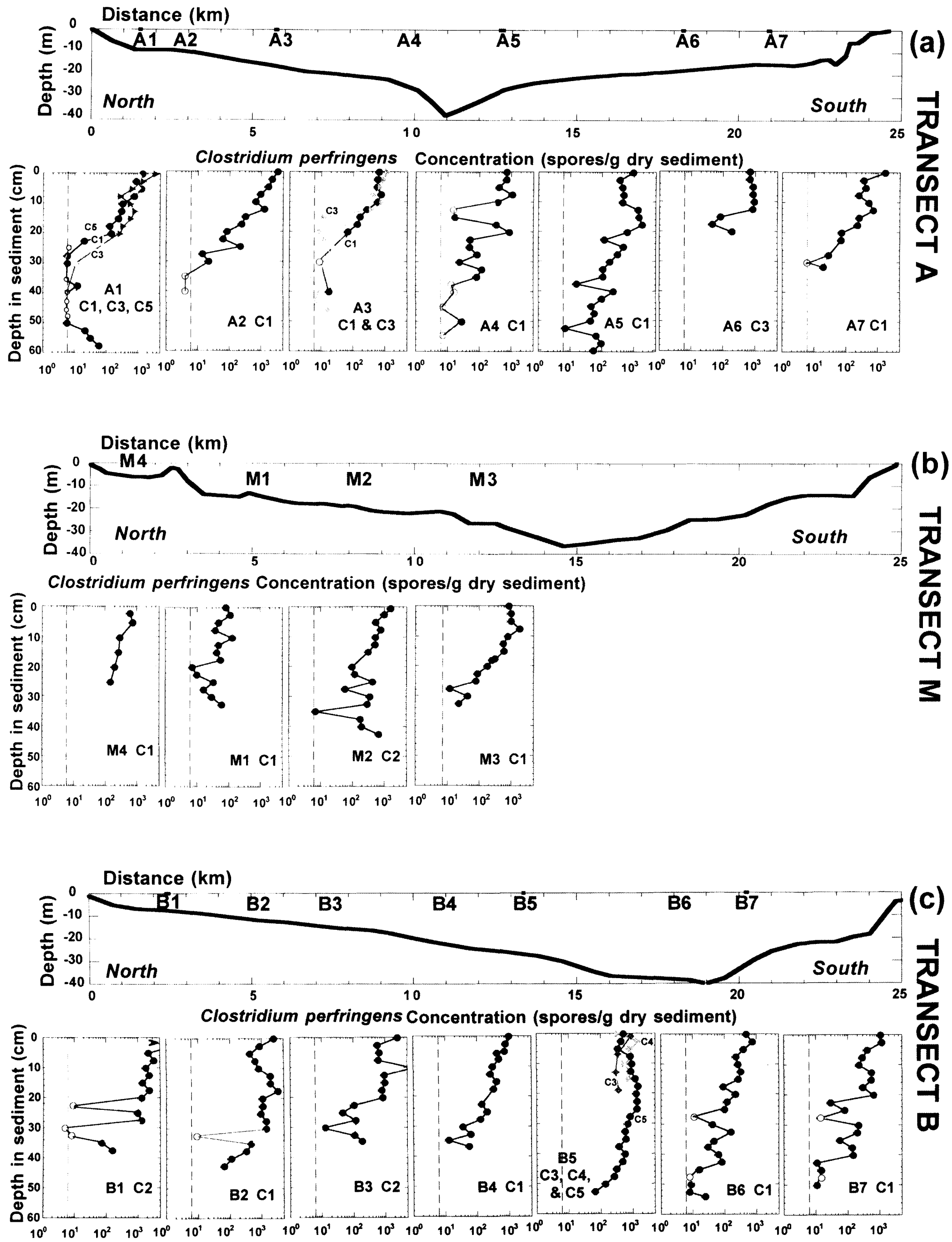


Figure 8. Profiles of *Clostridium perfringens* concentrations in cores shown with the bathymetry and location of each core along the N-S trending transects. From west to east, these are Transects (a–c) A, M and B; (d–f) C, D, and E; (g–i) F, G, and H; and (j–m) I, J, K, and L. Locations of transects and cores are in Figure 3 and Table 1. Maximum error bars (often within the symbol size) of ±25% are shown for *Clostridium perfringens* concentrations. Plotted sample depth in the sediment is ± 0.25 cm for cores sampled at 0.5-cm depth intervals, and ±1 cm depth for frozen cores that were sectioned in 2-cm intervals. Open symbols indicate samples measured as less than the detection limit, which is the value plotted. The dashed line indicates a representative, pre-contaminant background value. Depth and concentration scales are the same for all cores. Concentrations are shown on a log scale to allow comparison of values that range across three orders of magnitude.

dry sediment) occur at depths where low mercury concentrations (VAREKAMP *et al.*, this volume) and preliminary radiometric ages (BUCHHOLTZ TEN BRINK and MECRAY, 1998; BUCHHOLTZ TEN BRINK, unpublished data) indicate pre-pollutant, presumably pre-industrial, times where there was no measurable anthropogenic contribution of *Clostridium perfringens* to the sediment. The *Clostridium perfringens* concentrations gradually increase upwards, and then sharply in-

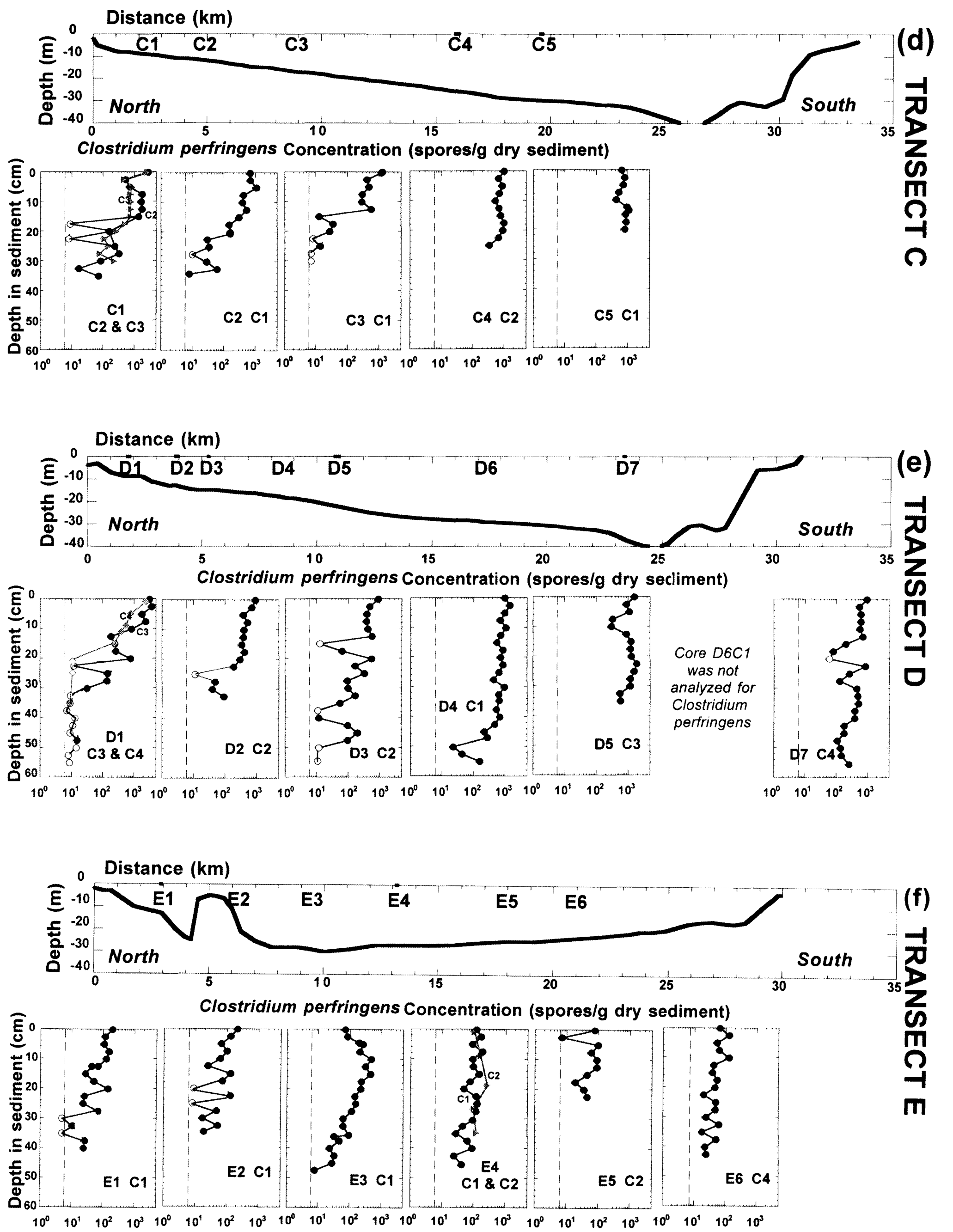
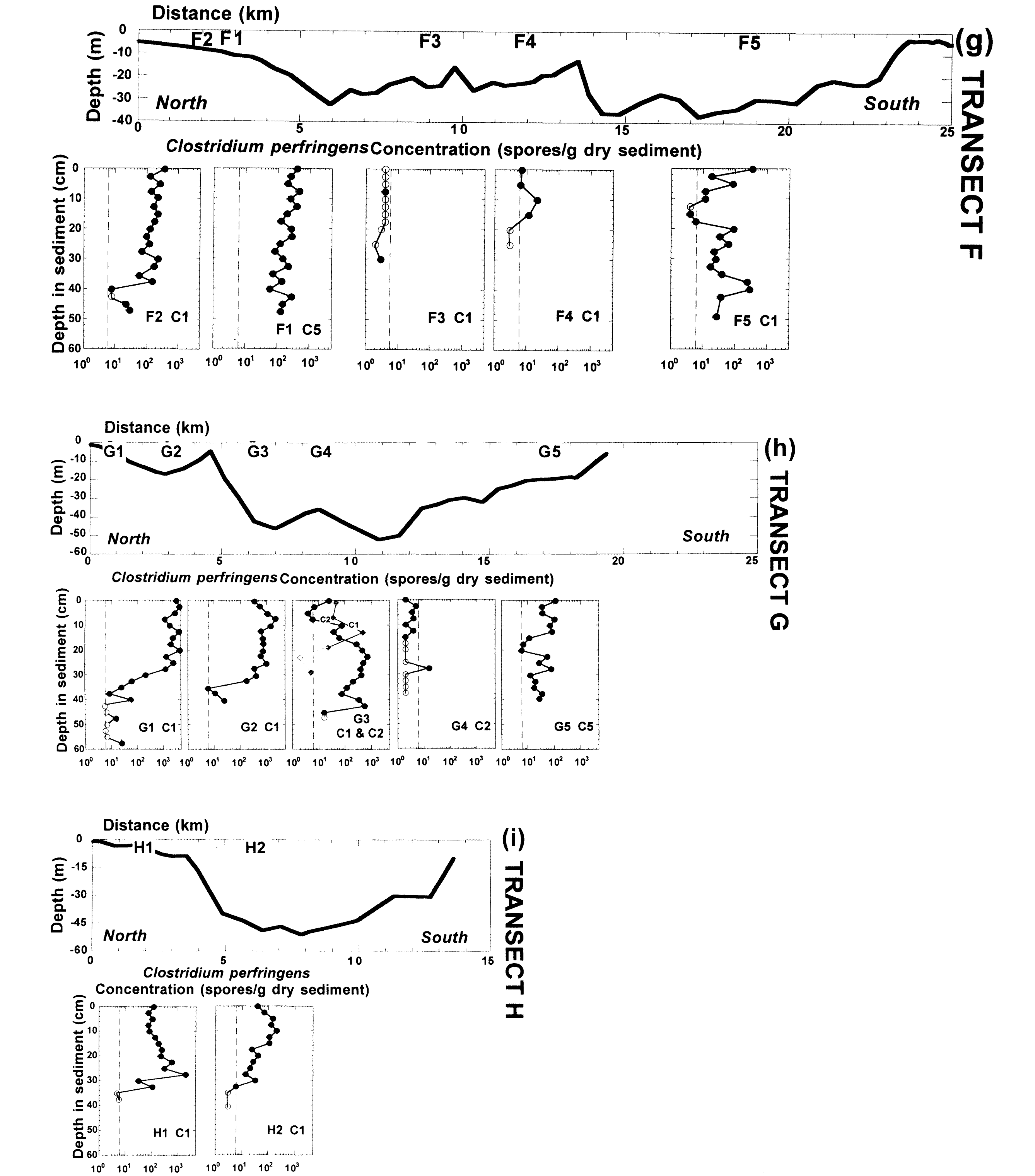


Figure 8. Continued.

crease to reach values 100 times greater than background in the upper section (10–40 cm in thickness) of the cores.

In the western and central basins (transects A, M, B, C, D, and E), cores from 31 out of 35 stations had *Clostridium perfringens* concentrations that increased or remained constant to the core top, *i.e.,* recent time, while four stations (A5, M3, B5, and E3) had values that dropped by an order of magnitude. The trend of *Clostridium perfringens* increasing to the present for most of the cores from Long Island Sound is unlike the trends of decreasing values seen for metal contaminants in sediments deposited in recent decades (MECRAY and BUCHHOLTZ TEN BRINK, this volume; VAREKAMP *et al.*, this volume). The cores from station B1 had particularly high concentrations of *Clostridium perfringens,* metal contaminants (although decreasing), and fast sediment accumulation. The proximity to the mouth of the Housatonic River and the city of Bridgewater are probable sources for the continued influx of these contaminants.



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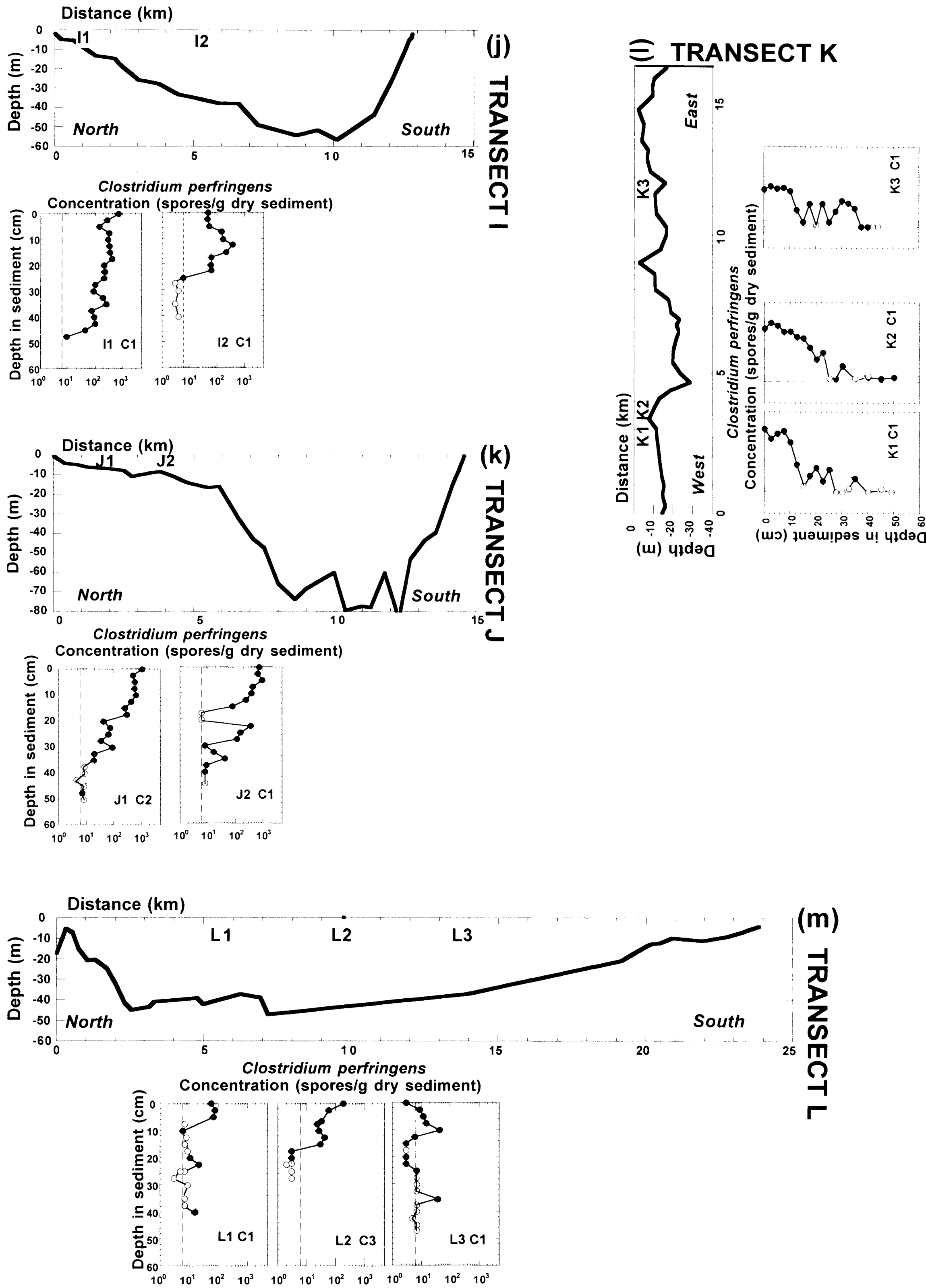

Figure 8. Continued.

In contrast to the muddy cores that are characteristic of depositional areas, sandy cores were collected from locations where bottom stress is high and sediment winnowing is active (*e.g.,* stations from Transects E and F in Figure 8). *Clostridium perfringens* concentrations in these cores range from background to values approximately 10 times greater than background, with *Clostridium perfringens* concentrations that are relatively constant with depth in the cores. The observed uniformity with depth is consistent with active reworking of the sea floor, which occurs in higher-energy environments, and suggests that the profiles reflect homogenization by physical mixing rather than the onset, or historical record, of *Clostridium perfringens* input. Cores from stations F3, F4, and G4 have spore concentrations that are consistently less than 25 spores/g dry sediment at all depths. We hypothesize that spores are not deposited in these cores since they are located in a region of bedload transport, and cores to the north and south (G3, G5, F5) have higher and irregular concentrations. Cores that were collected on transects H, J, and I (Figures 3 and 8) are located on the shallower, northern side of the bathymetric low that connects Long Island Sound to Block Island Sound. The profiles of these cores are similar to those in depositional areas to the west and all have background values of *Clostridium perfringens* at depth. Cores from stations H1, H2, and I2 decrease towards the surface after a mid-core maximum, while cores from stations I1, J1, and J2 have concentrations that continue to increase towards the surface.

Replicate cores were analyzed for selected sites in order to provide an assessment of limitations due to spatial heterogeneity and potential storage artifacts. Examination of x-radiographs and MSCL density measurements for replicate cores showed that the depositional character was comparable. Small variations in density or porosity were easily correlated between cores, although present at slightly different depths. The replicate stations also recorded little intra-station heterogeneity in profiles of *Clostridium perfringens* (Figure 8, *e.g.*, station A1), and long-term freezing did not result in significant loss of viability. In general, the ranges of concentrations and profile features found for *Clostridium perfringens* throughout the Sound were much greater than the range of uncertainty due to either intra-station variability or storage artifacts.

X-radiographs (Figure 9), density and water-content profiles, and grain-size analyses identify layers where sandier material was introduced into generally muddy, depositional cores. These layers are recorded in the *Clostridium perfringens* temporal record as low values, showing the effect of lithology on spore count (BUCHHOLTZ TEN BRINK and MECRAY, 1998). Sand layers carry a *Clostridium perfringens* signal that could be mistaken for a decrease in input if lithology is not noted. Normalization of these select profiles (which have steep gradients in *Clostridium perfringens* and ancillary data available) using lithological tracers such as grain size, % iron, or % aluminum, does not remove all of the observed variability. The remaining peaks in the profiles are attributed to either episodic increases in sewage input, or to biological processes that introduce more recently deposited sediment having higher *Clostridium perfringens* concentrations.

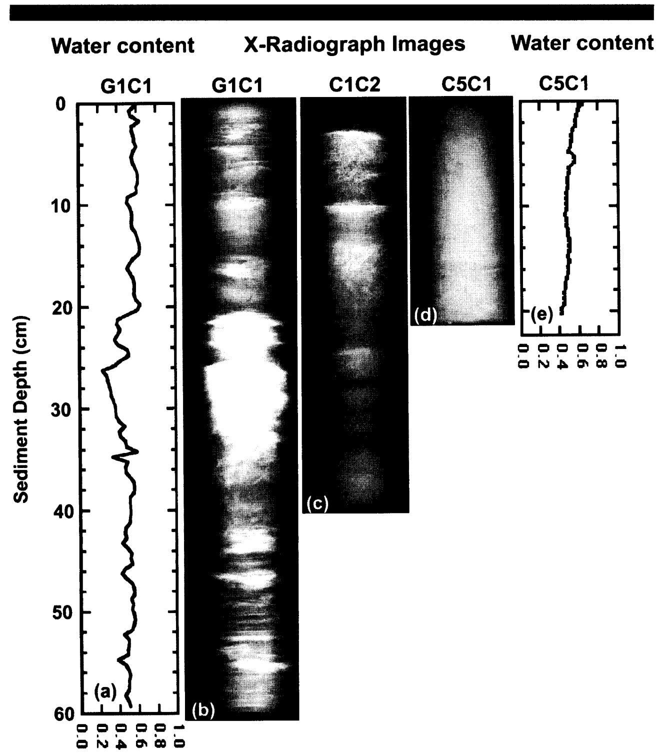

Figure 9. Water content (as weight fraction, *i.e.,* g water/g wet sediment) profiles and x-radiographs of selected cores: (a–b) G1C1, (c) C1C2, and (d–e) C5C1. Cores G1C1 and C1C2 are muddy with interspersed sand, gravel, or shell deposits.

Inventory of *Clostridium perfringens* in Long Island Sound Sediments

Measured concentrations and distributions of *Clostridium perfringens* in the sediments provide evidence of substantial sewage contamination in Long Island Sound. Inventories of total *Clostridium perfringens* in the cores range between 28 spores/cm^2 (no accumulation) and 70,000 spores/cm^2 (Table 1). At the majority of locations (Figure 10) in Long Island Sound, core inventories are less than 20,000 spores/cm^2 *Clostridium perfringens*. Local sources are probably responsible for the exceptionally high inventories ($>$55,000 spores/cm^2) found in Core B1C2, which is located off the Housatonic River, and core G1C1, which is located in a bay east of the of Connecticut River mouth. All of the inventories greater than 15,000 spores/cm^2 are in areas where fine-grained sediments predominate, and the sedimentary environment is classified as depositional. The inventories for the stations that are muddy and depositional, however, vary by a factor of 10. This variability probably reflects the dependence of *Clostridium perfringens* accumulation on the local sedimentation rate. Inventories calculated for the areas of non-deposition and reworking are estimated, as most of the cores do not reach background values at the bottom. For *Clostridium perfringens,* the median inventory in cores is 10,000 spores/cm^2 $\pm$ 100% for stations in Long Island Sound where values in the cores reached background, and $>$12,600 spores/cm^2 $\pm$ 90% for other stations. Where bathymetric and current gradients

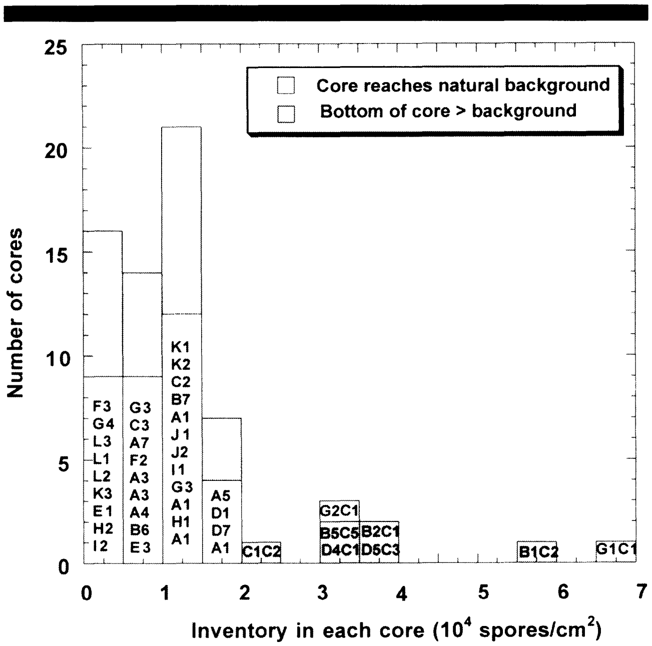

Figure 10. Histogram showing inventories (Table 1) of *Clostridium perfringens* calculated for each core. Core names are shown over the value bars for cores with large inventories and cores that reach background values at depth.

are high, there are also marked changes in *Clostridium perfringens* inventories over short distances. For example, cores that were collected on transects F and G have inventories that vary by three orders of magnitude over a distance of 17 km. Estimates of the total inventory for contaminants in Long Island Sound that are derived from spatial mapping must consequently include uncertainty attributable to (1) local sewage enrichment; (2) differing sedimentation rates within a common sedimentary environment; and (3) potentially large changes in inventories over short distances. The total inventory for the Sound, which has approximately 3,000 km^2 of sediment (KNEBEL and POPPE, this volume), is greater than 3.3×10^{17} *Clostridium perfringens* spores. This value is a *minimum* estimate as the spatial distribution of stations does not include the westernmost Sound, only minimum inventories were available for half the stations, and the distribution of stations may not representatively sample the diversity of inventories that are present.

Ages and Mixing of Contaminant Signals

Sediment profiles of naturally occurring isotopes (*e.g.*, ^{210}Pb) and radioactive fallout from nuclear-testing (*e.g.*, ^{137}Cs), combined with chemical and biological tracers (*e.g.*, *Clostridium perfringens*), indicate that mixing depths and sedimentation rates vary greatly within Long Island Sound. Mixing causes contaminants that were deposited in the past, such as *Clostridium perfringens,* to remain in the surface mixed-layer (BOTHNER *et al*, 1998; FULLER *et al.* 1999). At the surface, contaminants are accessible to biota and fine particles are subject to winnowing and sediment focusing.

The persistence of sandy laminations in muddy, depositional, cores (Figures 8 and 9) indicates that sediment mixing has not erased the historical record in these sediments. Conversely, the paucity of significant layering in cores that are sandier or have more uniform *Clostridium perfringens* values (Figure 9d–e), and the presence of wispy features that are characteristic of wave reworking, indicates that these are indeed well mixed by current activity. Mixing depths were inferred from constant ^{210}Pb values in the surface, ranging from 2 to 20 cm, with the deeper mixing occurring at sites with dynamic bottom currents. The sedimentation rate also varies markedly between locations of cores for which the accumulation rate can be determined. Preliminary results suggest that sediment accumulation rates range from 0.01 to 0.6 g/cm^2/yr. This range is in agreement with the average accumulation rate of 0.08 g/cm^2/y determined for the entire Sound (KNEBEL and POPPE, this volume). Age assignments for core A1C1 (BUCHHOLTZ TEN BRINK and MECRAY, 1998) show the onset of a sewage record in the late 1800's with a marked increase of *Clostridium perfringens* concentrations in the post-WWII period. A profile of mercury in a well-dated, nearby marsh core (KREULEN, 1999) has the same concentration features over this time period. During the last two centuries, the accumulation rate of *Clostridium perfringens* in the depositional cores, especially in the western region, appears to mirror the increase in population in the region over time (Figure 11; U.S. CENSUS BUREAU, 1999).

Regional Patterns

The distribution of *Clostridium perfringens* indicates that contamination of sediments by sewage is extensive in Long Island Sound. The upper 30 cm of sediment in cores collected throughout the Sound record the presence of this sewage tracer. The concentration values of *Clostridium perfringens* that were measured in this study of Long Island Sound sediments (<10 spores/g dry sediment to 15,000 spores/g dry sediment) are similar to those found in other urban estuaries. Values of 10,000 spores/g dry sediment were measured in Massachusetts Bay (PARMENTER and BOTHNER, 1993), and higher concentrations, up to 40,000 spores/g dry sediment, were reported for New York Bight's highly contaminated sediments (STUDHOLM *et al.*, 1995; BUCHHOLTZ TEN BRINK *et al.*, 1996). *Clostridium perfringens* concentrations in the sediments are also significantly lower than values of 10^6 spores/g found in sludge collected directly from sewage plants (PARMENTER and BOTHNER, 1993) or values of 10^9–10^{10} spores/g in feces (NRC, 1993).

The spatial coverage of core sites in the Sound provides data for a more complex assessment of the distribution and transport of *Clostridium perfringens* in Long Island Sound than is possible from surface samples alone. The surface map of *Clostridium perfringens* distribution (Figure 5b) closely follows the maps of sedimentary environments (KNEBEL and POPPE, this volume) and mean bedload transport (SIGNELL *et al.*, this volume); however, it masks the variations in *Clostridium perfringens* inventory that result from temporal differences in accumulation. Cores in depositional areas tend to record the history of *Clostridium perfringens* and sewage in-

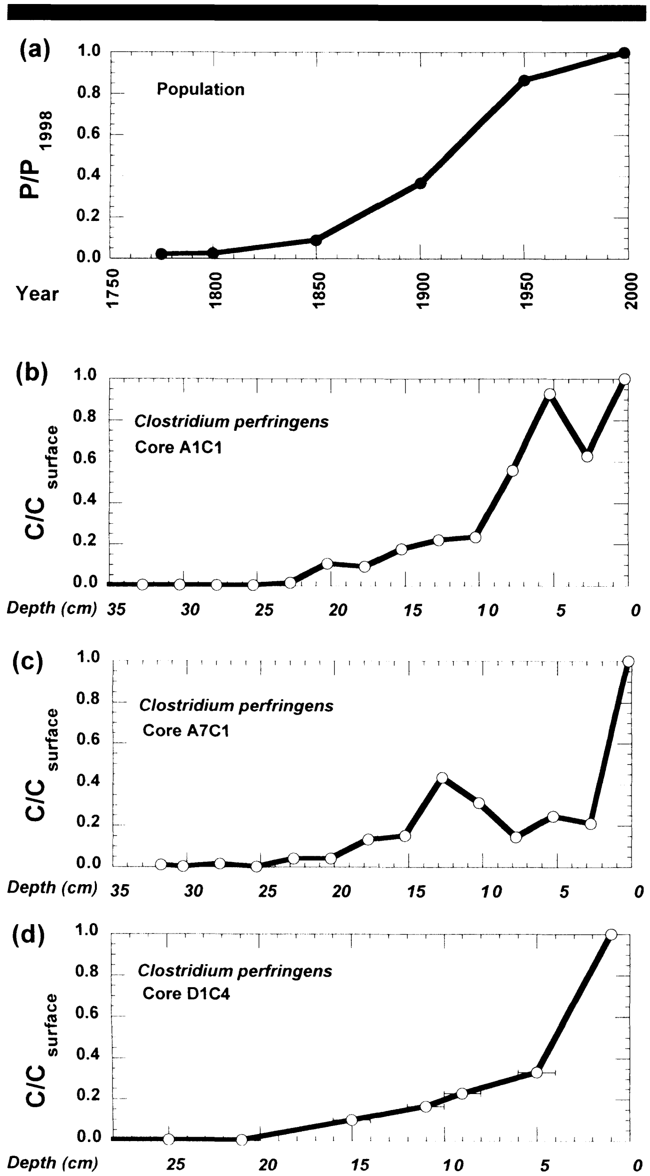

Figure 11. The increase in population around Long Island Sound, mirrored by the increase in *Clostridium perfringens* concentrations in sediment cores from depositional areas of western and northern Long Island Sound. (a) Population growth normalized to 1998 population (P/P_{1998}) vs. time (data from Figure 1). (b–d) *Clostridium perfringens* concentrations normalized to those at core top ($C/C_{surface}$) vs. depth in the sediment. Differences in depth scales between cores reflect differing sediment accumulation rates.

put; whereas, cores from regions that more frequently have high bottom stress integrate the historical signal over a variable mixed-layer depth. Cores from along the northern edge of the Sound (A1, C1, D1, and G1) have an increase in *Clostridium perfringens* gradient towards the core top that reflects population growth and its associated sewage signal. Locations of high velocity flow in both the eastern end of the Sound and along the bottom of the axial depression (SIGNELL *et al.*, this volume) have lower *Clostridium perfringens* concentrations and core inventories.

Individual core and radioisotope profiles (BUCHHOLTZ TEN BRINK, unpublished data) show that modern sedimentary conditions have persisted through most of the last century in Long Island Sound. Profiles from depositional areas generally have smoothly increasing *Clostridium perfringens* profiles from core depth to the surface, and repetitive features on several x-radiographs. Profiles and x-radiographs from sandier or reworked areas in the south and east (*e.g.*, Figure 9, C5C1) appear more homogenized. A third type of profile appears in a small group of cores (*e.g.*, B2, E3, G2 and G3) that are located at mid-depth on the northern side of the Sound. Many of these cores have broad, subsurface peaks in *Clostridium perfringens* that are not seen elsewhere in the Sound. These cores were not located within current or known historical disposal sites and no ages are available for them; thus, it cannot be determined whether this feature results from dispersal of the large volume of dredge-material that was disposed along this isobath in the 1950s (FREDETTE *et al.,* 1993). The cores F3, F4, and G4, which are located in the eastern portion of Long Island Sound, are the only cores that have *Clostridium perfringens* spore concentrations near background at all depths. This is consistent with the strong bottom currents that rework sediments and remove fine-grained material at these sites to create a sand-wave environment. Cores located elsewhere in the dynamic eastern Sound have peaks in the spore profile that indicate episodic sedimentation or *Clostridium perfringens* input, which may be related to the nearby Connecticut River.

Many estuaries receive a significant fraction of their net sediment from offshore, but under certain storm or tidal conditions, estuaries in the northeast can also be a source of contaminated-sediment to the offshore (BOTHNER *et al.,* 1994). LEWIS and DIGIACOMO-COHEN (this volume) suggest that the riverine supply of sediment into Long Island Sound is adequate to balance the mean sedimentation rate, without invoking significant sediment flux from or to Block Island Sound and offshore. Elevated *Clostridium perfringens* concentrations were found in cores from the L transect, which is located in Block Island Sound, and relatively distant from potential alternative sources of sewage contamination (Figure 2). These concentrations suggest that a portion of the sewage-derived contaminants in Long Island Sound may be exported to the open shelf.

Relation to Other Sewage-Derived Pollutants

Strong correlations of *Clostridium perfringens* with other anthropogenic contaminants can be used to predict probable sediment-loadings for other contaminants associated with sewage input (*e.g.*, BOTHNER *et al.*, 1993; BENOIT *et al.,* 1999; VAREKAMP *et al.*, this volume). Correlations of *Clostridium perfringens* with sediment grain size and sedimentary environments also allow predictions of the spore distribution from measured physical properties. Divergence in the relation between *Clostridium perfringens* and other contaminants in the sedimentary record exists where (1) the relationships between the two contaminants in the source change with time (*e.g.*, a factory is closed or the composition of a waste-stream is altered); or (2) fractionation occurs between the bacterial

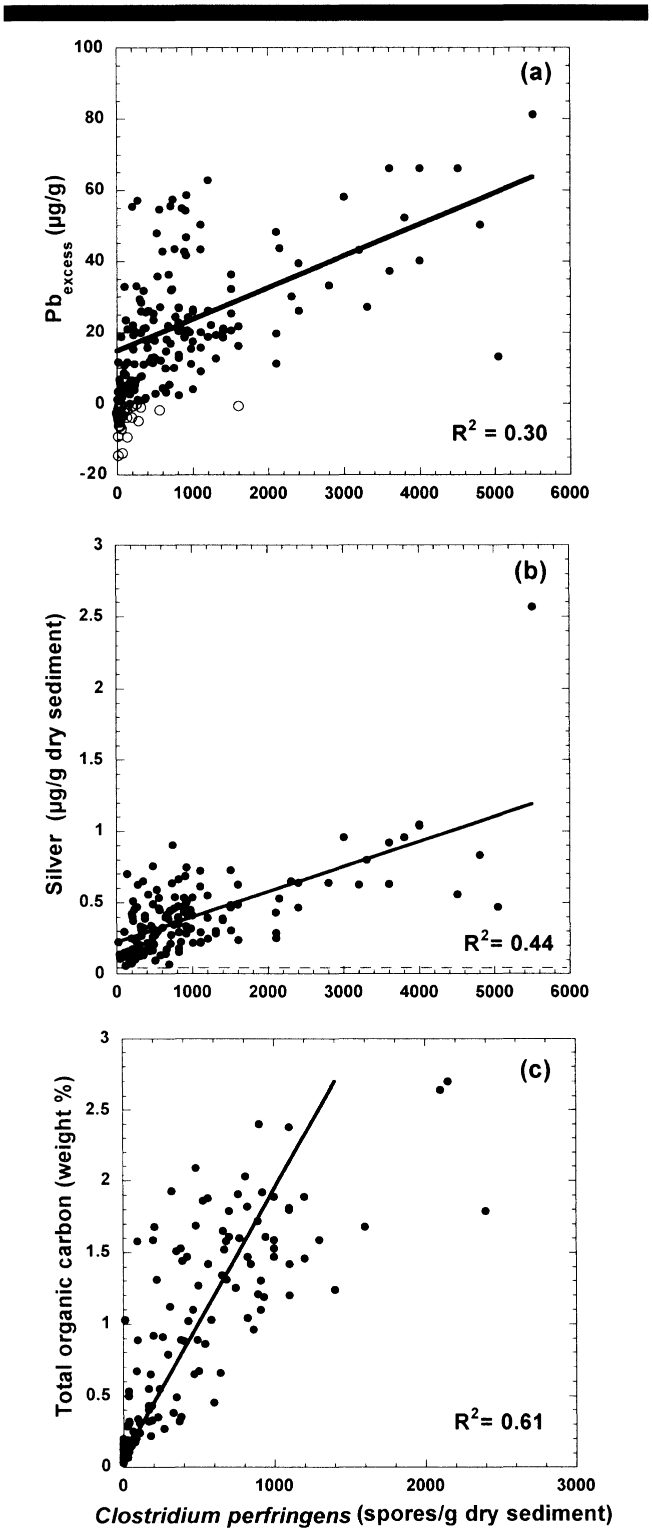

Figure 12. Correlations of *Clostridium perfringens* in surface sediments (0–2 cm) of Long Island Sound with other contaminants. (a) *Clostridium perfringens* vs. Excess lead (Pb_{excess}), which is defined as that in excess of naturally-occurring concentrations, taken here as a natural background value of 18.8 μg/g. Anthropogenic enrichment is present for values of excess Pb greater than 0 (solid points and line). (b) *Clostridium perfringens* vs. silver (Ag), with the natural Ag background of 0.5 μg/g indicated

contaminant and other types of contaminants, due to *in situ* transport or geochemical processes (*e.g.*, differential settling (DRAXLER *et al.*, 1996), die-off, or diagenesis).

Copper, lead (MECRAY and BUCHHOLTZ TEN BRINK, this volume) and mercury (VAREKAMP *et al.*, this volume) concentrations in Long Island Sound surface sediments show patterns that strongly resemble the *Clostridium perfringens* distribution. Summary statistics, and principle component analysis (PCA) by MECRAY and BUCHHOLTZ TEN BRINK (this volume) also indicate that *Clostridium perfringens* concentrations in Long Island Sound are greatest in depositional environments and when associated with fine-grained material; however an additional factor contributes to the *Clostridium perfringens* distribution. The positive correlation of *Clostridium perfringens* with metal contaminants in surface sediments is weaker for Long Island Sound than it is for Massachusetts Bay or the New York Bight. In Long Island Sound, linear correlations between *Clostridium perfringens* and excess metals (*e.g.*, Ag, Pb, Zn, and Hg; Figure 12a and b) have R^2 values of 0.25–0.5 (BUCHHOLTZ TEN BRINK and MECRAY, 1998), whereas those in New York Bight surface sediments have R^2 values between 0.90 and 0.96 (CABELLI *et al.*, 1984; BUCHHOLTZ TEN BRINK *et al.*, 1996; VAREKAMP *et al.*, this volume for Hg). Massachusetts Bay sediments have R^2 for Ag vs. *Clostridium perfringens* of 0.82, but much lesser correspondence to Pb and Zn values (BOTHNER *et al.*, 1993). In addition, the positive correlation between the fine-fraction of sediments and *Clostridium perfringens* in Long Island Sound surface sediments is less than that between the fine fraction and the anthropogenic metal contaminants (MECRAY and BUCHHOLTZ TEN BRINK, this volume). These poorer observed correlations may reflect multiple sources of sewage over a wider area in Long Island Sound rather than a single source as recorded at the other sites, or other non-sewage sources of metals in Long Island Sound. Both factors are probable since waste-disposal in Long Island Sound (Figure 2) is less centralized than in New York Bight or Massachusetts Bay.

Alternatively, the divergence between the *Clostridium perfringens*/metals ratio in surface sediments could reflect the fact that many of the sediments in Long Island Sound are mixed vertically and the ratios in the surface reflect changing values have been integrated over time. The concentrations of *Clostridium perfringens* in sediment cores have strong, positive, linear correlations with other pollutants in sediments deposited prior to prior to the 1980's (CABELLI *et al.*, 1984; BUCHHOLTZ TEN BRINK *et al.*, 1996). Our Long Island Sound cores (*e.g.*, KREULEN, 1999; VAREKAMP *et al.*, this volume for Hg) and others (*e.g.*, VALETTE-SILVER and SALOMONS, 1993; BOTHNER *et al.*, 1998) record a reduction in metals pollution due to clean air and clean water legislation in recent decades.

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Figure 12. Continued: by the dashed line. (c) *Clostridium perfringens* vs. total organic carbon (TOC). The TOC data is from POPPE *et al.* (1998), background values for Pb and Ag are from MECRAY and BUCHHOLTZ TEN BRINK (this volume), and values measured as less than detection limit are not plotted.

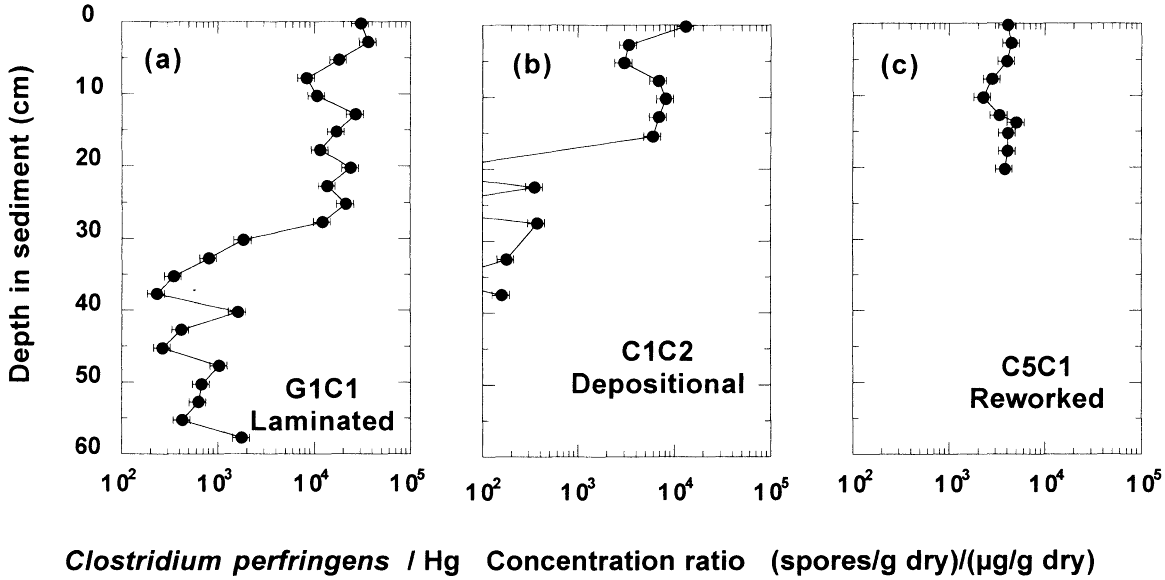

Figure 13. The concentration ratio of *Clostridium perfringens* and mercury (Hg) in unmixed and mixed sediment cores (See Figure 9 for x-radiographs). (a) A laminated core, G1C1, preserves the historical record in a depositional site in the eastern Sound. (b) Core C1C2, in the north, central Sound, is primarily depositional over time and preserves an historical record. (c) A reworked core, C5C1, integrates the historical signal over time and depth.

Clostridium perfringens concentrations do not decrease in a manner similar to the pollutant metals, but they continue to increase towards the surface. The sharp increase of the *Clostridium perfringens* ratio to many other contaminants (*e.g.*, Figure 13 for Hg) at depth in the cores records significant alterations in the per capita pollution term. This contrasts with the relatively constant ratio that is observed between concentrations of *Clostridium perfringens* and anthropogenic metals throughout the contaminated section of cores that have been vertically homogenized. At sites where the mixing regime is unknown, the use of *Clostridium perfringens* concentrations for predicting the concentrations of other contaminants in surface sediments is compromised by uncertainty in the contribution of older, or recycled, sediments to the surface values.

The value of *Clostridium perfringens* concentrations for predicting the load of other sewage-derived contaminants in the sediments depends on characterization of the processes that may deliver other contaminants differently or alter them *in situ.* Elevated organic carbon in sediments is often attributed to sewage disposal practices, and correlates with the *Clostridium perfringens* concentrations (Figure 12c) in surface sediments. Despite the potential for carbon to be utilized in the sediments, the TOC/*Clostridium perfringens* correlation is stronger than that for Ag (Figure 12b), which has few non-sewage sources. The Ag, in turn, has a higher correlation coefficient with *Clostridium perfringens* than does Pb (Figure 12a), which has more diverse sources and higher background values than Ag. Unlike metal contaminants, *Clostridium perfringens* spores do not interact chemically with mineral surfaces, nor is there any evidence of *in situ* die-off, grazing losses, or growth periods of *Clostridium perfringens* in these cores. Thus, the *Clostridium perfringens* distribution in Long Island Sound is dominated by advective processes and physical transport. Contaminant metals and organic compounds that are derived from the same sewage source can have an added component of chemical reaction that may affect their distribution in Long Island Sound sediments (*e.g.*, SALOMONS and FÖRSTNER, 1984). Extrapolation of the *Clostridium perfringens* distribution patterns to elements whose solid/solution partitioning is sensitive to redox conditions, or to chemical reactions with manganese, iron, sulfur, or organic carbon compounds, should be done with caution in locations having large variations in lithologic character.

Management Implications

Our ability to manage human interaction with the marine environment and strive towards a healthy, sustainable ecosystem in Long Island Sound benefits from improved understanding of the magnitude of sediment contamination in the Sound, and of the associated transport processes. The distribution of *Clostridium perfringens* indicates that contamination of sediments by sewage is extensive in Long Island Sound. The positive relationship of *Clostridium perfringens* concentrations with concentrations of other contaminants in surficial sediments (Figure 12; MECRAY and BUCHHOLTZ TEN BRINK, this volume) reflects both (1) the co-occurrence of population centers and industrial pollutants, and (2) the common transport of small *Clostridium perfringens* spores and contaminant-bearing fine-grained particles. The similarity between (1) the historical increase in population around Long Island Sound; (2) the increase in *Clostridium perfringens* recorded in depositional sediments; and (3) the elevated concentrations of contaminants in sediment cores all point to a widespread and long-standing anthropogenic impact in Long Island Sound.

The presence of *Clostridium perfringens* spores in these sediments is of minimal direct concern for human health because *in situ* conditions are probably inadequate to induce growth of *Clostridium perfringens* bacteria. The possibility exists, however, for ingested sediments to cause illness in marine fauna or higher trophic levels (WATKINS, 1996). Of greater concern is that elevated concentrations of *Clostridium perfringens* indicate that other parameters that may be deleterious to the environment are likely to be present (NRC, 1993; WATKINS and BURKHARDT, 1996). These spores, and the fine-grained sediment with which they are associated, are mixed laterally across the Sound. The continuing increase in *Clostridium perfringens* concentrations observed in the upper sections of sediment cores suggests that reductions of targeted metal pollutants in recent decades (VALETTE-SILVER and SALOMONS, 1993), and of direct sewage-sludge discharge, have not been mirrored by a reduction in overall sewage contamination to the sediments. The transport patterns for *Clostridium perfringens* in these sediments also suggest that much of the existing contaminated sediment will be redistributed within the Sound into the future. Correlations of particular contaminants with the abundance of *Clostridium perfringens* spores may prove useful in (1) estimating the fraction of sewage-derived pollutants and (2) monitoring the composition and magnitude of sewage effluents that reach the Sound.

CONCLUSIONS

Results from bacterial and lithological analysis of surface samples and sediment cores collected in Long Island Sound document the distribution and accumulation of contaminated sediments in Long Island Sound. *Clostridium perfringens* is a conservative tracer of sewage-derived pollution in Long Island Sound sediments. Its presence above background concentrations throughout the Sound indicates widespread sediment contamination. There is a close correspondence of *Clostridium perfringens* distributions with the spatial variations in grain size, sedimentary environment, and bottom stress in Long Island Sound. These relations occur because *Clostridium perfringens* spores are biologically and chemically inert *in situ*; therefore, their mobility is controlled by the physical transport and dispersal processes that occur in the Sound. The existence of relatively high concentrations and large inventories of *Clostridium perfringens* in fine-grained sediments results from simultaneous winnowing of fine material that occurs in regions of high bottom-stress, and the focusing of this material in regions of deposition. The *Clostridium perfringens* accumulation patterns are influenced by features characteristic of the sedimentary environment and, to a lesser degree, by the source proximity. Spore distribution and inventories in the sediments are modified, though, by mixing depths and accumulation rates within the Sound. Sediment cores from depositional areas provide an historical record of *Clostridium perfringens* concentrations, which reflect the rapid population growth and the impact of this growth on the coastal ecosystem. In depositional regions throughout the Sound, *Clostridium perfringens* core profiles record the onset of anthropogenic contamination in Long Island Sound, a regional population increase in the last two centuries, and increasing sewage contamination towards the present time.

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