Monitoring of *Vibrio harveyi* quorum sensing activity in real-time during infection of shrimp larvae – Supplementary information

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1. Calculation of the luminescence of bacteria associated with a single larva

The luminescence of bacteria associated with a single larva is calculated by subtracting the luminescence of bacteria in 200 µL water from that of the bacteria in a well containing 200 µL water plus one larva. See table below for examples of larvae challenged to the WT at the 6h and 12h time points. Based on this, one can calculate that the luminescence of shrimp-associated bacteria has increased 34-fold at the 12h time point.

Time point	Water plus larva (cps)	Water without larva (cps)	Bacteria associated with larva only (cps)
6h	60 ± 19	43 ± 3	17 ± 19
12h	1177 ± 115	591 ± 10	586 ± 115
			Ratio = 34

2. Calculation of the difference in bioluminescence on a per-cell basis between planktonic and shrimp-associated vibrios at the 12h time point

For cells in the shrimp culture water:
Luminescence = 591 cps for 200 µL
Cell number = 0.2 mL x 2.6 10^6 CFU/mL
= 5.1 10^5 CFU
Luminescence per cell = 1.15 10^{-3} cps/CFU

For shrimp-associated cells:
Luminescence = 586 cps/larva (see higher)
Cell number = 2.6 10^3 CFU/larva
Luminescence per cell = 2.25 10^{-1} cps/CFU

Ratio = 2.25 10^{-1} / 1.15 10^{-3}
= 195

Hence, there was approximately a 200-fold difference in luminescence on a per-cell basis between planktonic and shrimp-associated bacteria.

3. Calculation of the bacterial cell density in the brine shrimp gut

The gut volume was determined by Gunasekara et al. (2010, J Exp Mar Biol Ecol 393:78-82) to be in the order of 10^{-6} µm³. The number of shrimp-associated bacteria was 2.6 10^3. Hence, assuming that most shrimp-associated bacteria were located in the gut, the density of the bacteria in the gut was 2.6 10^3 CFU / 10^{-6} µm³ = 2.6 10^9 CFU/mL.