## Supplement 2. Supplementary Tables (S1–S14) and Figures (S1–S5)

Table S1: Intensity scale used to determine QPX weighted prevalence in hard clams.

| Intensity Scale | QPX copies/mg | QPX cells/mg |
|---|---|---|
| 0 = None | 0 = negative | 0 |
| 1 = Rare | < 75 = BLD | < 0.17 |
| 2 = Light | 440 | 1 |
| 3 = Mild | 2,200 | 5 |
| 4 = Moderate | 11,000 | 25 |
| 5 = Heavy | 55,000 | 125 |
| 6 = Very heavy | > 55,000 | > 125 |

BLD = below limit of detection

Table S2: Temperature gradient analysis performed on standards without any PCR additives. At each anneal temperature, the slope, linearity ($R^2$) and efficiency were determined. Based on acceptable qPCR parameters, the anneal optimum is shown in bold.

| qPCR Parameter | Anneal Temperature (°C) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 45.2 | 45.7 | 47 | 48.9 | 51.3 | **53.9** | **56.6** | 59.2 | 61.6 |
| **Slope** | 3.652 | 3.972 | 3.769 | 3.561 | 3.609 | **3.410** | **3.114** | 2.971 | 3.231 |
| **$R^2$** | 0.834 | 0.899 | 0.865 | 0.854 | 0.857 | **0.906** | **0.886** | 0.888 | 0.934 |
| **Efficiency** | 87.92 | 78.60 | 84.18 | 90.94 | 89.24 | **96.45** | **109.67** | 117.12 | 103.99 |

Table S3: Primer titration analysis performed on standards without any PCR additives. With each concentration, the slope, linearity ($R^2$) and efficiency were determined. The optimal primer concentration is shown in bold.

| qPCR Parameter | Primer Final Concentration (Forward/Reverse nM) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **100/100** | 200/200 | 50/50 | 100/200 | 100/50 | 200/100 | 200/50 | 50/100 | 50/200 |
| **Slope** | **-3.328** | -3.22 | -3.632 | -3.175 | -3.746 | -3.178 | -3.404 | -3.816 | -3.145 |
| **$R^2$** | **0.983** | 0.974 | 0.971 | 0.961 | 0.976 | 0.969 | 0.969 | 0.965 | 0.957 |
| **Efficiency** | **99.75** | 104.44 | 88.51 | 106.57 | 84.94 | 106.43 | 96.69 | 82.83 | 107.95 |

Table S4: Different combinations and concentrations of PCR additives (dimethyl sulfoxide; DMSO and glycerol) were supplemented to the Takyon Master Mix. The average and standard deviation are shown from conditions with more than one trial. With each combination of additives, the slope, linearity ($R^2$) and efficiency were determined, and the optimal PCR additives are shown in bold.

| qPCR Parameter | PCR Additives to Takyon Master Mix (final concentration) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | No Additives | 3% DMSO | 6% DMSO | 8% Glycerol | **1% DMSO + 8% Glycerol** | 2% DMSO + 8% Glycerol | 3% DMSO + 8% Glycerol | 6% DMSO + 8% Glycerol |
| **#Trials** | 4 | 1 | 1 | 4 | **2** | 2 | 2 | 1 |
| **Slope** | -3.664 ± 0.321 | -3.188 | -2.951 | -3.51 ± 0.31 | **-3.459 ± 0.122** | -3.46 ± 0.59 | -3.158 ± 0.05 | -3.1637 |
| **$R^2$** | 0.988 ± 0.007 | 0.98 | 0.968 | 0.978 ±0.019 | **0.996 ± 0.004** | 0.97 ±0.017 | 0.969 ±0.029 | 0.97 |
| **Efficiency** | 88 ± 10 | 106 | 118 | 93 ± 11 | **95 ± 5** | 97 ± 23 | 107 ± 2 | 107 |

Table S5: Mean $C_q$ values for the standard curve of linearized plasmid serial dilutions from 10 to $10^6$ QPX copies averaged over 12 independent trials (Figure 2) with 95% confidence intervals (CI), standard error (SE), and standard deviation (SD).

| Log initial copy number | Mean Cq | CI | SE | SD |
|---|---|---|---|---|
| 1 | 34.558 | 0.550 | 0.247 | 0.818 |
| 2 | 32.159 | 0.362 | 0.163 | 0.539 |
| 3 | 28.403 | 0.219 | 0.098 | 0.326 |
| 4 | 24.838 | 0.217 | 0.097 | 0.323 |
| 5 | 21.200 | 0.224 | 0.100 | 0.333 |
| 6 | 17.712 | 0.155 | 0.070 | 0.231 |

Table S6: Results from melt curve and gel electrophoresis analyses from the qPCR assay's analytical specificity testing.

| Species | Group | Number of Products | Melt Temperature (Tm °C) | Product Size (bp) |
|---|---|---|---|---|
| Quahog Parasite Unknown (QPX; *Mucochytrium quahogii*)* | Thraustochytrid | 1 | 79.5 | 190 |
| *Aurantiochytrium limacinum* (ATCC MYA-1381) | Thraustochytrid | 1 | 83.4 | 400 |
| *Schizochytrium aggregatum* (ATCC 28209) | Thraustochytrid | 1 | 85.5 | 400 |
| *Thraustochytrium aureum* (ATCC 34304) | Thraustochytrid | 1 | 84 | 400 |
| *Japanochytrium marinum* (ATCC 28207) | Thraustochytrid | 1 | 83.4 | 400 |
| *Oblongichytrium sp.* (isolate 606) | Oblongichytrid | none | none | none |
| *Aplanochytrium stocchinoi* (isolate GSB06) | Aplanochytrid | none | none | none |
| *Labyrinthula sp.* (isolate KIE13) | Labyrinthulid | none | none | none |

*multiple isolates were tested: 8BC7, 20AC1, MA, and VA

Table S7: DNA concentration and purity of samples as determined by Nanodrop ND-1000 spectrophotometry.

| **Sampling Site** | **Number of Samples** | **DNA concentration (ng/µl)** | | **Purity (A260/280)** | |
|---|---|---|---|---|---|
| | | Mean | Variance | Mean | Variance |
| Birch Creek | 72 | 31.8 | SE = 2.7<br>SD = 23<br>CI = 5.4 | 2.12 | SE = 0.05<br>SD = 0.4<br>CI = 0.09 |
| Peconic Estuary | 63 | 29.4 | SE = 2.9<br>SD = 22.6<br>CI = 5.7 | 1.85 | SE = 0.02<br>SD = 0.19<br>CI = 0.05 |
| Babylon Bay | 48 | 32.8 | SE = 3.36<br>SD = 23.3<br>CI = 6.8 | 2.02 | SE = 0.05<br>SD = 0.36<br>CI = 0.1 |
| Moriches Bay | 64 | 20.03 | SE = 2.01<br>SD = 16.1<br>CI = 4.02 | 1.88 | SE = 0.07<br>SD =0.58<br>CI = 0.24 |
| Oyster Bay | 48 | 18.7 | SE =2.05<br>SD = 14.2<br>CI = 4.13 | 2.21 | SE = 0.1<br>SD = 0.67<br>CI = 0.2 |
| Raritan Bay 8 | 64 | 32.2 | SE = 3.76<br>SD = 30.1<br>CI = 7.52 | 1.87 | SE = 0.1<br>SD = 0.78<br>CI = 0.2 |
| Raritan Bay 16 | 64 | 29.9 | SE = 4.8<br>SD = 33<br>CI = 9.7 | 1.88 | SE = 0.09<br>SD = 0.7<br>CI = 0.18 |
| **TOTAL** | **423** | **27.8** | SE = 1.18<br>SD = 24.4<br>CI =2.33 | **1.98** | SE = 0.03<br>SD = 0.57<br>CI = 0.05 |

SE = standard error; SD = standard deviation; CI = 95% confidence interval

Table S8: P-values of group comparisons of QPX in clams determined by qPCR by month and site (Kruskal-Wallis rank sum test), and disease history (Wilcoxon rank sum test).

| Parameter | Sampling Month | Sampling Site* | Sampling Site (individual) | Disease History |
|---|---|---|---|---|
| **% Total Positive** | 0.6947 | 0.2319 | 0.3127 | 0.5344 |
| **% Positive** | 0.7138 | 0.2673 | 0.2873 | 0.9016 |
| **% BLD** | 0.5855 | 0.6901 | 0.7853 | 0.8616 |
| **Weighted Prevalence** | 0.6471 | 0.2324 | 0.2861 | 0.767 |

*The two RB sites were included as 1 site, whereas in the individual sampling site comparison, they were compared as different sampling sites.

Table S9: Efficiency (E) and linearity ($R^2$) of spiked sample serial dilution series for seasonal inhibition testing using the Birch Creek sampling site. One positive, one below detection limit (BLD) and one negative sample was tested.

| Sampling Month | Positive Samples | | BLD Samples | | Negative Samples | | Average | |
|---|---|---|---|---|---|---|---|---|
| | E | $R^2$ | E | $R^2$ | E | $R^2$ | E | $R^2$ |
| June | 83.5 | 0.996 | 99.14 | 0.997 | n/a | n/a | 91.32 | 0.997 |
| July | 91.38 | 0.996 | 81.4 | 0.997 | 85 | 0.989 | 85.93 | 0.994 |
| September | 92.46 | 0.987 | 85.72 | 0.987 | 87.5 | 0.99 | 88.56 | 0.988 |
| October | 89.74 | 0.994 | 91.75 | 0.991 | 87.66 | 0.969 | 89.72 | 0.985 |
| **Average** | 89.27 | 0.993 | 89.5 | 0.993 | 86.72 | 0.983 | **88.66** | **0.990** |

n/a: not available because there were no negative samples in June

Table S10: Efficiency (E) and linearity ($R^2$) of spiked sample serial dilution series for inhibition testing of at least one positive sample from each site.

| Sample Site | Month | Efficiency (E) | Linearity ($R^2$) |
|---|---|---|---|
| Babylon Bay | September | 90.49 | 0.993 |
| Moriches Bay | July | 90.82 | 0.992 |
| Oyster Bay | September | 89.88 | 0.991 |
| Peconic Estuary | June | 84.01 | 0.992 |
| Raritan Bay site 8 | May | 85.61 | 0.992 |
| | August | 87.92 | 0.990 |
| Raritan Bay site 16 | May | 89.57 | 0.992 |
| | **Average** | **88.33** | **0.992** |

Table S11: Evaluation of differences in PCR amplification efficiency (E) and linearity ($R^2$) by Kruskal-Wallis rank sum test from clam tissue sample inhibition testing.

| Parameter | Grouped by | df | P-value |
|---|---|---|---|
| Efficiency (E) | Month | 5 | 0.8923 |
| | qPCR result | 2 | 0.5301 |
| | Site | 6 | 0.7716 |
| Linearity ($R^2$) | Month | 5 | 0.2617 |
| | qPCR result | 2 | 0.0964 |
| | Site | 6 | 0.9906 |

Table S12: Recovery rate of QPX through the DNA extraction kit and qPCR assay. The average and standard deviation is shown.

| Sample Site | Sample Month | Recovery Rate (%) |
|---|---|---|
| Birch Creek | June<br>July<br>September<br>October | 31.62<br>24.96<br>29.81<br>19.06 |
| Peconic Bay | June | 36.28 |
| Babylon Bay | September | 13.72 |
| Moriches Bay | July | 16.93 |
| Oyster Bay | September | 51.1 |
| Raritan Bay 8 | May<br>August | 25.41<br>45.09 |
| Raritan Bay 16 | May | 33.11 |
| | **Average** | **29.74 ± 11.52** |

Table S13: Histology results from selected clam samples after evaluation by qPCR. Only clam samples from Raritan Bay were positive for active QPX disease (in bold).

| Site | Month | Sample | QPX copies/mg | QPX cells/mg | Histology |
|---|---|---|---|---|---|
| RB8 | May | **15-011 #13** | **8845.29** | **20.10** | **Positive** |
| | | 15-011 #22 | 663.05 | 1.51 | Negative |
| | | 15-011 #27 | 97.84 | 0.22 | Negative |
| RB16 | May | 15-012 #4 | 371.74 | 0.84 | Negative |
| | | **15-012 #13** | **5366.30** | **12.20** | **Positive** |
| BB | June | 15-017 #14 | 92.83 | 0.21 | Negative |
| MB | June | 15-018 #10 | 88.07 | 0.20 | Negative |
| OB | June | 15-023 #23 | 267.58 | 0.61 | Negative |
| BC | June | 15-024 #5 | 897.24 | 2.04 | Negative |
| PE | June | 15-025 #20 | 1548.59 | 3.52 | Negative |
| | June | 15-025 #5 | 5437.34 | 12.36 | Negative |
| RB8 | July | 15-027 #8 | 282.03 | 0.64 | Negative |
| | | 15-027 #24 | BLD | BLD | Negative |
| | | 15-027 #27 | BLD | BLD | Negative |
| | | 15-027 #29 | 221.12 | 0.50 | Negative |
| RB16 | July | 15-032 #17 | BLD | BLD | Negative |
| | | 15-032 #24 | 106.57 | 0.24 | Negative |
| MB | July | 15-039 #17 | 274.71 | 0.62 | Negative |
| PE | July | 15-040 #14 | 102.45 | 0.23 | Negative |
| BC | July | 15-041 #9 | 146.12 | 0.33 | Negative |
| OB | Aug | 15-043 #24 | 145.17 | 0.33 | Negative |
| RB8 | Aug | **15-046 #1** | **1664.75** | **3.78** | **Positive** |
| | | 15-046 #2 | 3593.14 | 8.17 | Negative |
| RB16 | Aug | 15-047 #18 | 133.27 | 0.30 | Negative |
| BB | Sept | 15-048 #26 | 156.57 | 0.36 | Negative |
| MB | Sept | 15-049 #9 | 84.66 | 0.19 | Negative |
| PE | Sept | 15-050 #8 | 851.26 | 1.93 | Negative |
| BC | Sept | 15-051 #10 | 97.84 | 0.22 | Negative |
| OB | Sept | 15-053 #8 | 786.67 | 1.79 | Negative |
| BB | Oct | 15-055 #23 | 118.40 | 0.27 | Negative |
| MB | Oct | 15-056 #10 | 231.54 | 0.53 | Negative |
| PE | Oct | 15-057 #23 | 269.34 | 0.61 | Negative |
| BC | Oct | 15-058 #14 | BLD | BLD | Negative |
| RB8 | Oct | 15-062 #13 | BLD | BLD | Negative |
| | | 15-062 #20 | BLD | BLD | Negative |
| | | 15-062 #23 | 423.99 | 0.96 | Negative |
| | | 15-062 #30 | BLD | BLD | Negative |
| RB16 | Oct | 15-061 #3 | BLD | BLD | Negative |
| | | 15-061 #6 | Negative | Negative | Negative |
| | | 15-061 #7 | BLD | BLD | Negative |
| | | **15-061 #28** | **1092.90** | **2.48** | **Positive** |

Table S14: Technical comparison of the original assay by Liu et al. (2009) and the new, improved version of the assay.

| Parameter | Original Assay (Liu et al. 2009) | New Assay |
|---|---|---|
| **Sample preservation** | Ethanol | Frozen at -80°C |
| **DNA extraction (sample volume)** | 1 ml = 100 mg clam mantle tissue | 200 µl = 20 mg clam mantle tissue |
| **DNA extraction (QPX recovery)** | 8.6% ± 6.4% (SD) | 29.74% ± 11.52% (SD) |
| **Standard Curve Plasmid** | Circular plasmid | Linear plasmid |
| **PCR Efficiency** | 82.14% ± 6% (SD) | 95% ± 6.56% (SD) |
| **Standard Curve** | Run on every plate | 1 Robust standard curve with inter-run calibrator (IRC) |
| **qPCR Prep Time** | ~ 1 hour | ~ 30 minutes |
| **qPCR Thermocycler** | Stratagene MX300P | Eppendorf Mastercycler realplex4 ep gradient S |
| **qPCR Reagents** | Stratagene Custom Master mix using core reagent kit supplemented with 3% DMSO and 8% glycerol | Takyon Master Mix supplemented with 1% DMSO and 8% glycerol |
| **qPCR Reaction Volume** | 25 µl | 12.5 µl |
| **qPCR Anneal Temperature** | 55°C | 54°C |
| **qPCR Run Time** | 1 hour and 50 minutes | 1 hour and 30 minutes |
| **qPCR cost per sample** | Appx. $3 per sample with 2 replicates plus 2 replicate spiked samples + 24% of samples requiring rerun with 1:10 dilution | Appx. $1.15 per sample with 3 replicates; samples only rerun if Cq SD > 0.5 or amplification in no-template control |
| **# Samples per plate** | maximum of 20 | maximum of 30 |
| **# Technical replicates** | 2 replicates | 3 replicates |
| **Specificity testing** | *in silico* and against thraustochytrids only | against 7 species of labyrinthulomycetes, covering all 4 groups |
| **Inhibition Assessment** | Single point alien spike of sample with QPX plasmid | Clam tissue spiked with QPX cultured cells prior to DNA extraction and run in qPCR as dilution series |
| **Sample Inhibition** | 13.4% ± 20 (SD) of 56 samples; 18 samples had inhibition > 50% requiring dilution | 4.5% ± 4 (SD) of 18 representative samples* |
| **Conversion from copy number to cell** | 181 copies/cell (did not account for multinucleated cells) | 440 copies/mononucleate cell |

*Sample inhibition = Efficiency of standard curve – Efficiency of Samples = 93-88.5 = 4.5

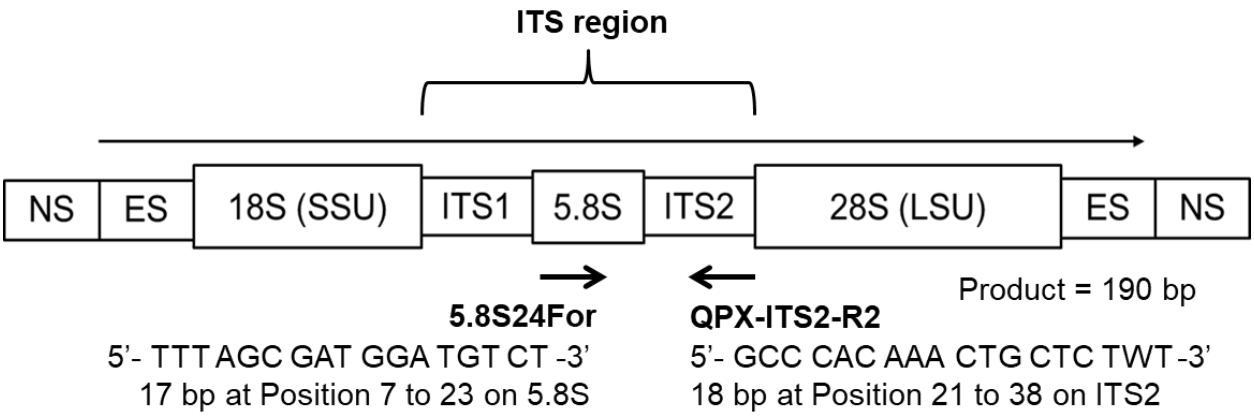



Figure S1: Schematic of the ribosomal RNA (rRNA) genes showing primers, respective locations and directions in which they prime. NS: non-transcribed region; ES: external transcribed spacer; SSU: small subunit or 18S rRNA gene; ITS1: internal transcribed spacer 1; 5.8S: 5.8S rRNA gene; ITS2: internal transcribed spacer 2; LSU: large subunit or 28S rRNA gene (modified from Qian et al. 2007; primers are from Liu et al. 2009). Primer positions are based on QPX isolate NY0400921C6, accession DQ641179 in GenBank.

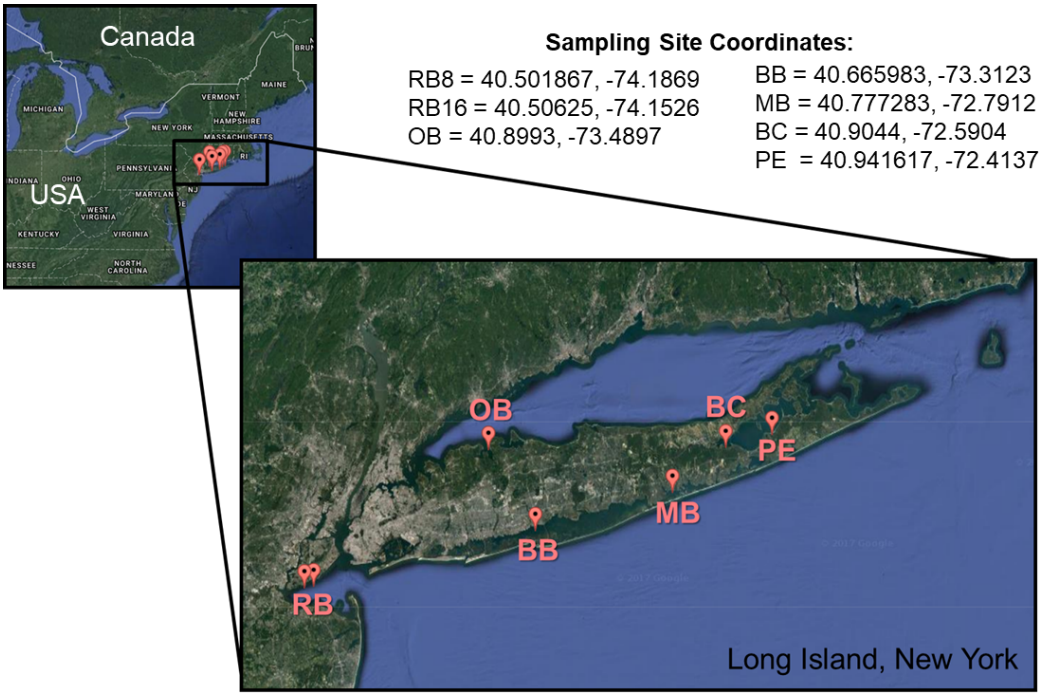
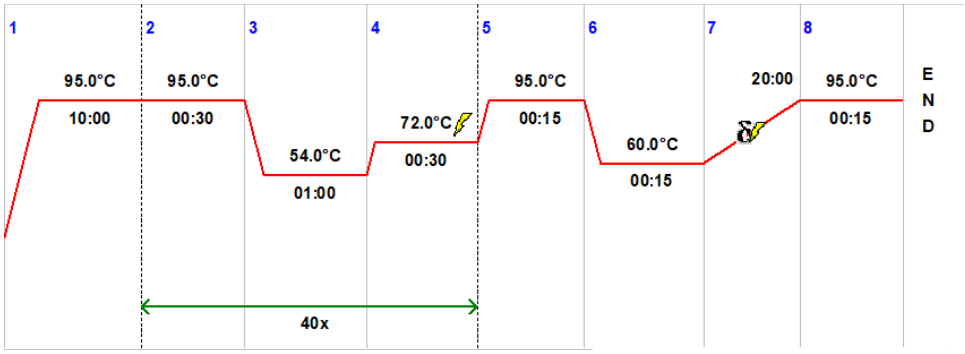



Figure S2: Field sites where wild hard clams were collected and tested for QPX using the new qPCR assay during the 2015 field season (May to October). RB = Raritan Bay; OB = Oyster Bay; BB = Babylon Bay; MB = Moriches Bay; BC = Birch Creek; PE = Peconic Estuary. Sites RB, OB, and BC are areas where QPX disease in clams has been previously identified. Sites BB, MB, and PE are areas that have never been screened for QPX. RB had two subsites (8 and 16).

| Reagent | Volume (µl) |
|---|---|
| 2X Takyon SYBR Master Mix (Eurogentec) | 6.25 |
| Nuclease-free $H_2O$ | 1.875 |
| 2 µM 5.8S24For primer | 0.625 |
| 2 µM QPX-ITS2-R2 primer | 0.625 |
| 50% Glycerol | 2 |
| Dimethyl sulfoxide (DMSO) | 0.125 |
| Sample DNA | 1 |
| Total Reaction Volume | 12.5 |




1 = Pre-denaturation; 2 = Denaturation; 3 = Anneal; 4 = Extension; 2-4 repeated 40 cycles; 5-8 = Melt Curve Analysis; the lightning bolt symbol represents a fluorescence measuring point.

Figure S3: Final qPCR cycling parameters and master mix (reagents and volume) determined from optimization trials.

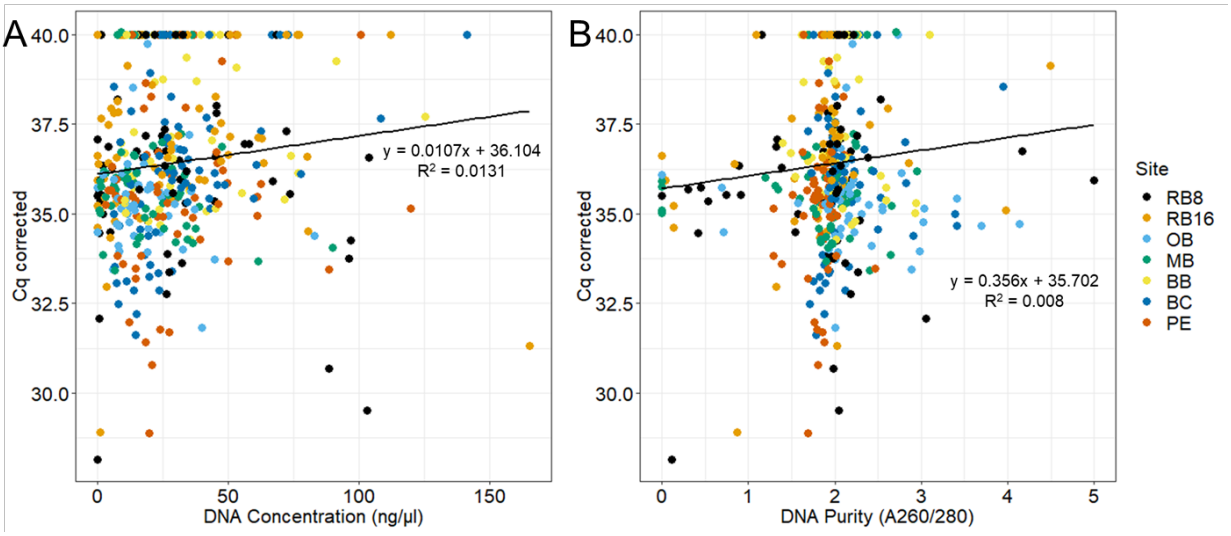



Figure S4: Corrected $C_q$ values with clam sample DNA concentration (A) and purity (B) of 423 samples.

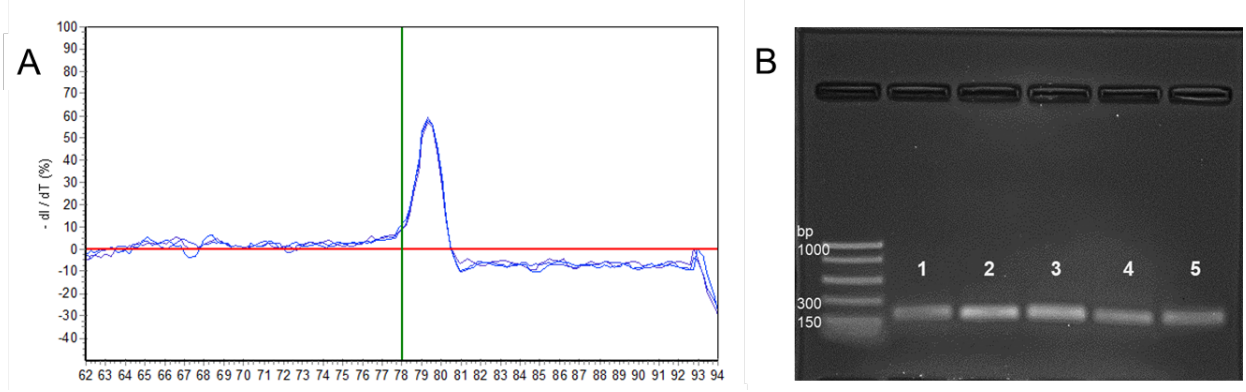



Figure S5: Melt curve analysis showing one product melting at 79.5°C in a positive clam sample from Raritan Bay site 16 (A). The red horizontal line is oriented at 0% dl/dT and the green vertical line is oriented at 78°C. Gel electrophoresis analysis of positive clam samples showing one product at approximately 190 bp (B). Samples are from (1) Raritan Bay site 16, (2) Peconic Estuary, (3) Birch Creek, (4) Oyster Bay, and (5) Moriches Bay.