Supplementary Figures and Tables

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Research Article

Mucochytrium quahogii (=QPX) is a commensal, opportunistic pathogen of the hard clam (*Mercenaria mercenaria*): evidence and implications for QPX disease management

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Supplementary Figures

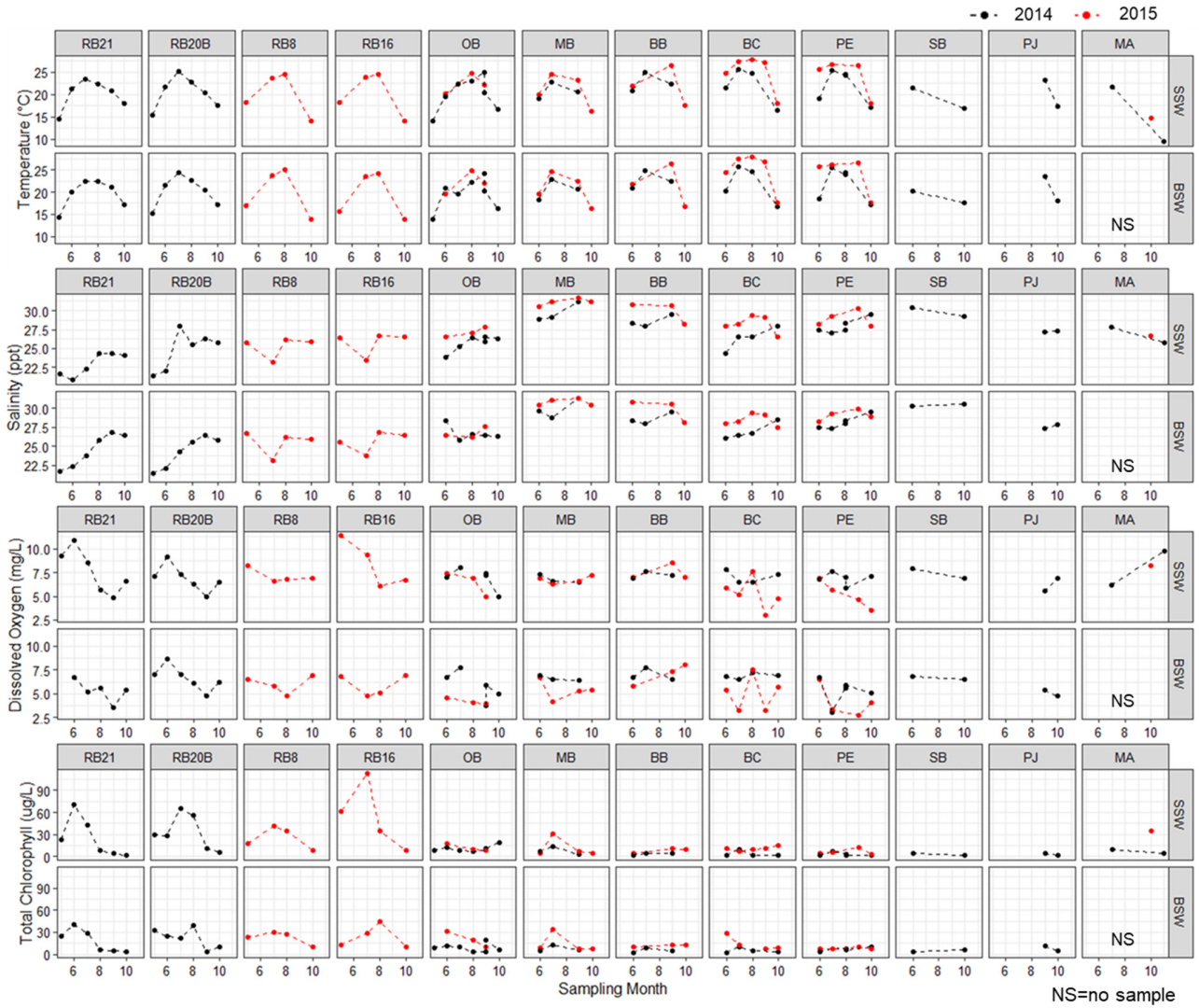

Figure S1: Measured environmental parameters by site and year for surface (SSW) and bottom (BSW) seawater.

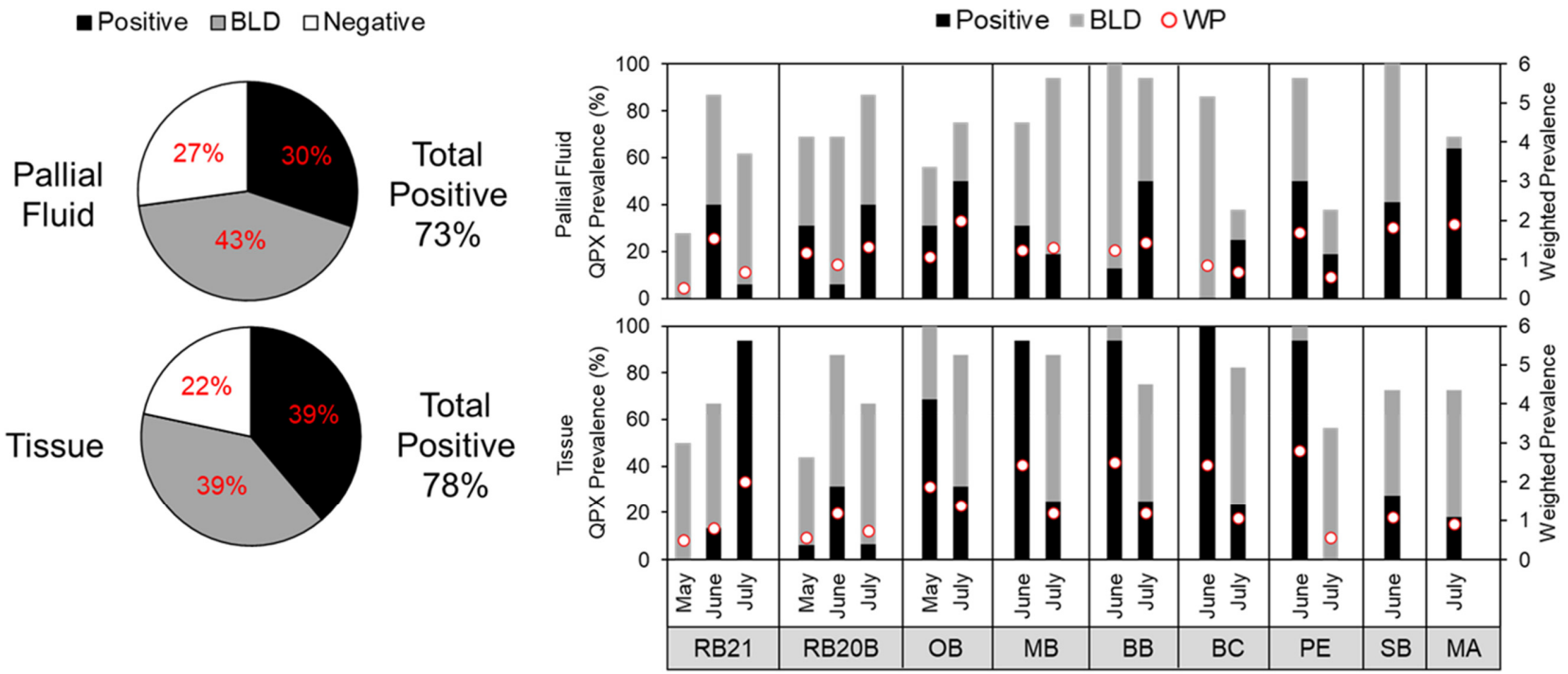

Figure S2: Summary of QPX prevalence in hard clam pallial fluid and mantle tissue field samples. Total QPX prevalence is the sum of positive and BLD samples.

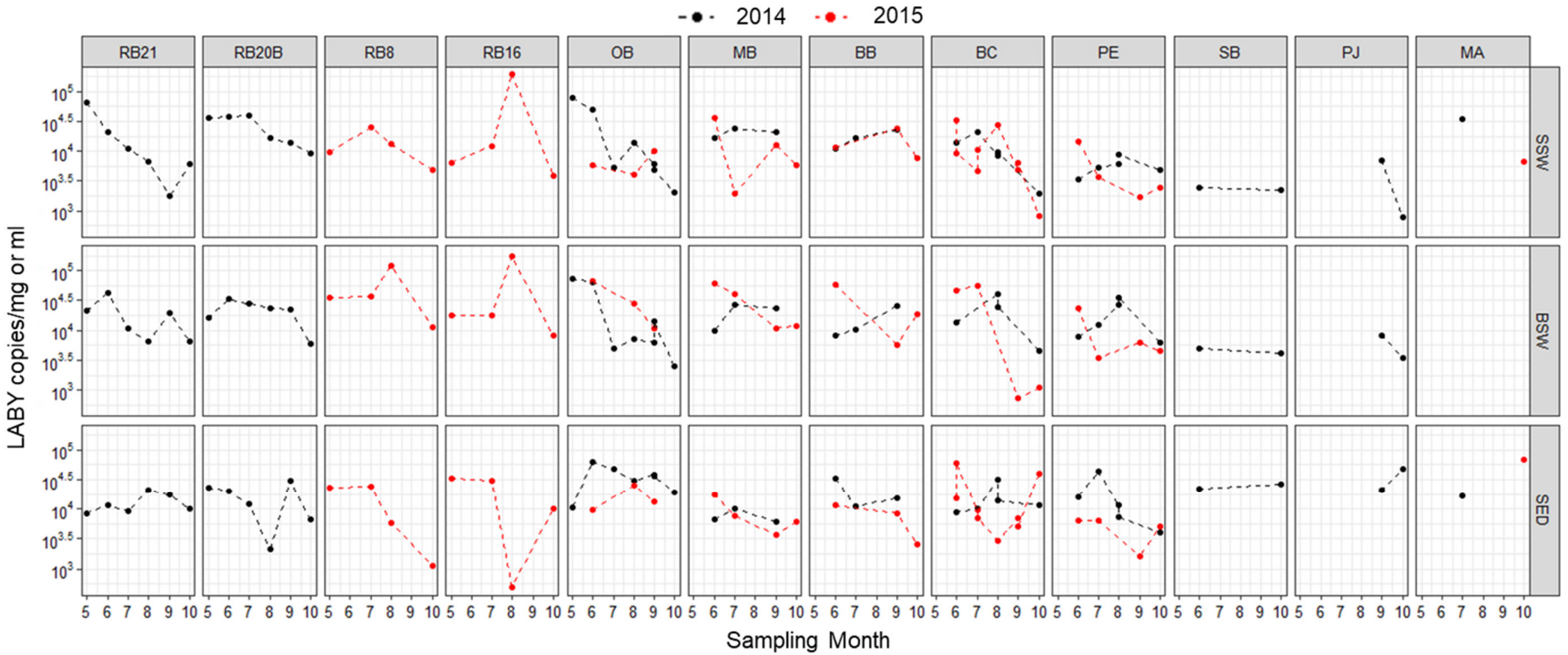

Figure S3: Labyrinthulomycete (LABY) abundance in environmental samples: surface seawater (SSW), bottom seawater (BSW), and sediment (SED), assayed using the labyrinthulomycete (LABY) qPCR. Values are expressed in terms of LABY gene copies per mL seawater or mg sediment on a log10 scale.

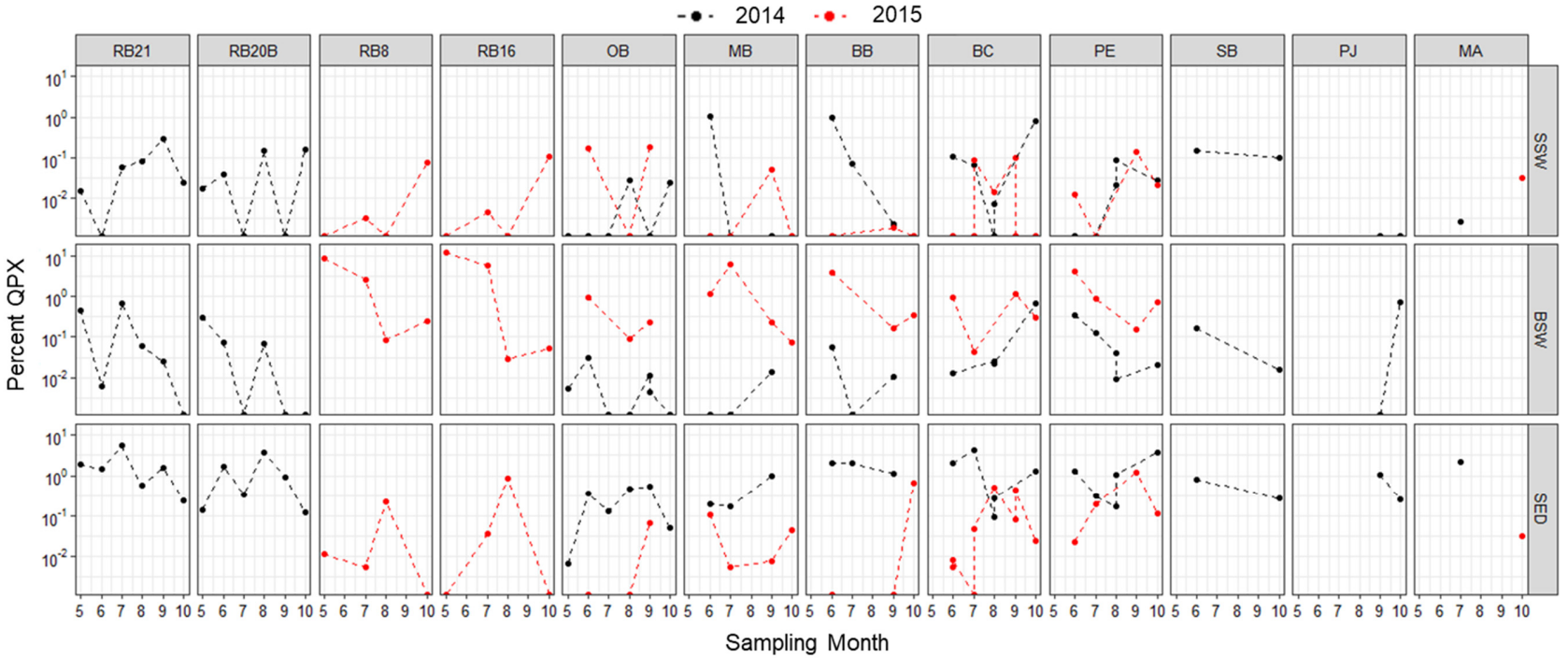

Figure S4: Percent contribution of *M. quahogii* (QPX) to total labyrinthulomycetes in environmental samples: surface seawater (SSW), bottom seawater (BSW), and sediment (SED) on a log10 scale.

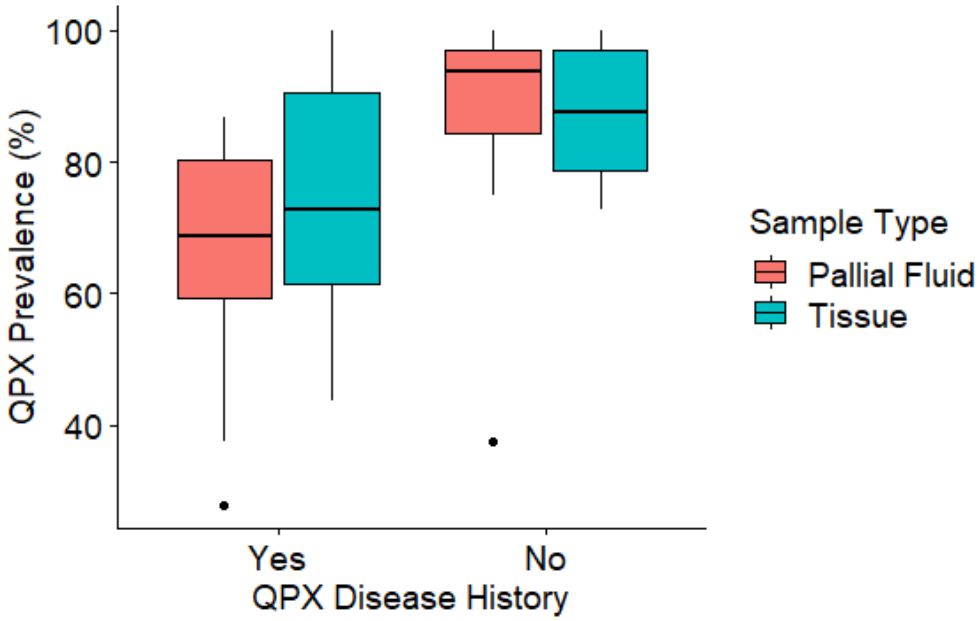

Figure S5: *M. quahogii* (QPX) prevalence (%TPOS = total positive included positive and BLD samples determined by qPCR) in hard clam pallial fluid and tissue (n = 291 subset only for tissue) grouped by sites with and without a history of QPX disease. The difference between pallial fluid samples was significant (p = 0.0229) without p-value adjustment by Wilcoxon rank sum test, while the difference between tissue samples was not (p = 0.201).

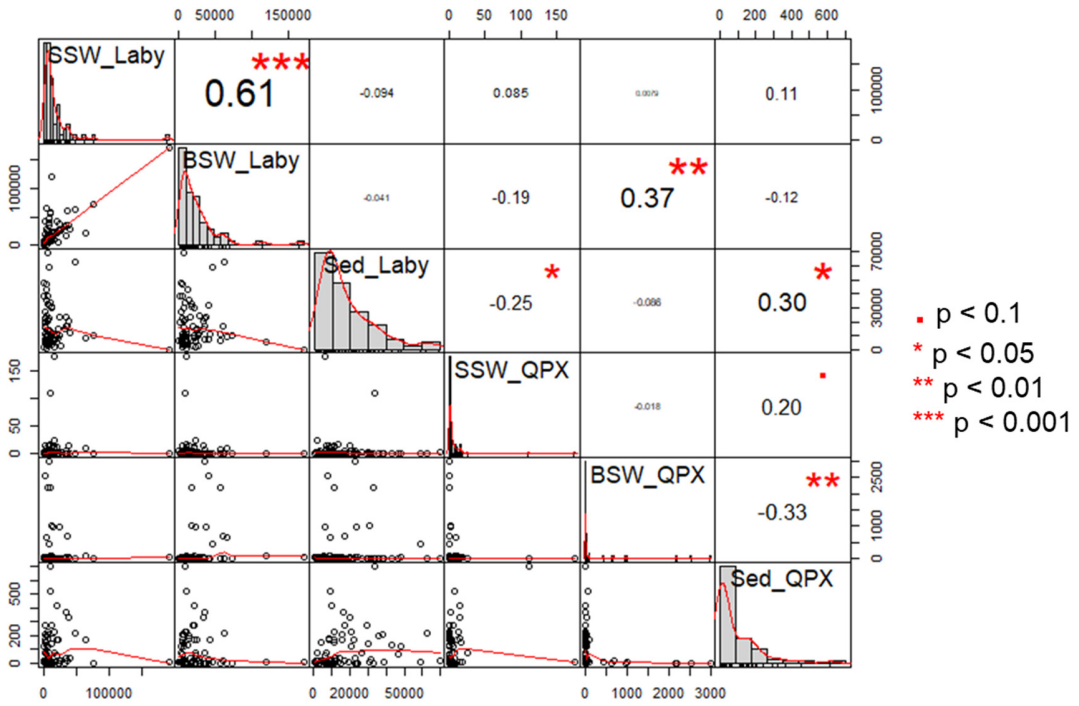

Figure S6: Spearman correlation coefficients (rho) correlogram with histograms for QPX and labyrinthulomycetes in the environment.

Supplementary Tables

Table S1: Sampling sites and samples collected during the field survey in 2014 and 2015.

Sample	Date	Site	Sample Type
1	5/13/2014	Oyster Bay	Clams + Environmental
2	5/20/2014	Raritan Bay 21	Clams + Environmental
3	5/20/2014	Raritan Bay 20B	Clams + Environmental
4	6/3/2014	Birch Creek	Clams + Environmental
5	6/3/2014	Peconic Estuary	Clams + Environmental
6	6/5/2014	Babylon	Clams + Environmental
7	6/5/2014	Moriches Bay	Clams + Environmental
8	6/10/2014	Oyster Bay	Environmental
9	6/17/2014	Raritan Bay 21	Clams + Environmental
10	6/17/2014	Raritan Bay 20B	Clams + Environmental
11	6/24/2014	Shinnecock Bay	Clams + Environmental
12	7/1/2014	Birch Creek	Clams + Environmental
13	7/1/2014	Peconic Estuary	Clams + Environmental
14	7/7/2014	Oyster Bay	Clams + Environmental
15	7/15/2014	MA	Clams + Environmental
16	7/17/2014	Moriches Bay	Clams + Environmental
17	7/17/2014	Babylon	Clams + Environmental
18	7/22/2014	Raritan Bay 21	Clams + Environmental
19	7/22/2014	Raritan Bay 20B	Clams + Environmental
20	8/4/2014	Oyster Bay	Environmental
21	8/5/2014	Peconic Estuary	Environmental
22	8/5/2014	Birch Creek	Environmental
23	8/19/2014	Raritan Bay 21	Clams + Environmental
24	8/19/2014	Raritan Bay 20B	Clams + Environmental
25	8/26/2014	Peconic Estuary	Clams + Environmental
26	8/26/2014	Birch Creek	Clams + Environmental
27	9/2/2014	Oyster Bay	Clams + Environmental
28	9/9/2014	Port Jefferson Harbor	Clams + Environmental
29	9/12/2014	Babylon	Environmental
30	9/12/2014	Moriches Bay	Environmental
31	9/16/2014	Raritan Bay 21	Clams + Environmental
32	9/16/2014	Raritan Bay 20B	Clams + Environmental
33	9/30/2014	Oyster Bay	Clams + Environmental
34	10/6/2014	Peconic Estuary	Environmental
35	10/6/2014	Birch Creek	Environmental
36	10/6/2014	Shinnecock Bay	Clams + Environmental
37	10/15/2014	Raritan Bay 21	Clams + Environmental
38	10/15/2014	Raritan Bay 20B	Clams + Environmental
39	10/21/2014	Oyster Bay	Clams + Environmental
40	10/21/2014	Port Jefferson Harbor	Environmental
41	11/14/2014	MA	Clams
42	5/27/2015	Raritan Bay 8	Clams + Environmental

Table S1 (cont'd): Sampling sites and samples collected during the field survey in 2014 and 2015.

Sample	Date	Site	Sample Type
43	5/27/2015	Raritan Bay 16	Clams + Environmental
44	6/4/2015	Birch Creek	Environmental
45	6/11/2015	Babylon Bay	Clams + Environmental
46	6/11/2015	Moriches Bay	Clams + Environmental
47	6/17/2015	Oyster Bay	Clams + Environmental
48	6/23/2015	Birch Creek	Clams + Environmental
49	6/23/2015	Peconic Bay	Clams + Environmental
50	7/7/2015	Raritan Bay 8	Clams + Environmental
51	7/7/2015	Raritan Bay 16	Clams + Environmental
52	7/14/2015	Birch Creek	Environmental
53	7/22/2015	Moriches Bay	Clams + Environmental
54	7/28/2015	Peconic Estuary	Clams + Environmental
55	7/28/2015	Birch Creek	Clams + Environmental
56	8/10/2015	Oyster Bay	Clams + Environmental
57	8/18/2015	Birch Creek	Environmental
58	8/26/2015	Raritan Bay 8	Clams + Environmental
59	8/26/2015	Raritan Bay 16	Clams + Environmental
60	9/2/2015	Babylon Bay	Clams + Environmental
61	9/2/2015	Moriches Bay	Clams + Environmental
62	9/9/2015	Peconic Estuary	Clams + Environmental
63	9/9/2015	Birch Creek	Clams + Environmental
64	9/22/2015	Oyster Bay	Clams + Environmental
65	9/29/2015	Birch Creek	Environmental
66	10/7/2015	Babylon Bay	Clams + Environmental
67	10/7/2015	Moriches Bay	Clams + Environmental
68	10/13/2015	Peconic Estuary	Clams + Environmental
69	10/13/2015	Birch Creek	Clams + Environmental
70	10/16/2015	MA	Clams + Environmental
71	10/20/2015	Raritan Bay 8	Clams + Environmental
72	10/20/2015	Raritan Bay 16	Clams + Environmental

Table S2: Intensity scale used to calculate *M. quahogii* (QPX) weighted prevalence in hard clam cohort mantle tissue and pallial fluid based on qPCR assays. Weighted prevalence was determined based on the sum of QPX load rated on the intensity scale for each individual clam, divided by the number of clams assayed for each sampling event or cohort. Scales differ due to different detection limit of the two assays.

Intensity	Tissue QPX copies/mg	Pallial Fluid QPX copies/ml
0 = None	0 = negative	0 = negative
1 = Rare	< 75 = BLD	< 500 = BLD
2 = Light	440	880
3 = Mild	2200	2200
4 = Moderate	11,000	11,000
5 = Heavy	55,000	55,000
6 = Severe	> 55,000	> 55,000

BLD = below limit of detection

Table S3: Local weather stations used to supplement measured metadata.

Sampling Site	Coordinates	Weather Station
Raritan Bay 21	40.498917, −74.1797	Newark, NJ (KEWR)
Raritan Bay 20B	40.542917, −74.1289	Newark, NJ (KEWR)
Raritan Bay 8	40.501867, −74.1869	Newark, NJ (KEWR)
Raritan Bay 16	40.50625, −74.1526	Newark, NJ (KEWR)
Oyster Bay	40.8993, −73.4897	Farmingdale, NY (KFRG)
Moriches Bay	40.777283, −72.7912	Shirley, NY (KHWV)
Babylon Bay	40.665983, −73.3123	Farmingdale, NY (KFRG)
Birch Creek	40.9044, −72.5904	East Quogue, NY (KFOK)
Peconic Estuary	40.941617, −72.4137	East Quogue, NY (KFOK)
Shinnecock Bay	40.86195, −72.4483	East Quogue, NY (KFOK)
Port Jefferson Harbor	40.953117, −73.0702	Ronkonkoma, NY (KISP)
Barnstable, MA	41.709798, −70.320348	Hyannis, MA (KHYA)

Table S4: Descriptive statistics of *M. quahogii* (QPX) prevalence (%) and concentration (copies/mg) in hard clam tissue at the cohort level (not individual clams, $n = 59$) determined by qPCR.

Hard Clam Tissue		Mean	Standard Error	Median	Standard Deviation	Range	Minimum	Minimum (non-zero)	Maximum	Confidence Interval (95.0%)
Prevalence	TPOS	74.7	3	81.25	23.07	85	15	15	100	6.01
	POS	30.9	4.03	18.75	30.97	100	0	5	100	8.07
	BLD	43.8	2.73	50	20.96	87.5	0	6.25	87.5	5.46
	NEG	25.3	3	18.75	23.06	85	0	6.25	85	6.01
	WP	1.15	0.08	1	0.64	2.66	0.15	0.15	2.81	0.17
Concentration	MEAN	1621	1045	150.29	8026	60,342	0	82.26	60,342	2092
	MIN	125.64	27.03	81.81	207.62	1254	0	75.3	1254	54.11
	MAX	13,616	11,249	246.34	86,406	66,0824	0	84.75	660,824	22,518
	RANGE	13,491	11,250	126.56	86,412	660,739	0	3.2	660,739	22,519

TPOS = % total positive (includes POS + BLD)
POS = % positive (quantifiable)
BLD = % below limit of detection (positive but unquantifiable)
NEG = % negative
WP = weighted prevalence
MIN = minimum concentration of QPX (copies/mg tissue)
MAX = maximum concentration of QPX (copies/mg tissue)
MEAN = mean concentration of QPX (copies/mg tissue) of positive samples only (excludes BLD and NEG)
RANGE = concentration range of QPX (copies/mg tissue)

Table S5: Hard clam samples positive for QPX disease by histopathology.

Sample	Site	Month	QPX copies/mg	QPX cells/mg	Intensity	Location
14-041-15	RB21	September	234.60	0.53	Moderate, multifocal	Visceral Mass and Gills
15-011-13	RB8	May	8845.29	20.10	Moderate, multifocal	Gills and Mantle
15-012-13	RB16	May	5366.30	12.20	Light, multifocal	Visceral Mass
15-046-01	RB8	August	1664.75	3.78	Rare, focal	Visceral Mass
15-061-28	RB16	October	1092.90	2.48	Light, multifocal	Mantle
15-059-08	MA	October	60338.27	137.13	Light, focal	Gills
15-059-13	MA	October	83826.37	190.51	Moderate, multifocal	Mantle

Table S6: Descriptive statistics of *M. quahogii* (QPX) prevalence (%) and concentration (copies/mL) in hard clam pallial fluid at the cohort level (not individual clams, n=18) determined by qPCR.

Hard Clam Pallial Fluid		Mean	Standard Error	Median	Standard Deviation	Range	Minimum	Minimum (non-zero)	Maximum	Confidence Interval (95.0%)
Prevalence	TPOS	73.19	5.21	75	22.11	72.22	27.78	27.78	100	10.99
	POS	28.66	4.48	31.25	19	63.64	0	6.25	63.64	9.45
	BLD	44.54	5.58	43.75	23.68	82.95	4.55	4.55	87.50	11.78
	NEG	26.81	5.21	25	22.11	72.22	0	6.25	72.22	10.99
	WP	1.21	0.11	1.25	0.48	1.72	0.28	0.28	2	0.24
Concentration	MEAN	1587	445	1199	1889	8655	0	515	8655	939
	MIN	615	113	552	480	2351	0	502	2351	239
	MAX	5121	2850	2442	12,092	53,080	0	515	53,080	6013
	RANGE	4506	2852	1727	12,102	52,537	0	410	52,537	6018

TPOS = % total positive (includes POS + BLD)
POS = % positive (quantifiable)
BLD = % below limit of detection (positive but unquantifiable)
NEG = % negative
WP = weighted prevalence
MIN = minimum concentration of QPX (copies/ml pallial fluid)
MAX = maximum concentration of QPX (copies/ml pallial fluid)
MEAN = mean concentration of QPX (copies/ml pallial fluid) of positive samples only (excludes BLD and NEG)
RANGE = concentration range of QPX (copies/ml pallial fluid)

Table S7: Descriptive statistics of *M. quahogii* (QPX) in environmental samples, determined by qPCR in QPX gene copies/mg sediment or ml seawater. SED = sediment; BSW = bottom seawater; SSW = surface seawater.

Statistic	SED	BSW	SSW
Count (N)	71	64	71
% Positive	89	83	56
Mean	93.15	241.44	7.36
Standard Error	16.06	78.81	2.92
Median	22.1	9.84	0.54
Standard Deviation	135.3	630.5	24.64
Range	702.61	2980	176.37
Minimum	0	0	0
Minimum (non-zero)	0.27	0.61	0.43
Maximum	702.61	2980	176.37
Confidence Interval (95.0%)	32.03	157.49	5.83

Table S8: *p*-values of group comparisons of *M. quahogii* in clams and environment by Wilcoxon rank sum test or Kruskal-Wallis rank sum test. *p*-values in bold were significant after Bonferroni (BF) correction* for each set of comparisons. *p*-values in red were significant without BF correction for exploratory analyses.

Sample	Parameter	Wilcoxon Rank Sum Test		Kruskal-Wallis Rank Sum Test	
		QPX Disease History	Year	Site	Month
Clam Tissue (sig at $p < 0.0125$)	TPOS	0.2456	0.1139	0.2186	0.1340
	POS	0.1693	0.1861	0.2257	0.0356
	BLD	0.9937	0.0662	0.8251	0.1519
	WP	0.0963	0.1824	0.2286	0.0411
Clam Pallial Fluid (sig at $p < 0.0125$)	TPOS	0.0229	n/a	0.362	0.0446
	POS	0.439		0.467	0.549
	BLD	0.364		0.433	0.0444
	WP	0.341		0.473	0.322
Environment (sig at $p < 0.0167$)	SED	0.8438	**6.25×10^{-11}**	0.5372	0.5563
	BSW	0.8694	**6.24×10^{-5}**	0.6601	0.0636
	SSW	0.4396	0.0866	0.7298	0.9564

*$p = 0.05/4$ comparisons for clams = 0.0125; $p = 0.05/3$ comparisons for environmental (ENV) = 0.0167; TPOS includes positive and BLD samples

Table S9: Significant *p*-values of metadata by Wilcoxon rank sum test grouped by sampling year without adjustment for multiple comparisons. *p*-values in bold were significant after Bonferroni* (BF) correction.

Metadata	***p*-value**
SSW Salinity	0.01326
Δ Salinity (SSW – BSW)	**0.0001487**
SSW Total chlorophyll	0.03183
BSW Total chlorophyll	0.01831
BSW Chlorophyll a	0.009892
Wind Degree Direction	0.0401
Precipitation (mean 3 month)	**0.001011**
Precipitation (mean 4 month)	**0.0001211**
Precipitation (sum 2 month)	0.04803
Precipitation (sum 3 month)	**0.0009504**
Precipitation (sum 4 month)	**50.6×10^{-5}**

*p = 0.05/47 comparisons = 0.00106

Table S10: Spearman's correlation coefficients (rho) between *M. quahogii* (QPX) in clams or the environment and environmental metadata. Only correlations with $p < 0.05$, expressed as rho, *p*-value are shown. Positive correlations are shaded blue and negative are shaded red. Significant correlations are in bold using Bonferroni correction at $p < 0.001$ for 48 correlations for each parameter with metadata. For environmental parameters with more than one metric, only the strongest correlation is shown. *M. quahogii* abundance in SSW was not correlated with any parameter. Explanation of abbreviations for environmental parameters with multiple metrics (monthly means, sums or lags) can be found in Table S11.

	Parameter	Day of Year	Month	Temperature	Salinity	DO	Chlorophyll	Precipitation	Wind Speed	Wind Direction
Clam Tissue (all)	%TPOS	NS	NS	LM3moT -0.376, 0.003	NS	NS	NS	NS	NS	NS
	%POS	−0.378, 0.003	−0.398, 0.0018	**LM3moT −0.414, 0.001**	NS	NS	NS	NS	NS	NS
	%BLD	NS	NS	NS	NS	NS	NS	**−0.487, 0.00009**	NS	NS
	WP	−0.395, 0.002	−0.398, 0.0017	**LM3moT −0.428, 0.0007**	NS	NS	NS	NS	NS	NS
	Min	NS	NS	NS	NS	NS	NS	NS	Mean −0.275, 0.04	NS
	Mean	−0.317, 0.01	−0.358, 0.006	M3moT −0.358, 0.005	NS	BSW 0.359, 0.005	BSW Chl b −0.275, 0.03	NS	NS	NS
	Max	NS	−0.374, 0.003	M3moT −0.384, 0.0026	NS	NS	NS	NS	NS	NS
	Range	−0.391, 0.002	**−0.416, 0.001**	**LM3moT −0.413, 0.001**	NS	NS	NS	NS	NS	NS
Clam Pallial Fluid (subset)	%TPOS	NS	NS	NS	SSW 0.554, 0.017	NS	NS	NS	Mean −0.654, 0.003	NS
	%POS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	%BLD	NS	NS	NS	NS	NS	NS	NS	Mean −0.596, 0.009	NS
	WP	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Min	NS	NS	NS	ΔSSW-BSW 0.549, 0.018	NS	NS	NS	NS	NS
	Mean	NS	NS	NS		NS	NS	NS	NS	NS
	Max	NS	NS	NS		NS	NS	NS	NS	NS
	Range	NS	NS	NS	BSW 0.47, 0.049	NS	NS	M4moP −0.532, 0.023	NS	NS
ENV	BSW	−0.29, 0.013	−0.317, 0.007	Max Air Temp 0.344, 0.003	NS	ΔSSW-BSW 0.359, 0.002	**BSW Chl a 0.444, 0.0001**	NS	Max 0.339, 0.0039	0.3, 0.01
	SED	NS	NS	NS	**ΔSSW-BSW −0.383, 0.001**	NS	BSW Chl a −0.28, 0.019	**S4moP 0.489, 0.00002**	NS	NS

NS = not significant at $p < 0.05$

Table S11: Descriptions and abbreviations of data used in the correlation analyses.

Full Description	Label	Quantitative (Q) or Category (C)
Clam QPX %Total Positive	TIS%Tpos	Q
Clam QPX %POS	TIS%Pos	Q
Clam %BLD	TIS%BLD	Q
Clam Minimum QPX copies/mg	TISMinQ	Q
Clam Maximum QPX copies/mg	TISMaxQ	Q
Clam Mean QPX copies/mg	TISAvgQ	Q
Clam Range QPX copies/mg	TISRangeQ	Q
Clam Weighted Prevalence	TISWPQ	Q
Site	Site	C
Embayment	Bay	C
Month	Month	C
QPX Disease History	QDisHis	C
Day of Year	Day	Q
Day of Both Years	DoBY	Q
Sediment QPX copies/mg	QPXsed	Q
BSW QPX copies/ml	QPXbsw	Q
SSW QPX copies/ml	QPXssw	Q
SSW Temperature (°C)	Tssw	Q
SSW Salinity (ppt)	Sssw	Q
SSW Dissolved Oxygen (mg/L)	DOssw	Q
BSW Temperature (°C)	Tbsw	Q
BSW Salinity (ppt)	Sbsw	Q
BSW Dissolved Oxygen (mg/L)	DObsw	Q
ΔTemp SSW-BSW	DelT	Q
ΔSalinity SSW-BSW	DelS	Q
ΔDO SSW – BSW	DelDO	Q
Depth (m)	Depth	Q
Depth	DepthC	C
SSW Total chl (ug/L)	TCssw	Q
SSW Chl a (ug/L)	CAssw	Q
SSW Chl b (ug/L)	CBssw	Q
SSW Chl c (ug/L)	CCssw	Q
BSW Total chl (ug/L)	TCbsw	Q
BSW Chl a (ug/L)	CAbsw	Q
BSW Chl b (ug/L)	CBbsw	Q
del Total Chl SSW-BSW	delTC	Q
Maximum Air Temperature	MaxAT	Q
Mean Air Temperature	MeanAT	Q
Min Air Temperature	MinAT	Q
Max Wind Speed (mph)	MaxWS	Q
Mean Wind Speed (mph)	MeanWS	Q

Table S11 (cont'd): Descriptions and abbreviations of data used in the correlation analyses.

Full Description	Label	Quantitative (Q) or Category (C)
Max Gust Speed (mph)	MaxGS	Q
Precipitation (in.)	Precip	Q
Cloud Cover	CloudC	Q
Weather Event	WEvent	C
Wind Direction (degrees)	WindDD	Q
Mean 1mo Temperature	M1moT	Q
Mean 2mo Temperature	M2moT	Q
Mean 3mo Temperature	M3moT	Q
Mean 4mo Temperature	M4moT	Q
Mean 1mo Precipitation	M1moP	Q
Mean 2mo Precipitation	M2moP	Q
Mean 3mo Precipitation	M3moP	Q
Mean 4mo Precipitation	M4moP	Q
Mean 1mo Wind Speed	M1moWS	Q
Mean 2mo Wind Speed	M2moWS	Q
Mean 3mo Wind Speed	M3moWS	Q
Mean 4mo Wind Speed	M4moWS	Q
Sum 1mo Precipitation	S1moP	Q
Sum 2mo Precipitation	S2moP	Q
Sum 3mo Precipitation	S3moP	Q
Sum 4mo Precipitation	S4moP	Q
Lag Mean 2mo Temperature	LM2moT	Q
Lag Mean 3mo Temperature	LM3moT	Q
Lag Mean 4mo Temperature	LM4moT	Q

Table S12: Descriptive statistics of *M. quahogii* (QPX), total labyrinthulomycetes (LABY), and percent QPX of total labyrinthulomycetes in environmental samples. Values are expressed in terms of gene copies per mg sediment or ml seawater.

Parameter	**QPX**			**LABY**			**%QPX**		
Sample Type	**SED**	**BSW**	**SSW**	**SED**	**BSW**	**SSW**	**SED**	**BSW**	**SSW**
Mean	93.15	241.44	7.36	17,666	24,612	16,578	0.71	0.85	0.08
Standard Error	16.06	78.81	2.92	1795	3584	3045	0.13	0.27	0.02
Median	22.1	9.84	0.54	11,667	15,389	9122	0.23	0.07	0
Standard Deviation	135.3	630.5	24.64	15,124	28,674	25,654	1.09	2.14	0.19
Range	702.61	2980	176.37	69,096	171,428	191,284	5.68	12.23	1.04
Minimum	0	0	0	487.48	720.94	780.83	0	0	0
Minimum (non-zero)	0.27	0.61	0.43	487.48	720.94	780.83	0.01	0	0
Maximum	702.61	2980	176.37	69,584	172,149	192,065	5.68	12.23	1.04
Count	71	64	71	71	64	71	71	64	71
Confidence Interval (95.0%)	32.03	157.49	5.83	3580	7162	6072	0.26	0.53	0.05

SED = sediment, BSW = bottom seawater, SSW = surface seawater