Supplemental Material

For

Spatial, Temporal and Matrix Variability of *Clostridium botulinum* Type E Toxin Gene (*bontE*) Distribution at Beaches in the Great Lakes

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17 pages
4 Figures
10 Tables

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Running Head: *C. botulinum* Distribution in the Great Lakes

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TABLE S1 Catchment and watershed parameters used for correlation and discriminant analysis

Sampling site	Catchment (ha)	Developed (%)	Barren land (%)	Forest & Shrub (%)	Grassland (%)	Agriculture (%)	Wetlands (%)	Population in 2000
Bay City State Recreation	0.1	4.5	0.0	47	0.0	36	7.6	0
Brimley	3.3	14	0.0	31	1.0	4.1	45	170
Hammond	2.5	62	30	1.3	0.0	0.2	2.3	206
Jeorse Park Beach	16	33	63	0.3	0.0	0.3	0.5	3,126
Linwood	3.5	19	0.0	17	0.3	1.7	55	581
Maumee	44	25	1.0	3.6	0.0	3.0	63	6,485
Portage Lake Front	12	58	1.5	20	0.7	0.6	15	6,886
Deland	10	90	8.6	0.3	0.4	0.9	0.2	20,118
Sleeping Bear	25	8.4	0.0	59	2.9	19	8.3	493
Waukegan	10	57	9.9	14	0.1	1.5	3.1	7,934

TABLE S2 Water quality data used for correlation and discriminant analysis

NAME	ID	Water Temp. ($^{\circ}$C)	Conducti-vity (μS)	TDS (ppm)	Salinity (ppm)	pH	DO (ppm)	ORP (mV)	Turbidity (NTU)	NO$_3$-N (mg/L)	PO$_4$ (mg/L)
Bay City	BC	21.5	378	266	202	8.4	11.4	220	61	0.58	0.21
Brimley	BSP	22.5	100	70	48	8.6	7.4	270	2.9	0.00	0.00
Hammond	HM	22.0	239	168	155	8.5	9.4	250	32	0.29	0.10
Jeorse Park	JP	24.1	310	215	154	9.0	7.5	180	49	0.00	0.00
Linwood	LW	23.2	354	260	183	8.3	7.9	200	33	0.49	0.24
Maumee Bay	MB	22.4	363	257	189	8.3	9.7	200	98	0.74	0.26
Portage Lake Front	PLF	26.4	586	404	291	9.3	4.5	150	4.0	0.00	0.05
Deland	DL	19.6	315	219	151	8.5	8.1	160	58	1.66	0.19
Sleeping Bear	SLBE	21.1	318	222	160	8.5	5.1	220	N/A	0.44	0.12
Waukegan	WK	21.8	258	198	136	8.7	8.9	150	5.6	1.8	0.22

All values are average of the sampling period of May – September 2012. N/A data not available

TABLE S3 Beach sanitary survey data used for discriminant analysis

NAME	ID	*E. coli* summer Avg.	Bird seasonal Avg.	Bird seasonal total	*Cladophora* onshore (score)	*Cladophora* in water (score)	*Chara* onshore (score)	*Chara* in water (score)	Dead round gobies onshore (score)	Mussel shells onshore (score)
Bay City	BC	29	39	1982	3.3	3.3	0.5	1.0	0.0	2.7
Brimley	BSP	276	16	916	0.0	0.0	0.0	0.0	0.0	0.0
Hammond	HM	374	18	1746	1.6	1.7	0.2	0.5	0.0	1.4
Jeorse Park	JP	822	98	4322	3.2	3.3	0.2	0.1	0.0	0.3
Linwood	LW	84	13	2323	2.5	1.9	0.1	0.9	0.0	2.2
Maumee Bay	MB	251	68	3870	1.7	N/A	0.3	0.2	0.03	2.5
Portage Lake Front	PLF	37	9	206	0.5	0.8	0.1	0.1	0.0	0.0
Deland	DL	32	126	7455	3.5	3.3	0.1	0.02	0.0	2.5
Sleeping Bear	SLBE	15	4	192	1.4	1.2	0.1	0.03	0.03	0.5
Waukegan	WK	175	32	7597	1.3	1.8	0.2	0.5	0.0	1.5

E. coli (CFU/100 mL), all values are average of the sampling period of May – September 2012. N/A: data not available

TABLE S4 Meteorological data used for discriminant analysis

NAME	ID	Wind speed score	Wave height score	Air Temp. (^{o}C)	Avg. cloud Cover (%)	Dew Point (^{o}C)	Water temp. high (^{o}F)	Water temp. Avg. (^{o}F)	Water temp. Low (^{o}F)	Wind high (mph)	Wind Avg. (mph)	Rainfall Avg. (inches)
Bay City	BC	1.3	0.5	22.0	19	16	74.0	64.2	54.7	26	5.0	0.10
Brimley	BSP	1.6	1.2	15.6	55	13	74.0	64.2	54.7	26	5.0	0.10
Hammond	HM	1.5	0.9	19.3	54	14	60.0	53.2	46.1	21	6.0	0.04
Jeorse Park	JP	1.4	0.9	23.0	46	17	84.0	74.6	65.1	14	7.6	0.11
Linwood	LW	1.4	0.5	18.4	27	13	68.9	59.6	50.5	10	4.0	0.07
Maumee Bay	MB	1.6	0.8	24.2	14	18	84.5	72.6	60.2	17	5.9	0.12
Portage Lake Front	PLF	1.0	1.0	23.1	35	18	84.3	75.1	65.6	14	7.5	0.11
Deland	DL	1.6	1.0	20.9	23	16	69.2	60.5	52.3	9	1.7	0.31
Sleeping Bear	SLBE	1.0	1.0	20.3	28	16	80.1	70.0	59.9	13	4.3	0.06
Waukegan	WK	1.0	1.0	20.7	4	15	68.9	62.5	56.3	19	11	0.12

All values are average of the sampling period of May – September 2012.

TABLE S5 Hydrological data used for discriminant analysis

NAME	ID	Wave height score	Surface Magnitude of velocity (m/s)	Magnitude Velocity depth Avg. (m/s)	Air Magnitude depth of velocity (m/s)
Bay City	BC	0.5	0.03	0.02	5.5
Brimley	BSP	1.2	N/A	0.07	5.0
Hammond	HM	0.9	0.04	0.03	5.8
Jeorse Park	JP	0.9	0.04	0.02	5.6
Linwood	LW	0.4	0.07	0.05	6.5
Maumee Bay	MB	0.8	N/A	0.02	4.7
Portage Lake Front	PLF	1.0	0.04	0.02	5.9
Deland	DL	1.1	0.08	0.04	4.9
Sleeping Bear	SLBE	1.0	0.04	0.01	4.9
Waukegan	WK	1.0	0.17	0.11	7.3

All values are average of the sampling period of May – September 2012. N/A: data not available

TABLE S6 Some quality control parameters of the qPCR standard curve

Parameters	Individual standard curve (range)	Compiled standard curve	Acceptable standard curve criteria
Efficiency	89.5 -101.7	98.2	90-110%
R^2	0.98 – 1.00	0.995	> 0.99
Slope	3.28 - 3.60	3.356	3.10 -3.58
LOD	3 gene copies	3 gene copies	
LOQ	10 gene copies	10 gene copies	

R^2: Correlation determination between gene copy numbers and No. of amplification cycles

TABLE S7 Hydrological, environmental and beach characteristics used in data analysis

Parameter	Categorical data	Numerical data	Data source
Beach Sources and conditions			
Cladophora onshore	yes		AMBLE survey
Cladophora in water	yes		AMBLE survey
Chara onshore	yes		AMBLE survey
Chara in water	yes		AMBLE survey
Dead round gobies onshore	yes		AMBLE survey
Mussel shells onshore	yes		AMBLE survey
Cloud cover on day of sampling	yes		AMBLE survey
Water temperature on day of sampling (°F)		yes	Beach sanitary survey
Air temperature on day of sampling (°C)		yes	AMBLE survey
pH		yes	Beach sanitary survey
Turbidity (NTU)		yes	Beach sanitary survey
Conductivity (µS)		yes	Beach sanitary survey
Oxidation reduction potential (mV)		yes	Beach sanitary survey
Catchment and watershed characteristics			
Population in catchment and watershed		yes	U. S. Census, 2010
Watershed area (Ha)		yes	Fry et al., 2011
Open water (%)		yes	Fry et al., 2011
Land cover (%)			
Developed		yes	Fry et al., 2011
Barren land: rock, sand, clay		yes	Fry et al., 2011
Forest and shrubs		yes	Fry et al., 2011
Grass land		yes	Fry et al., 2011
Agriculture		yes	Fry et al., 2011
Wetlands		yes	Fry et al., 2011
Rain (in)			
Catchment Mean 24 hour		yes	EnDDaT
Catchment Mean 48 hour		yes	EnDDaT
Catchment Mean 72 hour		yes	EnDDaT
Watershed Mean 24 hour		yes	EnDDaT
Watershed Mean 48 hour		yes	EnDDaT
Watershed Mean 72 hour		yes	EnDDaT

Hydro meteorological data

Daily beach wave height	yes		Beach sanitary survey
Wind speed	yes		Beach sanitary survey
Wind direction	yes		Beach sanitary survey
Height Above Model Sea Level (m)		yes	GLCFS
Eastward Water Velocity at Surface (m/s)		yes	GLCFS
Depth-Averaged Eastward Water Velocity (m/s)		yes	GLCFS
Northward Water Velocity at Surface (m/s)		yes	GLCFS
Depth-Averaged Northward Water Velocity (m/s)		yes	GLCFS
Wave Direction (degree)		yes	GLCFS
Significant Wave Height (m)		yes	GLCFS
Wave Period (S)		yes	GLCFS
Eastward Air Velocity (m/s)		yes	GLCFS
Northward Air Velocity (m/s)		yes	GLCFS
Air Temperature (°C)		yes	GLCFS
Cloud Cover (%)		yes	GLCFS
Dew Point (°C)		yes	GLCFS

Categorical rankings were expressed as integers (e.g., 1-5); EnDDat (Environmental Data Discovery and Transformation); GLCFS (Great Lakes Coastal Forecasting System)

TABLE S8 *bontE* gene copy numbers detection and quantification at Bay City

Date	*bontE* gene copies/ml of algae
6/14/2012	62748
6/21/2012	193377
6/28/2012	LOD
7/5/2012	LOD
7/11/2012	198559
7/19/2012	74151
7/26/2012	UND
8/2/2012	LOD
8/16/2012	53121
8/30/2012	41069
9/6/2012	74606

LOD: detectable but not quantifiable; UND: undetected

TABLE S9 Significant correlations between *bontE* detection and environmental parameters.

	bontE in water		*bontE* in AlgOn		*bontE* in muck[a]		*bontE* SUB		*bontE* SOS
Wind speed	-0.43	*bontE* in SUB	0.47	*bontE* in Water	0.95	Wind speed	-0.52	Wave height	0.5
	0.02		0.03		0.08		0.01		0.03
	30		21		4		23		19
Mean conductivity	0.41	Mean water temperature	0.29	*bontE* in SED	-0.8	Wave height	-0.35		
	0.04		0.01		0.08		0.09		
	26		67		5		23		
Mean salinity	0.48	Mean salinity	0.23	Mean TDS	0.6	Mussels	-0.42		
	0.01		0.07		0.08		0.04		
	25		63		9		23		
Mean turbidity	-0.49	Mean pH	0.3	Mean PO_4	0.81	Mean pH	0.4		
	0.01		0.01		0.001		0.06		
	25		65		9		22		
Mean NO_3-N	-0.37	Mean NO_3-N	-0.32						
	0.05		0.01						
	27		68						

Cell content: Correlation coefficient, P value, No. of samples. [a] Muck samples from BC only. AlgOn: algae onshore; SUB: algae submerged in water; SOS: sediment onshore; All *bontE* gene copies are in log scale; except turbidity, mean water temperature, PO_4 and TDS all other parameters are in scored valued ranked from 1-5.

TABLE S10 *bontE* gene copy numbers detection and quantification at Jeorse Park beach and the rainfall during the sampling period.

Date	Time	Catchment mean rainfall in inches			*bontE* gene copies/
		24 hour	48 hour	72 hour	1 ml algae
6/28/2012	9:00	0	0	0	49795
7/12/2012	9:00	0	0	0	43584
7/19/2012	9:00	1.063	0.545	0.363	UND
7/26/2012	9:00	0.031	0.025	0.137	UND
7/30/2012	9:15	0	0	0.014	175939
7/31/2012	10:00	0.424	0.299	0.17	30517
8/1/2012	9:30	0	0.2	0.167	LOD
8/2/2012	10:00	0	0	0.136	69077
8/14/2012	9:30	0.331	0.257	0.171	64144
8/15/2012	10:00	0.03	0.183	0.179	57525
8/17/2012	9:15	0.21	0.156	0.115	84946
8/18/2012	9:15	0	0.111	0.109	UND
8/23/2012	10:00	0	0	0.001	UND

LOD: detectable but not quantifiable; UND: undetected
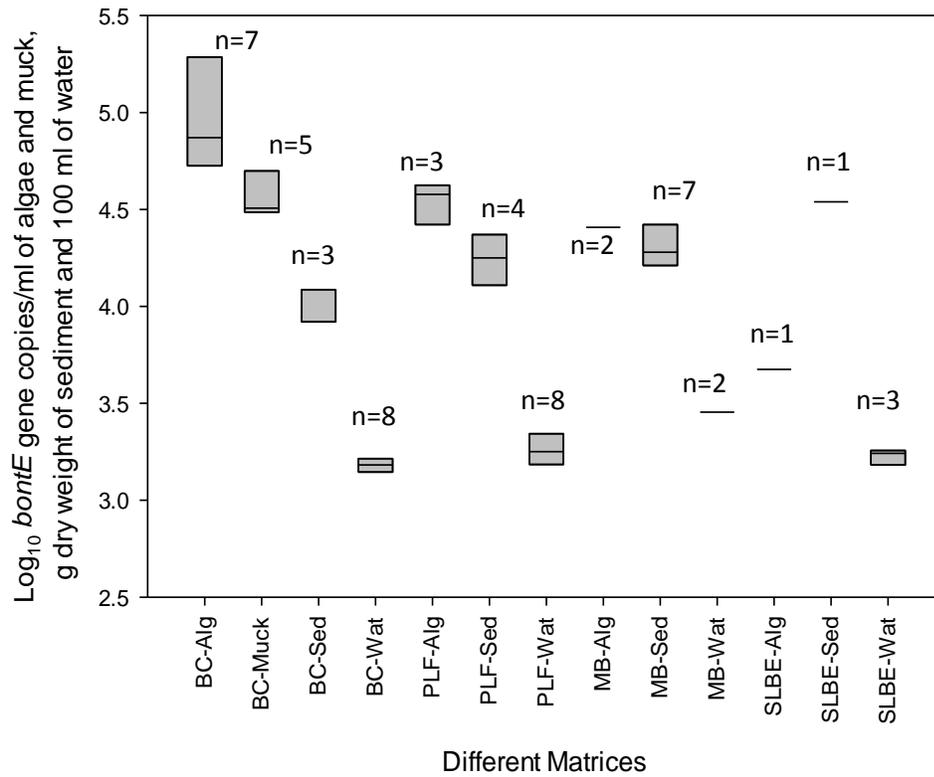
FIG S1 Distribution of *bontE* in the different matrices at Bay City (BC), Portage (PLF), Maumee Bay (MB) and SLBE. The horizontal line in the grey bars show the median value. Above each bar, the number shows the number of samples which were above the LOQ.
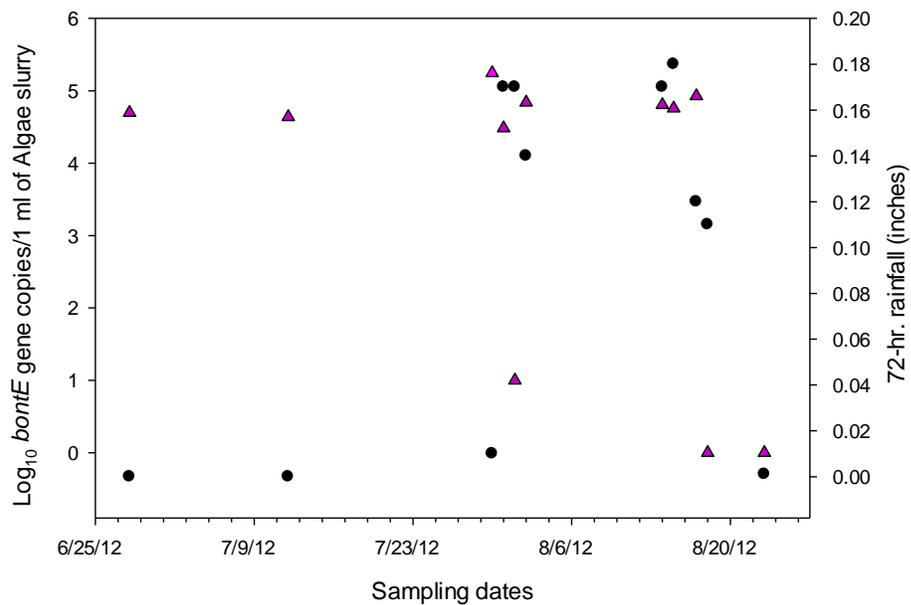
FIG S2 Temporal variability of *C. botulinum bontE* found at Jeorse Park Recreational Beach. Triangles represent concentration of $\log_{10}$ *bontE* gene copies found in AlgOn samples and circles represent 72-hr. rainfall in inches. $\log_{10}=1$ value was assigned to a sample which is detectable but non-quantifiable. $\log_{10}= 0$ values were assigned to two samples which were below limit of detection.
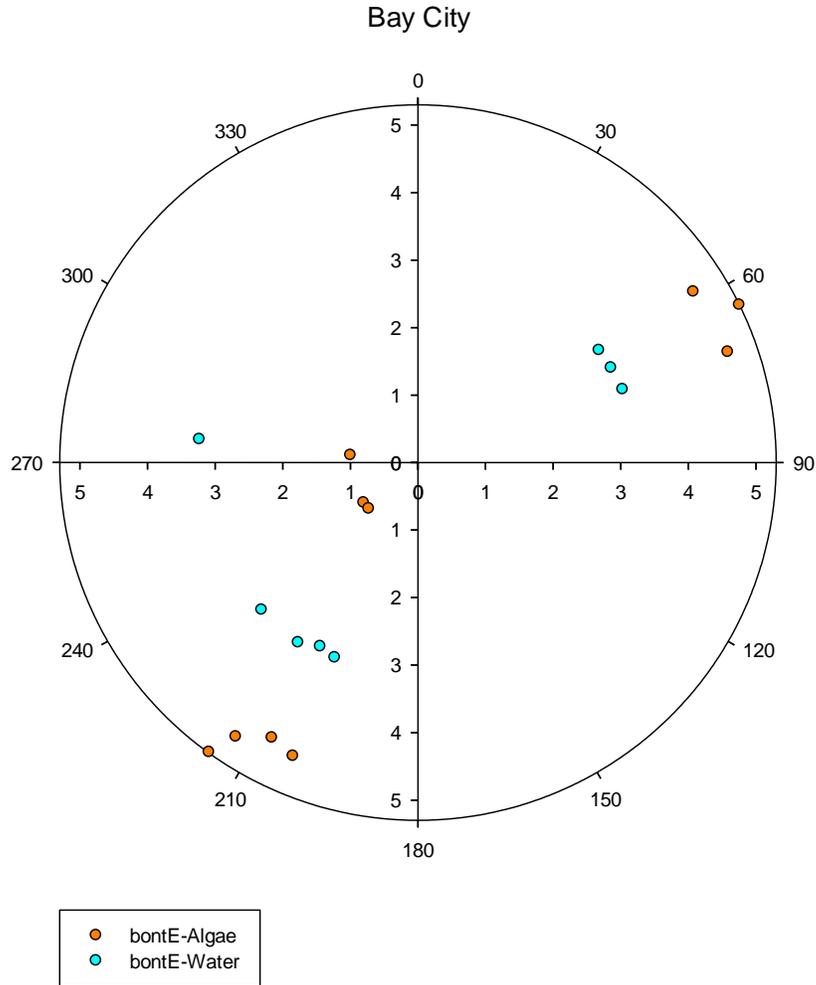
FIG S3 *bontE* detection vs. wind direction at Bay City during the swimming season. Both y and x axis are *bontE* gene copies in $\log_{10}$ scale, *bontE* concentration in algae is gene copies/1 ml while that of in water is gene copies/100 ml. $\text{Log}_{10}=1$ value was assigned to a sample which is detectable but non-quantifiable.
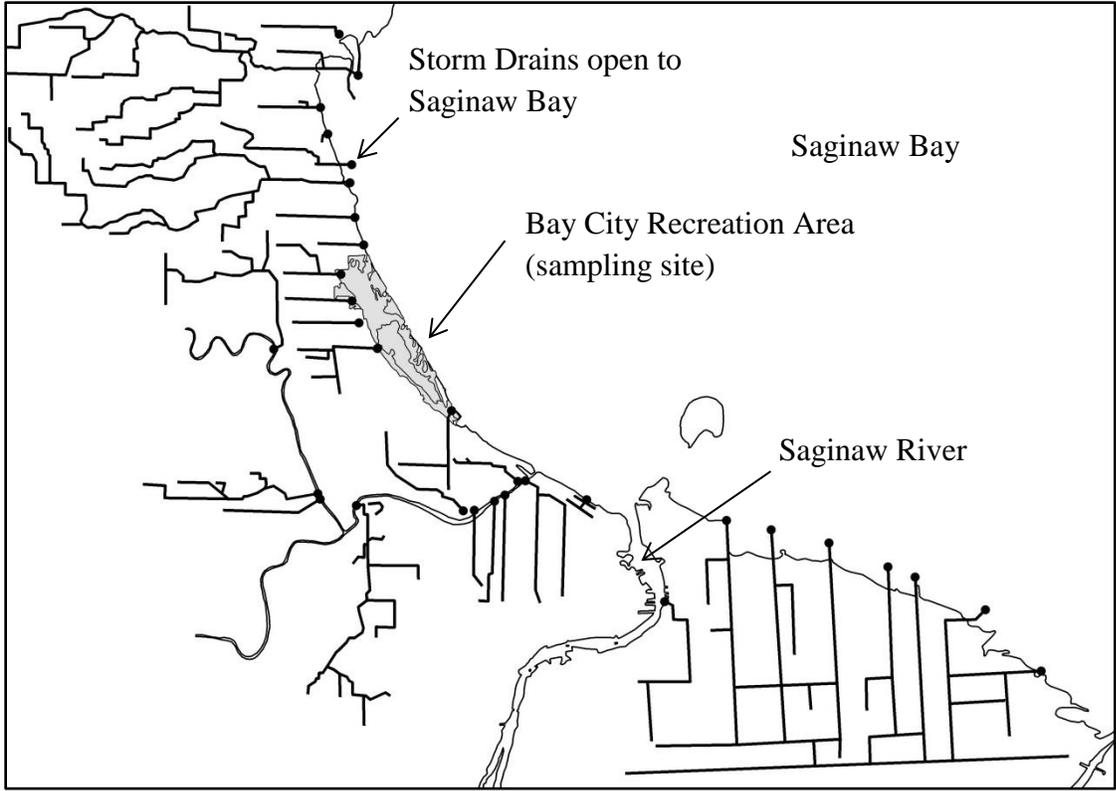
FIG S4 Locations of storm drains in the Bay City Recreation Area (highlighted in grey) (http://www.baycounty-mi.gov/DrainCommissioner/).

References

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