The following supplement accompanies the article

Detection and quantification of a keystone pathogen in a coastal marine ecosystem

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Supplement 1: Additional Methods for PCR/qPCR design and analysis

1) Selection and optimization of Nuc1 primer set.

Sequencing *Paramoeba invadens* and parasome ITS Regions

The nuclear SSU rDNAs of *Paramoeba invadens* and its parasome were reported previously (Feehan et al. 2013). To sequence the ITS1-5.8S rDNA-ITS2 regions (hereafter 'ITS regions') adjacent to each of these SSU rDNA regions, genomic DNA was extracted from seven different isolates of *P. invadens* (Supplementary Table 1), using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The *P. invadens* nuclear ITS region was amplified from the four strains reported in Feehan et al. 2013 by polymerase chain reaction (PCR) using a specific SSU rDNA primer, PARNUC-SSU-1512F (5`-TGC TAA TTA GTT GTG CAA ATG-3`) and the LSU rDNA primer GRev (5`-GGG ATC CAT ATG CTT AAG TTC AGC GGG T-3`; Coleman & Vacquier 2002). The parasome ITS region was amplified from all examined strains using a new parasome-specific SSU rDNA primer, PARP-SSU-1456F (5`-GTG TGC TCC TTG TCC T-3`) and the LSU rDNA primer ITS4 (5`-TCC TCC GCT TAT TGA TAT GC-3`; White et al. 1990). Both amplifications used the following thermocycling protocol: 94 °C for 5 min, 35 cycles of 94 °C for 20 s, 58 °C for 2 min, 72 °C for 3 min, and one final step of 72 °C for 10 min. The PCR products were gel-purified using the QIAquick Gel Extraction Kit (Qiagen), ethanol-precipitated, and re-suspended in distilled water, then cloned using the pGEM-T Easy Vector System (Promega, Madison, WI, USA). Five positive colonies were Sanger-sequenced (Genome Quebec) in both directions. Non-redundant ITS region sequences are accessioned in Genbank as MH934192-MH934209, and KP979793.

Design and testing of *P. invadens*-specific primers

The SSU rDNA and ITS region sequences of both nucleus and parasome of *P. invadens* were examined for potential species-specific primer sites. Three candidate primer sets intended to amplify fragments of 100 – 200 bp were designed using Primer3 (Koressaar & Remm 2007, Untergasser et al. 2012). Sets 'Nuc1' and 'Nuc2' were designed to amplify divergent regions of the *P. invadens* nuclear SSU rDNA while set 'IRE1' was designed against the parasome SSU rDNA (Supplementary Table 2). The specificity of these primers was checked *in silico* using nucleotide alignments of the equivalent SSU rDNAs from available *Paramoeba/Neoparamoeba* species.

Primer specificity was tested by PCR, using genomic DNA from *P. invadens* isolates SMB-8, SMB-60, and SP (Supplementary Table 1) as expected positives, and using as challenges i) genomic DNA from other *Paramoeba/Neoparamoeba* species (*P./N. branchiphila*, *P./N. aestuarina*, and two strains of *P./N. pemaquidensis*), and ii) genomic DNA from more distantly related marine eukaryotes (*Ancyromonas sigmoides*, *Cyranomonas australis*,

Developayella sp., *Notosolenus urceolatus*, *Neobodo designis*, and *Percolomonas cosmopolitus*). Reactions were conducted in 20 µl volumes containing 2 µl of 10X Reaction Buffer (Invitrogen); 0.2 mM each dNTP; 1.5 mM $MgCl_2$; 1 µM each primer, 1 µl template, and 1 U Taq DNA polymerase (Invitrogen), using the following thermocycling protocol: 94 °C for 2 min, 30 cycles of 94 °C for 15 s, 58 °C for 30 s, 72 °C for 15 s, then a final step at 72 °C for 1 min. Products were visualized using gel electrophoresis for this and all subsequent conventional PCR tests (1.5 % TAE agarose gel; 6 µl PCR product per well). All primer sets showed moderate-to-strong amplification from the *P. invadens* strains (strongest with Nuc1, weakest with IRE1), and no detectable amplification in the target size range from the challenge DNAs (very weak amplification of much larger fragments was seen in *P./N. aesturina* with Nuc1 and Nuc2, and *P./N. pemaquidensis* with Nuc2; Supplementary Fig. 1).

Then, primer sensitivity was tested using a dilution series of DNA extracted from a known number of *P. invadens* cells. Briefly, 2 ml of sterile seawater was pipetted onto a 7-d old monoxenic culture of *P. invadens* strain SMB-60 and gently scraped using a sterile scraper. The concentration of amoeba cells in this suspension was estimated using a hemocytometer and DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). DNA aliquots were prepared to represent 128, 64, 32, 16, 8, 4, 2, 1, 0.5 and 0.25 amoeba cells per PCR reaction and tested using the PCR protocol and gel electrophoresis conditions described above. Under these conditions, amplification was detectable visually down to 1 cell per reaction with Nuc1, 2 cells per reaction with Nuc2 and 32 cells per reaction with IRE1.

Based on these experiments, primer set Nuc1 was selected for further optimization, due to its better amplification intensity and sensitivity characteristics. Set Nuc1 consists of forward primer ParNssu905F (5`-TCA AGG CAA GCA TAA TTT TAA TG-3`) and reverse primer ParNssu1094R (5`-CAA GAA TTT CAC CTC TGA CAT C-3`), and amplifies a 190 bp region of the *P. invadens* nuclear SSU rDNA.

2) Optimisation of PCR protocols for different DNA samples

General optimization of PCR using Nuc1 primer set.

A general PCR protocol for amplification from *P. invadens* DNA using the Nuc1 primer set was optimized for primer annealing temperature and $MgCl_2$ concentration. The test DNA was from *P. invadens* isolates SMB-60 or PP-2012 (see above). Annealing temperatures tested were 50.0 °C, 52.4 °C, 53.8 °C, 56.7 °C, 58.1 °C, 59.6 °C, and 62.0 °C, with other PCR conditions retained as described in the previous section. The $MgCl_2$ concentrations tested were 2.8 mM, 2.4 mM, 2.0 mM, 1.7 mM, and 1.5 mM, with other PCR conditions as described above. Based on this, the annealing temperature of 58 °C was retained, as 58.1 °C was the highest tested temperature that reliably yielded strong amplification, while $MgCl_2$ concentration had little effect on amplification and 1.5 mM was retained to preserve stringency.

Sample-specific PCR optimization

To optimize PCR detection of *P. invadens* with the Nuc1 primer set in each of the different sample types examined, we separately tested $MgCl_2$ and primer concentration dilution series for the following DNA samples: Four radial nerve DNA samples from symptomatic sea urchins (*Strongylocentrotus droebachiensis*) from St Margarets Bay, plus two sediment DNA samples and four seawater samples taken from experimental aquaria housing diseased sea urchins (and thus *P. invadens*-positive). DNA was extracted as described in the main Methods. $MgCl_2$ was tested at final concentrations of 2.0 mM, 1.5 mM, 1.0 mM, and 0.5 mM (radial nerve DNA) or 2.5 mM, 2.0 mM, 1.5 mM, 1.0 mM, and 0.5 mM (sediment and seawater DNA), with all other PCR conditions as above. Primer concentrations tested were: 1

μM, 0.75 μM, 0.50 μM, and 0.25 μM of each primer, with other PCR conditions as above. Optimal $MgCl_2$ concentrations (lowest concentrations with no obvious loss of PCR efficiency) were 1.5 mM (radial nerve DNA) or 2.0 mM (sediment and seawater DNA). All primer concentrations other than 0.25 μM showed similar amplification levels for all DNAs, thus 0.5 μM was selected for all sample types.

Subsequently, we selected the amounts of sample DNA (i.e. dilutions) to be used for PCR reactions. DNA samples from four radial nerves and four sediment samples taken from St Margarets Bay during the disease outbreak of November 2014, and four seawater DNA samples sampled during October 2015 were examined. PCRs using primer set Nuc1 were conducted as described in the main Methods, except for DNA dilutions. A dilution series was examined that consisted of 1/1 (i.e. undiluted), 1/2, 1/4, and 1/8 of the elution from each DNA extraction. Parallel reactions were run for all combinations of samples × dilutions using the 'universal' SSU rDNA primer set of 514F (5`- TCT GGT GCC AGC AGC CGC GG-3`) and 1055R (5`-CGG CCA TGC ACC ACC-3`) with the same PCR protocol. All radial nerve DNA samples supported amplification using the Nuc1 primer set at 1/1 and 1/2 dilution at least, with evidence of inhibition (i.e. lower band intensity) at 1/1 in two cases (one corroborated by the universal primer set). All sediment DNA samples supported amplification using the Nuc1 primer set at 1/1 and 1/2 dilution at least, with (weak) evidence of inhibition at 1/1 in two cases (one marginally corroborated by the universal primer set). All seawater DNA samples supported amplification using the Nuc1 primer set at 1/1 and 1/2 dilution at least, but with unambiguously weaker (and difficult-to-detect) amplification at 1/2 relative to 1/1. On this basis 1/2 dilutions of radial nerve and sediment DNA samples were used for subsequent PCR detection, and 1/1 (i.e. undiluted) seawater DNA samples.

Sample-specific PCR sensitivity

The sensitivity of detecting *P. invadens* in environmental DNA samples using conventional PCR was inferred by adding DNA from known quantities of *P. invadens* cells to DNA extracted from radial nerve, sediment, and seawater samples (experiment 1) or, as a control, adding live *P. invadens* cells to such samples immediately prior to DNA extraction (experiment 2). Triplicate radial nerve, sediment, and seawater samples were used for both experiments.

In experiment 1, DNA extracted from radial nerve, sediment, and seawater that tested negative for *P. invadens* using PCR was spiked with *P. invadens* DNA extracted from a known number of cultured amoeba cells, corresponding to 16, 8, 4, 2, and 1 *P. invadens cells* per PCR reaction (plus an unspiked control). The *P. invadens* DNA was prepared as described earlier (see above: 'design and testing of *P. invadens*-specific primers'). Appropriate dilutions were then prepared from this *P. invadens* DNA stock, and added to radial nerve, sediment, and seawater DNA in a 1:1 ratio.

In experiment 2, radial nerve, sediment, and seawater expected to be *P. invadens*-negative were spiked with a known number of cultured *P. invadens* cells immediately prior to DNA extraction or immediately prior to filtration for seawater samples. To do this, an amoeba stock solution with a known *P. invadens* cell concentration was obtained as above (see 'Design and testing of *P. invadens*-specific primers'), and was added to radial nerve, sediment, and seawater samples immediately prior to DNA extraction (see main Methods) to yield a final concentration of 32 (radial nerve) or 16 (sediment and seawater) cell equivalents μl^{-1} of DNA solution. The DNA was diluted to 1/2 the original DNA concentration (i.e. to 16 or 8 cell equivalents per PCR reaction), and PCR was performed as reported in the main Methods. Products from experiment 1 and experiment 2 with the same estimated number of

cells per PCR reaction were then compared visually to identify possible biases in sensitivity estimates due to spiking with *P. invadens* DNA after DNA extraction.

In experiment 1, clear PCR amplification was detected down to 1 cell / PCR reaction in all radial nerve DNAs. Comparison of amplification in experiment 2 showed stronger amplification when the cells were co-extracted with the DNA sample. From this we inferred a very conservative detection limit of 1 cell/PCR for radial nerve DNA (= 10 cells / mg tissue using the DNA extraction and PCR protocols employed here). Later comparisons of PCR and qPCR results from the same samples suggests that the true detection limit could be an order of magnitude lower (see Results). For sediment DNA, PCR amplification was consistently detectable down to 2 cells / PCR reaction (experiment 1), again with stronger amplification in experiment 2, indicating a conservative detection limit of 2 cells/PCR in sediment DNA (= 40 cells / g sediment using our DNA extraction and PCR protocols). For seawater DNA, PCR amplification was consistently detected down to 1 cell/ PCR reaction in experiment 1, but amplification was much lower in experiment 2, suggesting loss of *Paramoeba* material during the sample collection process (for example, due to cell damage during filtration). This suggest that a conservative detection limit of 1 cell/PCR in seawater DNA inferred in experiment 1 (= 100 cells / L seawater using our DNA extraction and PCR protocols), could significantly overestimate sensitivity in real-world samples, depending on the form that *P. invadens* takes in the water column (see also Discussion, and qPCR optimization methods).

Confirmation of amplicon identity

Amplicons obtained from radial nerve, sediment, and seawater DNA samples using *P. invadens*-specific primer set Nuc1 (ParNssu905F and ParNssu1094R) were sequenced to confirm the specificity of the designed primers. PCR products from two positive radial nerve samples, four positive sediment samples, and three positive seawater samples (selected haphazardly from among positive samples from 2014 and 2015) were cloned using the pGEM-T Easy Vector System (Promega, Madison, WI, USA), or the StrataClone PCR Cloning Kit (Agilent Technologies, La Jolla, CA, USA) as per the manufacturer's protocols. 5 – 17 positive colonies per sample were identified by a PCR screen using vector primers, and 1 – 10 clones per sample were selected for sequencing. PCR products from the selected colonies were purified using the Qiagen MiniPrep Kit (Qiagen) and Sanger-sequenced (Genome Quebec). In total, 2 radial nerve clones, 25 sediment clones, and 8 seawater clones of the expected size were unidirectionally sequenced from the two, four, and three DNA samples, respectively. All were clearly derived from *P. invadens*, showing 98.3-100% sequence identity (i.e. a maximum of two mismatches) to one or other of the known sequence variants of the nuclear SSU rDNA of *P. invadens* (see Feehan et al. 2013).

Culturing versus molecular identification of *P. invadens* infection

A total of 19 sea urchins collected during and immediately after the disease outbreak in November 2014 were subjected to attempts to cultivate *P. invadens* from radial nerve tissue in parallel to DNA extraction (see main Methods). The goal was to allow comparison of using cultivation (e.g. Feehan et al. 2013) to detect *P. invadens* in sea urchins exposed to natural conditions versus detection using PCR. Logistical constraints on sampling and the timing of the disease outbreak resulted in unbalanced sampling, such that only 4 of these sea urchins proved to be PCR-positive for *P. invadens* (all sampled 6 November 2014, during the outbreak). 3 of these 4 urchins also yielded cultures of *P. invadens*. By contrast, none of the 15 PCR-negative sea urchins yielded *P. invadens* by culturing.

While limited, this data is consistent with PCR being no less sensitive (and probably more sensitive) than cultivation for detecting *P. invadens* in radial nerve samples. This conforms

with our observations: i) that conventional PCR using Nuc1 can detect *P. invadens* at low abundance (conservatively 1 cell per PCR reaction; see above), which would correspond to only a few tens of cells per radial nerve, and ii) evidence from our qPCR experiments that *P. invadens* load in infected urchins is usually very low (see Results). The detection limit using cultivation is unknown but it is plausible that cultivation would be unreliable at such low loads, especially given the possibility that some/most/all of the *P. invadens* DNA detected by PCR/qPCR may be from cells that are moribund or dead (e.g. due to immune activity by the sea urchin), and thus uncultivable.

3) Quantitative real-time PCR (qPCR)

Optimization of qPCR protocols.

As described in the main methods, quantitative real-time PCR (qPCR) using the Nuc1 primer set was performed using the StepOnePlus Real-Time PCR System (Applied Biosystems), with reactions carried out in 20 µl volumes containing 10 µl Power SYBR Green PCR Master Mix (Applied Biosystems), 900 nM of each primer, and different amounts of bovine serum albumin (BSA) depending on the sample. Here we briefly describe how the primer concentrations, BSA levels and DNA sample dilutions used were selected.

The primer concentrations used (900 nM / 900 nM) were selected in a preliminary qPCR experiment that tested a 3 x 3 matrix with primer concentrations of 50 nM, 300 nM, or 900 nM for each primer. DNA from cultured *P. invadens* SMB-60 (50 cells / reaction) was used as template, with triplication for each treatment (except the 300 nM / 300 nM condition, which was a duplicate due to a failed run). Optimal primer concentrations were determined based on critical threshold (C_t) value and reaction efficiency (target ≥ 90 %), estimated using LineRegPCR 11.0 (Ramakers et al. 2003). Primer combinations with one or both primers at 50 nM concentration had low efficiency (< 90 %) and C_t values 3 – 8 cycles higher than other combinations. Combinations containing one or both primers at 300 nM or 900 nM all showed average efficiency >90 % and similar average C_t (20.17 – 20.55). The highest efficiency (96.12 %) was obtained with both primers at a concentration of 900 nM.

The BSA levels and dilutions of sample DNA were co-optimized for each sample type examined in the study. Treatments of 1.2 µg, 2.5 µg, and 5.0 µg BSA per reaction were tested. Template DNA (added as 1 µL aliquots) was tested at final concentrations of 1/4, 1/2, and 1/1 the original DNA concentration (sediment DNA was also tested at final concentrations of 1/16 and 1/8). Radial nerve, sediment, and seawater DNA samples that had tested negative for *P. invadens* by conventional PCR were spiked with DNA from cultured *P. invadens* (10 cells / reaction), and used as template DNA for these experiments. Based on a target efficiency ≥ 90 %, radial nerve DNA was suitable for qPCR at all DNA and BSA treatments tested. Average efficiency mostly increased with increasing BSA level, though tended to be anomalously high with 5.0 µg BSA (≥100.9 %; Supplementary Table 3). The C_t values for radial nerve samples 19.55 – 21.93, with a positive correlation between DNA sample concentration and C_t (Pearson's r > 0.95). A template DNA concentration of 1/2 and BSA concentration of 2.5 µg were selected as the best compromise for detection/quantitation of *P. invadens* in radial nerve DNA (corresponding to an in-principle lower detection limit of 0.05 cells mg-1 tissue with our calibrations). Seawater DNA was unsuitable for qPCR in all reactions containing 5.0 µg of BSA and all reactions with a DNA concentration of 1/1 (Supplementary Table 3). Other combinations were suitable for qPCR (average efficiency 90.2 – 91.9 %; C_t 22.92 – 23.38). A DNA sample concentration of 1/2 was selected to maximize the number of cells in a single reaction (in-principle detection limit 0.5 cells L-1 seawater), then a BSA concentration 1.2 µg / reaction was selected as giving (marginally) the best measured average efficiency (90.5%). Sediment DNA was unsuitable for qPCR at all

DNA and BSA levels tests, except when background DNA was diluted 1/16 and 2.5 μg BSA (average efficiency 90.5%, C_t 23.91). At natural abundances, *P. invadens* was not reliably detected in sediment at DNA concentrations of 1/8 (by conventional PCR – see 'Sample-specific PCR optimization' above). Thus, qPCR using the specifications described appeared unsuitable to detect *P. invadens* in sediment DNA, and was not pursued further.

Reliability of cell number estimates using qPCR

For logistical reasons, the calibration curves for qPCR were generated by adding sample-specific background DNA to DNA extracted from known numbers of *P. invadens* cells. Cell numbers may be under- or over-estimated with this method if there is a difference in results obtained when spiking DNA before vs. after extraction (e.g. if the recovery of cells differs when spiking with *P. invadens* before DNA extraction). To identify the reliability of cell number estimates obtained by qPCR, radial nerve and seawater samples expected to be *P. invadens*-negative were spiked with cultured *P. invadens* cells prior to DNA extraction as described previously (see 'experiment 2' from 'Sample-specific PCR sensitivity', above), to yield DNA samples containing DNA from 16 (radial nerve) or 8 (seawater) *P. invadens* cells per reaction (each treatment performed in triplicate; plus no-spike controls). Reaction conditions and generation of calibration curves were as described in the main methods. The slope, R^2, and efficiency values were -3.524, 0.995, 92.2 % and -3.57, 0.982, 90.6 % for radial nerve and seawater calibration curves, respectively. Estimates of *P. invadens* cell number in radial nerve samples spiked with 16 cells/reaction of *P. invadens* prior to DNA extraction ranged from 24.4 – 29.6 cells/reaction, suggesting a modest overestimation (factor of ~1.5-2) of true cell number using our protocols. Estimates of *P. invadens* cell number in seawater samples spiked prior to DNA extraction with 8 cells/reaction ranged from 0.62 – 0.92 cells. This indicates a significant underestimation, of around an order of magnitude, when inferring numbers of *P. invadens* cells in seawater samples, given the filtration and DNA extraction protocols followed here. These results were consistent with the qualitative observations with conventional PCR (see 'Sample-specific PCR sensitivity', above)

Genomic copy number of SSU rRNA gene in *P. invadens*

To help assess the plausibility of observations of very low cell abundances in our experimental qPCR results, we estimated the SSU rDNA copy number in the *P. invadens* nuclear genome. The 190 bp fragment of *P. invadens* SSU rDNA amplified by the Nuc1 primer set was cloned using the pGEM-T Easy Vector System (Promega, Madison, WI, USA). Recombinant plasmid DNA was purified using the Qiagen MiniPrep Kit (Qiagen). After purification, a conventional PCR using the Nuc1 primers was conducted using the plasmid DNA as a template (general protocol; see 'Design and testing of *P. invadens*-specific primers' above), the DNA concentration in the resultant product was determined using Nano-drop (Thermo Fisher Scientific), and from this, the number of 190 bp construct copies in the PCR product was calculated. A calibration curve was generated using a 10-fold dilution series ranging from 10^5 to 10^1 copies per PCR, run in triplicate. DNA from 20 and 50 cells of cultured *P. invadens* strain SP was run against these copy number calibration curves (in duplicate) using the qPCR protocol described in the main Methods (with 1.2 μg BSA / reaction). The slope, R^2, and efficiency values were -3.392, 1.0, and 97.168 %, respectively. Averaged estimates derived from 20 cell equivalents and 50 cell equivalents were 474 and 471 copies of the nuclear SSU rDNA sequence per cell respectively. This estimate of ~475 SSU rDNA copies per cell demonstrates the plausibility of detecting *P. invadens* DNA by qPCR at abundances around 10^{-2} cell equivalents per reaction (see Results), even though it is several times lower than similarly derived estimates for *P./N. perurans* (2900 copies per cell; Bridle et al. 2010).

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Table S1. *Paramoeba invadens* isolates used to sequence ITS regions of the nuclear genome and parasome nuclear genome, and to test the specificity of designed primers. Isolates were obtained from sea urchins during disease outbreaks in 2011 and 2012 at various locations in Nova Scotia (see Fig. 1).

Year Isolated	Isolate Name	Location	Depth (m)	Source
2011	SMB-8	St. Margaret's Bay	8	Feehan et al. 2013
2011	SMB-60	St. Margaret's Bay	60	Feehan et al. 2013
2011	SPS	Sandy Point, Shelburne	8	Feehan et al. 2013
2011	SP	Splitnose Point	8	Feehan et al. 2013
2012	PP-2012	Point Pleasant	3-6	C. Feehan unpublished data
2012	SP-2012	Splitnose Point	8	C. Feehan unpublished data
2012	GI-2012	Gravel Island	8	C. Feehan unpublished data

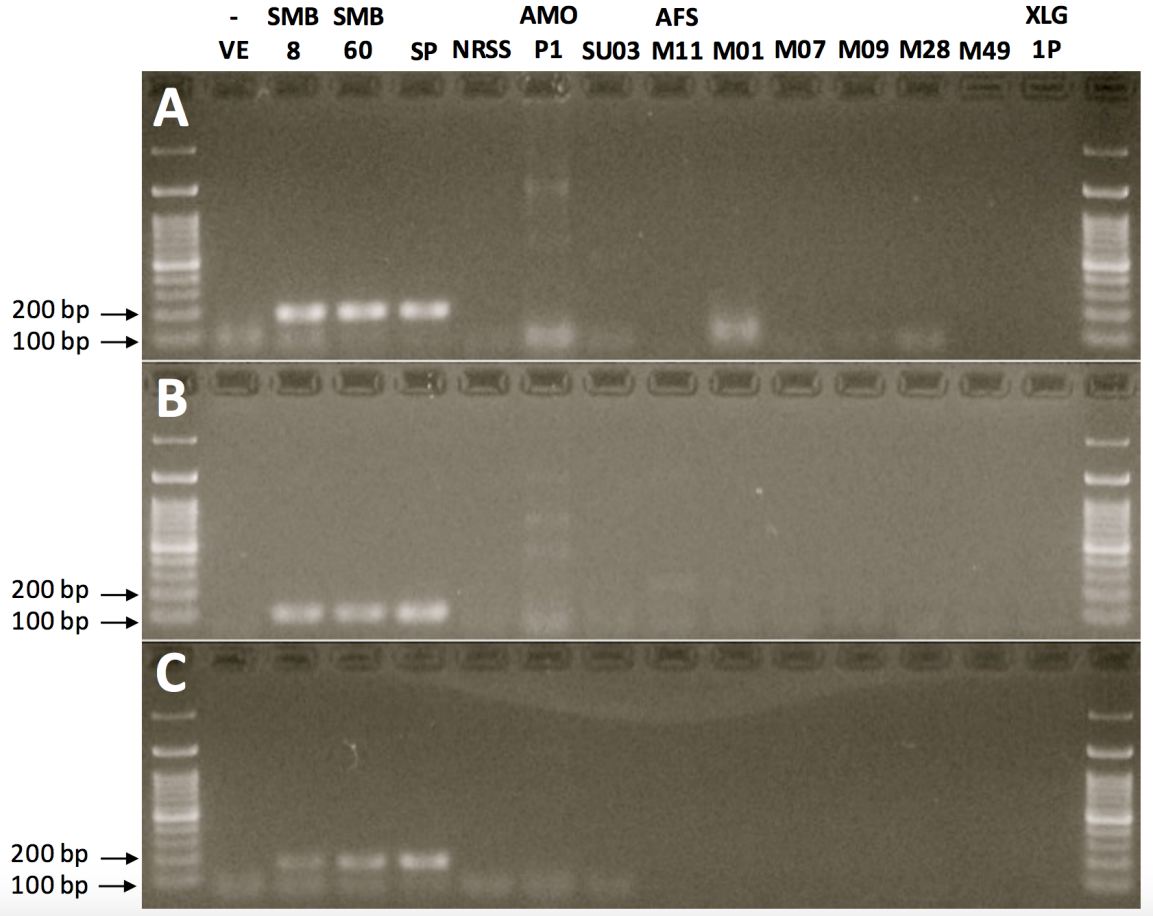
Table S2. Details of primer sets designed against the *Paramoeba invadens* nuclear SSU rRNA gene and parasome SSU rRNA gene. Numbers embedded in primer names indicate the binding position of the primer, from its 5` end, in the relevant SSU rRNA sequence from *P. invadens* isolate SP (Genbank accession numbers KC790385.1 and KC790388.1).

Primer Set	Genome type	Amplicon size (bp)	Primer Name	Primer Direction	Primer sequence
Nuc1	*P. invadens* nuclear	190	ParNssu904F	Forward	5`-TCAAGGCAAGC ATAATTTTAATG-3`
			ParNssu1093R	Reverse	5`-CAAGAATTTCA CCTCTGACATC-3`
Nuc2	*P. invadens* nuclear	107	ParNssu1570F	Forward	5`-GGAAGGGGGTT TCTTTGTT-3`
			ParNssu1676R	Reverse	5`-ATTATCGGAAT CRTCAARCGT-3`
IRE1	Parasome nuclear	172	ParPssu878F	Forward	5`-AGAGGTGAAAT TCTGTGATCTTC-3`
			ParPssu1049R	Reverse	5`-CCCATCGGAAT AAGATGCTA-3`

Table S3. Optimization of DNA concentration and bovine serum albumin (BSA) levels in quantitative real-time PCR (qPCR) experiments for detection/quantification of *Paramoeba invadens* using the Nuc1 primer set. Key results (efficiency; critical threshold - C_t) are shown for different dilutions of *P. invadens*-negative radial nerve and seawater DNA samples spiked with DNA from 10 *P. invadens* cells. DNA concentration is based on the addition of 1 µl DNA solution to each reaction. Averages for radial nerve samples were calculated from triplicate reactions and averages for seawater samples were calculated from duplicate reactions. Similar experiments with sediment DNA showed average efficiencies << 90% under all conditions unless DNA was over-diluted (1/16), and are not shown.

DNA Type	DNA Concentrations	BSA Concentration (mg / reaction)	Average Efficiency (%)	Average Ct Value
Radial Nerve	1/4	1.2	96.3	19.58
		2.5	101.4	19.55
		5	100.9	19.95
	1/2	1.2	96.5	20.26
		2.5	100.3	20.12
		5	108.2	20.47
	1/1	1.2	93.9	21.67
		2.5	99.4	21.93
		5	102.2	21.78
Seawater	1/4	1.2	91.9	23.14
		2.5	91.2	22.92
		5	89.3	23.14
	1/2	1.2	90.5	23.38
		2.5	90.2	23.2
		5	88.9	23.44
	1/1	1.2	86.7	22.86
		2.5	87.7	21.81
		5	85.3	22.79

Fig. S1. Testing the specificity of *P. invadens*-specific primer sets: A) Nuc1 (ParNssu905F & ParNssu1094R; 190 bp fragment) designed to a region of the *Paramoeba* nuclear SSU rRNA gene, B) Nuc2 (ParNssu1571F & ParNssu1677R; 107 bp fragment) designed to a region of the *Paramoeba* nuclear SSU rRNA gene, and C) IRE1 (ParPssu1017F & ParPssu1188R; 172 bp fragment) designed to a region of the parasome nuclear SSU rRNA gene. Primer sets were tested against 3 strains of *P. invadens* [SMB8; SMB60; SP], 4 strains representing 3 other *Paramoeba* species [NRSS: *P. branchiphila*; AMOP1: *P. aestuarina*; SU03 & AFSM11: two strains of *P. pemaquidensis*], and various other marine eukaryote species [M01: *Ancyromonas sigmoides*; M07: *Neobodo designis*; M09: *Cyranomonas australis*; M28: *Developayella* sp.; M49: *Notosolenus urceolatus*; XLG1P: *Percolomonas cosmopolitus* 'group 1'].



Supplement 2: Predicted urchin mortality based on hurricane activity

Table S4. Hurricanes passing between 35°N and the Atlantic coast of Nova Scotia, and between 55 and 70°W, from 2009 to 2017. Date is when a storm was closest to coast. Wind (W) is maximum sustained wind speed of a storm at the closest distance (D); T_m is mean temperature at 8 m depth in St. Margarets Bay in the 2-wk period following a storm. P_m is probability of a disease outbreak causing mass mortality of *Strongylocentrotus droebachiensis* following a storm, as predicted by the Scheibling & Lauzon-Guay (2010) logistic regression model: $P_m = 1/(1 + e^{-z})$, $z = -14.352 + 0.082W - 0.069D^2 + 4.966T$, where T is a dummy variable for a temperature threshold based on T_m (T = 1 if $T_m > 12.2$°C, T = 0 if $T_m \leq 12.2$°C).

Year	Storm	Date	Wind (km h^{-1})	Distance (km)	T_m (°C)	P_m
2014	Arthur	5 Jul	111	113	6.9	0.005
	Bertha	6 Aug	83	493	13.6	0.014
	Cristobal	28 Aug	130	647	12.0	0.166
	Gonzalo	19 Oct	148	418	13.8	0.824
2015	Joaquin	6 Oct	117	804	14.2	0.014
	Kate	11 Nov	105	943	9.5	0.000
2016	Nicole	14 Oct	113	1110	14.6	0.000
2017	Gert	17 Aug	167	580	11.7	0.048
	Jose	21 Sep	93	493	17.8	0.051
	Maria	29 Sep	102	647	17.1	0.014
	Lee	29 Sep	120	418	17.1	0.001