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Starvation of the bacterium *Vibrio atlanticus* induces simultaneous attacks on the dinoflagellate *Alexandrium pacificum*

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eLife Assessment

This **important** study **convincingly** shows that Vibrio bacteria act as predators of ecologically significant algae that contribute to harmful blooms in the lab and in their natural habitat, and that predation is induced by starvation. The authors suggest a working model that can be the basis for future work on this system. The study will be very impactful to those interested in the diversity of microbial predator-prey interactions and controlling toxic algal bloom.

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Abstract

Phytoplankton serve as a source of nutrients for bacteria in the marine environment. The interactions between algae and bacteria are known to include mutualism, commensalism, competition or antagonism. This occurs in the microenvironment surrounding phytoplankton cells, the phycosphere. An interface rich in nutrients and organic molecules exuded by the cell. Here, based on *in situ* observation and on an *in vitro* interaction study, we report on a novel form of starvation-induced hunting that the cells of selected *Vibrio* species exert on dinoflagellates. The results showed that *Vibrio atlanticus* was capable of attacking and killing the dinoflagellate *Alexandrium pacificum* ACT03. Briefly, the observed mechanism of algal-killing consists of first, the 'immobilization stage' involving the secretion of algicidal metabolites that disrupt the flagella of the algae. The 'attack stage' when *Vibrios* simultaneously surrounds algal cells at high density for a brief period without invading them. Finally, the 'killing stage' induces the lysis and consumption of the dinoflagellates.

By using a combination of biochemical, proteomic, molecular and fluorescence microscopy approaches, we showed that this relationship is not related to the decomposition of algal organic matter, *Vibrio* quorum sensing pathways, toxicity of the algae or pathogenicity of the bacterium but is conditioned by nutrient stress, iron availability and link to the iron-vibrioferrin transport system of *V. atlanticus*. This is the first evidence of a new mechanism that could to be involved in regulating Alexandrium spp. blooms and giving Vibrio a competitive advantage in obtaining

nutrients from the environment. The interaction model we propose here suggests that Vibrio could play a role in regulating the proliferation of Alexandrium spp., giving it a competitive advantage in obtaining nutrients from the environment.

Introduction

Harmful algal blooms (HABs) have experienced an increase in their occurrence, intensity, and geographical distribution on a global scale, resulting in adverse environmental, health, and socioeconomic impacts (Marampouti et al., 2021). HABs have a considerable impact on human health as a result of direct exposure to volatile toxins or by toxic seafood consumption (Burkholder et al., 2018). From an ecological point of view, the expansion of HABs can result in the erosion of biodiversity, because they cause massive mortality of marine species and they are generally monospecific in nature (Chai et al., 2020). In coastal areas, understanding the biological interactions that control toxic algal blooms is therefore a major ecological challenge. Among HAB-causing organisms, a number of *Alexandrium* species have been placed on the list of invasive Mediterranean species. Among them, *Alexandrium pacificum* is a flagellated eukaryotic unicellular organism that together with *Alexandrium tamarense* and *Alexandrium fundyense* form the "Alexandrium tamarense" complex responsible for paralytic shellfish poisoning worldwide (Hadjadji et al., 2020). Since 1998, *A. pacificum* (former *A. catenella*) was monitored by the French phytoplankton observation and monitoring network (Rephy) in the Thau lagoon (French Mediterranean) because it produces paralytic shellfish toxins (PSTs) resulting in paralytic shellfish poisoning (PSP) syndrome.

Laania (Laanaia et al., 2013) showed that in Thau lagoon, a water temperature around 20°C for several days and organic and inorganic nutrients in sufficient concentrations are parameters favoring the development of *A. pacificum*, whose massive blooms occur in autumn. Algal blooms are seasonal events resulting in a rapid increase in the concentration of a species of algae in an aquatic environment.

Depending on the species of phytoplankton, tolerance to physicochemical parameters varies, which influences when blooms occur (Leblad et al., 2020). Interestingly, although the collapse of phytoplankton blooms has been previously attributed to viruses (Pal et al., 2020), some ecological studies have suggested an important role of algicidal bacteria (Su et al., 2007; Wang et al., 2010). Among them several are belonging to the Vibrio genus (Li et al., 2014; Wang et al., 2020).

Vibrio (class γ-proteobacteria) are common microorganisms in marine systems worldwide (Baker-Austin et al., 2017; Mavian et al., 2020), where they are important components of the food chain, particularly in biodegradation, nutrient regeneration and biogeochemical cycles (Oberbeckmann et al., 2012). Vibrio is one of the most studied bacterial taxa due to their ubiquity in coastal marine systems and their capacity to cause infections in humans and animals, leading sometimes to epizootic or zoonotic epidemics (LeRoux et al., 2015; Mavian et al., 2020). *Vibrio* are extremely adaptable to their environment (Johnson, 2013). The main factors influencing their occurrence and distribution in water are temperature, salinity, nutrient availability (Wang et al., 2020), multiple strategies such as biofilm formation on biotic and abiotic surfaces (Espinoza-Vergara et al., 2020), or interactions with a multitude of other organisms such as eukaryotic predators (Drebes Dörr and Blokesch, 2020) or plankton (Lopez-Joven et al., 2018) are used by *Vibrio* in the environment. There is also evidence that global climate change has increased *Vibrio*-associated illnesses affecting humans and animals (Brumfield et al., 2021; Muhling et al., 2017). However, the drivers and dynamics of *Vibrio* survival and propagation in the marine environment are not yet fully understood.

A substantial number of research articles have highlighted the potential of γ-proteobacteria to exert algicidal activity against dinoflagellates, supporting the hypothesis that γ-proteobacteria such as *Vibrio* play a role in the control of algae blooms *in situ* (Coyne et al., 2022). However, the mechanisms behind *Vibrio*-driven algal lysis in the environment remain to be elucidated. Particularly, it is unclear how in the water column, algicidal compounds secreted by bacteria can concentrate around the algae to exert their lytic effect.

This study aims to describe observations made in the natural environment between Vibrio bacteria and Alexandrium algal blooms, and to determine in vitro the main factors involved in this relationship. Using a combination of biochemical, proteomic, molecular, and fluorescence microscopy approaches, we explored the role of algal toxicity, bacterial pathogenicity and the quorum sensing pathway on this relationship and showed the important role of nutrient stress and the iron uptake pathway in this unique Vibrio/Alexandrium interaction.

Methods

Quantification of *Alexandrium* algae and *Vibrio* bacteria in the environment by Qpcr

Seawater samples were collected in the Thau Lagoon (southern France, a shallow Mediterranean ecosystem open to the sea (Abadie et al., 1999), during spring and autumn 2015. Briefly, samples were collected from the subsurface (-50 cm) near an oyster table at a phytoplankton surveillance site (part of the REPHY network, N 43°26.058' and E 003°39.878'). Once a week during spring and autumn 2015, during field sampling campaigns, 20 L of water was filtered on board through a 180 µm pore-size nylon membrane. At the laboratory, according to Lopez-Joven et al. (Lopez-Joven et al., 2018) seawater was fractionated into two size classes as follows: 2 L of the above filtrate was filtered through a 0.8 µm pore-size polycarbonate Whatman Nuclepore membrane to obtain organisms in the 0.8–180 µm range corresponding to plankton-associated *Vibrio* and living *Alexandrium* forms. Then, the filtrate from the 0.8 µm membrane was filtered again through a 0.2 µm pore-size polycarbonate Whatman Nuclepore membrane until the membrane was saturated. *Alexandrium* cells, ranging from 25 to 40 µm, belong to the microphytoplankton and are therefore retained in the 0.8–180 µm fraction. Any *Vibrio* cells potentially associated with or attached to *Alexandrium* cells will also be retained in this fraction. *Vibrio* cells are approximately 0.5-0.8 µm-thick. The fraction between 0.2 and 0.8 µm therefore includes the free-living *Vibrio*. The bacterial population collected on 0.8-µm-pore-size filters was designated the particle-associated community, and the population on 0.2-µm-pore-size filters was designated as the free-living community. Membranes (in triplicate) were then conserved in 500 µL of 100% EtOH at -20°C. Environmental DNA (eDNA) was extracted from the MF Millipore membrane using the Macherey-Nagel NucleoSpin Tissue Kit and resuspended in 100 µL of water. The samples were then stored at -20°C after eDNA quantity and purity were assessed using a NanoDrop system (NanoDrop Technologies, Wilmington, DE, USA). PCR amplification reactions were done on a Roche LightCycler 480 Real-Time thermocycler (qPHD platform, University of Montpellier, France) using specific primer pairs (Table 3). Typically, the reactions contained 1 µL of template DNA (the DNA concentration for all samples varied from 1 to 40 µg mL^{-1}), 0.5 µL of each primer (3.33 µM) and 4 µL of reaction mixture (SYBR Green Master Mix) in a total volume of 6 µL. The reaction parameters were as follows: 5 minutes at 95°C (initial denaturation) and 40 cycles of 10 s at 95°C (denaturation), 10 s at the corresponding hybridization temperature (Table 3) and 10 s at 72°C (elongation). Melting curve profiles were generated by increasing the temperature from 65°C to 95°C at 0.5°C s^{-1}. Amplification products were analysed using LightCycler software (Roche Diagnostics). *Vibrio spp*. and *A. pacificum* and *A. tamarense* were quantified by constructing calibration curves based on DNA from the *V. atlanticus* LGP32 reference strain (former *V. tasmaniensis* LGP32) and from the *A. pacificum* reference strain (ACT03: *A. catenella* strain isolated form Thau in 2003) and the *A. tamarense* reference strain (ATT07: *A. tamarense* isolated from Thau in 2007) (not shown).

Strains and growth conditions

Vibrio strains

Wild-type and isogenic mutants of *Vibrio atlanticus* LGP32 (Table 1) were used in this study. Deletion-mutants included Δ*luxR*, Δ*luxP*, Δ*luxM* and Δ*pvuB* isogenic strains. The Δ*pvuB* mutant was constructed here by allelic exchange as described previously by Le Roux (Le Roux et al., 2007). We also used *V. atlanticus* LGP32 carrying the pSW3654T-GFP plasmid (Le Roux et al., 2007), hereafter referred to as *V. atlanticus* LGP32-GFP. Bacterial strains were grown at 22 ± 1°C in Zobell medium (0, 38 µM iron (III)). When needed, 25 µg mL^{-1} chloramphenicol (Cm) was added to cultures of *V. atlanticus* LGP32-GFP (Le Roux et al., 2007).

Phytoplancton strains

Non-axenic phytoplankton species (Table 2) were grown in batch culture in enriched natural sea water (ENSW, 6,55 µM iron (III)) with a salinity of 36 practical salinity units (PSU) at 22 ± 1°C under cool white fluorescent illumination (100 µmol photons m^{-2} s^{-1}) and a 12 h:12 h light:dark cycle (Harrison et al., 1980). The algae were used for experiments in their exponential growth phase.

Co-culture assay

For each tested phytoplankton species (Table 2), 2.10^4 cells harvested in their exponential growth phase (doubling time between 5 and 7 days) were placed in 20 mL of ENSW medium in a 50 mL suspension culture flask (Cellstar® PS, Greiner bio-one). After incubation for 24 h at 22 ± 1°C under cool white fluorescent illumination, 40 µL of *Vibrio* strains (Table 1) grown for 12, 36, 60 and 156 h in Zobell medium or in Zobell medium supplemented with Fecl3 (6 µM iron (III)) or with H3BO3 (0.47 mM) or the corresponding culture supernatant were added to the phytoplankton cells (Table 2). After incubation at 22 ± 1°C for 0, 15, 30, 45 and 60 min under cool white fluorescent lights, living, non-swimming, attacked and lysed phytoplankton cells were counted in a sedimentation chamber under an inverted microscope. The number of lysed cells corresponded to phytoplankton cells showing disrupted membranes. Non-swimming algae were not counted as lysed cells. For *Vibrio* analysis, 100 µL of a 1:10 serial dilution mixture in ENSW (from 10^{-2} to 10^{-10}) was plated on *Vibrio* Selective TCBS (thiosulfate-citrate-bile salts-sucrose) agar (in triplicate). After incubation for 24 h at 22 ± 1°C, the number of living *Vibrio* cells was determined by counting colony-forming units (CFUs). The data come from three independent experiments using independent phytoplankton cultures and independent bacterial cultures.

Microscope observations

The dynamic of the interaction between *A. pacificum* ACT03 isolated from the French Thau Lagoon, south of France (Laabir et al., 2011) and *V. atlanticus* LGP32, which is an oyster pathogen isolated from the French Atlantic coast (Gay et al., 2004) and present in the Thau Lagoon (Lopez-Joven et al., 2018) was surveyed. As the *A. pacificum* ACT03 strain (table 2) used in the study is not axenic, there is potential for bacteria other than *V. atlanticus* LGP32 to be present in the experiments. To elucidate the interaction without thoroughly accounting for the non-axenic cells, interaction was observed under a Zeiss Axio upright fluorescence microscope equipped with an AxioCamMRm 2 digital microscope camera using *V. atlanticus* LGP32 tagged with green fluorescent protein (GFP). Lasers were used at excitation wavelength (λex) 488 nm for GFP (emission wavelengths (λem): 505–530 nm) and λex 532 nm for plankton chloroplasts (λem 560–630 nm). Images were taken sequentially to avoid cross-contamination between fluorochromes. Sequences of images were merged during the *Vibrio-Alexandrium* interaction using ZEN 2012 (blue edition) software. Interaction events between *Vibrio* strains and phytoplankton strains were also observed under a Leica TCS SPE confocal laser scanning system connected to a Leica DM 2500 upright microscope camera (Montpellier RIO Imaging Platform, University of Montpellier, France).

Vibrio species	Strains	Virulence for fish or invertebrates	References	Attack *A. pacificum*	Lyse *A. pacificum*
Vibrio atlanticus LGP32	WT	Yes	(Gay et al., 2004)	+	+
//	WT + pSW3654T-GFP	Yes	(Le Roux et al., 2007)	+	+
//	ΔLuxM	ND	Ifremer Institute, France	+	+
//	ΔLuxS	ND	//	+	+
//	ΔLuxR	ND	//	+	+
//	ΔPvuB	ND	This work	-	+
Vibrio tasmaniensis	J5-9	Yes	(Lemire et al., 2015)	+	+
//	LMG20012^T	No	(Thompson et al., 2003)	+	+
Vibrio crassostreae	J2.9	Yes	(Lemire et al., 2015)	+	+
//	J2-8	No	//	+	+
Vibrio fischeri	ES114	ND	(Mandel et al., 2008)	+	+
Vibrio harveyi	ATCC14126	Yes	(Liu et al., 1996)	+	+
Vibrio aestuarianus	janv-32	Yes	(Labreuche et al., 2010)	+	+

Table 1. Ability of Vibrio strains to attack and to lyse *Alexandrium pacificum*.

ND: not determined.

Dinoflagellates species	Strains	Toxicity for human	References	Flagella degraded	Cells Attacked	Cells Lysed
Alexandrium pacificum	ACT03, Thau, France	Yes	(Laabir et al., 2011)	+	+	+
Alexandrium catenella	Bizerte, Tunisia	Yes	(Fertouna-Bellakhal et al., 2015)	+	+	+
//	F3-9F, Tarragona, Spain	ND	//	+	+	+
//	C10-5, Annaba, Algeria	Yes	(Hadjadji et al., 2020)	+	+	+
Alexandrium tamarense	ATT07, Thau, France	No	(Rolland et al., 2012)	+	+	+
Alexandrium spp.	Golf of Tunis, Tunisia	ND	Algal collection university of Montpellier, France	+	+	+
//	Bizerte, Tunisia	ND	//	+	+	+
//	Mediterranean coast, Morocco	ND	//	+	+	+
Prorocentrum lima	PLBZT14, Bizerte, Tunisia	Yes	(Ben-Gharbia et al., 2016)	-	-	-
Coolia monotis	CMBZT14, Bizerte, Tunisia	ND	//	-	-	-
Vulcanodinium rugosum	IFR-VRU-01, Ingril, France	Yes	(Abadie et al., 2015)	-	-	-
Karenia selliformis	Golf of Gabes, Tunisia	ND	Algal collection university of Montpellier, France	-	-	-
Scripsiella trochoidae	Mellah Lagoon, Algeria	-	//	-	-	-
Gyrodinium impudicum	Golf of Tunis, Tunisia	-	//	-	-	-
Amphidium carterae	SAMS, Scotland	ND	SAMS laboratory, Scotland	-	-	-
Gymnodinium catenatum	M'diq Bay, Morocco	+	(Leblad et al., 2020)	+	+	+

Table 2. Ability of *Vibrio Atlanticus* LGP32 to degrade flagella, attack and lyse the targeted dinoflagellates *spp*. commonly found in the Mediterranean Sea.

ND not determined.

Comparative proteomic analysis

Vibrio sampling and protein extraction

V. atlanticus LGP32 was grown for 60 h at 22°C in artificial seawater (high nutrient stress, cond. 1) or 12 h at 22°C in Zobell media (low nutrient stress, cond. 2). After 10 min centrifugation at 8000 rpm, crude protein extracts of *V. atlanticus* LGP32 in each culture condition (triplicates) were obtained by sonication on ice at 20% amplitude for 20 s in 200 µL of ice-cold denaturing buffer (7 M urea, 2 M thiourea, 4% CHAPS in 30 mM Tris-HCl, pH 8.5) and clarified by centrifugation at 2000 x *g*, 15 min, 4°C. The protein concentration of the supernatant was estimated using the 2D Quant Kit (Cytiva™, MERCK) and samples were stored at -80°C until use.

Bi-dimensional gel electrophoresis (2D gel)

Proteins extracts were individually analysed on 2D gel electrophoresis (6 gels per condition each corresponding to different biological replicates). To do so, 100 µg of proteins from each extract was added to rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS, 65 mM DTT) for a total volume of 350 µL. They were then individually loaded onto 17 cm isoelectric focusing strips (Bio-Rad) with a stabilized non-linear pH ranging from 3 to 10. Due to the high complexity of the protein profile in the acidic part (left) of the gel pH 3–10 (Fig. S3), we conducted additional 'close-up' analyses in gels using 17 cm isoelectric focusing strips (Bio-Rad) with a narrower, stabilized pH gradient ranging from 4 to 7. Strips were rehydrated passively for 5 h at 22°C, followed by active rehydration for 14 h under a 50 V current at 22°C (to help large proteins enter the strips).

Thereafter, isoelectrofocusing was carried out using the following programme: 50 V for 1 h, 250 V for 1 h, 8000 V for 1 h and a final step at 8000 V for a total of 140 000 V.h with a slow ramping voltage (quadratic increasing voltage) at each step. Focused proteins were reduced by incubating the strip twice in equilibration buffer (1.5 M Tris, 6 M urea, 2% SDS, 30% glycerol; bromophenol blue, pH 8.8) containing DTT (130 mM) at 55°C. Then, they were alkylated by incubation with equilibration buffer containing iodoacetamide (135 mM) on a rocking agitator (400 rpm) at room temperature protected from light.

Proteins were also separated according to their molecular weight (second dimension) on 12% acrylamide/0.32% piperazine diacrylamide gels run at 25 mA per gel for 30 min followed by 75 mA per gel for 8 h using a Protean II XL system (Bio-Rad). Gels were stained using an MS-compatible silver staining protocol and scanned using a ChemiDoc MP Imaging System (Bio-Rad) associated with Image Lab software version 4.0.1 (Bio-Rad).

Comparative bioinformatics analysis of 2D gels

Twelve gels (six per condition) were selected for comparative analysis on PD-Quest v. 7.4.0 (Bio-Rad) to identify changes in protein abundance between the proteomic profiles of *V. atlanticus* LGP32 cultured in contrasting nutrient conditions (ENSW/Zobell). Spots whose mean intensity across six replicates per strain was two times higher or lower than those from the other strain, with a P <0.01 (Mann-Whitney U-test), were considered significantly different in terms of abundance between the two conditions (quantitative difference). Differentially represented spots were then excised from the gels, destained, trypsin-digested and the obtained peptides were identified by tandem mass spectrometry (MS-MS) using the PISSARO platform facility (University of Rouen, France). To identify protein(s) present in each spot, the obtained peptides were compared with *V. atlanticus* LGP32 reference genome *(https://vibrio.biocyc.org/)*. The genes whose peptides matched strongly were retrieved and used for an BLASTx query against non-redundant databases to determine the protein identity of the best match. A gene was considered as strongly matched when at least two peptides matched the sequence with a coverage of> 6%. Their theoretical isoelectric point (pI) and molecular weights were also calculated using the Expasy server (*https://www.expasy.org/*) to compare them with the location of the spot on the gel. Altogether, these complementary analyses made it possible to characterize the protein identity of each spot with confidence.

Gene expression analysis

Vibrio sampling and RNA extraction

V. atlanticus LGP32 was grown in Zobell media for 36 h and 60 h at 22°C (decline phase of growth, nutrient stress) or for 12 h at 22°C (exponential grow phase, poor nutrient stress). Total RNA was isolated from *V. atlanticus* LGP32 using the standard TRIzol method (Invitrogen Life Technologies SAS, Saint-Aubin, France) and then treated with DNase (Invitrogen) to eliminate genomic DNA contamination. After sodium acetate precipitation, the quantity and quality of the total RNA were determined using a NanoDrop spectrophotometer and agarose gel electrophoresis. Following heat denaturing (70°C for 5 minutes), reverse transcription was performed using 1 µg of RNA prepared with 50 ng μL^{-1} oligo-(dT) 12–18 mer in a 20-µL reaction containing 1 mM dNTPs, 1 unit μL^{-1} RNAseOUT and 200 units μL^{-1} Moloney murine leukaemia virus reverse transcriptase (M-MLV RT) in reverse transcriptase buffer, according to the manufacturer's instructions (Invitrogen Life Technologies SAS, Saint-Aubin, France).

PCR amplification

Amplification reactions were analysed using a Roche LightCycler 480 Real-Time thermocycler (Bio-Environnement platform, University of Perpignan, France). In this study, several PCR primer pairs were designed using Primer3 software (optimal primer size: 20 bases; Tm: 60°C; primer GC%: 50; 2GC clamp and product size range: 150–200 bp) and calibrated with *V. atlanticus* LGP32 genomic DNA (Table 1). To determine the qPCR efficiency of each primer pair used, standard curves were generated using seven serial dilutions of genomic DNA (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-5}, 10^{-7} and 10^{-8}) (not shown); the qPCR efficiencies of the tested genes varied between 1.85 and 2.08 (Table 3). For gene expression, reverse transcription was performed with 1 µg of total RNA using random hexamers and SuperScript IV reverse transcriptase (Invitrogen). The total qPCR reaction volume was 10 µL and consisted of 5 µL of cDNA (diluted 1:5), 2.5 µL of SensiFAST SYBR No-ROX Mix (Bioline) and 100 nM or 300 nM PCR primer pair (Table 3). The reaction parameters were as follows: 2 min at 95°C (initial denaturation) and 40 cycles of 5 s at 95°C (denaturation), 10 s at 59°C (annealing) and 20 s at 72°C (elongation). The specificity of each PCR was checked by measuring fluorescent signals during melting curve analysis (PCR product heated from 65°C to 95°C continuously and slowly at 0.1°C s^{-1}). Relative expression was calculated by normalization to the expression of two constitutively expressed housekeeping genes, namely, 6PKF (VS_2913) and CcmC (VS_0852), using the delta-delta threshold cycle (ΔΔCt) method(Pfaffl, 2001).

Detection of quorum-sensing signalling molecules

Vibrio culture

To detect the QS molecules (AI-2, AI-1 and CAI-1) *V. atlanticus* LGP32 was grown in Zobell media for 12 h (exponential growth phase, control) and 60 h (decline phase of growth, nutrient stress).

AI-2 analysis

Bioluminescence assay using the QS bioluminescent of *Vibrio campbellii* MM32 (*luxN*::Cm, *luxS*::Tn5Kan) was used to detect AI-2 molecules in culture supernatants. Briefly, *Vibrio* cultures were centrifuged at 17,000 x *g* for 10 min, and the resulting supernatants were filtered on 0.22 µm. Then 20 µL of the filtrates were mixed with 180 µL of *V. campbellii* MM32 diluted 1:5000 then incubated at 30°C and 100 rpm. Luminescence and cell density (OD620) were collected in triplicate and analysed according to Tourneroche et al.(Tourneroche et al., 2019).

AI-1 and CAI-1 extraction and LC-MS analysis

Chemical analyses were conducted with a Q Exactive Focus Orbitrap System coupled to an Ultimate 3000™ ultrahigh-performance liquid chromatography (UHPLC) system (Thermo Fisher Scientific) according to Rodrigues et al. (Rodrigues et al., 2022).

Briefly, ethyl acetate (2 mL) was added into each culture (2 mL). This mixture was shaken overnight at room temperature (150 rpm). The two phases were then separated and the aqueous phase was extracted once again. The two obtained organic phases were pooled and the solvent

Species	genes	Primers Sequences	Tm (°C)	Efficiency	References
Alexandrium pacificum (ACT03)	18S – 28S rRNA ITS region	TGATATTGTGGGCAACTGTAA AACATCTGTTAGCTCACGGAA	54		(Genovesi et al., 2011)
Alexandrium tamarense (ATT07)	18S – 28S rRNA ITS region	TGGTAATTCTTCATTGATTACAATG AACATCTGTTAGCTCACGGAA	54		//
Vibrios spp.	16S	CGGTGAAATGCGTAGAGAT TTACTAGCGATTCCGAGTTC	62		(Kitatsukamoto et al., 1993)
Vibrio atlanticus LGP32	LuxN (VS_II0260)	CACTTGCTAGTATCATCGC ATCGAGTTAGCAAGAGCAC	60	1,92	This work
//	LuxM (VS_II0261)	TCCACTTATCACAAACAGG ACTGTACTTCCATTTGTCG	60	1,91	//
//	LuxP (VS_II0355)	AAGTTCAGGATGAACCTATC CAAAGAGATACTTTGCTGAG	60	1,89	//
//	LuxS (VS_2562)	ACTCTCGAGCACCTATACG GAAGGCGTACCAATCAAGC	60	1,85	//
//	CqsS (VS_1725)	GACATCTATTGATGTTATGC TCACCCACTTCACGTAACTG	60	1,91	//
//	PvsA (VS_II0355),Vibrioferrin biosynthesis protein	CAGAGCAAGAGCTAGAACC TCGTTGAGAACCTGACGAG	59	1,91	//
//	PvuB (VS_II1126), ABC transporter vibrioferrin uptake FecB	TAGTGCAACCATGGGAATCG TAAACCGTACGTAGACGCTC	57	2,01	//
//	PvuA2 (VS_II1127), Vibrioferrin receptor FecA	GGAGCTACAAGCATTCGTTC TTCGTCATATGGTCGCTTCG	57	2,08	//
//	Housekiping gene 1, CcmC (VS_0852)	ATTGCCGCCTTTATCGGTTT CAAGCACCCCACATTGGTTT	60		(Vanhove et al., 2016)
//	Housekiping gene 2, 6-phosphofructokinase (VS_2913)	GCCGTCACTGTGGTGACCTT TGCTTCTTGCCTTTCGCAAT	60		//

Table 3. **Oligonucleotide sequences of primers used for RNA expression analysis.**

was evaporated under vacuum. The crude extracts were dissolved in 500 µL LC-MS grade methanol for analysis. The experiments were performed using biological triplicates, each of which was analyzed in triplicate. Analyses of extracts and standards (3 µL injected) were performed in electrospray positive ionization mode in the 50–750 m/z range in centroid mode. The parameters were as follows: spray voltage: 3 kV; sheath flow rate: 75; aux gas pressure: 20; capillary temperature: 350°C; heater temperature: 430°C. The analysis was conducted in Full MS data-dependent MS2 mode (Discovery mode). Resolution was set to 70,000 in Full MS mode, and the AGC (automatic gain control) target was set to $1x10^6$. In MS2, resolution was 17,500, AGC target was set to $2x10^5$, isolation window was 0.4 m/z, and normalized collision energy was stepped to 15, 30 and 40 eV. The UHPLC column was a Phenomenex Luna Omega Polar C18 1.6 µm, 150 x 2.1 mm. The column temperature was set to 42°C, and the flow rate was 0.4 mL min^{-1}. The solvent system was a mixture of water (A) with increasing proportions of acetonitrile (B), with both solvents modified with 0.1% formic acid. The gradient was as follows: 1% B 3 min before injection, then from 1 to 15 min, a gradient increase of B up to 100% (curve 5), followed by 100% B for 5 min. The flow was injected into the mass spectrometer starting immediately after injection. All data were acquired and processed using FreeStyle 1.5 software (Thermo Fisher Scientific).

Chemicals and solvents

N-acyl-homoserine lactones (AHL) were obtained from Cayman Chemical (Ann Arbor, MI, USA). Stock solutions were obtained by dissolving standards in methanol or dichloromethane (C18-AHL) at a concentration of 1 mg mL^{-1} and stored at -80°C. Standard solutions for UHPLC-high-resolution tandem mass spectrometry (HRMS) analyses were prepared by diluting each individual standard solution with methanol in order to obtain a concentration range from 2000 to 20 ng mL^{-1}. LC-MS grade methanol, acetonitrile and formic acid were purchased from Biosolve (Biosolve Chimie, Dieuze, France), analytical-grade ethyl acetate was obtained from Sigma-Aldrich. Pure water was obtained from Elga Purelab Flex System (Veolia LabWater STI, Antony, France).

Nature of lytic compounds secreted by *V. atlanticus* LGP32

To determine the temperature-sensitivity of the lytic compounds secreted, *V. atlanticus* LGP32 grown for 60 h in Zobell media at 22 °C was filtered through a 10 kDa membrane (Amicon® Ultra-4 filter unit). The eluate containing molecules with MW below 10 KDa was then incubated in a water bath at 100°C for 30 min. Boiled filtrates (0.1 mL) were subsequently used to inoculate *A. pacificum* (ACT03 strain) cultures, then lytic activity was observed under the Leica TCS SPE confocal laser scanning system. Zobell media with the same treatment was used as control.

Statistical analysis

Environmental data

Statistical analyses were performed using R 3.6.3 software. The relationship between *Alexandrium* and *Vibrio* was explored separately in spring and autumn. We used a generalized linear model specifying a Gaussian family. For spring, the dataset for salinity and temperature was complete (14 periods of observation). An influential period was detected and removed from the dataset because no dead *Alexandrium* cells were observed. The effects of explanatory variables such as log10 (Vibrio+1), salinity and temperature were centred, reduced and tested as fixed effects with a linear relationship. Model selection was performed using the Akaike information criterion corrected for small sample size (AICc). Models were considered different whenever the difference between their AICc value and the lowest AICc value (ΔAICc) was lower than 2 (Burnham and Anderson, 2002). *Alexandrium* distribution and model residuals were checked for normal distribution assumptions (QQ plot and Shapiro-Wilk test). For autumn, the dataset was complete for 10 periods of observation. Salinity and temperature were missing for three periods. We explored the relationship between *Alexandrium* and *Vibrio* alone using the method detailed above for spring.

In vitro data

Statistical analyses were performed using one-way ANOVA (analysed by pair) followed by Tukey's test (Statistica 10.0 software, StatSoft, Maison-Alfort, France). *P <0.05, **P <0.01, ***P <0.001

Results

Concomitant occurrence of *A. pacificum* ACT03, *A. tamarense* ATT07 and free-living *Vibrio spp.* in the Thau lagoon

In the spring and autumn of 2015, in the Thau Lagoon (Fig. 1A), we detected *Alexandrium* algae (*A. pacificum* ACT03 *and A. tamarense* ATT07, both alive and in degraded cell forms) and free-living *Vibrio*, but no plankton-associated *Vibrio* were observed (Fig. 1B, Data S1). Using model selection based on AICc, we found no significant relationship between *Alexandrium* (*A. pacificum* ACT03, *A. tamarense*) and *Vibrio Spp.* abundances in autumn. This result is consistent with the difficulty Vibrio has in growing at temperatures below 20°C and with the many environmental factors that can influence the dynamics of algae proliferation (Laanaia et al., 2013). Interestingly, in spring 2015, the mean densities of all *Alexandrium* cells (degraded and alive) and of free-living *Vibrio* were positively correlated. The lowest AICc was obtained with the model explaining degraded form of *Alexandrium* density based on the free *Vibrio* density (Fig. 1C). Given that, this model is not so different from the model with only the intercept, but better than any other linear combination with other potentially interfering drivers, such as temperature and salinity (Fig. 1D), we searched for evidence of a relationship between *Vibrio* and *Alexandrium* by studying their interaction *in vitro*.

Vibrio atlanticus LGP32 feeds on Alexandrium pacificum ACT03

To investigate whether *Alexandrium* interacts with *Vibrio*, we incubated in Enriched Natural SeaWater (ENSW) *A. pacificum* ACT03 (2.0×10^4 cells) with *V. atlanticus* LGP32 previously grown for 12 hours in Zobell media (initial concentration of 8.8×10^7 cells mL^{-1}). In interaction with *V. atlanticus* LGP32, *A. pacificum* ACT03 cell abundance decreased significantly from $2.1\ 10^4$ cells mL^{-1} after 1h of exposure to 1.1×10^4 cells mL^{-1} after 48 h of exposure (Fig. 2A), while the *V. atlanticus* LGP32 concentration grew significantly after 26 h of interaction, reaching a maximum peak density of 7.6×10^7 CFU mL^{-1} at 34 h (Fig. 2B). In the control experiment where *A. pacificum* ACT03 was cultured alone in ENSW, the algal concentration remained stable over time (Fig. 2A) and no bacteria were on the corresponding TCBS plates (*Vibrio* selective medium). In the control where *V. atlanticus* LGP32 was grown alone in ENSW, the bacterial concentration decreased from 7.0×10^5 CFU/mL after 1 hour of incubation to 1.1×10^5 CFU/mL after 48 hours of incubation (Fig. 2B). These results show that the interaction between *V. atlanticus* LGP32 and *A. pacificum* ACT03 leads to a decline in the algal population and promotes the growth of *V. atlanticus* LGP32. This suggests that *V. atlanticus* LGP32 is able to feed on *A. pacificum* ACT03.

V. atlanticus LGP32 performs attacks on *A. pacificum* ACT03

Epifluorescence microscopy observation of GFP-labelled *V. atlanticus* LGP32 (previously grow in Zobell medium) in interaction showed that *V. atlanticus* LGP32 cells are capable to simultaneously attacks *A. pacificum* ACT03 cells (Fig, 2C and Video 1). The attacks were extremely rapid, with empty thecae (algal envelopes) observed in the medium after less than 60 s (Fig 2D and Video 2). During the attack, *V. atlanticus* LGP32 did not invade the algal cell but remained clustered on the cell surface (Fig. 2C).

Attack of *A. pacificum* ACT03 is activated by *V. atlanticus* LGP32 starvation

In 2002, Martin hypothesized that nutritional stress induces bacteria to lyse algae (Martin, 2002). To test this hypothesis, we monitored *V. atlanticus* LGP32 behaviour in response to starvation (Fig. 3). We observed that *V. atlanticus* LGP32 in exponentially growth phase (12 h of culture in Zobell medium, 8.8×10^7 cells mL^{-1}) did not interact with *A. pacificum* ACT03 cells for the first hour of contact (Fig. 3A, B, C). In contrast, *V. atlanticus* LGP32 in the decline phase (36 h of culture in Zobell medium, $1.3\ 10^7$ cells mL^{-1}) induced a significant decrease in the number of motile algae cells by 8.9% after 15 min and by 43.3% after 60 min (Fig. 3A). This phenomenon corresponded

Figure 1. Dynamics of *Alexandrium* and *Vibrio* in the environment.

(A) Location of the monitoring station in the Thau Lagoon (southern France). **(B)** Mean abundance (DNA equiv.) of *Vibrio spp*. (16S) and Alexandrium *spp*. *(A. pacificum* ACT03 + *A. tamarense* ATT07). *Vibrio* cells (black line with diamond dot) and degraded *Alexandrium* cells (grey line with round dot) were evidence in the 0.2–0.8 µm fraction (free *Vibrio* fraction) in spring and autumn 2015. Living *Alexandrium* cells (grey line with roundot) but no plankton-associated *Vibrio spp*. (black line with diamond dot) were evidence in the 0.8–180 µm in spring and autumn. **(C)** Result of Akaike information criterion (AICc) models tested to explain the mean value of degraded Alexandrium cells (dead cells) in spring. **(D)** Wald test of the AICc model attributing the mean value of degraded cells of Alexandrium in spring to free *Vibrio*.

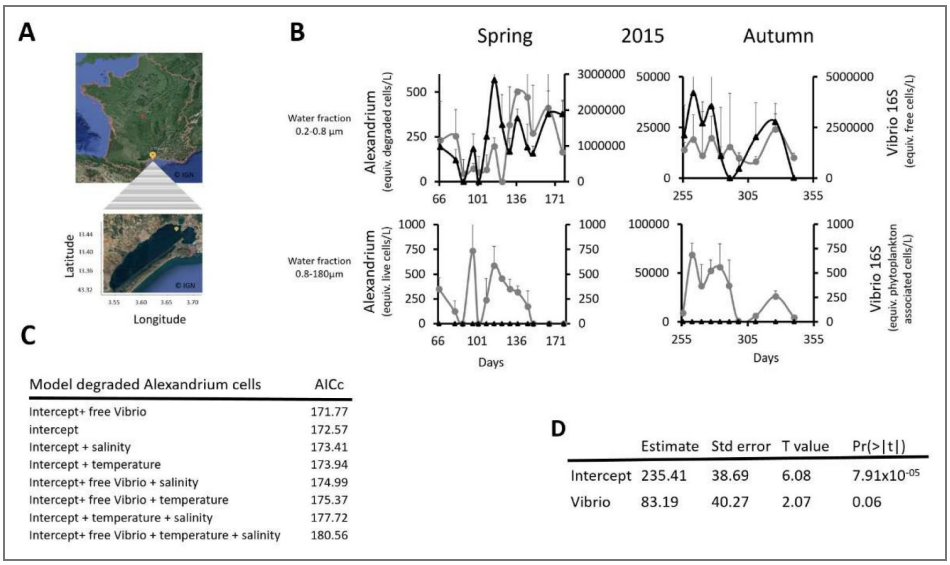
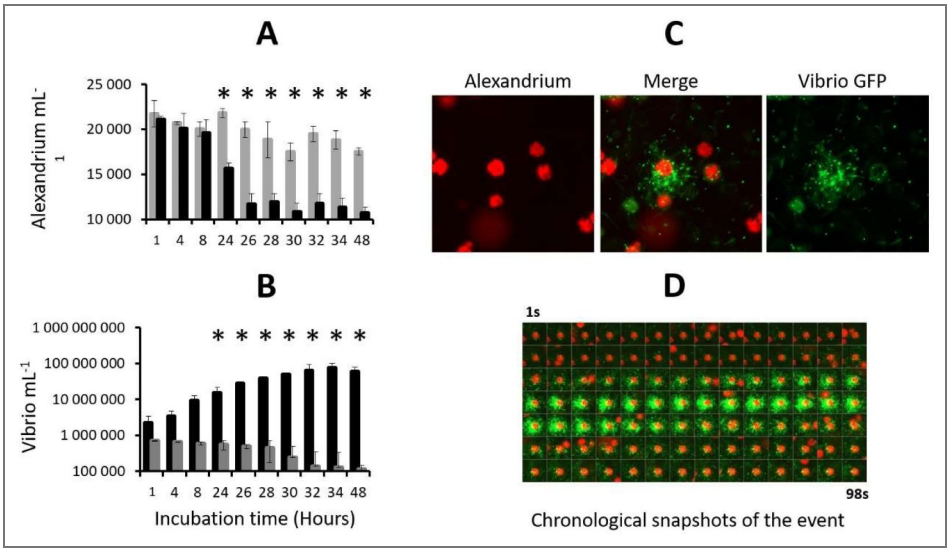


Figure 2. Incubation of *Vibrio atlanticus* LGP32 and *Alexandrium pacificum* ACT03 in enriched natural seawater (ENSW).

(A) *A. pacificum* ACT03 cultured alone (grey bar) and incubated with *V. atlanticus* LGP32 (black bar) in ENSW. **(B)** *V. atlanticus* LGP32 cultured alone (grey bar) and incubated with *A. pacificum* ACT03 (black bar) in ENSW.**(C)** Snapshot of the interaction between *V. atlanticus* LGP32-GFP cells (60-hour culture) and one cell of *A. pacificum* ACT03 taken at 8h00 of co-culture. **(D)** Chronological snapshots of the interaction (98 pictures, one per second). *V. atlanticus* LGP32 (small green cells) and *A. pacificum* ACT03 cell (large red cell). All experiments were done in triplicates. Asterisks indicate significant differences in a multiple comparison test (One-way ANOVA with post hoc Tukey test), *P ≤ 0.05.



to the degradation and/or disruption of algal flagella (Video 3). The flagella no longer functioned correctly, which caused irregular swimming of the algae (Video 3, left cell). This was followed by a complete cessation of swimming. When the flagellum detached from the algae (Video 3, right cell), the attack occurred. With starved *V. atlanticus* LGP32 (60 h of culture in Zobell medium, 0.6 10^7 cells mL^{-1}), algae immobilization was fast and significant (91.4% in 15 min, Fig. 3A), and algae were attacked individually being targeted by *V. atlanticus* LGP32 cells (Video 4). The percentage of cells attacked and killed peaked at 30% after 15–30 min of contact (Fig. 3B and 3B1) and then decreased. After 1 h, attacks had stopped with approximately 40% of the algal cells still alive (Fig. 3B1 and 3C). Although it remains unclear whether the attacks occur during a specific phase of growth, it is evident that the cells are already weakened before attack as they have all lost their flagella.

An old-starved culture of *V. atlanticus* LGP32 (126 h of culture in Zobell medium, < 0.1 10^7 cells mL^{-1}) significantly immobilized *A. pacificum* ACT03 cells within a few minutes, with lysis occurring immediately (Fig. 3A and 3C), making it impossible to detect attacks by *V. atlanticus* LGP32 (Fig. 3B). The lysis phase corresponded to initial vesicle formation followed by the bursting of *A. pacificum* ACT03 cells (Fig. 3C and 3C1).

We next tested whether this lytic effect was linked to *Vibrio* culture supernatant and mediated by thermostable molecule (s) secreted by *Vibrio*. The culture supernatant of starved culture of *V. atlanticus* LGP32 (36 h) filtered through a 10 kDa membrane and then incubated at 100°C for 30 min still possessed its lytic properties, indicating that the algicidal compounds produced by *V. atlanticus* LGP32 are small thermostable molecules unlikely to be lytic enzymes, or lysins able to digest the algae cell. However, these experimental observations clearly show the key role of nutrient limitation in triggering the attack behaviour and the secretion of lytic compounds of *V. atlanticus* LGP32.

Attack occurs on *A. pacificum* ACT03 in exponential phase of growth

Here, we wondered whether the live/dead status of algae is important for *V. atlanticus* LGP32-mediated attacks targeting. To this end, *A. pacificum* ACT03 in exponential growth phase was first exposed for 30 minutes to the supernatant of a 126-hour culture of *V. atlanticus* LGP32, which induced lysis of 70% of the *A. pacificum* ACT03 cells (Figures 3C and 3C1 (arrow 2) and Video 4). Next, cells of *V. atlanticus* LGP32 from a 60-hour culture, capable of attacking *A. pacificum* ACT03 cells (Fig. 3B), were added. For 1 hour of exposure, no attack was observed on the previously lysed algae. This result is similar to what is observed on the Video 1 with flash attacks only on immobilized, but not degraded *A. pacificum* ACT03 cells (red), and not on lysed cells (green). In addition, no attacks occurred on cells from an old *A. pacificum* ACT03 culture (1-month culture). Together, with the very short duration of attacks (Video 1), these results indicate that *V. atlanticus* LGP32 attacked exponentially growing cells of *A. pacificum* ACT03, but not decomposing algae, suggesting that this behaviour is not just an opportunistic response of heterotrophic bacteria to organic substrates.

Attack is independent of quorum sensing

Considering the simultaneous action of *V. atlanticus* LGP32 in attacks on *A. pacificum* ACT03, we tested whether the attack process depended on the key physiological mechanism that regulates many functions in marine microbial cells, quorum sensing (QS) (Lami, 2019; Papenfort and Bassler, 2016), a type of cell-cell communication. Although QS is a cell-density-dependent mechanism, our results showed no attack from a 12 h culture of *V. atlanticus* LGP32 up to a concentration of 4.10^6 *Vibrio* mL^{-1} (Fig 4A). Attacks were only observed with *V. atlanticus* LGP32 from a 60 h culture at low concentration of 5.10^3 *Vibrio* mL^{-1} to the highest concentration tested of 5.10^5 *Vibrio* mL^{-1} (Fig 4A), consistent with the hypothesis that the attacks were independent of *Vibrio* density.

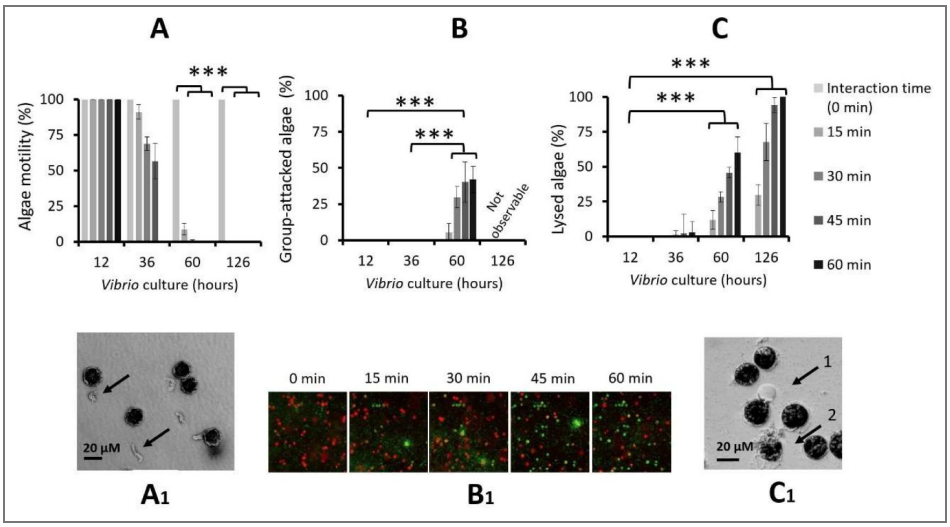


Figure 3. Role of *Vibrio atlanticus* LGP32 starvation in the interspecific interaction process.

Experiments were conducted by incubating*A. pacificum* ACT03 with *V. atlanticus* LGP32 previously grown for 12, 36, 60 and 126 h in Zobell medium. **(A)** Cumulative percentage of motile *A. pacificum* ACT03 cells. **(B)** Cumulative number of cells attacked by *V. atlanticus* LGP32 and **(C**) Cumulative cell lysis after 0, 15, 30, 45 and 60 minutes of interaction. Corresponding pictures showing **(A1)** Black arrows indicate unhooked and degrade flagellum from *A. pacificum* ACT03 flagellum, **(B1)** Chronological sequence of five snapshots showing *V. atlanticus* LGP32-GFP cells (60-hour culture) and *A. pacificum* ACT03 cells, during the first hour of their interaction. *V. atlanticus* LGP32 (small green cells), living *A. pacificum* ACT03 (large red cells) and dead *A. pacificum* ACT03 (large green cell). **(C1)** Black arrow 1 indicate vesicle formation on *A. pacificum* ACT03 cell and black arrow 2 indicate lysed *A. pacificum* ACT03 cell. All percentages were determined based on a minimum of 2,000 cells of *A. pacificum* ACT03. All experiments were done in triplicates. Asterisks indicate significant differences in a multiple comparison test (One-way ANOVA with post hoc Tukey test), ***P ≤ 0.001

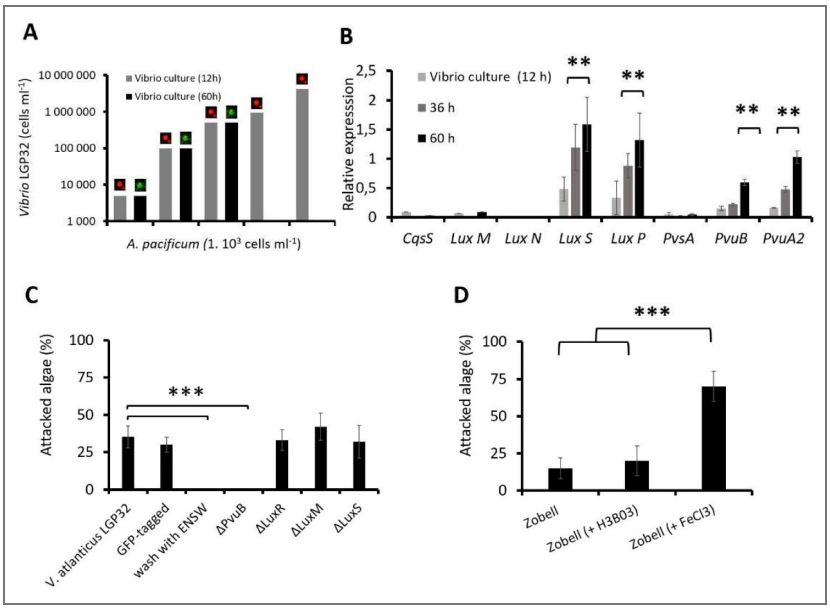


Figure 4. Role of quorum sensing and the vibrioferrin iron uptake pathway in the interaction process.

(A) Effect of *V. atlanticus* LGP32 cell density on the attack process. *A. pacificum* ACT03 cells (1.10^3 cells mL^{-1}) were incubated with *V. atlanticus* LGP32 grown for 60 hours in Zobell medium at concentrations ranging from 5.10^3 to 5.10^5 cells mL^{-1} (black bars). For comparison, *A. pacificum* ACT03 incubated with *V. atlanticus* LGP32 grown for 12 hours in Zobell medium at concentrations ranging from 5.10^3 to 4.10^6 cells mL−1 (grey bars). The image on the bars indicates either unaffected algae (live red algae) or algae attacked by vibrio's (algae covered with green vibrio's) during the interaction **(B)** CqsS, luxM, luxN, luxS, and luxP quorum sensing and PvsA, PvuB and PvuA2 vibrioferrin pathway genes expression in *V. atlanticus* LGP32 grown for 12, 36 and 60 h in Zobell medium. **(C)** Effect of *V. atlanticus* LGP32 mutants on the attacked process. Experiments were conducted by incubating *A. pacificum* ACT03 with *V. atlanticus* LGP32, *V. atlanticus* LGP32 tagged with GFP, *V. atlanticus* LGP32 washed with ENSW or *V. atlanticus* LGP32 mutant ΔPvuB, ΔluxM, ΔluxR and ΔluxS previously grown 60 h in Zobell media (control), The percentage of *A. pacificum* ACT03 attacked was determined during the first 30 min of exposure. **(D)** Effect of *V. atlanticus* LGP32 cultures media composition on the attacked process. Experiments were conducted by incubating *A. pacificum* ACT03 with *V. atlanticus* LGP32 grown 60 h in Zobell media supplemented with H3BO4 or FeCl3. The results were compared with an exposure to *V. atlanticus* LGP32 grown 60 h in Zobell media. All percentages were determined based on a minimum of 2,000 cells of *A. pacificum* ACT03. All experiments were done in triplicates. Asterisks indicate significant differences in a multiple comparison test (One-way ANOVA with post hoc Tukey test), **P ≤ 0.01, ***P ≤ 0.001

The analysis of the expression of genes involved in the known QS pathways in *Vibrio* cell (Fig. S3A), highlighted that only the AI-2 pathway was induced during nutrient stress of *V. atlanticus* LGP32, because only the expression of the AI synthase (*LuxS*) and its receptor (*LuxP*) increased significantly (Fig. 4B; ANOVA p <0.05). This was confirmed by a QS bioluminescence assay, which showed a AI-2 molecules (unquantified) in the Zobell culture supernatant of *V. atlanticus* LGP32 after 60 h of culture but not after 12 h of culture and not in the ENSW supernatant of *V. atlanticus* LGP32 after 12 or 60 h of culture.

UHPLC-HRMS/MS provided no evidence of detectable HAI-1) and CAI-1 in any experiments.

Targeted mutagenesis of key genes involved in two of the three known QS pathways in vibrios (Fig. S3), ΔluxM (HAI-1 production), ΔluxS (AI-2 production), and ΔluxR (main high-density QS regulator), did not result in any changes in the attack behavior of *V. atlanticus* LGP32 (Fig. 4C). Combined with the absence of overexpression of the CqsS gene (inducible by CAI-1) involved in the last known QS pathway in Vibrio (Fig. S3), these results indicated that the attack by *V. atlanticus* LGP32 is most likely unrelated to QS.

Attack related to the availability of iron

The comparative analysis of the proteome of *V. atlanticus* LGP32 incubated 60 h in artificial seawater (ENSW) versus *V. atlanticus* LGP32 grown 12 h in Zobell nutrient-rich medium revealed 10 proteins modulated by nutrient stress (Fig. S2). The two most down-regulated proteins correspond to be ß-ketoacyl-(acyl-carrier-protein) synthase II (-22-fold in ENSW compared to Zobell), a key regulator of bacterial fatty acid synthesis, and the dihydroorotase (-6.6-fold in ENSW compared to Zobell), an enzyme essential for pyrimidine biosynthesis and thus bacterial proliferation and growth. The low expression of these proteins in ENSW is consistent with *V. atlanticus* LGP32 nutritional starvation. The most up-regulated protein in starved *V. atlanticus* LGP32, with an increase of more than 6-fold, was glucosamine-6-phophate deaminase, an enzyme involved in bacterial energy metabolism probably necessary for its survival. Among the other up-regulated proteins, one was an iron siderophore-binding protein (Spot 4413, Fig. S2A) corresponding to the vibrioferrin outer membrane receptor PvuB, whose gene is part of the *pvu* operons involved in iron transport (Fig. S3B). Interestingly, the corresponding gene *pvuB* as well as the vibrioferrin membrane receptor gene *pvuA2* (Fig. S3B) were both significantly induced in *Vibrio* under nutrient stress (Fig. 4B; ANOVA p <0.01) but not the one involved in the vibrioferrin biosynthesis, *pvsA* (Fig. 4B). Remarkably, among the 10 proteins identified by proteomic analysis only *V. atlanticus* LGP32 mutant lacking *pvuB* failed to attack *A. pacificum* ACT03 (Fig. 4C; ANOVA p <0.001). In the absence of the *pvuB* gene, *V. atlanticus* LGP32 was unable to attack simultaneously *A. pacificum* ACT03. In addition, *V. atlanticus* LGP32 cells that had been washed with ENSW to remove their culture supernatant metabolites also failed to attack *A. pacificum* ACT03 (Fig. 4C; ANOVA p <0.001), which is congruent with the hypothesis that attacks depend on the *V. atlanticus* LGP32 vibrioferrin transport system. Finally, attacks increased significantly, when FeCl3 was added to the *Vibrio* culture medium (Fig. 4D) but not with H3BO4 (Fig. 4D) Finally, attacks increased significantly, when FeCl3 was added to the Vibrio culture medium but not with H_3BO_4 known also to be capable of being transported by vibrioferrin (see Fig. 4D).. Taken together, those results are consistent with the hypothesis that attacks are regulated by iron.

Attack is a *Vibrio spp.* behaviour specific to *Alexandrium spp*

To evaluate the dinoflagellates specificity of the attack behaviour, a selection of *Vibrio spp.* was co-cultured with a selection of dinoflagellate strains commonly found in the Mediterranean Sea. The results showed that, among the *Vibrio spp*. tested (pathogenic or not) all, under nutrient stress, were able to secrete algicidal compounds, immobilize, attack and lyse *A. pacificum* ACT03 cells (Table 1) and no linked to their pathogenesis for fish of invertebrates was observed (Table 1). Among the sixteen dinoflagellates species tested, only *Alexandrium spp*. (non-toxic and paralytic

shellfish toxin (PST) producers) and *Gymnodinium catenatum* (PST producer) were immobilized, attacked and lysed by *V. atlanticus* LGP32 (Table 2), but no linked to PSTs was revealed (Table 2).

Discussion

Predation is a widespread mode of interaction for survival in the natural world (Finke and Denno, 2004; Sinclair et al., 2003). Among predators, predatory bacteria are found in a wide variety of environments and, like bacteriophages and predatory protists, feed primarily on other bacteria, although a few cases of predation on microbial eukaryotes have also been reported (Johnke et al., 2014; Perez et al., 2016).

Consider a predator as a free-living organism that kills its prey and feeds on it, this study provides data suggesting the ability of Vibrios to develop an original predator-like behaviour to kill and feed on algae.

In fact, the strategy developed by *Vibrio* to kill algae may be reminiscent of strategies previously described in the prokaryotes (Johnke et al., 2014). As shown in Video 1, the interaction between *V. atlanticus* LGP32 and *A. pacificum* ACT03 proceeds in three stages (Fig. 5). The first stage, the '**immobilization stage**', recalls the strategy used by *Streptomyces* to immobilize its prey (Kumbhar et al., 2014) based on the secretion of algicidal metabolites that disrupt the flagella. The second stage, the '**attack stage**' corresponding to the physical contact between Vibrios and Alexandrium, is similar to the strategy used by *Myxococcus xanthus* and Lysobacter. These bacteria must be in close proximity to their prey in order to cause lysis and utilize their biomass, regardless of the prey's species (Martin, 2002; Genovesi et al., 2013; Perez et al., 2016; Zhang et al., 2020). *V. atlanticus* LGP32 also surrounds *A. pacificum* ACT03 cells at high density for a very short time, but does not invade the algal cell. Visually, this phenomenon resembles bacteria clustering around lysed ciliate cells (Blackburn et al., 1998). The third stage, the '**killing stage**', is similar to that of epibiotic bacterial predators, which induces the lysis of bacteria from the outside (Rashidan and Bird, 2001). Overall, these observations suggest that *V. atlanticus* LGP32 can exhibit a predator-like behaviour.

The attack behaviour of *V. atlanticus* LGP32 was linked to iron absorption, as mutants with impaired iron absorption completely lost its ability to attack the algae. Iron is an essential element for growth in most organisms, including phytoplankton (Martin and Fitzwater, 1988) and bacteria (Neilands, 1981), and its concentration in seawater is known to be very low with measurements in the open ocean surface at 0.2 nM and in deep waters at 0.6 nM (Millero, 1998).

Moreover, Its low solubility in seawater limits its availability (Bruland et al., 1994; Wu and Luther, 1994). To acquire iron, bacteria have developed systems based on the secretion (and subsequent uptake) of iron-chelating siderophores to obtain this element from the environment (Amin et al., 2009a). Therefore, many *Vibrio spp*. produce a siderophore known as vibrioferrin, which is synthesized and secreted by proteins encoded by the *pvsABCDE* gene cluster (Fig. S3). Given that boron is known for its role in regulating a global bacterial cellular response to phytoplankton and to bind to vibrioferrin (Romano et al., 2013; Weerasinghe et al., 2013), we tested its potential involvement in simultaneous vibrio attacks. Compared to the Zobell control, no effect on the number of attacks was observed. For iron-vibrioferrin uptake, *Vibrio parahaemolyticus* uses a membrane siderophore receptor, called PvuA, which is coupled to an inner membrane ATP-binding cassette (ABC). This ABC transporter system comprised of proteins encoded by the pvuABCDE gene cluster (Fig. S3) is required for transporting the siderophore across the inner membrane (Tanabe et al., 2003). Siderophores are not only iron carriers but also important regulators of virulence (Miethke and Marahiel, 2007) and mediators of bacterial interaction with phytoplankton (Amin et al., 2009b; Kramer et al., 2020). We showed here a pivotal role of iron in the interaction between *V. altlanticus* LGP32 and *A. pacificum* ACT03. This mirrors the mutualistic interaction observed between *Gymnodinium catenatum* and *Marinobacter* (Amin et al., 2009b). In fact, in natural settings, the co-occurence of *Marinobacter* and *G. catenatum* is suggested to depend on a mutually beneficial utilization of iron and carbon resources (Bolch et al., 2011). As in the present study, iron seems to play a key role in the interaction.

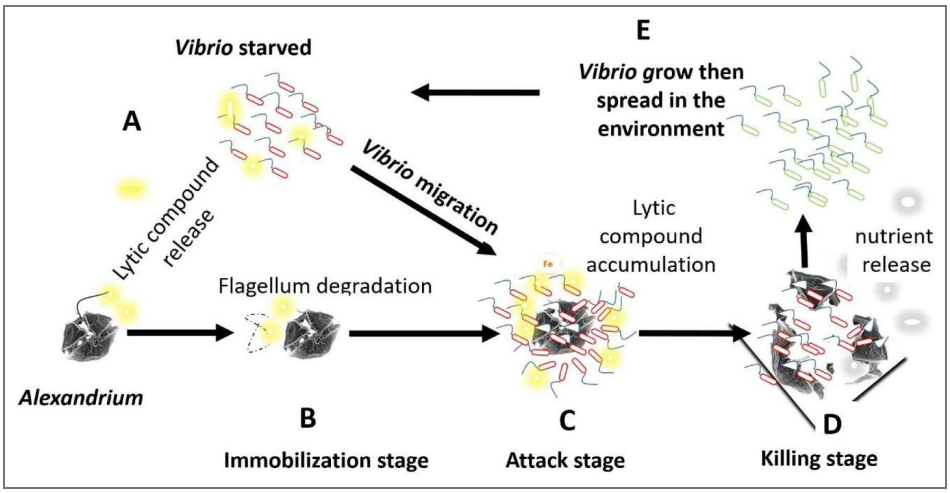


Figure 5. Schematic representation of a putative strategy developed by *Vibrio spp*. to feed on Alexandrium *spp*. and *G. catenatum* in the environment.

(A) *Vibrio* in the environment when subjected to starvation secrete non-protein lytic compounds. **(B)** Some of these lytic compounds degrade the flagella, immobilizing the alga (immobilization stage). **(C)** Then *Vibrio* swims and clusters around its prey (attack stage). **(D)** Lytic compounds released by *Vibrio* where able to concentrate around the algae cells, thereby lysing the algae (killing stage). **(E)** Feeding on the released nutrients, *Vibrio* multiply and then spread in the environment. Yellow clouds: Lytic compound release by *Vibrio,* Grey clouds: Algal nutrients released upon lysis.

Indeed, the labile iron released through the photolysis of ferric chelates with vibrioferrin providing a crucial iron source for phytoplankton, which need substantial amounts of iron to support carbon fixation through photosynthesis (Amin et al., 2009a; Yang et al., 2021). This fixed carbon, in turn, sustains the growth of both the phytoplankton and their bacterial counterparts (Amin et al., 2009b; Kramer et al., 2020). Interestingly, if a general nutrient deficiency causes attacks, iron supplementation increases this number of attacks (Figure 4D), suggesting the importance of iron absorption in the attack behavior.

Future studies should determine whether nutrient deficiency increases the iron absorption capacity of Vibrio bacteria and whether this could plays a major role in the attack mechanism.

This study showed that quorum sensing is not involved in microbial attacks. Thus, *Vibrio atlanticus* LGP32 mutants lacking the genes involved in known quorum sensing pathways exhibit the same phenotype as wild-type *Vibrio atlanticus* LGP32, and Vibrio density does not induce attacks. However, V. atlanticus LGP32 produces AI-2 during attacks. Quorum sensing and iron acquisition are sometimes interconnected in Vibrio (McRose et al., 2018). For example, in *Vibrio vulnificus*, the production of vulnibactin (a siderophore) is known to be controlled by AI-2 (Kim and Shin, 2011). Similarly, AI-2 could be involved in the production of vibriferin in *Vibrio atlanticus* LGP32.

In the natural environment, associations between bacteria and algae have already been observed (Lopez-Joven et al., 2018; Miller et al., 2005; Rosales et al., 2022; Xu et al., 2022). We have shown here that algal attacks by vibrios can be carried out in vitro. Environnemental data collected in the Thau lagoon showed a correlation between the presence of Alexandrium and that of Vibrio, suggesting that such interactions could also occur in the marine environnement. If that were the case, this behaviour would provide an important ecological advantage to *Vibrio* to obtain nutrients in environment, where *Alexandrium spp*. and *Gymnodinium catenatum* form blooms.

With more than 30 species distributed all over the world (Anderson et al., 2012; Hallegraeff et al., 2021), *Alexandrium spp.* and *Gymnodinium spp*. considered as invasive species by the Delivering Alien Invasive Species Inventories for Europe (*http://www.europe-aliens.org*)), could play an unexpected and important role in maintaining, structuring and regulating *Vibrio* populations in the ecosystem. In turn, *Vibrio* could contribute to the regulation and control of their blooms.

To conclude, this study reveals the capacity of some *Vibrio spp*. to be facultative predators-like that hunt specific algae. In the current context of climate change favourable to their development monitoring the invasive algae, *Alexandrium spp*. and *Gymnodinium catenatum* should be considered not only for their potent harmful effect on humans and animals, but also as a potential source of nutrients for the expansion of *Vibrio*, particularly pathogenic species (Lemire et al., 2015).

Supplementary figures

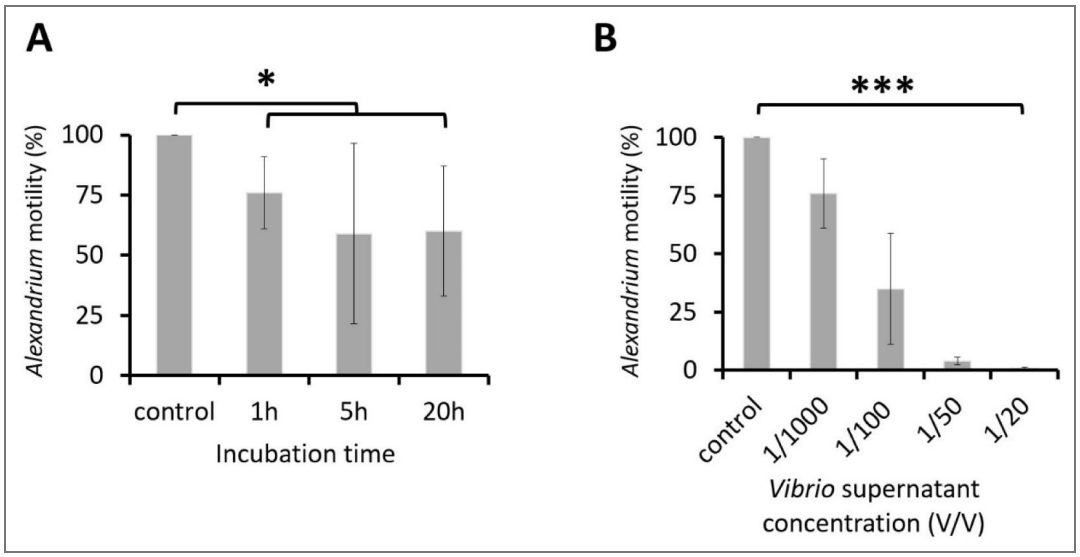
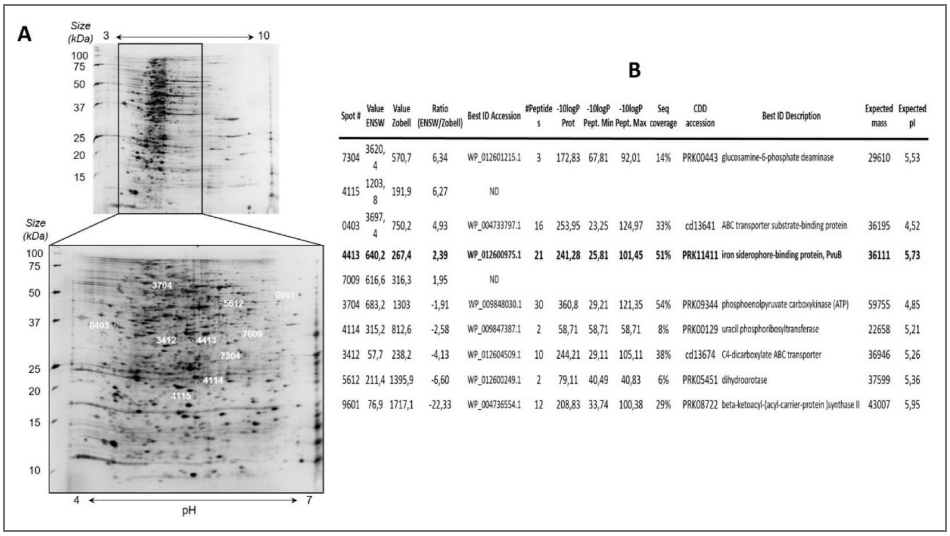


Figure S1. Time and dose-dependent effects of the *V. atlanticus* LGP32 culture supernatant on *A. pacificum* ACT03 motility. (A) A time dependence experiment was conducted by incubating *A. pacificum* ACT03 for 1, 5 or 20 h with 1/1000 v/v (1 µL/mL) of culture supernatant from *V. atlanticus* LGP32 previously grown for 60 h in Zobell culture media. **(B)** A dose dependence experiment was conducted by incubating *A. pacificum* ACT03 for 1 h with 1/1000 to 1/20 v/v (1-50 µL/mL) of culture supernatant from *V. atlanticus* previously grown for 60 h in Zobell media. The percentage of motile *A. pacificum* ACT03 was determined after 1 hours of exposure. All percentages were determined based on a minimum of 2,000 cells of *A. pacificum* ACT03. Error bars represent the standard deviation of the mean of three independent experiments. Asterisks indicate significant differences in a multiple comparison test (One-way ANOVA with post hoc Tukey test), *P ≤ 0.05, ***P ≤ 0.001.

Figure S2. ***Vibrio atlanticus*** **LGP32 proteome analysis following nutrient stress.**

(A) Example of 2D gel, the numbers in white on the gel 4-7 correspond to the number and position of the protein spots analyzed. **(B)** Proteins identified by LC-MS/MS as differentially represented in the 2D gel comparative approach following nutrient stress. ND: Not determined; ENSW (artificial seawater).

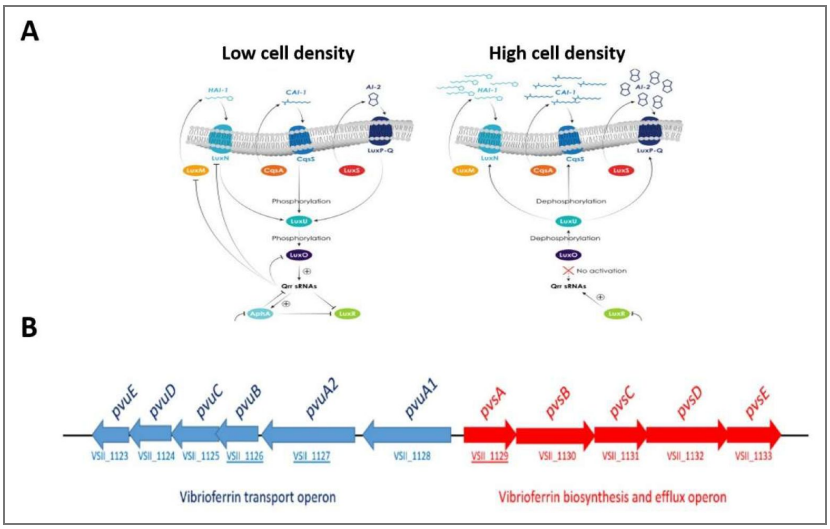


B

Spot #	Value ENSW	Value Zobell	Ratio (ENSW/Zobell)	Best ID Accession	#Peptides	-10logP Prot	-10logP Pept. Min	-10logP Pept. Max	Seq coverage	CDD accession	Best ID Description	Expected mass	Expected pI
7304	3620,4	570,7	6,34	WP_012601215.1	3	172,83	67,81	92,01	14%	PRK00443	glucosamine-6-phosphate deaminase	29610	5,53
4115	1203,8	191,9	6,27	ND									
0403	3697,4	750,2	4,93	WP_004733797.1	16	253,95	23,25	124,97	33%	cd13641	ABC transporter substrate-binding protein	36195	4,52
4413	**640,2**	**267,4**	**2,39**	**WP_012600975.1**	**21**	**241,28**	**25,81**	**101,45**	**51%**	**PRK11411**	**iron siderophore-binding protein, PvuB**	**36111**	**5,73**
7009	616,6	316,3	1,95	ND									
3704	683,2	1303	-1,91	WP_009848030.1	30	360,8	29,21	121,35	54%	PRK09344	phosphoenolpyruvate carboxykinase (ATP)	59755	4,85
4114	315,2	812,6	-2,58	WP_009847387.1	2	58,71	58,71	58,71	8%	PRK00129	uracil phosphoribosyltransferase	22658	5,21
3412	57,7	238,2	-4,13	WP_012604509.1	10	244,21	29,11	105,11	38%	cd13674	C4-dicarboxylate ABC transporter	36946	5,26
5612	211,4	1395,9	-6,60	WP_012600249.1	2	79,11	40,49	40,83	6%	PRK05451	dihydroorotase	37599	5,36
9601	76,9	1717,1	-22,33	WP_004736554.1	12	208,83	33,74	100,38	29%	PRK08722	beta-ketoacyl-[acyl-carrier-protein]synthase II	43007	5,95

Figure S3. **Quorum sensing and the vibrioferrin iron uptake pathway in** ***Vibrio*****.**

(A) Putative quorum sensing (QS) pathways at low and high cell density in *Vibrio* according to Lami et al.(Lami, 2019). **(B)** Genetic organization of the vibrioferrin utilization gene cluster on *V. atlanticus* LGP32 chromosome 2. The Pvu and Pvs operons are involved in the secretion and the transport of ferric vibrioferrin and biosynthesis of vibrioferrin, respectively. Arrows indicate the transcriptional directions of the genes. VSII1126, VSII1137 and VSII1129 corresponding to PvuB, PvuA2 and PvsA genes respectively.



Data availability

Data generated or analysed during this study are included in the manuscript (tables, supplementary materials, videos).

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Additional files

Data S1.

Video 1. Dynamics of *Vibrio atlanticus* LGP32-*Alexandrium pacificum* ACT03 interaction. GFP-tagged *V. atlanticus* (small green cells); living *A. pacificum* (large red cells); lysed *A. pacificum* (large green cells) filmed under an epifluorescence microscope.

Video 2. Second-by-second timing of *Vibrio Atlanticus* LGP32 attacking *Alexandrium pacificum* ACT03. GFP-tagged *V. atlanticus* (small green cells); *A. pacificum* living cell (large red cells) filmed under an epifluorescence microscope.

Video 3. Degradation and disruption of *Alexandrium pacificum* ACT03 flagella. Effect of *Vibrio* supernatant on the first stage of the interaction filmed under a confocal microscope.

Video 4. Attacks of *Vibrio atlanticus* LGP32 on target *Alexandrium pacificum* ACT03. This video, recorded under a confocal microscope, shows Vibrios simultaneously attacking a first immobilized Alexandrium cell, then moving on to attack a second cell without ever targeting the other cells present, suggesting active communication between the Vibrio bacteria. *V. atlanticus* LGP32 (small cells); *A. pacificum* ACT03 (large cells).

Video 5 Vesicle formation and bursting of an *Alexandrium pacificum* ACT03. Direct effect of *Vibrio* supernatant on *Alexandrium* after 126 h of culture filmed under a confocal microscope.

References

Abadie E., Amzil Z., Belin C., Comps M.A., Elzière-Papayanni P., Lassus P., le Bec C., Baut M.L., Nezan E., Poggi R. (1999) *Contamination de l'étang de Thau par Alexandrium tamarense (No. Rapport 884)* Ifremer.

Abadie E., Kaci L., Berteaux T., Hess P., Sechet V., Masseret E., Rolland J.L., Laabir M (2015) Effect of Nitrate, Ammonium and Urea on Growth and Pinnatoxin G Production of Vulcanodinium rugosum. *Mar. Drugs* **13**:5642-5656 https://doi.org/10.3390/md13095642 | PubMed

Amin S.A., Green D.H., Hart M.C., Kuepper F.C., Sunda W.G., Carrano C.J (2009a) Photolysis of iron-siderophore chelates promotes bacterial-algal mutualism. *Proc. Natl. Acad. Sci. U. S. A* **106**:17071-17076 https://doi.org/10.1073/pnas.0905512106 | PubMed

Amin S.A., Green D.H., Kuepper F.C., Carrano C.J (2009b) Vibrioferrin, an Unusual Marine Siderophore: Iron Binding, Photochemistry, and Biological Implications. *Inorg. Chem* **48**:11451-11458 https://doi.org/10.1021/ic9016883 | PubMed

Anderson D.M., Alpermann T.J., Cembella A.D., Collos Y., Masseret E., Montresor M (2012) The globally distributed genus Alexandrium: Multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae* **14**:10-35 https://doi.org/10.1016/j.hal.2011.10.012 | PubMed

Baker-Austin C., Trinanes J., Gonzalez-Escalona N., Martinez-Urtaza J (2017) Non-Cholera Vibrios: The Microbial Barometer of Climate Change. *Trends Microbiol* **25**:76-84 https://doi.org/10.1016/j.tim.2016.09.008 | PubMed

Ben-Gharbia H., Yahia O.K.-D., Amzil Z., Chomerat N., Abadie E., Masseret E., Sibat M., Triki H.Z., Nouri H., Laabir M (2016) Toxicity and Growth Assessments of Three Thermophilic Benthic Dinoflagellates (Ostreopsis cf. ovata, Prorocentrum lima and Coolia monotis) Developing in the Southern Mediterranean Basin. *Toxins* **8**:297 https://doi.org/10.3390/toxins8100297 | PubMed

Blackburn N., Fenchel T., Mitchell J (1998) Microscale nutrient patches in planktonic habitats shown by chemotactic bacteria. *Science* **282**:2254-2256 https://doi.org/10.1126/science.282.5397.2254 | PubMed

Bolch C.J.S., Subramanian T.A., Green D.H (2011) The toxic dinoflagellate *Gymnodinium catenatum* (Dinophyceae) requires marine bacteria for growth[1]. *J. Phycol* **47**:1009-1022 https://doi.org/10.1111/j.1529-8817.2011.01043.x | PubMed

Bruland K., Orians K., Cowen J (1994) Reactive Trace-Metals in the Stratified Central North Pacific. *Geochim. Cosmochim. Acta* **58**:3171-3182 https://doi.org/10.1016/0016-7037(94)90044-2

Brumfield K.D., Usmani M., Chen K.M., Gangwar M., Jutla A.S., Huq A., Colwell R.R (2021) Environmental parameters associated with incidence and transmission of pathogenic *Vibrio spp*. *Environ. Microbiol* **23**:7314-7340 https://doi.org/10.1111/1462-2920.15716 | PubMed

Burkholder J.M., Shumway S.E., Glibert P.M. (2018) Food Web and Ecosystem Impacts of Harmful Algae. In: Shumway S.E., Burkholder J.M., Morton S.L. (Eds). *Harmful Algal Blooms* John Wiley & Sons, Ltd. pp. 243-336 https://doi.org/10.1002/9781118994672.ch7

Burnham K.P., Anderson D.R (2002) Summary. In: *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach* New York, NY: Springer. pp. 437-454 https://doi.org/10.1007/978-0-387-22456-5_8

Chai Z.Y., Wang H., Deng Y., Hu Z., Tang Y.Z (2020) Harmful algal blooms significantly reduce the resource use efficiency in a coastal plankton community. *Sci. Total Environ* **704**:135381 https://doi.org/10.1016/j.scitotenv.2019.135381 | PubMed

Coyne K.J., Wang Y., Johnson G (2022) Algicidal Bacteria: A Review of Current Knowledge and Applications to Control Harmful Algal Blooms. *Front. Microbiol* **13**:871177 https://doi.org/10.3389/fmicb.2022.871177 | PubMed

Drebes Dörr N.C., Blokesch M. (2020) Interbacterial competition and anti-predatory behaviour of environmental *Vibrio cholerae* strains. *Environ. Microbiol* **22**:4485-4504 https://doi.org/10.1111/1462-2920.15224 | PubMed

Espinoza-Vergara G., Hoque M.M., McDougald D., Noorian P (2020) The Impact of Protozoan Predation on the Pathogenicity of Vibrio cholerae. *Front. Microbiol* **11**:17 https://doi.org/10.3389/fmicb.2020.00017 | PubMed

Fertouna-Bellakhal M., Dhib A., Fathalli A., Bellakhal M., Chomérat N., Masseret E., Laabir M., Turki S., Aleya L (2015) Alexandrium pacificum Litaker sp. nov (Group IV): Resting cyst distribution and toxin profile of vegetative cells in Bizerte Lagoon (Tunisia, Southern Mediterranean Sea). *Harmful Algae* **48**:69-82 https://doi.org/10.1016/j.hal.2015.07.007 | PubMed

Finke D.L., Denno R.F (2004) Predator diversity dampens trophic cascades. *Nature* **429**:407-410 https://doi.org/10.1038/nature02554 | PubMed

Gay M., Renault T., Pons A.M., Le Roux F. (2004) Two Vibrio splendidus related strains collaborate to kill Crassostrea gigas: taxonomy and host alterations. *Dis. Aquat. Organ* **62**:65-74 https://doi.org/10.3354/dao062065 | PubMed

Genovesi B., Mouillot D., Laugier T., Fiandrino A., Laabir M., Vaquer A., Grzebyk D (2013) Influences of sedimentation and hydrodynamics on the spatial distribution of Alexandrium catenella/tamarense resting cysts in a shellfish farming lagoon impacted by toxic blooms. *Harmful Algae* **25**:15-25 https://doi.org/10.1016/j.hal.2013.02.002

Genovesi B., Shin-Grzebyk M.-S., Grzebyk D., Laabir M., Gagnaire P.-A., Vaquer A., Pastoureaud A., Lasserre B., Collos Y., Berrebi P., *et al.* (2011) Assessment of cryptic species diversity within blooms and cyst bank of the Alexandrium tamarense complex (Dinophyceae) in a Mediterranean lagoon facilitated by semi-multiplex PCR. *J. Plankton Res* **33**:405-414 https://doi.org/10.1093/plankt/fbq127

Hadjadji I., Laabir M., Frihi H., Collos Y., Shao Z.J., Berrebi P., Abadie E., Amzil Z., Chomérat N., Rolland J.L., *et al.* (2020) Unsuspected intraspecific variability in the toxin production, growth and morphology of the dinoflagellate Alexandrium pacificum R.W. Litaker (Group IV) blooming in a South Western Mediterranean marine ecosystem, Annaba Bay (Algeria). *Toxicon* **180**:79-88 https://doi.org/10.1016/j.toxicon.2020.04.005 | PubMed

Hallegraeff G.M., Anderson D.M., Belin C., Bottein M.-Y.D., Bresnan E., Chinain M., Enevoldsen H., Iwataki M., Karlson B., McKenzie C.H., *et al.* (2021) Perceived global increase in algal blooms is attributable to intensified monitoring and emerging bloom impacts. *Commun. Earth Environ* **2**:117 https://doi.org/10.1038/s43247-021-00178-8 | PubMed

Harrison P., Waters R., Taylor F (1980) A Broad-Spectrum Artificial Seawater Medium for Coastal and Open Ocean Phytoplankton. *J. Phycol* **16**:28-35 https://doi.org/10.1111/j.1529-8817.1980.tb00724.x

Johnke J., Cohen Y., de Leeuw M., Kushmaro A., Jurkevitch E., Chatzinotas A. (2014) Multiple micro-predators controlling bacterial communities in the environment. *Curr. Opin. Biotechnol* **27**:185-190 https://doi.org/10.1016/j.copbio.2014.02.003 | PubMed

Johnson C.N (2013) Fitness Factors in Vibrios: a Mini-review. *Microb. Ecol* **65**:826-851 https://doi.org/10.1007/s00248-012-0168-x | PubMed

Kim C.M., Shin S.H (2011) Modulation of Iron-Uptake Systems by a Mutation of luxS Encoding an Autoinducer-2 Synthase in Vibrio vulnificus. *Biol. Pharm. Bull* **34**:632-637 https://doi.org/10.1248/bpb.34.632 | PubMed

Kitatsukamoto K., Oyaizu H., Nanba K., Simidu U (1993) Phylogenetic-Relationships of Marine-Bacteria, Mainly Members of the Family Vibrionaceae, Determined on the Basis of 16s Ribosomal-Rna Sequences. *Int. J. Syst. Bacteriol.* **43**:8-19 https://doi.org/10.1099/00207713-43-1-8 | PubMed

Kramer J., Oezkaya O., Kuemmerli R (2020) Bacterial siderophores in community and host interactions. *Nat. Rev. Microbiol* **18**:152-163 https://doi.org/10.1038/s41579-019-0284-4 | PubMed

Kumbhar C., Mudliar P., Bhatia L., Kshirsagar A., Watve M (2014) Widespread predatory abilities in the genus Streptomyces. *Arch. Microbiol* **196**:235-248 https://doi.org/10.1007/s00203-014-0961-7 | PubMed

Laabir M., Jauzein C., Genovesi B., Masseret E., Grzebyk D., Cecchi P., Vaquer A., Perrin Y., Collos Y (2011) Influence of temperature, salinity and irradiance on the growth and cell yield of the harmful red tide dinoflagellate Alexandrium catenella colonizing Mediterranean waters. *J. Plankton Res* **33**:1550-1563 https://doi.org/10.1093/plankt/fbr050

Laanaia N., Vaquer A., Fiandrino A., Genovesi B., Pastoureaud A., Cecchi P., Collos Y (2013) Wind and temperature controls on Alexandrium blooms (2000-2007) in Thau lagoon (Western Mediterranean). *Harmful Algae* **28**:31-36 https://doi.org/10.1016/j.hal.2013.05.016

Labreuche Y., Le Roux F., Henry J., Zatylny C., Huvet A., Lambert C., Soudant P., Mazel D., Nicolas J.-L. (2010) Vibrio aestuarianus zinc metalloprotease causes lethality in the Pacific oyster Crassostrea gigas and impairs the host cellular immune defenses. *Fish Shellfish Immunol* **29**:753-758 https://doi.org/10.1016/j.fsi.2010.07.007 | PubMed

Lami R. (2019) Quorum Sensing in Marine Biofilms and Environments. In: Tommonaro T. (Ed). *Quorum Sensing* Academic Press. Elsevier. pp. 55-96 https://doi.org/10.1016/B978-0-12-814905-8.00003-4

Le Roux F., Binesse J., Saulnier D., Mazel D. (2007) Construction of a *Vibrio splendidus* Mutant Lacking the Metalloprotease Gene *vsm* by Use of a Novel Counterselectable Suicide Vector. *Appl. Environ. Microbiol* **73**:777-784 https://doi.org/10.1128/AEM.02147-06 | PubMed

Leblad B.R., Amnhir R., Reqia S., Sitel F., Daoudi M., Marhraoui M., Abdellah M.K.O., Veron B., Er-Raioui H., Laabir M (2020) Seasonal variations of phytoplankton assemblages in relation to environmental factors in Mediterranean coastal waters of Morocco, a focus on HABs species. *Harmful Algae* **96**:101819 https://doi.org/10.1016/j.hal.2020.101819 | PubMed

Lemire A., Goudenege D., Versigny T., Petton B., Calteau A., Labreuche Y., Le Roux F. (2015) Populations, not clones, are the unit of vibrio pathogenesis in naturally infected oysters. *Isme J* **9**:1523-1531 https://doi.org/10.1038/ismej.2014.233 | PubMed

LeRoux F., Wegner K.M., Baker-Austin C., Vezzulli L., Osorio C.R., Amaro C., Ritchie J.M., Defoirdt T., Destoumieux-Garzon D., Blokesch M., *et al.* (2015) The emergence of Vibrio pathogens in Europe: ecology, evolution, and pathogenesis (Paris, 11-12th March 2015). *Front. Microbiol.* **6** https://doi.org/10.3389/fmicb.2015.00830 | PubMed

Li D., Zhang H., Fu L., An X., Zhang B., Li Y., Chen Z., Zheng W., Yi L., Zheng T (2014) A Novel Algicide: Evidence of the Effect of a Fatty Acid Compound from the Marine Bacterium, Vibrio sp BS02 on the Harmful Dinoflagellate, Alexandrium tamarense. *Plos One* **9**:e91201 https://doi.org/10.1371/journal.pone.0091201 | PubMed

Liu P.-C., Lee K.-K., Yii K.-C., Kou G.-H., Chen S.-N (1996) News & Notes: Isolation of Vibrio harveyi from Diseased Kuruma Prawns Penaeus japonicus. *Curr. Microbiol* **33**:129-132 https://doi.org/10.1007/s002849900087 | PubMed

Lopez-Joven C., Rolland J.-L., Haffner P., Caro A., Roques C., Carre C., Travers M.-A., Abadie E., Laabir M., Bonnet D., *et al.* (2018) Oyster Farming, Temperature, and Plankton Influence the Dynamics of Pathogenic Vibrios in the Thau Lagoon. *Front. Microbiol* **9**:2530 https://doi.org/10.3389/fmicb.2018.02530 | PubMed

Mandel M.J., Stabb E.V., Ruby E.G (2008) Comparative genomics-based investigation of resequencing targets in Vibrio fischeri: Focus on point miscalls and artefactual expansions. *Bmc Genomics* **9**:138 https://doi.org/10.1186/1471-2164-9-138 | PubMed

Marampouti C., Buma A.G.J., De Boer M.K. (2021) Mediterranean alien harmful algal blooms: origins and impacts. *Environ. Sci. Pollut. Res* **28**:3837-3851 https://doi.org/10.1007/s11356-020-10383-1 | PubMed

Martin J., Fitzwater S (1988) Iron-Deficiency Limits Phytoplankton Growth in the Northeast Pacific Subarctic. *Nature* **331**:341-343 https://doi.org/10.1038/331341a0

Martin M.O (2002) Predatory prokaryotes: An emerging research opportunity. *J. Mol. Microbiol. Biotechnol* **4**:467-477 PubMed

Mavian C., Paisie T.K., Alam M.T., Browne C., Beau De Rochars V.M., Nembrini S., Cash M.N., Nelson E.J., Azarian T., Ali A., *et al.* (2020) Toxigenic Vibrio cholerae evolution and establishment of reservoirs in aquatic ecosystems. *Proc. Natl. Acad. Sci* **117**:7897-7904 https://doi.org/10.1073/pnas.1918763117 | PubMed

McRose D.L., Baars O., Seyedsayamdost M.R., Morel F.M.M (2018) Quorum sensing and iron regulate a two-for-one siderophore gene cluster in *Vibrio harveyi*. *Proc. Natl. Acad. Sci* **115**:7581-7586 https://doi.org/10.1073/pnas.1805791115 | PubMed

Miethke M., Marahiel M.A (2007) Siderophore-Based Iron Acquisition and Pathogen Control. *Microbiol. Mol. Biol. Rev* **71**:413-451 https://doi.org/10.1128/MMBR.00012-07 | PubMed

Miller S.D., Haddock S.H.D., Elvidge C.D., Lee T.F (2005) Detection of a bioluminescent milky sea from space. *Proc. Natl. Acad. Sci. U. S. A* **102**:14181-14184 https://doi.org/10.1073/pnas.0507253102 | PubMed

Millero F.J (1998) Solubility of Fe(III) in seawater. *Earth Planet. Sci. Lett* **154**:323-329 https://doi.org/10.1016/S0012-821X(97)00179-9

Muhling B.A., Jacobs J., Stock C.A., Gaitan C.F., Saba V.S (2017) Projections of the future occurrence, distribution, and seasonality of three Vibrio species in the Chesapeake Bay under a high-emission climate change scenario. *Geohealth* **1**:278-296 https://doi.org/10.1002/2017GH000089 | PubMed

Neilands J (1981) Iron-Absorption and Transport in Microorganisms. *Annu. Rev. Nutr* **1**:27-46 https://doi.org/10.1146/annurev.nu.01.070181.000331 | PubMed

Oberbeckmann S., Fuchs B.M., Meiners M., Wichels A., Wiltshire K.H., Gerdts G (2012) Seasonal Dynamics and Modeling of a Vibrio Community in Coastal Waters of the North Sea. *Microb. Ecol* **63**:543-551 https://doi.org/10.1007/s00248-011-9990-9 | PubMed

Pal M., Yesankar P.J., Dwivedi A., Qureshi A (2020) Biotic control of harmful algal blooms (HABs): A brief review. *J. Environ. Manage* **268**:110687 https://doi.org/10.1016/j.jenvman.2020.110687 | PubMed

Papenfort K., Bassler B.L (2016) Quorum sensing signal-response systems in Gram-negative bacteria. *Nat. Rev. Microbiol* **14**:576-588 https://doi.org/10.1038/nrmicro.2016.89 | PubMed

Perez J., Moraleda-Munoz A., Javier Marcos-Torres F., Munoz-Dorado J (2016) Bacterial predation: 75 years and counting! Environ. *Microbiol* **18**:766-779 https://doi.org/10.1111/1462-2920.13171 | PubMed

Pfaffl M.W (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**:e45 https://doi.org/10.1093/nar/29.9.e45 | PubMed

Rashidan K.K., Bird D.F (2001) Role of predatory bacteria in the termination of a cyanobacterial bloom. *Microb. Ecol* **41**:97-105 https://doi.org/10.1007/s002480000074 | PubMed

Rodrigues A.M.S., Lami R., Escoubeyrou K., Intertaglia L., Mazurek C., Doberva M., Pérez-Ferrer P., Stien D (2022) Straightforward *N*-Acyl Homoserine Lactone Discovery and Annotation by LC–MS/MS-based Molecular Networking. *J. Proteome Res* **21**:635-642 https://doi.org/10.1021/acs.jproteome.1c00849 | PubMed

Rolland J.-L., Pelletier K., Masseret E., Rieuvilleneuve F., Savar V., Santini A., Amzil Z., Laabir M (2012) Paralytic Toxins Accumulation and Tissue Expression of alpha-Amylase and Lipase Genes in the Pacific Oyster Crassostrea gigas Fed with the Neurotoxic Dinoflagellate Alexandrium catenella. *Mar. Drugs* **10**:2519-2534 https://doi.org/10.3390/md10112519 | PubMed

Romano A., Trimble L., Hobusch A.R., Schroeder K.J., Amin S.A., Hartnett A.D., Barker R.A., Crumbliss A.L., Carrano C.J (2013) Regulation of iron transport related genes by boron in the marine bacterium Marinobacter algicola DG893. *Metallomics* **5**:1025 https://doi.org/10.1039/c3mt00068k | PubMed

Rosales D., Ellett A., Jacobs J., Ozbay G., Parveen S., Pitula J (2022) Investigating the Relationship between Nitrate, Total Dissolved Nitrogen, and Phosphate with Abundance of Pathogenic Vibrios and Harmful Algal Blooms in Rehoboth Bay, Delaware. *Appl. Environ. Microbiol* **88**:e00356-22 https://doi.org/10.1128/aem.00356-22 | PubMed

Sinclair A.R.E., Mduma S., Brashares J.S (2003) Patterns of predation in a diverse predator-prey system. *Nature* **425**:288-290 https://doi.org/10.1038/nature01934 | PubMed

Su R.Q., Yang X.R., Zheng T.L., Tian Y., Jiao N.Z., Cai L.Z., Hong H.S (2007) Isolation and characterization of a marine algicidal bacterium against the toxic dinoflagellate Alexandrium tamarense. *Harmful Algae* **6**:799-810 https://doi.org/10.1016/j.hal.2007.04.004

Tanabe T., Funahashi T., Nakao H., Miyoshi S.-I., Shinoda S., Yamamoto S (2003) Identification and Characterization of Genes Required for Biosynthesis and Transport of the Siderophore Vibrioferrin in *Vibrio parahaemolyticus*. *J. Bacteriol* **185**:6938-6949 https://doi.org/10.1128/JB.185.23.6938-6949.2003 | PubMed

Thompson F.L., Thompson C.C., Swings J (2003) Vibrio tasmaniensis sp. nov., isolated from Atlantic Salmon (Salmo salar L.). *Syst. Appl. Microbiol* **26**:65-69 https://doi.org/10.1078/072320203322337326 | PubMed

Tourneroche A., Lami R., Hubas C., Blanchet E., Vallet M., Escoubeyrou K., Paris A., Prado S (2019) Bacterial–Fungal Interactions in the Kelp Endomicrobiota Drive Autoinducer-2 Quorum Sensing. *Front. Microbiol* **10**:1693 https://doi.org/10.3389/fmicb.2019.01693 | PubMed

Vanhove A.S., Rubio T.P., Nguyen A.N., Lemire A., Roche D., Nicod J., Vergnes A., Poirier A.C., Disconzi E., Bachere E., *et al.* (2016) Copper homeostasis at the host vibrio interface: lessons from intracellular vibrio transcriptomics. *Environ. Microbiol* **18**:875-888 https://doi.org/10.1111/1462-2920.13083 | PubMed

Wang X., Li Z., Su J., Tian Y., Ning X., Hong H., Zheng T (2010) Lysis of a red-tide causing alga, Alexandrium tamarense, caused by bacteria from its phycosphere. *Biol. Control* **52**:123-130 https://doi.org/10.1016/j.biocontrol.2009.10.004

Wang Y., Li S., Liu G., Li X., Yang Q., Xu Y., Hu Z., Chen C.-Y., Chang J.-S (2020) Continuous production of algicidal compounds against Akashiwo sanguinea via a Vibrio sp. co-culture. *Bioresour. Technol* **295**:122246 https://doi.org/10.1016/j.biortech.2019.122246 | PubMed

Weerasinghe A.J., Amin S.A., Barker R.A., Othman T., Romano A.N., Parker Siburt C.J., Tisnado J., Lambert L.A., Huxford T., Carrano C.J., *et al.* (2013) Borate as a Synergistic Anion for *Marinobacter algicola* Ferric Binding Protein, FbpA: A Role for Boron in Iron Transport in Marine Life. *J. Am. Chem. Soc* **135**:14504-14507 https://doi.org/10.1021/ja406609s | PubMed

Wu J., Luther G (1994) Size-Fractionated Iron Concentrations in the Water Column of the Western North-Atlantic Ocean. *Limnol. Oceanogr* **39**:1119-1129 https://doi.org/10.4319/lo.1994.39.5.1119

Xu Q., Wang P., Huangleng J., Su H., Chen P., Chen X., Zhao H., Kang Z., Tang J., Jiang G., *et al.* (2022) Co-occurrence of chromophytic phytoplankton and the Vibrio community during Phaeocystis globosa blooms in the Beibu Gulf. *Sci. Total Environ* **805**:150303 https://doi.org/10.1016/j.scitotenv.2021.150303 | PubMed

Yang Q., Feng Q., Zhang B., Gao J., Sheng Z., Xue Q., Zhang X (2021) Marinobacter alexandrii sp. nov., a novel yellow-pigmented and algae growth-promoting bacterium isolated from marine phycosphere microbiota. *Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **114**:709-718 https://doi.org/10.1007/s10482-021-01551-5 | PubMed

Zhang W., Wang Y., Lu H., Liu Q., Wang C., Hu W., Zhao K (2020) Dynamics of Solitary Predation by Myxococcus xanthus on Escherichia coli Observed at the Single-Cell Level. *Appl. Environ. Microbiol* **86**:e02286-19 https://doi.org/10.1128/AEM.02286-19 | PubMed

Peer reviews

Reviewer #1 (Public review):

Summary:

Rolland and colleagues investigated the interaction between Vibrio bacteria and Alexandrium algae. The authors found a correlation between the abundance of the two in the Thau Lagoon and observed in the laboratory that Vibrio grows to higher numbers in the presence of the algae than in monoculture. Timelapse imaging of Alexandrium in coculture with Vibrio enabled the authors to observe Vibrio bacteria in proximity to the algae and subsequent algae death. The authors further determine the mechanism of the interaction between the two and point out similarities between the observed phenotypes and predator prey behaviours across organisms.

Strengths:

The study combines field work with mechanistic studies in the laboratory and uses a wide array of techniques ranging from co-cultivation experiments to genetic engineering, microscopy and proteomics. Further, the authors test multiple Vibrio and Alexandria species and claim a wide spread of the observed phenotypes.

Comments on revisions:

I thank the authors for their additional work on the manuscript. My comments were addressed to my satisfaction.

https://doi.org/10.7554/eLife.107221.2.sa2

Reviewer #2 (Public review):

Goal summary

The authors sought to (i) demonstrate correlations between the dynamics of the dinoflagellate Alexandrium pacificum and the bacterim Vibrio atlanticus in natural populations, ii) demonstrate the occurrence of predation in laboratory experiments, iii) demonstrate that predation is induced by predator starvation, and iv) test for effects of quorum sensing and iron-uptake genes on the predation process.

Strengths include

- Data indicating correlated dynamics in a natural environment that increase the motivation for study of in vitro interactions

- Experimental design allowing clear inference of predation based on population counts of both prey and predators in addition to microscopy-based evidence

- Supplementation of population-level data with molecular approaches to test hypotheses regarding possible involvement of quorum sensing and iron update in predation

Weaknesses include

- A quantitative analysis of effects of manipulating V. atlanticus density on rates of predation would have been valuable

- Lack of clarity in some of the methodological descriptions

Appraisal

The authors convincingly demonstrate that V. atlanticus can prey on A. pacificum, provide strongly suggestive evidence that such predation is induced by starvation and clearly demonstrate that both iron availability and correspondingly the presence of genes involved in iron uptake strongly influence the efficacy of predation.

Discussion of impact

This paper will interest those interested in the diversity of forms of microbial predation and how microbial predatory behavior responds to environmental fluctuations. It will also interest those investigating bacteria-algae interactions and potential ecological controls of algal blooms. It may also interest researchers of microbial cooperation in light of the suggestion of communication between predator cells.

https://doi.org/10.7554/eLife.107221.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***Reviewer #1 (Public review):***
>
> *Weaknesses:*
>
> *In my view, the presentation of the data is in some cases not ideal. The phrasing of some conclusions (e.g., group-attacks and wolf-pack-hunting by the bacteria) is in my opinion too strong based on the herein provided data.*

We agree with your comment and have replaced the terms "Group-attacks" and "wolf-pack-hunting by "attacks" throughout the manuscript.

> ***Reviewer #1 (Recommendations for the authors):***
>
> *(1) Figure 2AB, please add the name of the statistical test and the number of replicates that the data is based on to the figure legend.*

We thank Reviewer#1 for highlighting the need for more detail. We have revised the manuscript accordingly. The captions of figures 2, 3, 4 and S1 were revised to include the name of the statistical test and the number of replicates. Asterisks indicate significant differences in a multiple comparison test (One -way ANOVA with post hoc Tukey test),* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

> *(2) Figure 2C is this figure referred to in the text?*

We apologize for this oversight. Figure 2C was replaced by new figures 2C and 2D and the old figure 2C is now referenced in the manuscript as Fig 3B1.

> *(3) Movie 1, could the movie please also be provided as .mp4? I suggest including individual images across time in the main figure so that readers do not rely on opening a supplementary file for this key finding of the study.*

In the revised manuscript, all the videos were converted to mp4 format and individual images across time were included in Figure 2C and 2D (Chronological snapshots of one attack) and in figure 3B1 (Chronological snapshots of the complete event), thereby improving the readability of the manuscript.

> *(4) Figure 3A2 (text l. 355), I am afraid I do not find this figure.*

Fig. 3A2 which previously corresponded to Fig. 3B1, correspond now to Fig. 2C and Fig. 2D. This has been corrected in the revised version of the manuscript.

> *(5) Lines 356ff, I am afraid that I find it hard to follow what the authors refer to as the right cell or the left cell. I suggest either adding labels to the movies or providing individual images across multiple timepoints into the main figure that can be labelled and bring across the point.*

Arrows have been added to videos 3–5 to clearly indicate the cells referred to in the text and facilitate tracking across time.

> *(6) In general, for all the microscopy, on how many cells have these phenomena been observed? What is n=x? Has this been quantified?*

We thank the reviewer for pointing this out.

In caption of Fig. 3, the sentence "(A) Percentage of motile *A. pacificum* ACT03. (B) *A. pacificum* ACT03 attacked by *V. atlanticus* LGP32 and (C) *A. pacificum* ACT03 lysis after 0, 15, 30, 45 and 60 min of interaction. "was replaced by "(A) Cumulative percentage of motile *A. pacificum* ACT03 cells. (B) Cumulative number of cells attacked by *V. atlanticus* LGP32 and (C) Cumulative cell lysis after 0, 15, 30, 45 and 60 minutes of interaction.". In Fig. 3 caption, the sentence "All percentages were determined based on a minimum of 2,000 cells of *A. pacificum* ACT03." was also added.

In Fig. 4 caption, the sentence "All percentages were determined based on a minimum of 2,000 cells of *A. pacificum* ACT03." was added.

In Fig. S1 caption, the sentence "All percentages were determined based on a minimum of 2,000 cells of *A. pacificum* ACT03." was added.

> *(7) Figure S1A, does this figure show means plus/minus standard deviation? If yes, please add this to the figure legends.*

In Fig. S1 caption, the sentence "Error bars represent the standard deviation of the mean of three independent experiments" was added.

> *How do the authors explain the big variation in the test condition and not in the control?*

Regarding the higher variation observed in the test condition compared to the control, this may, on the one hand, reflect biological variability between independent batches of 60-h *V. atlanticus* cultures used to prepare the supernatants, and, on the other hand, a heterogeneity in the physiological status of independent algal batches (N = 3 ; 2 × 10^4 cells ; see Materials and Methods, Co-culture assay), which may not be perfectly synchronized . In contrast, the control condition consists of *A. pacificum* cultures incubated in fresh medium without bacterial supernatant, for which algal motility is highly reproducible and thus shows very little variation.

> *(8) Line 375, "The lysis phase corresponded to initial vesicle formation followed by the bursting of A. pacificum ACT03 cells (Movie 5) and was induced by the old-starved culture supernatant of V. atlanticus LGP32 (Fig. S1)." Is this reference to Figure S1 correct? S1*

> *shows motility, doesn't it? I don't see how this data supports the statement made in this sentence.*

We apologize for this unclear message.

"The lysis phase corresponded to initial vesicle formation followed by the bursting of *A. pacificum* ACT03 cells (Video 5) and was induced by the old-starved culture supernatant of *V. atlanticus* LGP32 (Fig. S1)." was replaced by "The lysis phase corresponded to initial vesicle formation followed by the bursting of *A. pacificum* ACT03 cells (Fig. 3C and 3C1).

And “We next tested whether this lytic effect was mediated by thermostable molecule (s) secreted by *Vibrio*. “was replaced by “We next tested whether this lytic effect was linked to *Vibrio* culture supernatant and mediated by thermostable molecule (s) secreted by *Vibrio*.

> *(9) Line 388ff, "Group attacks were observed on non-degraded A. pacificum ACT03 cells, but not on previously lysed cells." No reference to a figure is provided. I am afraid I don't see the data that this statement is based on.*

As it is impossible to show a lack of attack, we just clarified the basis of our experiment.

“To this end, *A. pacificum* ACT03 in exponential growth phase was first exposed for 30 minutes to the supernatant of a 60-hour culture of *V. atlanticus* LGP32, which induced 25% lysis of *A. pacificum* ACT03 cells. Next, the corresponding *V. atlanticus* LGP32 cells were added. During exposure, attacks were observed only on undegraded *A. pacificum* ACT03 cells, but not on previously lysed cells” was replaced by “To this end, *A. pacificum* ACT03 in exponential growth phase was first exposed for 30 minutes to the supernatant of a 126-hour culture of *V. atlanticus* LGP32, which induced lysis of 70% of the *A. pacificum* ACT03 cells (Figures 3C and 3C1, arrow 2 and video 4). Next, cells of *V. atlanticus* LGP32 from a 60-hour culture, capable of attacking *A. pacificum* ACT03 cells (Fig. 3B), were added. For 1 hour of exposure, no attack was observed on the previously lysed algae.”

> *(10) Figure 4a, Based on the labeling of the figure, in particular the x-axis, it is not fully clear to me what I am looking at.*

Figure 4A has been reworked and its legend modified. We hope that this graph is clearer now.

> *(11) Line 428, did the authors consider complementing the pvuD deletion mutant and testing for gain of function when providing the gene in trans?*

We did not investigate pvuD in this study and did not construct a pvuD deletion mutant. We therefore assume that the recommendation refers to pvuB, which was the focus of our work. Unfortunately, we did not perform this experiment. However, several lines of evidence support the implication of PvuB and the vibrioferrin uptake system in this process: (i) the loss of attack behaviour is specific to the mutant in the vibrioferrin uptake pathway and (ii) our expression and proteomic data show a strong induction of vibrioferrin uptake components under starvation and iron-manipulated conditions, which correlate with the attack phenotype.

> *(12) Use of the term "group attack" in parentheses in the text, but in the section header and title. Is there really sufficient actual data to say that this is a "group attack"? What exactly are the indications for this being a behaviour of a group?*

We agree with you. The terms “group attacks” and “wolf-pack hunting” were replaced by the more neutral term “attacks” throughout the manuscript.

> *(13) Table S1 and S2, those tables give a nice overview. Do the authors provide the raw data based on which they make a claim on "+" and "-" in the individual categories? I would prefer to see the actual data or at least have the possibility to look into this.*

In the revised versions of Tables 1 and 2, we have improved the captions and clarified the meaning of each column in order to avoid any ambiguity between the results of this study and the bibliographic information.

Specifically regarding Table 2 :

We do not present any visuals of the interaction between Vibrio and Alexandrium because these species all look alike. Regarding the other algae species tested in interaction with Vibrio, phenomena other than lysis or cell attack have been observed and are the subject of specific laboratory studies.

> *(14) Line 456 "first study", line 40f "first evidence of a new mechanism". I suggest toning this down a bit and being clearer in the abstract about this being a working model that can be suggested based on individual bits of data.*

We thank Reviewer #1 for this helpful suggestion.

In the summary:

“This is the first evidence of a new mechanism that could to be involved in regulating Alexandrium spp. blooms and giving Vibrio a competitive advantage in obtaining nutrients from the environment.” was replaced by “The interaction model we propose here suggests that Vibrio could play a role in regulating the proliferation of Alexandrium spp., giving it a competitive advantage in obtaining nutrients from the environment.”

In the discussion:

Considering predator as a free organism that feeds at the expense of another, this study is the first evidence of the capacity of some *Vibrio* to develop a predatory strategy against an alga. This behaviour differs from parasitism, because the survival of *Vibrio* is not exclusively dependent on algae in environment” was replaced by “Consider a predator as a free-living organism that kills its prey and feeds on it, this study provides data suggesting the ability of Vibrios to develop an original predator-like behaviour to kill and feed on algae.”

> *(15) Line 469 "Overall, these observations show that V. atlanticus LGP32 is able of wolf-pack hunting behaviour." I see the similarities. I feel that the term "show" is a bit too strong here, or I suggest referring to "wolf-pack-like behaviour".*

The sentence “Overall, these observations show that *V. atlanticus* LGP32 is able of wolf-pack hunting attack behaviour” was replaced by “Overall, these observations suggest that *V. atlanticus* LGP32 can exhibit a predator-like behaviour”

> ***Reviewer #2 (Public review):***
>
> *As Weaknesses Reviewer #2 include:*
>
> *(1) A lack of early, clear definitions for several important terms used in the paper, including 'predation', 'coordination' and 'coordinated action', 'group attack', and 'wolf-pack hunting', along with a corresponding lack of criteria for what evidence would warrant use of some of these labels. (For example, does mere simultaneity of attacks of an A. pacificum cell by many V. atlanticus cells constitute "coordination"? Or, as it seems to us, does coordination require some form of signalling between predator cells?)*

The term “Coordinate” was replaced by “simultaneous” throughout the manuscript

The terms “Group attack” and “wolf pack hunting” were replaced by “attack” throughout the manuscript

> *(2) Absence of controls for cell density in the test for starvation effects on predatory behaviour; unclear how the length of incubation affects the density of V. atlanticus cells.*

We thank the reviewer for pointing this out.

Cells density experiment was already performed (cf. Fig. 4A).

The sentence. ”All percentages were determined based on a minimum of 2,000 cells of *A. pacificum* ACT03.“ was added in captions of Fig. 3, Fig. 4 and Fig S1

> *(3) Lack of clarity in some of the methodological descriptions*

The Methodology has been checked and some improvements have been made.

> ***Reviewer #2 (Recommendations for the authors):***
>
> *(A) Title*
>
> *(1) Could 'induces' be better than 'promotes'?*

We agree with Reviewer #2. The initial title, “Starvation of the bacterium *Vibrio atlanticus* promotes lightning group-attacks on the dinoflagellate *Alexandrium pacificum*”, was replaced by “Starvation of the bacterium *Vibrio atlanticus* induces simultaneous attacks on the dinoflagellate *Alexandrium pacificum*”.

> *(B) Abstract*
>
> *(1) Perhaps define pycosphere in the abstract - many readers might not know this word.*

We have revised the abstract to define the term phycosphere and added the sentence “This occurs in the microenvironment surrounding phytoplankton cells, the phycosphere. An interface rich in nutrients and organic molecules exuded by the cell.”

> *(2) Perhaps "on dinoflagellates".*

We thank Reviewer #2 for this suggestion. We have revised the abstract by replacing “on the dinoflagellates species” with “on dinoflagellates”.

> *(3) Line 33 - The word 'prey' is used without a claim of predation having yet been made; only killing has been claimed so far.*

We agree and have replaced the word “prey” by “algae” in the abstract.

> *(4) Line 34 - It is unclear whether the description refers to the 'attack stage' or to 'wolf-pack attack' in general. The sentence is written in such a way that it seems to refer to 'wolf-pack attack'. However, this would seem to be incorrect, with the description being specific to V. atlanticus.*

To avoid this ambiguity, we have removed the sentence “resembles the ‘wolf-pack attack’ strategy” from the abstract.

> *(5) Line 35 - Should there be a 'consumption phase'?*

We agree with the reviewer #2, “degradation” was replaced by “consumption”.

> *(6) If predation is claimed later in the manuscript (which it is), it should be explicitly claimed in the abstract.*

We thank Reviewer #2 for this helpful suggestion.

We have revised the abstract. The sentence “Results showed that *Vibrio atlanticus* was able to coordinate lightning group attacks then kill the dinoflagellate *Alexandrium pacificum* ACT03” was replaced by “The results showed that *Vibrio atlanticus* was capable of attacking and killing the dinoflagellate *Alexandrium pacificum* ACT03”.

> *(C) Main text*
>
> *(1) Line 54 - Perhaps "Among HAB-causing organisms...".*

We agree with the reviewer’s suggestion and have revised the wording.

> *(2) Line 56 - "that, together with..., form the "Alexandrium tamarense" complex".*

We agree with the reviewer’s suggestion and have revised the sentence.

> *(3) Line 57 - What this "complex" is and its significance should be explained.*

“Among them, *Alexandrium pacificum* is a flagellated eukaryotic unicellular organism that together with *Alexandrium tamarense* and *Alexandrium fundyense* form the "Alexandrium tamarense" complex (Hadjadji et al., 2020)” was replaced by

“Among them, *Alexandrium pacificum* is a flagellated eukaryotic unicellular organism that together with *Alexandrium tamarense* and *Alexandrium fundyense* form the "Alexandrium tamarense" complex, responsible for paralytic shellfish poisoning worldwide (Hadjadji et al., 2020)”

> *(4) Line 58 - What is a Rephy survey?*

We clarified this point, “by rephy survey” was replaced by “by the French phytoplankton observation and monitoring network (Rephy)”

> *(5) Line 59 - 'resulting in' instead of 'resulting of'.*

We agree with the reviewer and have replaced “resulting of” with “resulting in”.

> *(6) Line 65 - It seems that ', influencing the time of appearance of blooms' would be more correct than the current phrasing. The current phrasing is unclear regarding the relation between species, tolerance range, and the time of appearance of blooms.*

To address this point, “Depending on the phytoplankton species, the tolerance range of physicochemical parameters is different and influences the time of appearance of blooms” was replaced by “Depending on the species of phytoplankton, tolerance to physicochemical parameters varies, which influences when blooms occur.”

> *(7) Line 76 - Run-on sentence which should probably be split after the reference to Wang et al., 2020.*

We agree with the reviewer and have split the sentence.

> *(8) Line 89 - What are these observations?*

This sentence was reformulated.

“Based on observations from the natural environment showing a potent relationship between Vibrio and Alexandrium algae bloom events, this study aim to determine *in vitro*, the main factors implicated in this relationship” was replaced by ”This study aims to describe observations made in the natural environment between Vibrio bacteria and Alexandrium algal blooms, and to determine in vitro the main factors involved in this relationship.”

> *(9) Line 94 - This is the first clear reference to a predator-prey interaction, and it is stated as if it's established. Is it not a central goal of the study to demonstrate that predation is even happening?*
>
> *Based on the title and abstract, I would have expected the major claims of the paper highlighted in the abstract to be:*
>
> *(i) that predation of algae by bacteria occurs in this system,*
>
> *(ii) there is a social component of predation,*
>
> *(iii) claims about what induces this predatory behaviour.*

The summary has been amended accordingly, and the term “predation” has been removed, along with all sentences referring to it.

> *(10) Line 99 - What does n.d. mean?*

This point was addressed in the revised version.

> *(11) Line 97 section - specify qPCR.*

This point was clarified in the revised version.

> *(12) Line 139 - Mentioning the oligonucleotides in this part of the methods seems out of place. Would this not fit better in the section on Gene expression analysis?*

This sentence was discarded from this paragraph.

> *(13) Line 147 - Where did the co-cultured phytoplankton species come from?*

To answer this point, reference to Table 2 was added

> *(14) Line 149 - Is it known if the phytoplankton strains had all grown to the same density after 24 hours?*

The doubling time of dinoflagellates in laboratory culture is between 5 and 7 days. During the duration of the experiments, the dinoflagellate concentration did not change significantly.

The sentence “(doubling time between 5 and 7 days)” was added

> *(15) Line 150 - Was the density of the Vibrio cultures at the different incubation times measured? Density might play an important role in predation, and so it would be important to control for density in these assays.*

The concentrations of live vibrio in each individual culture were not actually measured. However, the role of vibrio density in attacks was measured and is shown in Figure 4A and observed in Fig 2B.

> *(16) Line 153 - How long was the co-incubation?*

The incubation times were added in the revised version.

> *(17) Line 158 - What is mean by "independent experiments", more exactly?*

To clarify this point, “Data are the means of three independent experiments” was replaced by “The data come from three independent experiments using independent phytoplankton cultures and independent bacterial cultures.”

> *(18) Line 161 - Perhaps give the source information about the Vibrio strain at its first mention.*

A reference has been added in the revised preprint.

> *(19) Line 163 - line 141 refer to multiple non-axenic species, whereas here "the algal strain" is referred to.*
>
> *And*
>
> *(20) Line 164 - language phrasing throughout the manuscript could use some polishing, e.g., "this means that additional bacteria...".*

To address this comment, “As the algal strain used in the study is not axenic, means that additional bacteria, other than the *V. atlanticus* LGP32, are potentially present in the experiments.” was replaced by “As the *A. pacificum* ACT03 strain (table 2) used in the study is not axenic, there is potential for bacteria other than *V. atlanticus* LGP32 to be present in the experiments.”

> *(21) Line 208 - Why were both magnitude and p-value criteria used rather than just p-values?*

In the present proteomic approach each experimental condition was measured six times, and the average (mean) value was used to reduce random noise. Then we selected differences that had to be large enough to matter biologically, this is a central criterion and at least a 2-fold change was considered to focus exclusively on biologically relevant differences, which allowed us to control for the effect size. However, the differences also had to be statistically significant, we applied a statistical confidence at $P < 0.01$, to be sure that there is less than a 1% chance the result happened randomly. In the present proteomic approach each experimental condition was measured six times, and the average (mean) value was used to reduce random noise.

Then we selected differences that had to be large enough to matter biologically, this is a central criteria and at least a 2-fold change was considered to focus exclusively on biologically relevant differences, which allowed us to control for the effect size. However, the differences also had to be statistically significant, we applied a statistical confidence at $P < 0.01$, to be sure that there is less than a 1% chance the result happened randomly. We considered that using both criteria makes the results meaningful and trustworthy, not just a small or random fluctuation.

> *(22) Line 270 - Were these three replicate experiments also "independent"; if yes, in what sense?*

“All experiments were conducted in triplicate” was replaced by “The experiments were performed using biological triplicates, each of which was analyzed in triplicate.”

> *(23) Line 296 - Perhaps "the temperature-sensitivity (or resistance) of" rather than "the nature of".*

The modification was made in the new manuscript.

> *(24) Line 307 - The sentence mentions only one influential period that was removed from the dataset, but the word 'whenever' suggests multiple occurrences.*

We agree, “whenever” was replaced by “because”.

> *(25) Line 325 - line 327 - The rationale behind the first part of the following sentence isn't clear to me, and what is meant by the second part is also not clear.*

To clarify this point, “This result is consistent with the difficulty that Vibrio has in growing at temperatures below 20°C and with the complex interacting factors driving bloom dynamics (Laanaia et al., 2013)” was replaced by “This result is consistent with the difficulty Vibrio has in growing at temperatures below 20°C and with the many environmental factors that influence the dynamics of algae proliferation (Laanaia et al., 2013)."

> *(26) Line 327 - line 328 - Hard to interpret; does this refer to living algal cells, or all algal cells, living and degraded?*

To improve clarity, “Interestingly, in spring 2015, the mean densities of all *Alexandrium* cells and of free-living *Vibrio* were positively correlated” was replaced by “Interestingly, in spring 2015, the mean densities of *Alexandrium* cells (living and degraded) and of free-living *Vibrio* were positively correlated”

> *(27) Figure 2 - These results strongly point to predation, but why the Vibrio population would already be elevated in the co-culture treatment relative to the control immediately after inoculation (0 hrs) is not clear.*

The experiments were not conducted at the same time, and the first value on the graphs corresponds to the concentration of vibrio determined after 1 hour of exposure/incubation and not at time 0. Figures 2A and 2B have been modified accordingly, and substantial changes have been made to the relevant section of the results.

> *(28) Line 348 - There's no mention of Figure 2C in the main text, or of the statistical test associated with it in the Figure 2 legend.*

To address this comment, Figure 2C has now been cited in the main text, and the statistical analysis method has been added to the Figure 2 caption.

> *(29) Line 352 - Text descriptions of videos are not easy to connect with the video content. Label the file names the same as how they are referred to in the text.*

We agree with you, the sentence “Epifluorescence microscopy observation of GFP-labelled *V. atlanticus* LGP32 (previously grown in Zobell medium) in interaction showed that *A. pacificum* ACT03 cells that had lost their motility were attacked individually by *V. atlanticus* LGP32 before being lysed (Fig, 2C and Video 1). “was rephrased and replaced by “Epifluorescence microscopy observation of GFP-labelled *V. atlanticus* LGP32 (previously grow in Zobell medium) in interaction showed that *V. atlanticus* LGP32 simultaneously attacks *A. pacificum* ACT03 cells (Fig, 2C and Video 1).”

> *(30) Movie 1 could be cut to remove uninteresting footage at the start. What indicates lysis? Is the deformation of the cells an indication of lysis?*

To respond to this comment, Video 1 has been shortened and in the caption, “degraded” was replaced by “lysed”

> *(31) Line 353 - Video could be zoomed in more on a few typical attacks to remove visual noise.*

A chronological overview of an attack has been added to Figure 2 corresponding to Figure 2D, and a chronological overview of the overall event has been added to Figure 3 corresponding to Figure 3B1.

> *(32) Line 355 - There does not seem to be a Figure 3A2.*

To address this point, the Fig. 2 and Fig. 3 has been revised for more clarity. See above

> *(33) Figure 3 - Can the authors fully exclude an effect of bacterial density as distinct from an effect of growth/starvation phase? It would be helpful to determine bacterial viable population densities at 12, 36, 60, and 126 hrs of incubation in Zobell medium, and to control for density in testing for effects on algae.*

Information on Vibrio densities incubated in Zobell medium for 12, 36, 60, and 126 hours has been now included in the results section "Attack of *A. pacificum* ACT03 is activated by *V. atlanticus* LGP32 starvation."

> *(34) Line 363 - It is unclear how the degradation of the flagella is apparent from movie 3. It would be helpful to have a comparison with healthy flagella.*

Alexandrium cells with intact flagella move so quickly that it is impossible for us to follow them and film their flagella with the tools at our disposal.

For greater clarity, arrows have been added to videos 3, 4 and 5.

> *(35) Line 364 - Sudden change from referring to the recording as 'video' instead of movie. What is meant by erratic swimming? The cell does not seem to move much.*

To address this comment, "Movie" was replaced by "Video" throughout the manuscript and "erratic swimming" was replaced by "irregular swimming"

> *(36) Line 365 - How did you observe the detachment of the flagellum?*

The detachment of the flagellum can be observed using a confocal microscope. This process was filmed and presented in Video 3. Arrows have been added to the video to clearly indicate the flagellum detachment.

> *(37) Line 368 - Perhaps this is due to it not being clear regarding which movie is meant, but there is no clear attack visible in movie 4.*

To make this clearer, arrows have been added to the video 4 to indicate attached cells.

And the sentence in the caption of the video 4 "*Vibrio*, filmed under a confocal microscope, attacks in groups one immobilized *Alexandrium* cell then moves on to attack — still as a group — another cell without touching the other whole cells, suggesting active communication between *Vibrio* cells" was rewritten and replaced by "This video, recorded under a confocal microscope, shows Vibrios simultaneously attacking a first immobilized Alexandrium cell, then moving on to attack a second cell without ever targeting the other cells present, suggesting active communication between the Vibrio bacteria."

> *(38) Line 369 - It seems the peak attach % was reached at 45 minutes, not 15-30 minutes.*

Sorry for the confusion. In fig. 3 for more clarity, the sentence "(A) Percentage of *A. pacificum* ACT03 motile cells. (B) cells attacked by *V. atlanticus* LGP32 and (C) cells lysis after 0, 15, 30, 45 and 60 min of interaction" was replaced by "(A) Cumulative percentage of motile *A. pacificum* ACT03 cells. (B) Cumulative number of cells attacked by *V. atlanticus* LGP32 and (C) Cumulative cell lysis after 0, 15, 30, 45 and 60 minutes of interaction."

> *(39) Line 382 - "clearly show role of nutrient limitation", see comment re controlling for any role of bacterial density.*

To address this point, information's on Vibrio densities were added in the manuscript. See cf comment 33.

> *(40) Line 385 - line 386 - Phrasing unclear.*

We have revised the text accordingly, “To this aim, *A. pacificum* ACT03 in exponential growth phase was first exposed for 30 min to supernatant from 60 hours starved *V. atlanticus* LGP32 Zobell media that induced 25% lysis of *A. pacificum* ACT03 cells and next to the corresponding *V. atlanticus* LGP32 cells. Group attacks were observed on non-degraded *A. pacificum* ACT03 cells, but not on lysed cells.“ was replaced by “To this end, *A. pacificum* ACT03 in exponential growth phase was first exposed for 30 minutes to the supernatant of a 126-hour culture of *V. atlanticus* LGP32, which induced lysis of 70% of the *A. pacificum* ACT03 cells (Figures 3C and 3C1, arrow 2 and video 4). Next, cells of *V. atlanticus* LGP32 from a 60-hour culture, capable of attacking *A. pacificum* ACT03 cells (Fig. 3B), were added. For 1 hour of exposure, no attack was observed on the previously lysed algae.”

> *(41) Line 413 - Is this the only pathway for quorum sensing in V. atlanticus?*

Indeed, the last two sentences of this paragraph are unclear.

To address this point:

“By targeted mutagenesis of key genes involved in QS pathways Δ*luxM* (HAI-1 production), Δ*luxS* (AI-2 production) and Δ*luxR* (high-density QS master regulator) did not lead to any change in the attack behaviour of *V. atlanticus* LGP32 (Fig. 4C).” was replaced by “Targeted mutagenesis of key genes involved in two of the three known QS pathways in vibrios (Fig. S3), ΔluxM (HAI-1 production), ΔluxS (AI-2 production), and ΔluxR (main high-density QS regulator), did not result in any changes in the attack behavior of *V. atlanticus* LGP32 (Fig. 4C).”

And “Taken together these results showed that attack by *V. atlanticus* LGP32 is not link to QS.” was replaced by. “Combined with the absence of overexpression of the CqsS gene (inducible by CAI-1) involved in the last known QS pathway in Vibrio (Fig. S3), these results indicated that the attack by *V. atlanticus* LGP32 is most likely unrelated to QS.”

> *(42) The references to tropism aren't clear.*

You're right, there's no reason to use the term tropism here. We have removed it.

> *(43) Line 439 - Why was H3BO4 used as a control for the addition of FeCl3?*

For clarity, the sentence “Boron being known to be a regulator or capable of being transported by vibrioferrin (Romano et al., 2013; Weerasinghe et al., 2013), we tested its potential involvement in the interaction but no effect was evidenced here.” was replaced by “Given that boron is known for its role in regulating a global bacterial cellular response to phytoplankton and to bind to vibrioferrin (Romano et al., 2013; Weerasinghe et al., 2013), we tested its potential involvement in simultaneous vibrio attacks. Compared to the Zobell control, no effect on the number of attacks was observed”

> *(44) Line 441 - line 449 - Should explicitly say in text that no attacks were observed for any species other than the Alexandrium and Gymnodinium species.*

We agree and have explicitly stated in the text that no attacks were observed for any species other than *Alexandrium* and *Gymnodinium*.

> *(45) Line 454 - line 455 - The last part of this sentence seems a strange statement, since*

> *(i) it has long been know that predatory bacteria can eat a wide range of eukaryotes, ii) one of the cited papers (Perez et al) actually highlights a case of bacterial predation on algae, and iii) in the next paragraph the authors themselves highlight Streptomyces predation of algae.*

To make this clearer, « Among predators, predatory bacteria are found in a wide variety of environments, and like bacteriophages and predatory protists, they have been reported to prey exclusively on other bacteria » was replaced by “Among predators, predatory bacteria are found in a wide variety of environments and, like bacteriophages and predatory protists, feed primarily on other bacteria, although a few cases of predation on microbial eukaryotes have also been reported.”

> *(46) Line 455 - Better to clarify the authors' definition of a predator at the start of the paper. The offered definition seems more like a definition of 'consumer' than 'predator', as the latter normally involves both the killing and consumption of other organisms, not just consumption with some kind of "expense".*

To address this comment:

- “predator behaviour” was replaced by “predator-like behaviour”

- and “Considering predator as a free organism that feeds at the expense of another, this study is the first evidence of the capacity of some *Vibrio* to develop a predatory strategy against an alga. This behaviour differs from parasitism, because the survival of *Vibrio* is not exclusively dependent on algae in environment” was replaced by “Consider a predator as a free-living organism that kills its prey and feeds on it, this study provides data suggesting the ability of Vibrios to develop an original predator-like behaviour to kill and feed on algae.”

> *(47) Line 457 - Don't see the benefit of trying to distinguish from parasitism here, especially since parasitism can be facultative, whereas the authors' phrasing suggests that it is always obligate.*

You are right, this sentence has been deleted.

> *(48) Line 463 - line 464 - The authors should clearly explain exactly what detailed aspects of Myxococcus and Lysobacter predation they think the "attack stage" of V. atlanticus resembles.*

Accordingly, “The second stage, the ‘attack stage’ corresponding to physical contact between Vibrio and Alexandrium resembles the ‘wolf-pack attack’ strategy described for *Myxococcus xanthus* and *Lysobacter regardless* of the prey species used, *M. xanthus* must be in close proximity to prey cells in order to induce their lysis and to benefit from their biomass (Martin, 2002; Perez et al., 2014)” was replaced by “The second stage, the ‘attack stage’ corresponding to the physical contact between Vibrios and Alexandrium, is similar to the strategy used by Myxococcus xanthus and Lysobacter. These bacteria must be in close proximity to their prey in order to cause lysis and utilize their biomass, regardless of the prey's species (Martin, 2002; Genovesi et al., 2013; Perez et al., 2016; Zhang et al., 2020)”

> *(49) Line 466 - line 467 - The comparison to bacteria clustering around lysed cells is surprising since the authors show that V. atlanticus does not attack already lysed cells.*

The sentence was rephrased, “This phenomenon is comparable to that of bacteria clustering around lysed ciliate cells “was replaced by “Visually, this phenomenon resembles bacteria clustering around lysed ciliate cells.”

> *(50) Line 469 - Missing is a statement of exactly what criteria constitute "wolf-pack hunting behaviour" and exactly how V. atlanticus meets those criteria.*

To address this point, “wolf-pack hunting behaviour” was replaced by “predator-like behaviour”

> *'Able of' should be corrected to 'Capable of'.*

We agree and have reworded the sentence.

> *(51) Line 470 - Consider starting a new paragraph for the material on quorum sensing.*

Accordingly, we have separated the section concerning QS pathway from the section concerning iron pathway.

> *(52) As part of their discussion on the role of iron uptake, can the authors comment on any relationship between starvation and iron uptake, and in particular the observations that, while general nutrient deprivation induces attacks, supplementation with a specific nutrient (iron) also induces attacks (Figure 4D)? Do bacteria starved for general growth substrates take up more iron than growing bacteria?*

To respond to this comment, “Future study could demonstrate further the role of vibrioferrin in group attack, by adding iron-saturated vibrioferrin to algae-Vibrio co-cultures.” was replaced by “Interestingly, if a general nutrient deficiency causes attacks, iron supplementation increases the number of attacks (Figure 4D), suggesting the importance of iron absorption in the attack behavior. Future studies should determine whether nutrient deficiency increases the iron absorption capacity of Vibrios and whether this plays a major role in the attack mechanism.”

> *(53) Line 486 - Of what is boron known to be a regulator?*

To respond to this comment, “Given that boron is known for its regulatory properties and for being transportable by vibrioferrin“ was replaced by “Given that boron is known for its role in regulating a global bacterial cellular response to phytoplankton and to bind to vibrioferrin”.

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