

***Tetracapsuloides bryosalmonae* abundance in river water**

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Table S1. Results from the qPCR primer optimisation using different volume combinations of each primer (F = forward and R = reverse) using a sample of a single *Tetracapsuloides bryosalmonae* spore. The results presented are the mean quantification cycle (C_q) values and respective standard deviation (SD).

F primer volume (μl or μM)	R primer volume (μl or μM)	C_q value \pm SD
0.05	0.05	38.27 \pm 0.69
0.05	0.50	33.95 \pm 0.81
0.05	1.00	33.75 \pm 2.68
0.50	0.05	31.78 \pm 2.27
0.50	0.50	29.49 \pm 2.37
0.50	1.00	28.54 \pm 2.79
1.00	0.05	33.71 \pm 3.41
1.00	0.50	29.51 \pm 2.11
1.00	1.00	28.15 \pm 3.01

Table S2. Results from the qPCR inhibition test using an internal positive control (IPC) using large-volume (LV) and small-volume (SV) samples. The results presented are the mean quantification cycle (C_q) values and respective standard deviation (SD) of reactions without eDNA (IPC control) and with eDNA spiked (LV and SV samples).

Sample		C_q value \pm SD
IPC control		16.34 \pm 0.407
LV	Avon Root 3 Week 1	16.80 \pm 0.262
	Dun Root 3 Week 4	16.54 \pm 0.012
	Itchen Root 3 Week 3	16.81 \pm 0.172
SV	Avon Root 3 Week 1	18.27 \pm 0.289
	Dun Root 2 Week 2	18.70 \pm 0.525
	Dun Root 2 Week 3	18.23 \pm 0.004
	Itchen Root 2 Week 3	16.83 \pm 0.078

Table S3. Results from the *Tetracapsuloides bryosalmonae* qPCR sensitivity test. A 1:10 serial dilution of two positive samples (the three negative samples tested were negative at any dilution) was tested. The results presented are the mean quantification cycle (C_q) values and respective standard deviation (SD) in PCR reactions with and without the addition of bovine serum albumin (BSA). There was no significant difference between C_q values obtained with and without BSA (Paired t -test: $t = -0.837$, $df = 5$, $P = 0.441$). SV = small-volume, LV = large-volume.

Sample	Dilution factor	C_q value \pm SD	
		No BSA	BSA
Itchen SV Root 3 Week 5	0	21.81 \pm 7.88	21.12 \pm 0.24
	1:10	25.88 \pm 0.18	25.67 \pm 0.34
	1:100	31.00 \pm 0.20	30.76 \pm 0.92
	1:1,000	-	-
	1:10,000	-	30.58 \pm 0.87
	1:100,000	-	-
Avon LV Root 3 Week 4	0	26.13 \pm 0.12	26.82 \pm 0.09
	1:10	27.46 \pm 0.77	27.64 \pm 0.16
	1:100	-	30.38 \pm 1.23
	1:1,000	-	31.29 \pm 0.03
	1:10,000	-	-
	1:100,000	-	-

Table S4. Inter-assay variation (reproducibility) of the *Tetracapsuloides bryosalmonae* assay using all seven reference standard points. Calculated mean concentration, standard deviation (SD) and coefficient of variation (CV) are given (n = 4 runs).

Expected standard concentration (mol l⁻¹)	Calculated mean concentration (mol l⁻¹)	SD	CV (%)
1e-12	1.14e-12	9.56e-14	8.40
1e-13	1.12e-13	1.07e-14	9.60
1e-14	8.92e-15	5.28e-16	5.91
1e-15	7.87e-16	4.15e-17	5.28
1e-16	1.02e-16	9.03e-18	8.88
1e-17	9.25e-18	9.28e-19	10.03
1e-18	1.26e-18	1.11e-19	8.78

Table S5. *Tetracapsuloides bryosalmonae* abundance according to sampling method, river, root and trip. Abundance is presented as mean quantification cycle (C_q) values and respective standard deviation (SD). SV = small-volume, LV = large-volume.

Sampling method	River	Root	C_q value \pm SD				
			Week 1	Week 2	Week 3	Week 4	Week 5
SV	Avon	1	-	-	29.21 \pm 0.48	-	-
		2	29.80 \pm 0.18	-	29.35 \pm 0.16	-	30.22 \pm 0.13
		3	-	30.58 \pm 0.61	29.51 \pm 1.22	29.97 \pm 0.51	-
	Dun	1	-	-	-	28.27 \pm 0.30	-
		2	-	30.50 \pm 0.59	30.54 \pm 0.05	-	-
		3	29.63 \pm 0.32	-	-	-	-
	Itchen	1	-	-	-	-	30.27 \pm 0.37
		2	-	-	-	-	-
		3	-	-	-	-	25.63 \pm 0.27
LV	Avon	3	29.38 \pm 0.50	27.51 \pm 0.37	29.03 \pm 0.42	26.17 \pm 0.16	29.35 \pm 0.53
	Dun	3	-	30.62 \pm 0.05	30.13 \pm 0.35	29.20 \pm 0.13	28.28 \pm 0.64
	Itchen	3	-	-	-	30.75 \pm 0.61	28.48 \pm 0.43

Figure S1. Standard curve derived from a 1:10 serial dilution of an 18S *Tetracapsuloides bryosalmonae* DNA template showing parasite DNA concentration vs. quantification cycle (C_q) value. Each standard was run in duplicate. The curve is significantly linear over seven references from $1e-18$ to $1e-12$ mol l^{-1} .

