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# Quorum Sensing Regulation of Virulence Gene Expression in *Vibrio harveyi* during its Interaction with Marine Diatom *Skeletonema marinoi*

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## Abstract

**Communication between species from different kingdoms may be as important as intra-kingdom communication. It has recently been confirmed that co-existing bacteria and phytoplankton in aquatic ecosystems do cross-talk. This study examined the signs of possible cross signalling between *V. harveyi,* one of the predominant bacterial species of the marine ecosystem and a dominant diatom species, *S.marinoi,* to understand communication over species borders. It is known that *V.harveyi* employ quorum sensing for cell-to-cell communication, bioluminescence (*luxR*), and the regulation of the virulence gene (*vhp, chiA*). Former studies have also shown, this kind of interactions being disrupted by compounds secreted by a few algal species existing in the aquatic ecosystem. We investigated the QS communication by quantifying the expression levels of virulence regulator *luxR* and virulence factors metalloprotease (*vhp*) and chitinase (*chiA*) in four different *V. harveyi* strains grown in the presence of *S. marinoi* strain. Results obtained in this study indicate that quorum sensing was activated in strains of *V. harveyi* analysed but did not regulate the expressions of *vhp* and *chiA* virulence factors. This observation suggests that the existence of *S. marinoi* did not interfere with the QS behavior of *V. harveyi* and its interaction with marine diatom; it may be due to the commensalism relationship.**

**Keywords:** Quorum sensing (QS), diatom-bacteria relationship, Skeletonema, harveyi clade vibrios, virulence gene expression

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## INTRODUCTION

In aquatic ecosystems, algae and bacteria coexist together, and there are many interactions between them, which are of particular interest to marine ecology.[1-4] Such interactions influence the physiology of these organisms, alter ecological and biogeochemical events, and shape the diversity of microbial communities in the ecosystem.[5] Quorum sensing (QS) is one such interaction through which density-dependent communication between bacterial cells takes place through the formation, release and acquisition of small signal molecules[1,3,6,7] to control the expression of certain genes.[1] *Vibrio harveyi* secretes three different molecules of the quorum sensing [Harveyi Autoinducer 1; Autoinducer 2 and Cholerae Autoinducer 1] that function synergistically in the regulation of gene expression.[8-11] With the aid of these QS molecules, bacteria induce changes in downstream gene regulation and control many biological functions beneficial for bacterial fitness and virulence.[1,12]

Further, QS also modulates gene expression, essential for bacterial cell development, survival and pathogenicity.[13,14] The successful colonization and invasion of host by the pathogenic bacteria known to be mediated by QS.[13,15-18] Therefore, QS has numerous influences on bacterial physiology and function as well as promotes contact-mediated communication with their symbionts.[7]

Research on species communication has been focusing primarily on bacteria, with inadequate focus on algae.[19] Cross-communication between bacteria and algae has only recently become an area of scientific interest. That inter-kingdom signaling takes place has been confirmed just over the last decade.[4,17,20] To date, three forms of inter-kingdom signaling molecules/ mechanisms have been identified. Bacterial QS molecule is one such compound involved in cell to cell signalling, similarly, algal pheromones released by diatoms has similar structures and functionality as QS molecules. This similarity may facilitate their use in signalling over species borders.[21,22] Many eukaryotes can actively interfere with the QS behaviour of the bacteria by generating compounds that mimic the bacterium's own signals.[2,23,24] Given the potential of these molecules to interfere with QS allows for the biological control of several bacterial pathogens, as there is a correlation between QS and their pathogenesis.[25,26]

Algae and bacterial biofilms have been found to interact via QS[27,28] and marine macro-algae prevent bacterial colonization by disrupting QS.[15,29,30] The potential of *Delisea pulchra*, a marine macroalga, to limit bacterial colonization by releasing halogenated furanones has been extensively explored.[15,31,32] The red macro-alga *Ahnfeltiopsis flabelliformis* has been found to have three new AHL antagonists.[33] It inhibits AHL-regulated QS in a dose-dependent manner through a mixture of compounds.[34] Likewise, few minor QS-interfering activity has been detected in macro-algae from the families *Caulerpaceae, Rhodomelaceae* and *Galaxauraceae*.[35] Different marine bacteria have also been reported to produce QS-inhibitory metabolites.[36-38]

Unlike macro-algae and marine bacteria interaction, little is known about the impact of secondary metabolites generated by micro-algae on bacterial QS. *Chlamydomonas reinhardtii* is the first micro-algae reported to develop QS-interfering compounds.[39,40] Micro-algal interference of QS regulated virulence of many aquaculture pathogens, might constitute a new bio-control strategy for bacterial disease in pisciculture. In a study by Natrah et al[30] the effect of different strains of micro-algae, commonly used in aquaculture, from the families *Chlorophyceae, Trebouxiophyceae, Eustigmatophyceae, Prymnesiophyceae, Bacillariophyceae* and *Coscinodiscophyceae* was evaluated on QS-regulated gene expression. Different strains of bacteria, including *Vibrio harveyi* an important pathogen of aquaculture was used to evaluate the ability of these micro-algae to interfere with AHL QS.

The members of the harveyi clade vibrios have been studied for virulence factors necessary for the induction of pathogenesis in susceptible host.[41,42] And these virulence factors are under the control of several regulatory systems, including QS and the transmembrane transcriptional regulator ToxR.[43,44]

Virulence factors and its processes in many pathogens, including *Vibrio* species, are frequently studied using simplified model systems. Environmental conditions and mechanisms that

occur *in vivo* might be distinct from those that occur in these simplified systems.[45] Therefore, it is important to also carry out experiments under conditions that are similar to what the bacteria experience during interaction. So far, most studies reporting on virulence regulation in *Vibrio* species were performed mainly with macroalgae including green algae. Very few focuses on *Vibrio's* interaction with diatoms.[30,46] Several groups of diatoms are the major source of atmospheric oxygen responsible for 20-30% of all carbon fixations on the planet. In this study, species of *Skeletonema*, a globally widespread microalgal genus[47,48] was used to study the QS regulation of the virulence gene expression of Harveyi clade vibrios in the presence of a dominant presence phytoplankton species, *S. marinoi*. The expression level of virulence regulator *luxR* gene and of the virulence factors metalloprotease (*vhp*) gene and chitinase (*chiA*) gene in a group of Harveyi clade vibrios was quantified. QS mediated regulation of selected virulence genes were analyzed in four different strains of *V. harveyi* (one non-virulent, one strongly virulent and 2 different wild type strains) during co-culturing with axenic cultures of a marine diatom *S. marinoi.*

## MATERIALS AND METHODS

The transcription levels of quorum sensing regulator (*luxR*) and the genes for virulence such as metalloprotease (*vhp*) and chitinase (*chiA*) in a group of harveyi clade vibrios (Wild type; BB120 and LMG21363 two QS mutants; QS+ i.e quorum sensing maximally active mutant and QS- i.e quorum sensing inactive mutant) was quantified during co-culturing with axenic cultures of *S. marinoi*.

### Growing *S. marinoi* and harveyi clade vibrios

Wild type strains BB120 and LMG21363, and quorum sensing mutants JAF 483 (Quorum sensing maximum activity mutant- QS+) and JAF 548 (Quorum sensing inactive mutant- QS-) belonging to the harveyi clade were used in the study. The bacterial strains used in this experiment were obtained from the Laboratory of Aquaculture and Artemia Reference Center, Ghent University, Belgium (Table 1). Single colonies of each bacterial strains were grown in marine broth (Himedia, India) at 28°C under continuous agitation of 150 rpm and cells were subsequently harvested at a concentration of 104 and 107 CFU $ml^{-1}$.

*S. marinoi*, strain HakH from GUMACC (University of Gothenburg Marine Algal Culture Collection) was made axenic following the protocol in65. *S. marinoi*, and subsequently *S. marinoi* together with *Vibrio*, were grown in f/2 medium,[66] 30 PSU, with a light intensity of 50 µmol photon $m^{-2}$ $s^{-1}$ and a 12:12h light dark cycle. The temperature maintained was 26-27°C during the day and 22-23°C during night.

Before starting the experiment, growth curves were established for *S. marinoi* and the four *vibrio* strains. Further, to confirm whether there is an impediment of algal growth by bacteria or vice versa was checked in another experiment. BB120, wild type strain of *V. harveyi*, in different concentrations ($10^2$-$10^7$ CFU $ml^{-1}$) was added to a fixed density of *S. marinoi*. At the time of inoculation, both *Vibrio* and *Skeletonema* were in exponential growth phase. After 24 and 48 h of incubation, subsamples were extracted and *Skeletonema* cell abundance was estimated by using a Sedgewick Rafter chamber and an inverted Axiovert Zeiss microscope. The microscopic counts were compared to the growth curve under controlled conditions. Growth status of bacteria, on the other hand, was assessed by plate count on agar plates.

Specificity of the *V. harveyi* primers (*rpoA, luxR, vhp, chiA*) used in the study were checked in order to confirm the absence of primer binding sites in *Skeletonema* genome by running PCR reactions using *Skeletonema* DNA. *Skeletonema* genomic DNA was extracted using the PCI method.[67] All the PCR reactions was performed as per the previously described protocol in which BB120 *vibrio* strain was used as a positive control.[67] The amplified amplicons were then electrophoresed on a 1.5% agarose gel, stained with ethidium bromide and the results observed using a gel documentation system.

### Experimental set up

Two density ranges of *S. marinoi* (1-2 x$10^4$ and 4-8 x$10^5$ cells $ml^{-1}$) and two concentrations ($10^4$ and $10^7$ CFU $ml^{-1}$) of *V. harveyi* were selected for the gene expression study. To each of the two *S. marinoi* densities, *Vibrio* strains were added at low and high concentration. All combinations (Table 2)

were run in triplicates. *Vibrio* cells were harvested and washed in sterile distilled water before adding to the *S. marinoi* cultures and the cell numbers were determined spectrophotometrically (Shimadzu UV-1601, Kyoto, Japan) at 600 nm. Both *S. marinoi* and *V. harveyi* cells were grown together for a period of 48 hours and cells were harvested, at different time points, for RNA extraction.

**RNA extraction and Reverse transcription**

For quantification of gene expression, bacterial cells were collected at 0, 12, 24, 36 and 48 h after inoculation of different isolates of *Vibrio* strains to the *S. marinoi* cultures. Sampled cells were washed with autoclaved seawater and suspended in bacterial RNA protective reagent (Qiagen, Germany) according to the manufacturer's guidelines. The pellets were stored at -80°C until RNA extraction. Before the RNA extraction, tissue debris were homogenized using Qiashredder (Qiagen, Germany) and RNA was extracted using the commercially available RNA extraction Kit (Qiagen, Germany) according to the producer's instructions. Extracts were subsequently treated with DNase I, according to the manufacturer's protocol to remove the contaminating DNA. RNA quantity was checked spectrophotometrically. The extracted RNA was converted to cDNA using the reverse transcriptase (Fermentas International Inc., Canada) as per the standard protocol.

**Real-time PCR**

Real-time PCR was used to examine the expression levels of *luxR*, *vhp* (metalloprotease) and *chiA* (chitinase) genes. Specific primers were used for these genes (Table 3) at a concentration of 200 nM. The *rpoA* (RNA polymerase A subunit)

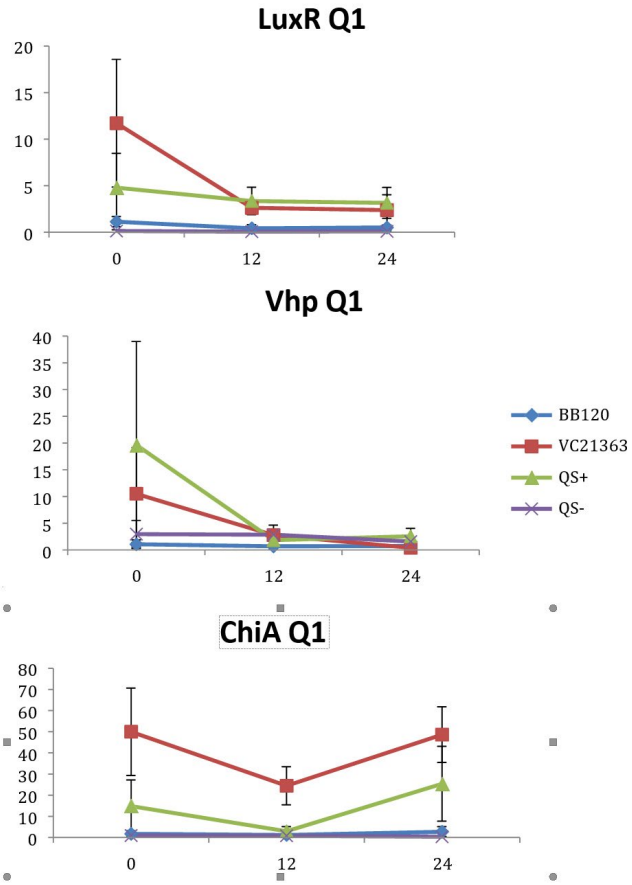


**Fig. 1.** Relative gene expression levels of *luxR, vhp* and *chiA* over a 24h period in treatment with $8x10^5$ cells ml of *Skeletonema* and *Vibrio* $10^7$ cells $ml^{-1}$. Y-axis= Relative expression to *RpoA,* X-axis= Time elapsed (hr). Error bars represent standard error of mean.

gene, which is considered to be a housekeeping gene, was used as a control.[68] Real-time PCR was performed in StepOne™ Real-Time PCR System (Applied Biosystems, USA) according to the protocol followed by Ruwandeepika et al.[55] Data acquisition was performed at the end of each extension step and analysis was performed using 2-ΔΔCt method.[69]

**Statistical analysis of gene expression data**

Three-way ANOVA (SPSS statistics v. 23, IBM) was used to determine whether there was a three-way interaction between the three independent variables: *V. harveyi* strain, *V. harveyi* cell concentration and *S. marinoi* cell concentration in our experiment. The net effect of such an interaction was tested separately on *luxR*, *vhp* and *chiA* gene expression. *V. harveyi* cell concentration (high vs. low), *S. marinoi* cell concentration (high vs. low), *V. harveyi* strains (QS+, QS-, BB120 and LMG21363) were treated as fixed factors. Significance levels were set at $p < 0.05$. The maximum expression values of the

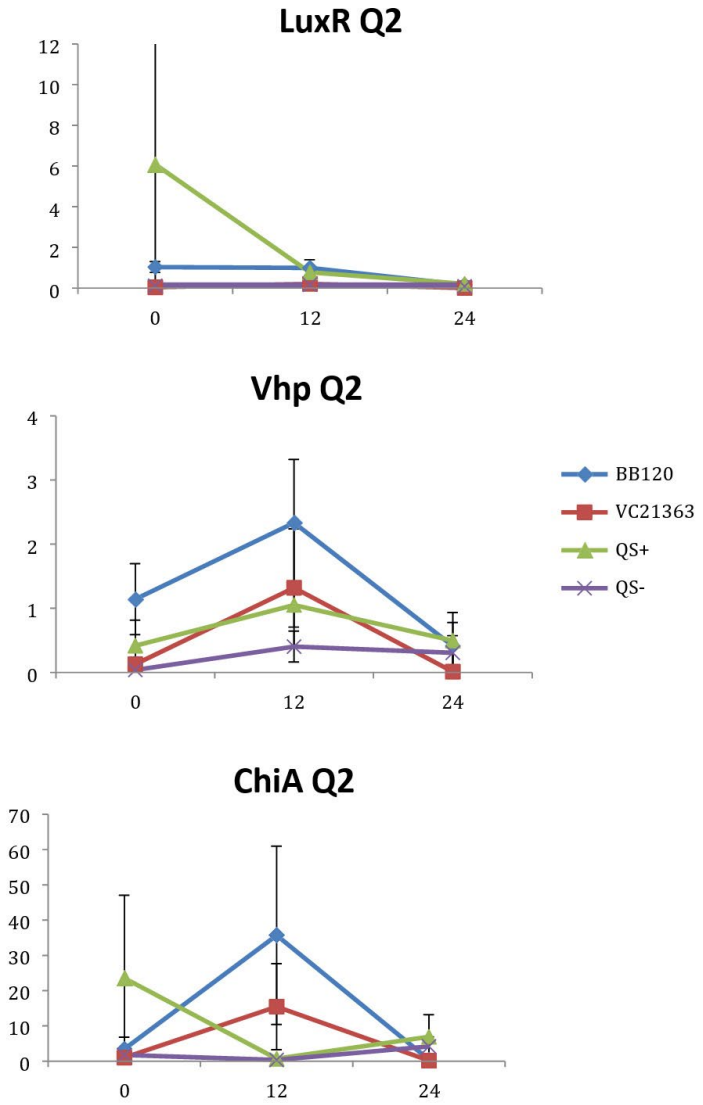


**Fig. 2.** Relative gene expression levels of *luxR, vhp* and *chiA* over a 24h period in treatment with *Skeletonema* $4x10^5$ cells per ml and *Vibrio* $=10^4$ cells per ml). Y-axis= Relative expression to *RpoA,* X-axis= Time elapsed (hr). Error bars represent standard error of mean.

genes in focus were used as response variables in these tests, and before running the analyses; these were checked for homogeneity of variance and normality. Since all the three response variables were found to have heterogeneous variances, attempts were made to transform the data. Data for *vhp* successfully passed Levene's test of variance upon log transformation, but *luxR* and *chiA* values failed to pass this test even after log transformation or square-root transformation. However, as the interaction output (p-value of the ANOVA) for the gene *vhp* was found to be similar for both transformed and non-transformed data, we decided to use the ANOVA approach for all three genes using non-transformed data in all analyses so as to avoid any discrepancies in comparisons.

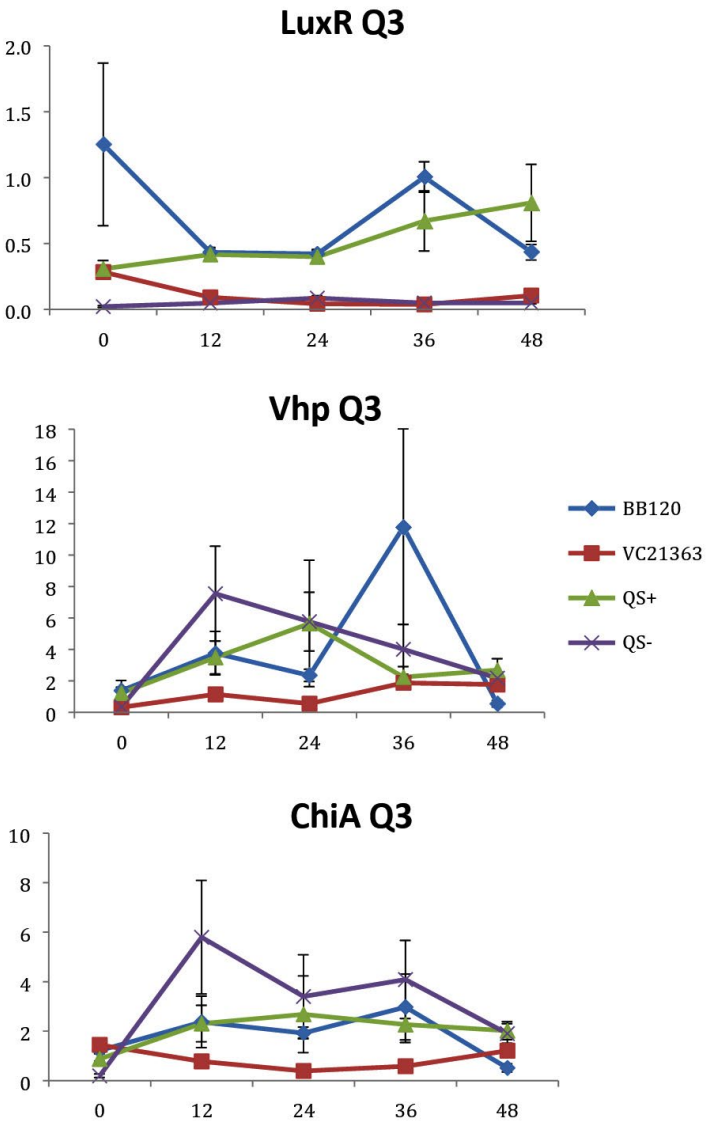


**Fig. 3.** Relative gene expression levels of *luxR, vhp* and *chiA* over a 48h period in treatment *Skeletonema* $2x10^4$ cells per ml and *Vibrio* $=10^7$ cells per ml. Y-axis= Relative expression to *RpoA,* X-axis= Time elapsed (hr). Error bars represent standard error of mean.

## RESULTS

### Controls for growth and primer specificity

A control experiment was performed to test if the bacteria impeded *S. marinoi* growth and vice versa. This experiment confirmed that the microscopic counts of *S. marinoi* in the presence of *Vibrio* corresponded to the growth curve without bacteria present, and plate counts of *Vibrio* wild type strain BB120 in the presence of *S. marinoi* were similar to the counts in the absence of the diatom. It was, therefore, concluded that neither of the two inhibited each other's growth.

A specificity test of the PCR primers used for amplifying *rpoA*, *luxR*, *vhp*, *chiA* genes in *Vibrio* BB120 genomic DNA and DNA from axenic *S. marinoi*, as templates for PCR, showed no bands for *S. marinoi* when PCR products were resolved on an agarose gel. The absence of bands for *S. marinoi* confirmed the bacterial specificity of these primers.

### Gene expression

Relative gene expression varied over the temporal scale of the experiment for all three genes, *luxR, vhp and chiA*, and in particular for

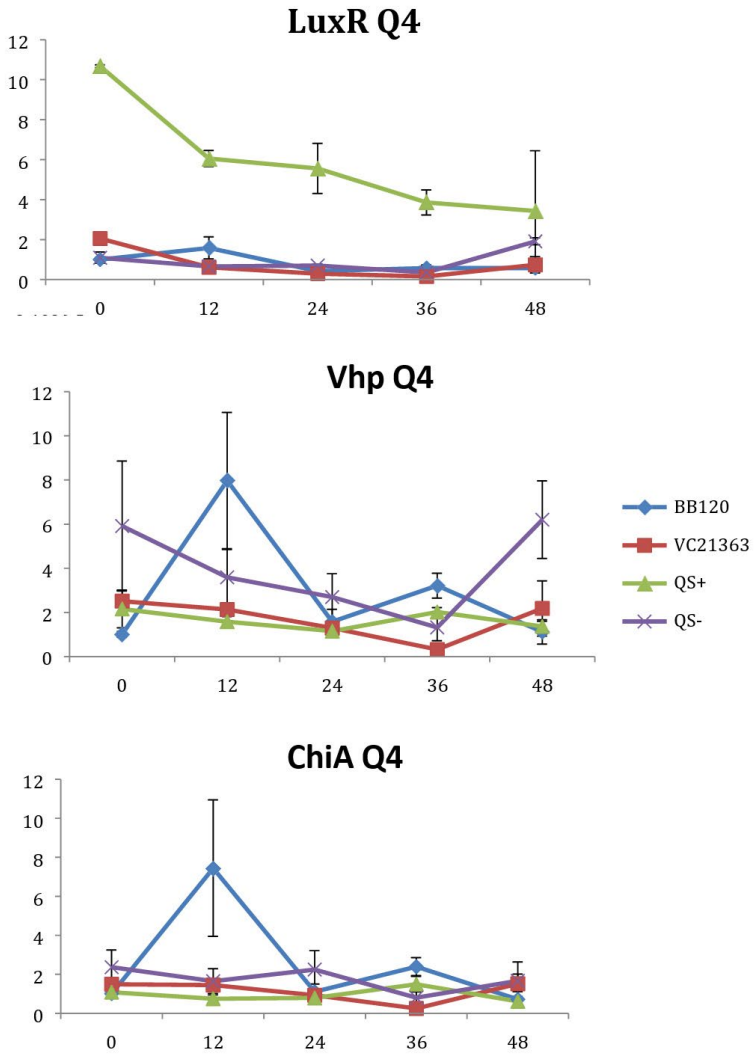


**Fig. 4.** Relative gene expression levels of *luxR, vhp* and *chiA* over a 48h period in treatment *Skeletonema marinoi*=1x10$^4$ cells ml$^{-1}$, and *Vibrio* =10$^4$ cells ml$^{-1}$. Y-axis= Relative expression to *RpoA*, X-axis= Time elapsed (hr). Error bars represent standard error of mean.

QS+ and the BB120 strains of *V. harveyi* (Fig. 1-4). Indeed, a few values were exceptionally high (see Fig. 1-2, Q1 and Q2). To compare the effects of the combination of the three different treatment factors (high and low *Skeletonema* concentration, high and low *Vibrio* concentration, and strain of *Vibrio*; QS+, QS-, BB120 and LMG21363) we applied a three-way ANOVA for each of the three genes. Essentially, a three-way ANOVA primarily looks for interactions among the three factors affecting the gene expression. With no interaction, gene expression is to some extent consistent for different levels for at least one of the factors (*Vibrio* strain), while with a significant three-way interaction the effects are strongly idiosyncratic for the various combinations of concentration levels.

We found a statistically significant three-way interaction between the factors *V. harveyi* strain, *V. harveyi* concentration and *S. marinoi* cell concentration for the *luxR* gene ($F(3,23) = 5.483$, $p = 0.005$). This means that the *luxR* gene expression was statistically different among all the four *Vibrio* strains, $F(3,23) = 7.270$, $p = 0.001$. To disentangle the different effects of the different strains, we continued with pairwise comparisons (Table 4), and found that the expression of *luxR* was statistically different between the QS- and QS+ strains ($p = 0.003$) and between the QS- and BB120 strains ($p = 0.002$), with the QS- strain having lower expression than the other two strains for all combinations of *Skeletonema* and *Vibrio* concentrations (Fig. 1-4). However, *luxR* expression in QS- strain was not found to be statistically different from its expression in strain LMG21362 ($p = 0.751$). So, *luxR* expression being higher and statistically different indicates that quorum sensing was initiated in strains QS+ and BB120, though the wild type strain LMG21362 showed relatively low *luxR* expression. In QS- strain, *luxR* expression level remained constant.

There was no clear difference in the expression of gene *vhp*, in the *Vibrio* strains. That is, we found no statistically significant three-way interaction between *V. harveyi* strains, *V. harveyi* concentration and *S. marinoi* cell concentration for

**Table 1.** Wild type and quorum sensing mutants of *V. harveyi* used in this study

| Strain | Genotype | References |
|---|---|---|
| BB120 | Marine isolate from which strains JAF483 and JAF548 are derived | (Bassler et al)[70] |
| JAF483 | Encodes LuxO locked in high cell density conformation (QS+) | (Freeman and Bassler)[71] |
| JAF548 | Encodes LuxO locked in low cell density conformation (QS-) | (Freeman and Bassler)[71] |
| LMG21363 | Isolate from the lymphoid organ of diseased shrimp (P. monodon) juveniles | (Soto-Rodriguez et al)[72] |

**Table 2.** Concentration of *S. marinoi* and *V. harveyi* used in this experiment

| Treatment | *S. marinoi* concen. (cells $ml^{-1}$) | Vibrio concen. (CFU $ml^{-1}$) |
|---|---|---|
| Q1 | $8x10^5$ | $10^7$ |
| Q2 | $4x10^5$ | $10^4$ |
| Q3 | $2x10^4$ | $10^7$ |
| Q4 | $1x10^4$ | $10^4$ |

**Table 3.** Primers used for PCR and real-time PCR amplification

| Gene | Primer Sequence (5'-3') | Amplicon size (bp) | References |
|---|---|---|---|
| *luxR* | F: TCAAGATTGCAAAGAGACCTCG<br>R: AGCAAACACTTCAAGAGCGA | 84 | (Defoirdt et al)[68] |
| metalloprotease (*vhp*) | F: CTGAACGACGCCCATTATTT<br>R: CGCTGACACATCAAGGCTAA | 201 | (Ruwandeepika et al)[55] |
| Chitinase A (*chiA*) | F: GGAAGATGGCGTGATTGACT<br>R: GGCATCAATTTCCCAAGAGA | 232 | (Defoirdt et al)[59] |
| RNA Polymerase subunit (*rpoA*) | F: CGTAGCTGAAGGCAAAGATGA<br>R: AAGCTGGAACATAACCACGA | 197 | (Defoirdt et al)[68] |

this gene (F (3,23) = 2.305, p = 0.104). Thus, *Vibrio* strains had no significant effect on the *vhp* gene's expression level, F (3,23) = 2.382, p = 0.096. This was further confirmed when pairwise comparisons were applied (Table 4), as these showed that in strain QS+ gene *vhp* did not express differently from strains QS- (p = 0.493), BB120 (p = 0.080) or LMG21363 (p = 0.783). Similarly, the expression levels of gene *chiA* did not differ significantly among the four *Vibrio* strains, as shown by the non-significant three-way interaction between *V. harveyi* strain, *V. harveyi* concentration and *S. marinoi* cell concentration for this gene F (3,24) = 1.760, p = 0.182). The different *Vibrio* strains showed no significant effect on the expression of *chiA*, F (3,24) = 1.743, p = 0.185, and this was also obvious from the pairwise comparisons (Table 4) showing that in QS+ strain, gene *chiA* did not express differently than in QS- strains (p = 0.844), BB120 (p = 0.073) or VC21363 (p = 0.563). This suggests that even though quorum sensing was activated in strains QS+, BB120, and LMG21363, it did not change the expression levels of *vhp* and *chiA* in the presence of the algae.

## DISCUSSION

According to previous studies (*in vivo* and in vitro), *luxR* gene is activated in the presence of sufficient amount of signal molecules[49-51] and the activation of *luxR* gene is an indication of the activation of quorum sensing in Harveyi clade. In *V. harveyi*, *luxR* directly activates the lux operon,[52] whereas majority of other QS-regulated genes and some virulence factors appear to be indirectly controlled by *luxR*.[53]

**Table 4.** Pairwise comparison (p-value) of gene expression (*luxR, vhp, chiA*) between different *Vibrio* strains (BB120, LMG21363, QS+, QS-)

| Gene | Strain | | p-value |
|---|---|---|---|
| *luxR* | QS- | BB120 | 0.002 |
| | | LMG21363 | 0.751 |
| | QS+ | | 0.003 |
| *vhp* | QS+ | BB120 | 0.080 |
| | | LMG21363 | 0.783 |
| | QS- | | 0.493 |
| *chiA* | QS+ | BB120 | 0.073 |
| | | LMG21363 | 0.563 |
| | QS- | | 0.844 |

Metalloprotease (*vhp*) and chitinase (*chiA*) genes are two such virulence factors. QS has been shown to regulate metalloprotease expression in *V. harveyi* positively,[54,55] *V. cholerae* and other marine vibrios as well.[56–58] QS regulation of chitinase has been seen in *V. harveyi*[59] with a negative regulation on chitinase A gene expression.

In our study, gene *luxR* was activated in all the strains. However, the expression in the QS maximally activated (QS+) and wild type strain (BB120) was higher and significantly different from its expression in the QS- strain. This confirmed the activation of quorum sensing in these strains, inferring that *S. marinoi* did not produce any QS interfering signals. *LuxR* gene expression in strain LMG21363 (wild type), however, was not seen to be significantly different from its expression in strain QS-. There is a possibility of time-based lag between the presence of both autoinducers and the *luxR* gene expression of in LMG21363. As suggested by Mok et al,[54] an extended presence of both autoinducers is required to stimulate the expression of bioluminescence in this pathogen. So, despite QS being initiated in strain LMG21363, the *luxR* signal wasn't strong enough to be significantly different from its expression in QS inactivated strain (QS-).

*In vitro* and *in vivo* studies of *V. harveyi* show that while metalloprotease (*vhp*) is positively regulated by *luxR*,[51,54] chitinase gene (*chiA*) is negatively regulated.[59] As per our observations, even though QS was activated in strains QS+ and BB120, it did not regulate the expression levels of *vhp* and *chiA*. The expression of these genes was not significantly different in these strains compared to quorum sensing inhibited strain QS-. One possible explanation for this result is that these virulence factors were not expressed as they would be as part of *Vibrio* species's pathogenesis or defense mechanism. However, this response of *V. harveyi* might be specific for this particular *S. marinoi* strain. The process of interaction of *V. harveyi* is still not fully understood and may well be different in different strains or populations of *S. marinoi* as the response of the *V. harveyi* strain has been shown to be host-strain specific.[60] *vhp* gene response in *V. harveyi* has been studied only during the infection of brine shrimp larvae.[51,55] Studies on *V. harveyi* clade infection of crustaceans, molluscs and fish have investigated responses

at physiological level.[61-63] The expression levels of genes coding for virulence factors and/or QS regulated genes among these organisms is yet to be studied.

Moreover, different genes may be involved and expressed at different stages of the infection. So, the virulence genes included in our experiment may not have been activated until after 48 hours, unlike the *V. harveyi*-brine shrimp system where 48 hours of infection observed a consequent virulence gene response.[51] Running our experiment longer than 48 hours might have seen regulation by *luxR* in a different manner at some later point. However, based on the gene expression recorded during the first 48 hours of exposure, with different concentration combinations of *Vibrio* and *Skeletonema*, it seems that the *Vibrio* strains BB120, LMG21363 and JAF483 do not use this *S. marinoi* strain in a potential host pathogen relationship, but rather form a in commensalism relationship. This would eliminate the possibility of using *S. marinoi* as a potential bio-control agent against aquaculture pathogens. Another possibility is that the *luxR* transcriptional regulator levels were not sufficiently high to allow expression of the virulence factors.[50] This, perhaps, can be the case as the expression of the quorum sensing-regulated genes is proportional to the levels of the signal molecules.[64]

Indeed, we may be looking for the needle in the haystack, as the quorum sensing regulation is fundamentally complex, involving different virulence factors with tentatively specific roles, and involving an essentially unknown time-dimension in relation to the stage of the infection and production and activation of virulence factors. Finally, whether or not different virulence genes are responsible for infection or interaction with different hosts remains yet another challenging question.

Our study shows how bacterial communication functions in the presence of a diatom. It adds on to the existing knowledge on inter-kingdom signaling, highlighting the quorum sensing regulation in *V. harveyi* clade in the presence of a diatom species. It paves the way for future studies investigating the communication and signaling between bacteria and diatoms.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

GK applied the methodology and carried out the experiments. BKK and TD performed the data analysis and did the validation. TD did the interpretation work. BKK performed experiment supervision and wrote the first draft. HDR performed formal analysis and edited the draft. INK conceptualized and administered the project, performed final review and edited the manuscript. All listed authors have read and approved the final manuscript for publication.

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## DATA AVAILABILITY

The datasets generated during the current study are available from the corresponding author on reasonable request.

## ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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