FINAL CONFERENCE

OPEN TO ALL STAKEHOLDERS

Brest (F)

26-28 November 2019

Pole Numérique Brest Iroise :
26-27 November

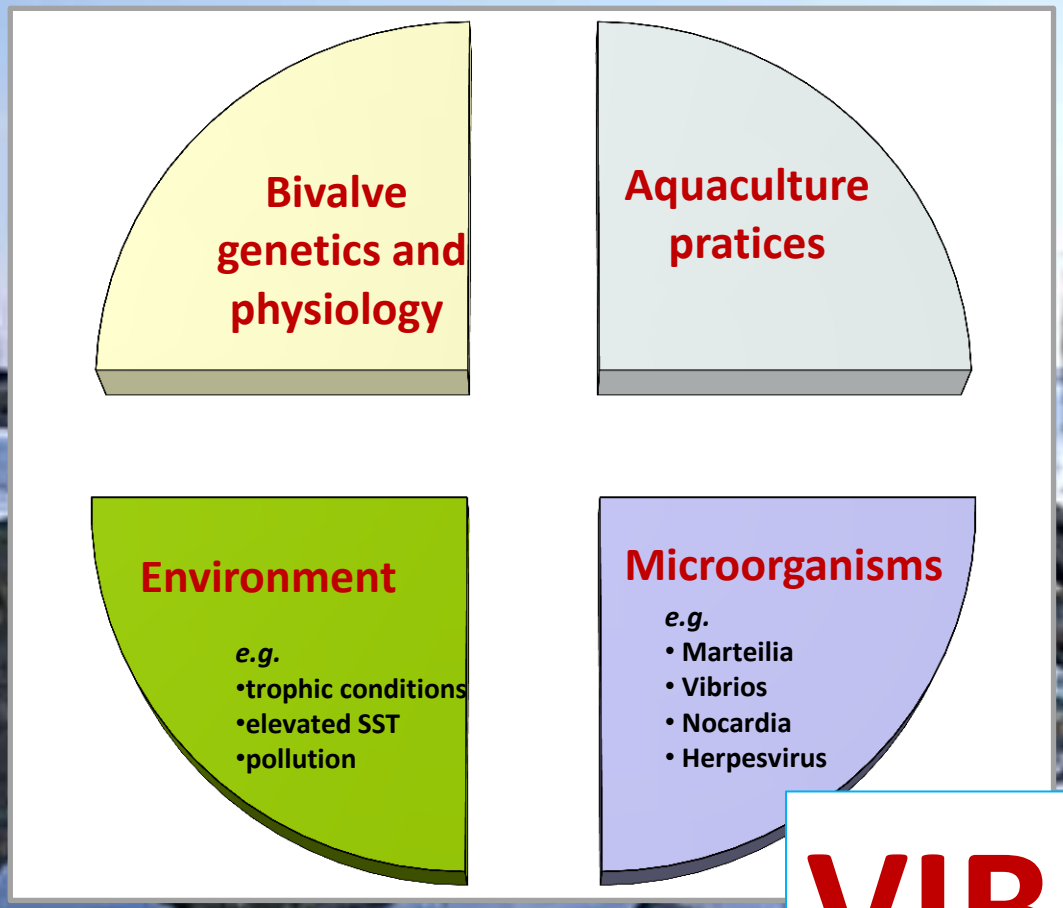
Océanopolis :
28 November

VIVALDI

Role of plankton in mediating Vibrio infections in bivalves

Luigi Vezzulli
University of Genoa

BIVALVE SUSCEPTIBILITY TO INFECTION



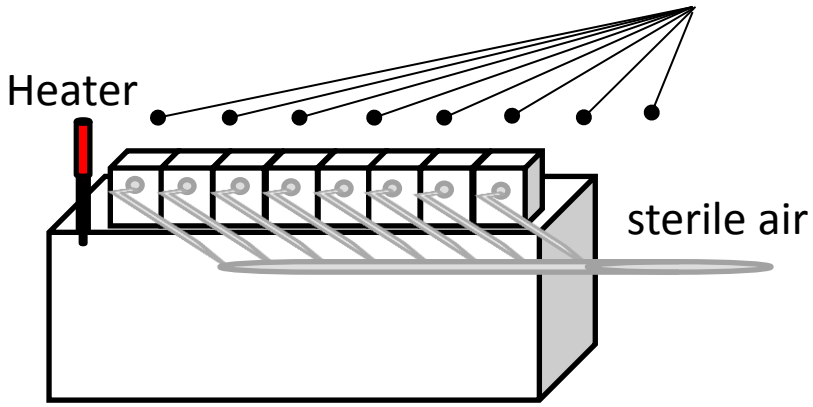
VIBRIOS

VIBRIO AESTUARIANUS
VIBRIO TASMANIENSIS
(SPLENDIDUS CLADE)

VIBRIO SURVIVAL IN THE MARINE ENVIRONMENT

VIABILITY & CULTURABILITY IN WATER AND SEDIMENT

MICROCOSM EXPERIMENTS



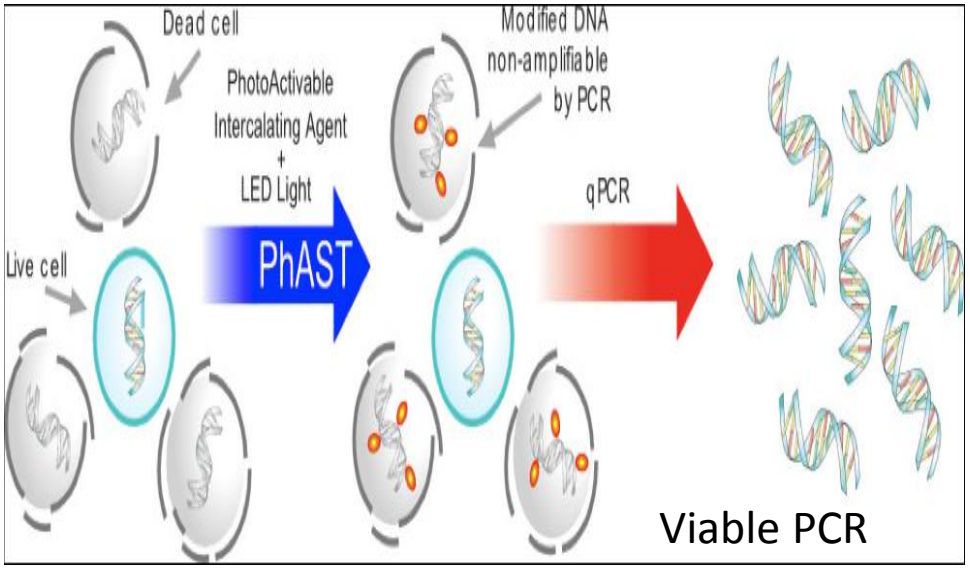
VIBRIO AESTUARIANUS **01/32**

VIBRIO TASMANIENSIS **LGP32**

Inoculum: 10^7cells/ml in conditions mimicking those of coastal waters

- **5°C and 25°C temperature**
- **20‰ and 35‰ salinity**

- **CULTURABLE:** Luria–Bertani Agar + NaCl (3%) and/or Marine Agar
- **TOTAL:** Real time PCR
- **VIABLE:** Real time PCR after propidium monoazide treatment (00 µM) and photoactivation with a 300W (3200K) halogen lamp



Vt, Va SURVIVAL IN SEAWATER

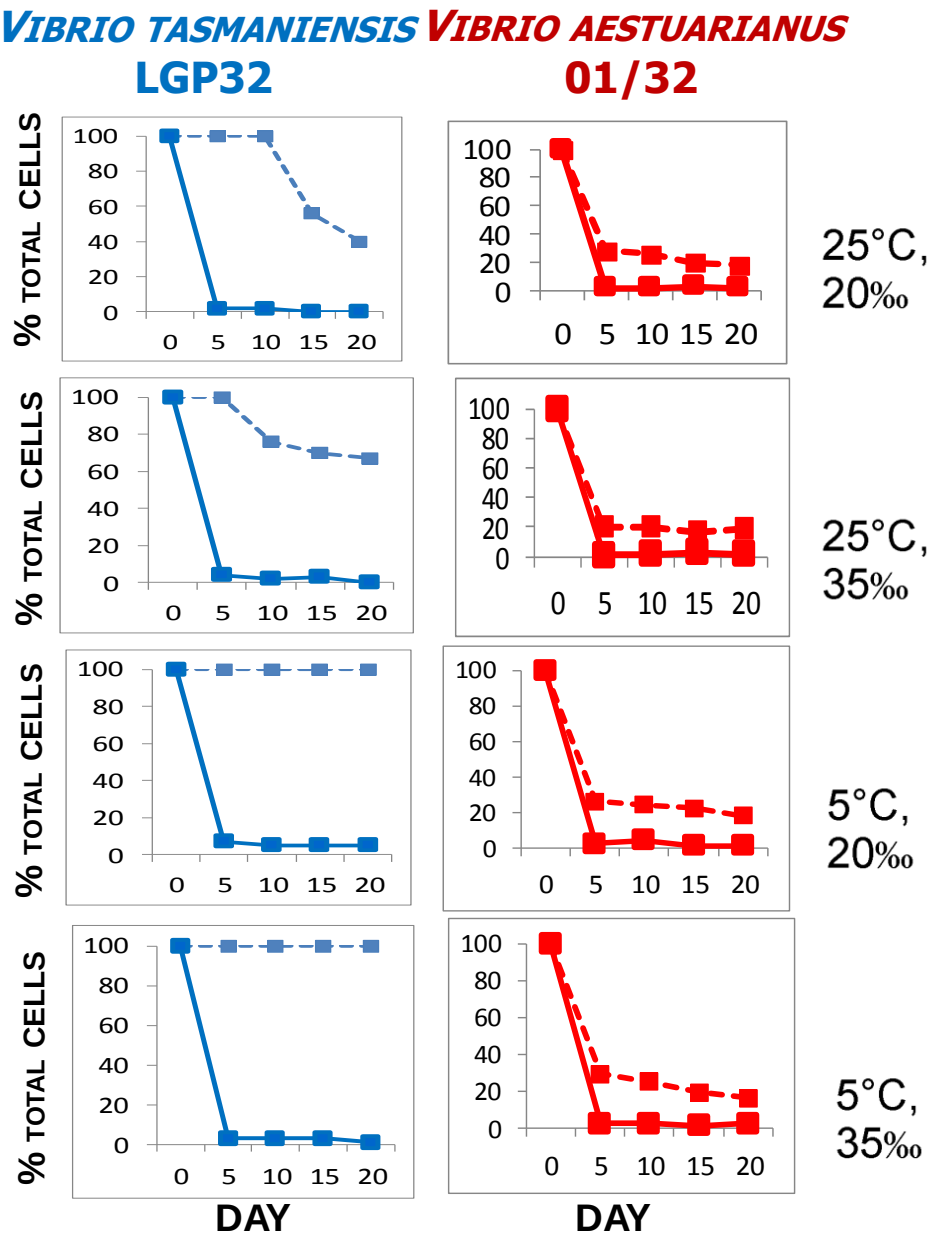
Seawater

VIBRIO STRAINS RAPIDLY LOSE CULTURABILITY IN SEAWATER

VIABLE

CULTURABLE

RESULTS ARE % OF TOTAL BACTERIA

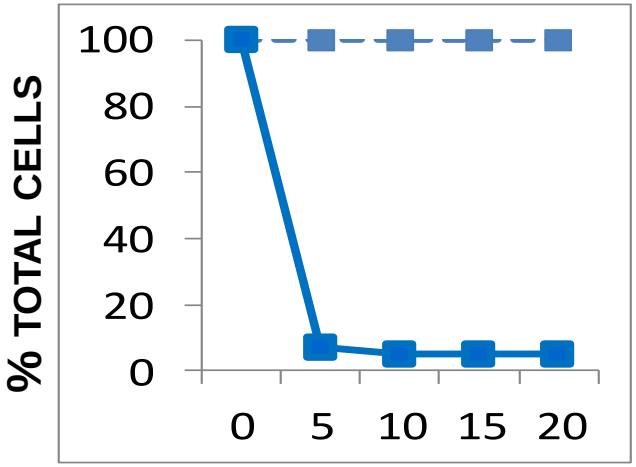


Vt, Va SURVIVAL IN THE SEDIMENT

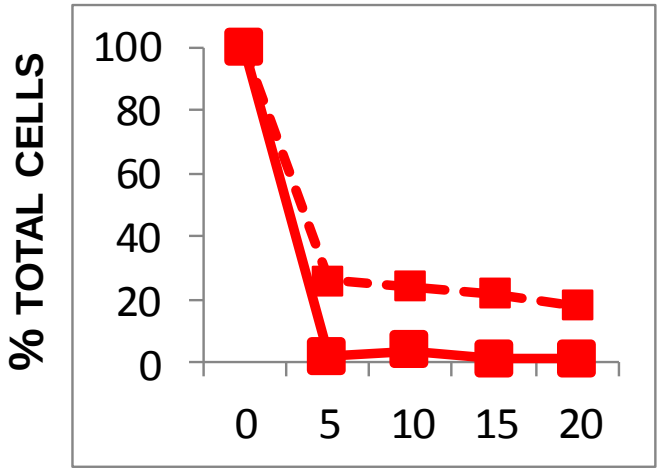
Seawater

ASW
5°C, 20 ‰
SALINITY

VIBRIO TASMANIENSIS
LGP32

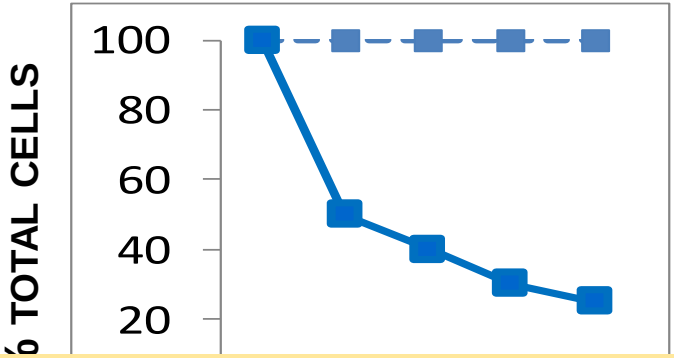


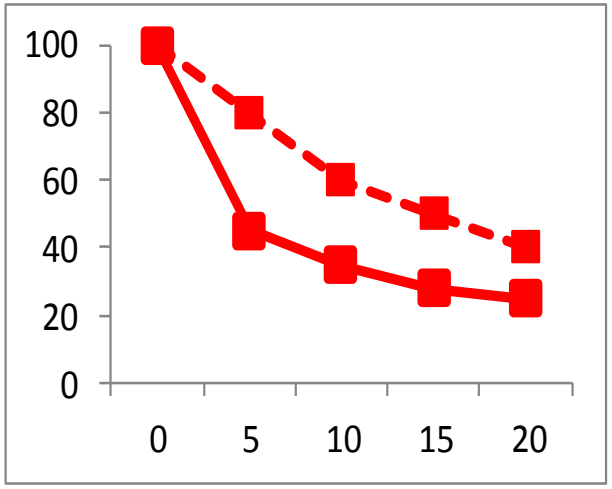
VIBRIO AESTUARIANUS
01/32



Sediment

SEDIMENT
5°C, 20 ‰
SALINITY



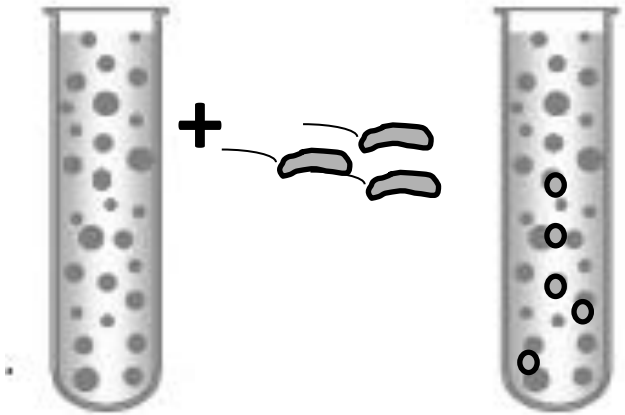


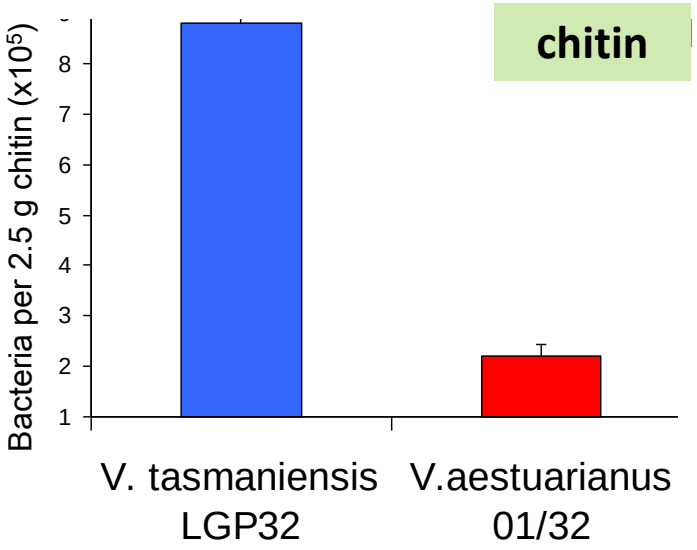
VIABLE

IN SEDIMENT VIBRIO STRAINS MAINTAIN VIABILITY AND CULTURABILITY FOR LONGER TIMES THAN IN SEA WATER

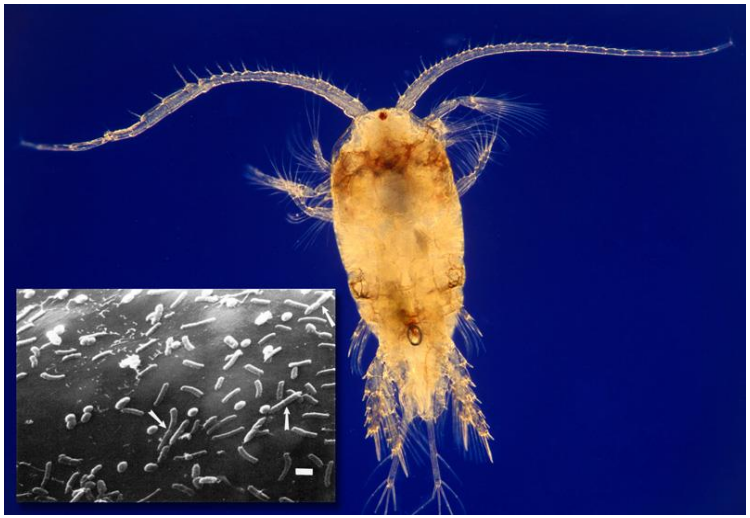
Vt, Va INTERACTIONS WITH:

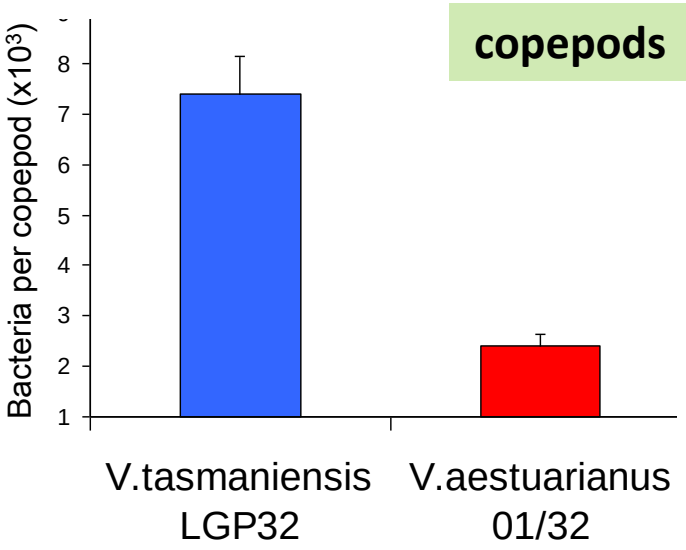
CHITIN PARTICLES



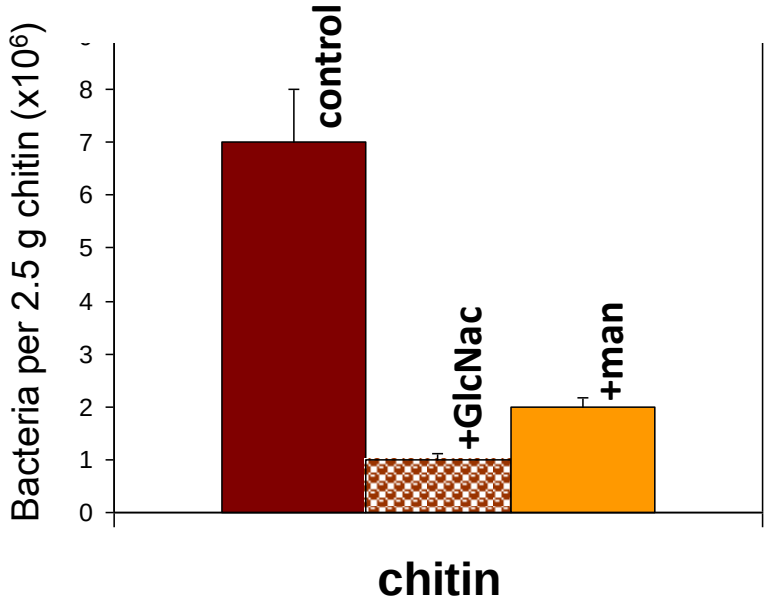


COPEPODS (*T. fulvus*)





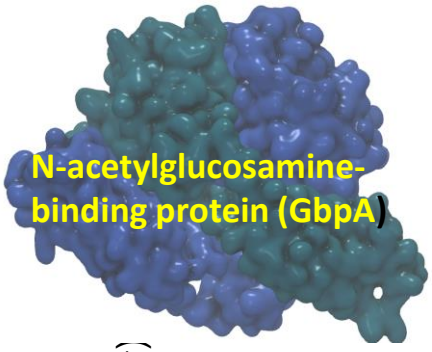
ROLE OF MSHA and GBPA ligands

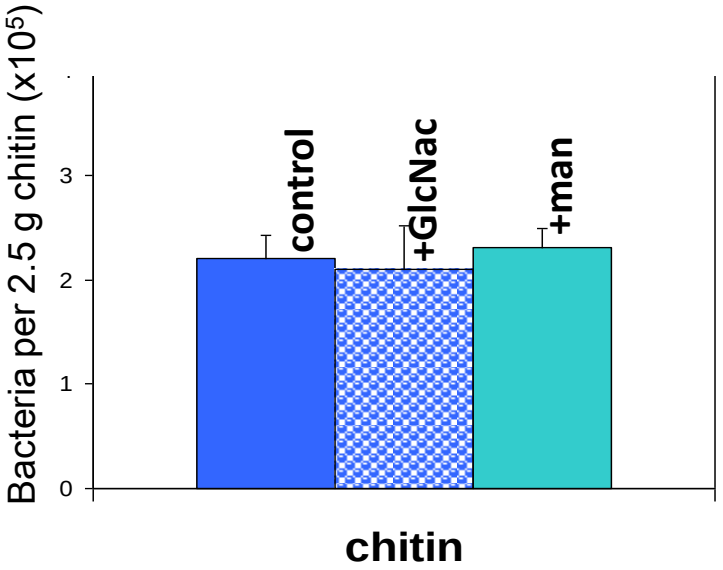


***V. CHOLERAE ELTOR* 14034**

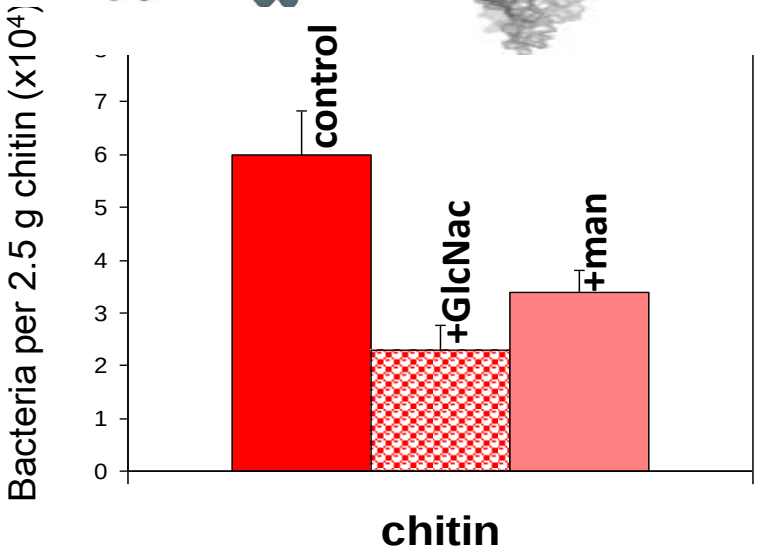
mshA+, gbpA+

(Zampini *et al.*, 2005; Stauder *et al.*, 2012)



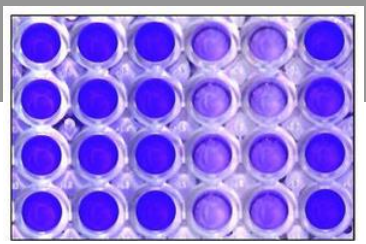


***V. TASMANIENSIS* LGP32** *mshA+*

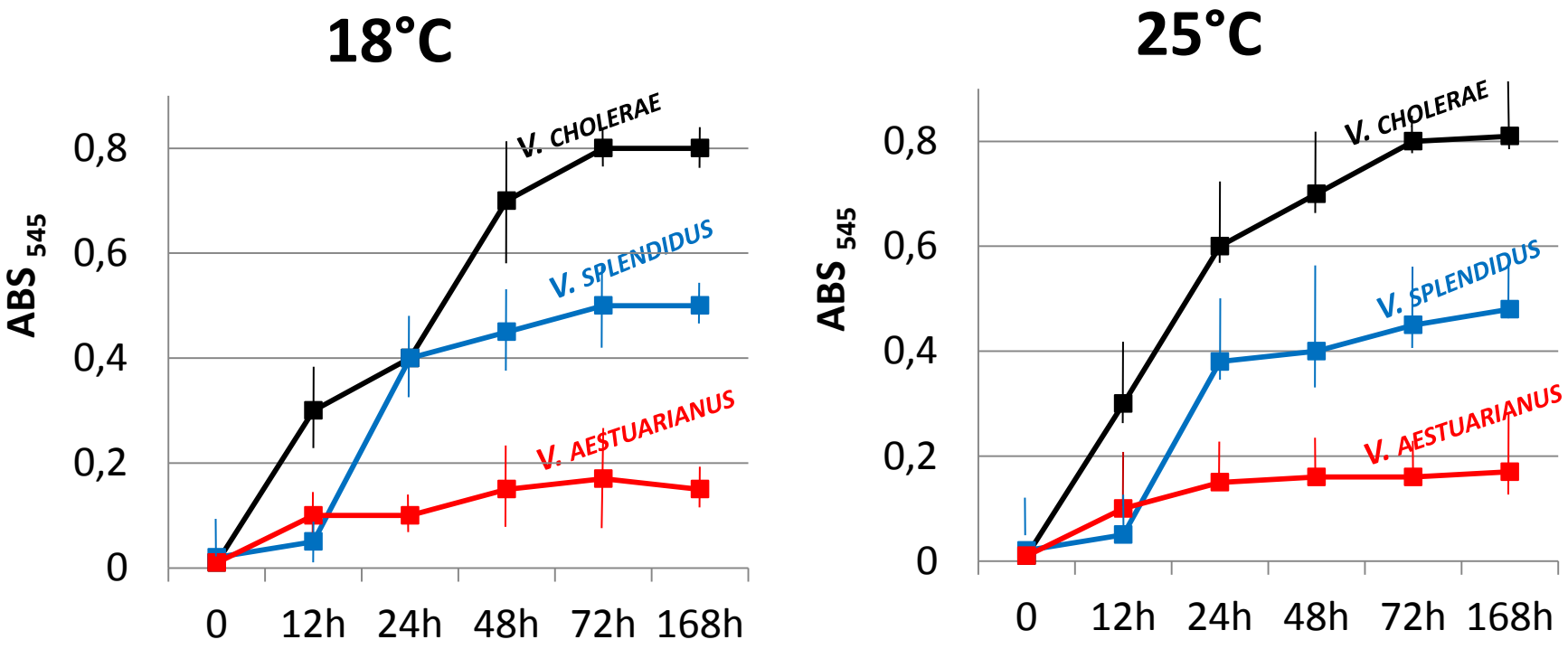


***V. AESTUARIANUS* 01/032** *mshA+, gbpA+*

BIOFILM FORMATION



MICROTITER PLATE BIOFILM ASSAY



FOLD DIFFERENCE BETWEEN ABS_{545} AT 72 H		
	VS V. TASMANIENSIS	*VS V. CHOLERAE*
V. TASMANIENSIS		1.6
V. AESTUARIANUS	3.0	4.7

Conclusions I

V. aestuarianus 01/32 and *V. tasmaniensis* LGP32

- Maintain culturability and viability for longer time in the sediment
- Attach to chitin particles and interact with zooplankton *T. fulvus*
- develop biofilm on microtiter plates

Vt, Va ENTERING THE VBNC STATE

ARTIFICIAL SEA WATER

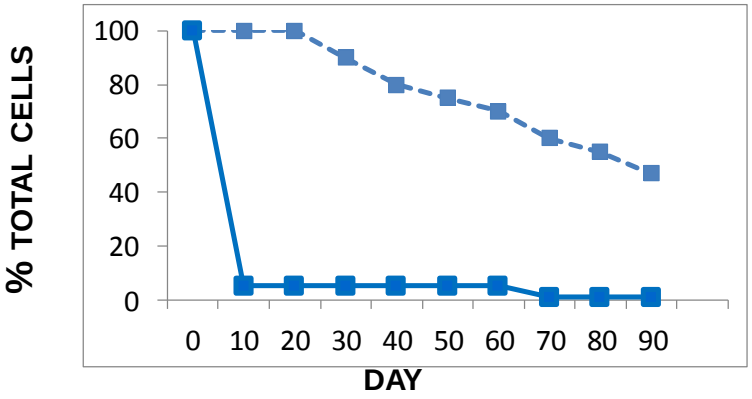
INOCULUM: 10^6/ML

5°C

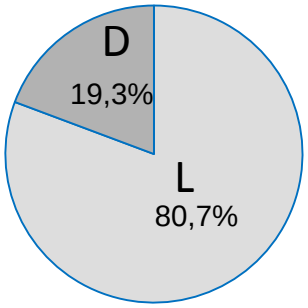
20 ‰ SALINITY

90 DAY INCUBATION

VIBRIO TASMANIENSIS LGP32

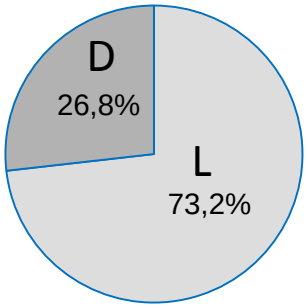


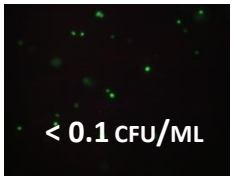
>0.2 um



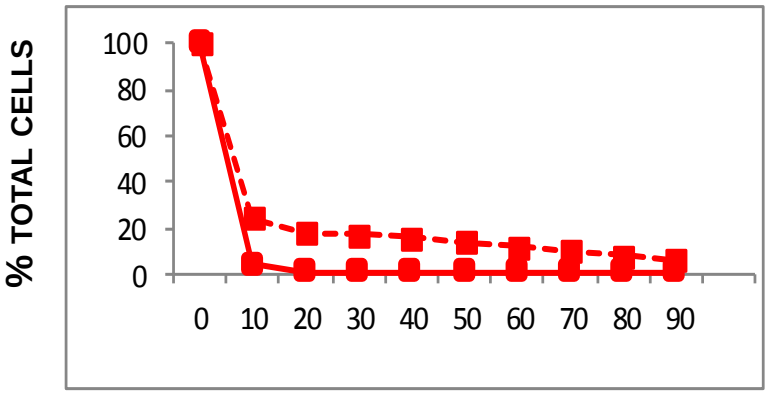
10^4 CFU/ML

<0.2 um





VIBRIO AESTUARIANUS O1/32



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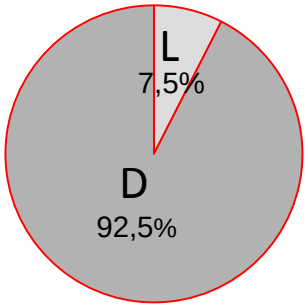
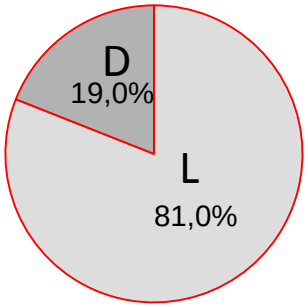
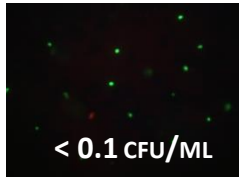
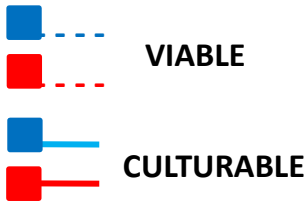


10^1 CFU/ML

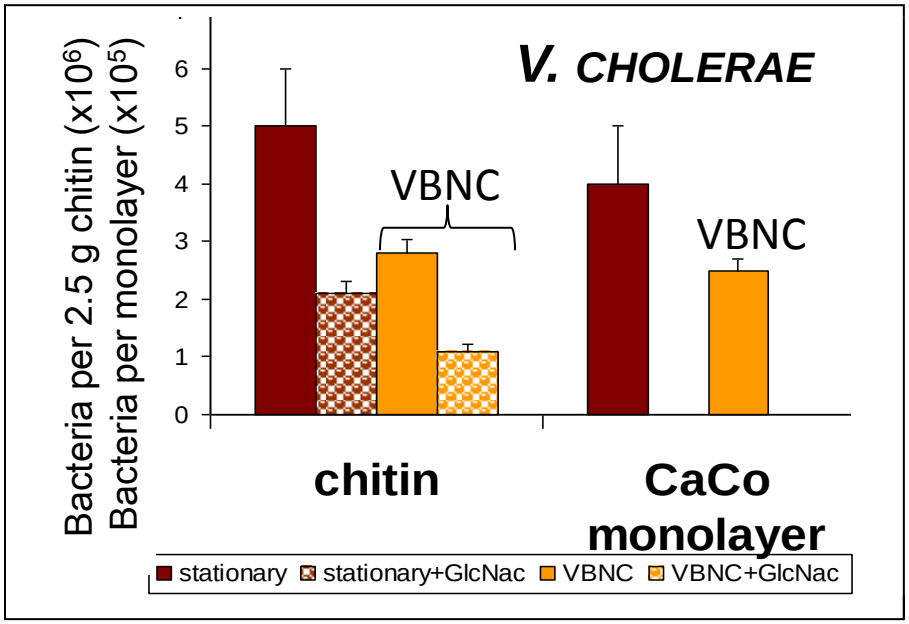
<0.2 um

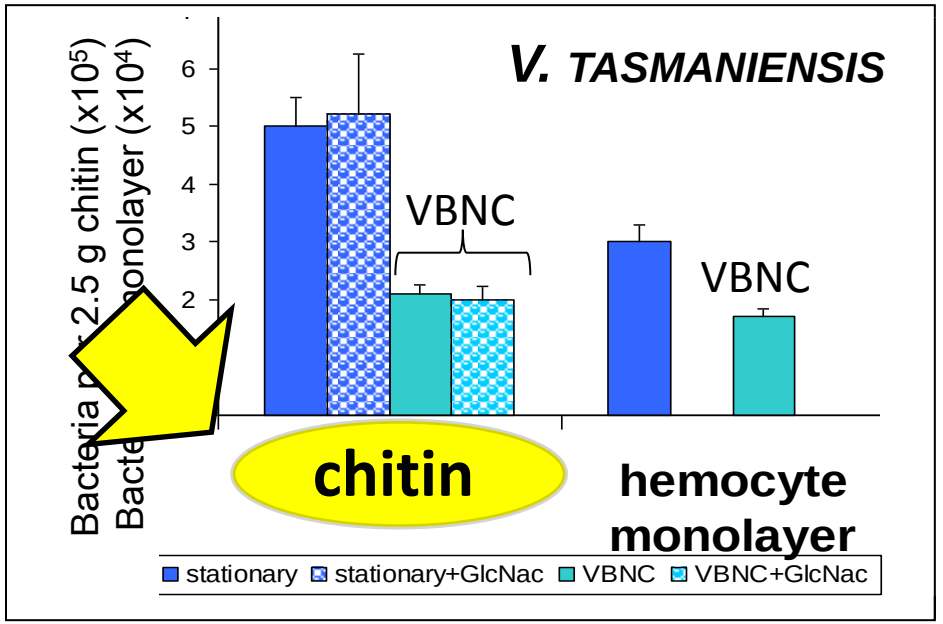






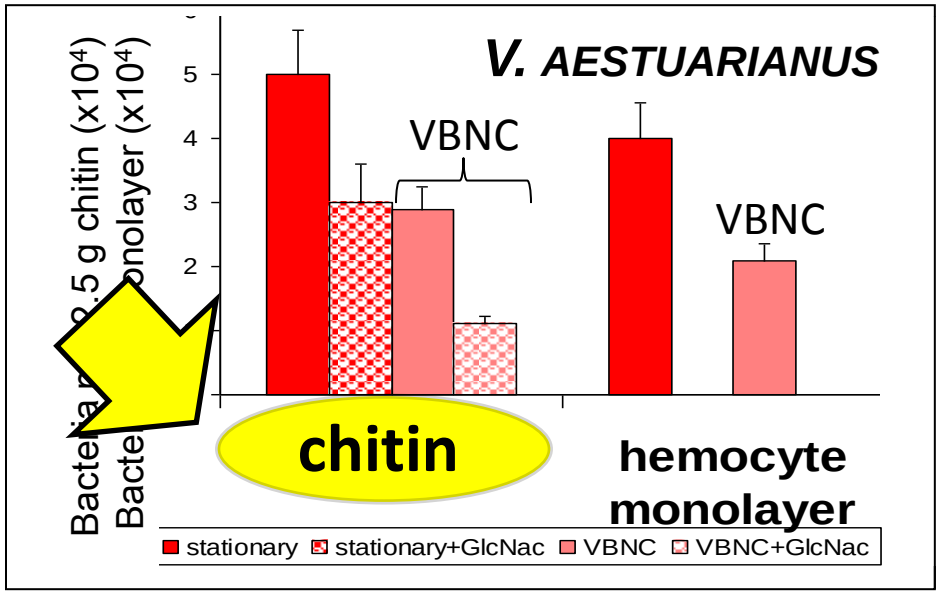
Vt, Va VBNC INTERACTIONS WITH CHITIN AND CELLS





Pruzzo *et al*. 2003. Environ. Microbiol. *5*:850

	% reduction VBNC *vs* stationary	
	chitin	monolayer
V. splendidus	58%	43%
V. aestuarianus	42%	48%
V. cholerae	44%	38%



Conclusions II

V. AESTUARIANUS 01/32 AND *V. TASMANIENSIS* LGP32

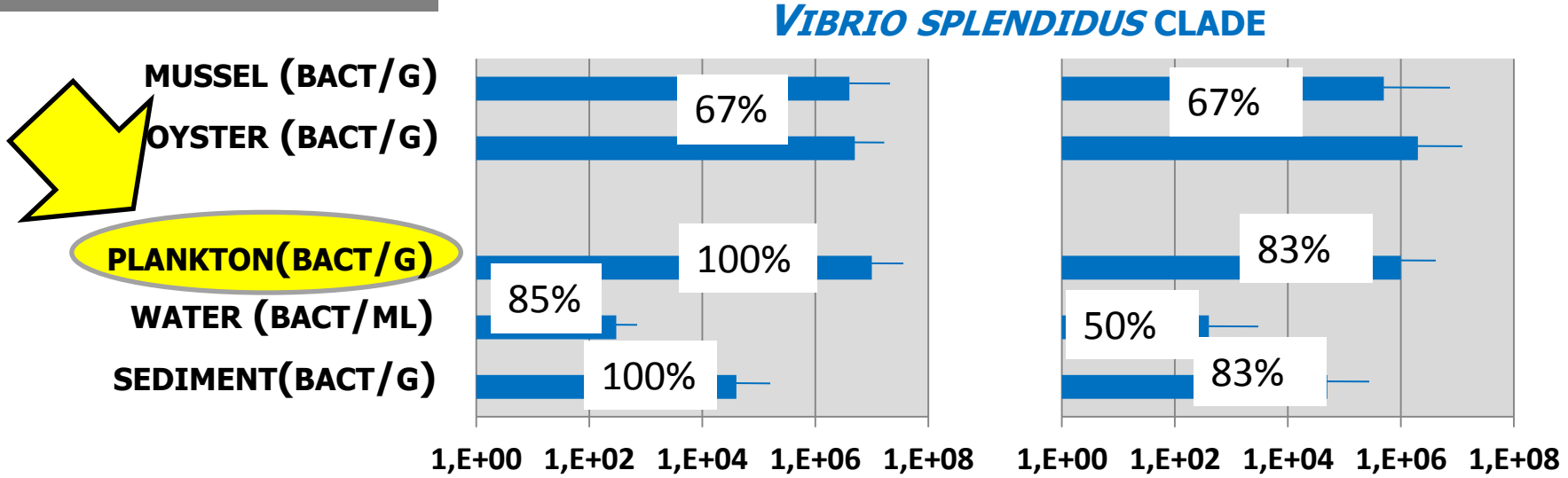
- CAN ENTER A VBNC STATE AFTER EXTENDED INCUBATION AT 5°C A CONDITION THAT COULD BE MET DURING THE WINTER SEASON IN ENVIRONMENTS WHERE BIVALVE FARMING GENERALLY TAKES PLACE
- VBNC CELLS MAINTAIN ADHESIVE PROPERTIES TOWARDS CHITIN AND BIVALVE CELLS

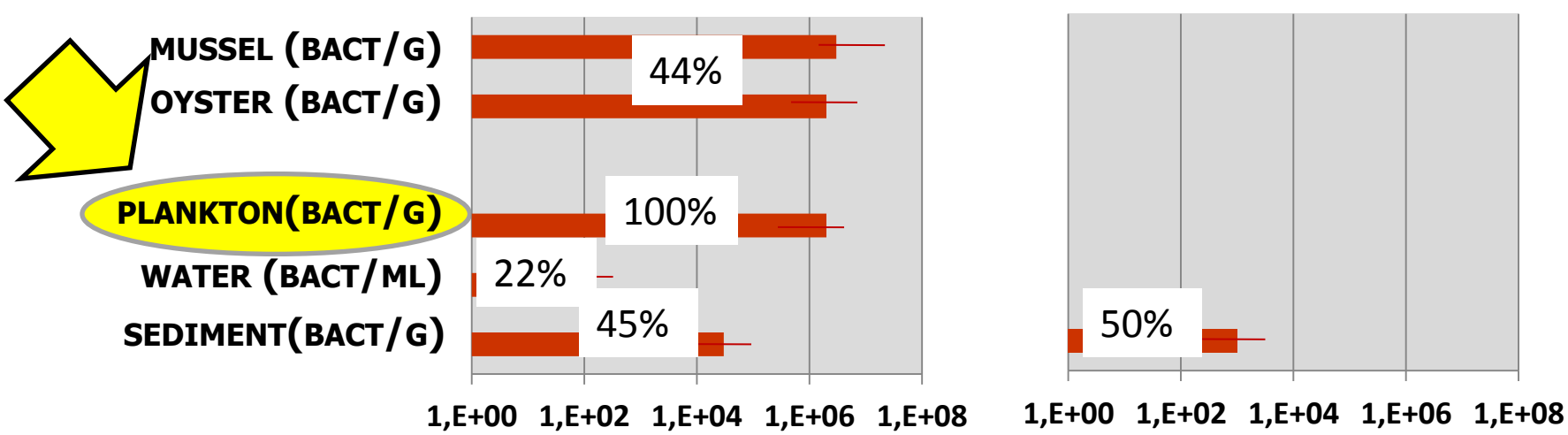
PERCENTAGES OF SAMPLES WITH TOTAL VS, VA BACTERIA ARE SHOWN

IN FIELD STUDIES (GORO LAGOON)

WARM MONTHS (18-27°C; 21-24 ‰ SALINITY)

COLD MONTHS (4-14°C; 25-29 ‰ SALINITY)





55 samples

REAL-TIME PCR COUNTS

Conclusions III

PLANKTON REPRESENT AN ENVIRONMENTAL RESERVOIR FOR *V. AESTUARIANUS* 01/32 AND *V. SPLENDIDUS* (CLADE)

ROLE OF PLANKTON AS VECTOR FOR Va INFECTIONS

12X

Stabulation:
2L ASW, 31ppt, 18°C

After
24h

12X

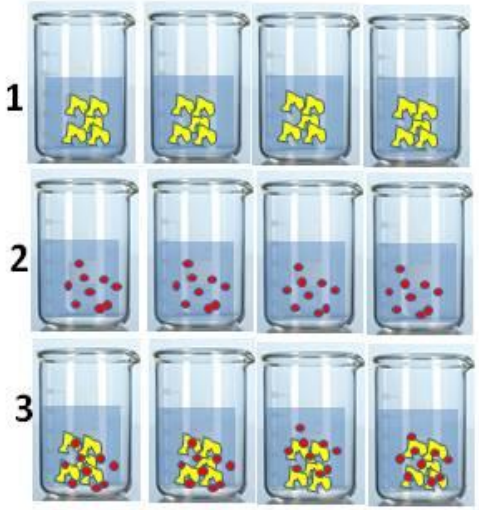
Salinity stress:
2L ASW, 15ppt, 18°C

After
24h

INFECTION

350ml ASW, 15ppt, 18°C
1. **Tested vectors** [1x10^5 aggr/ml]
2. *Vibrio aestuarianus* 02/041 [1x10^8 cell/ml]
3. **Tested vectors** [1x10^5 aggr/ml]+ *Vibrio aestuarianus* [1x10^8 cell/ml]

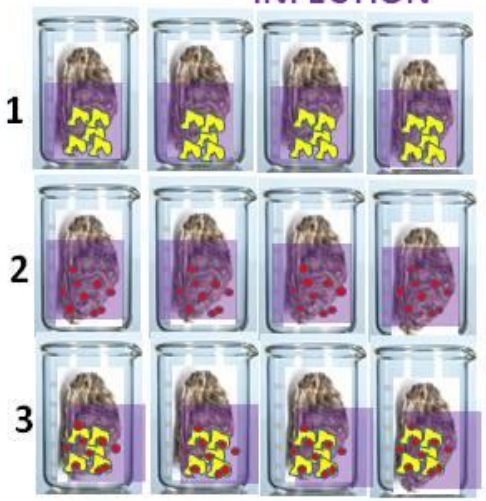
After 2h



VECTORS

Nannochloropsis gaditana
Chitin particles
Marine Snow particles

THE EXPERIMENTS WERE RUN IN TRIPLICATE



After 4h

12X

Post-infection:
2L ASW, 15ppt, 18°C, 36-48h

Laboratory analysis

- Lysosomal membrane stability
- Enumeration of culturable vibrios in hemolymph
- Enumeration of total *V. aestuarianus* and *Vibrio spp* bacteria in hemolymph

EXPERIMENTAL INFECTIONS (CHITIN FRAGMENTS)

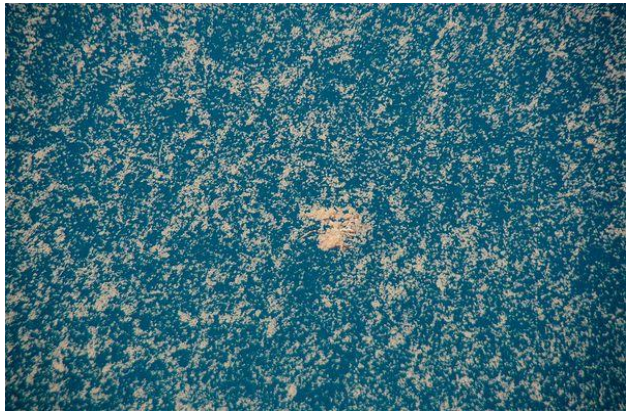
INFECTION EXPERIMENTS

RESULTS

V. aestuarianus 02/041 and *Vibrio*-like bacteria concentration in hemolymph of oysters infected with either *V. aestuarianus* 02/041 bacteria alone, or chitin fragments alone, or mixture of bacteria plus chitin

Oyster infection with	*V. aestuarianus* bacteria/ml	*V. aestuarianus* CFU/ml	*Vibrio spp* bacteria/ml	Vibrio-like CFU/ml
Only chitin	0	0	$3.3x10^4 \pm 9{,}1x10^3$	$5.1x10^3 \pm 9.7x10^2$
Only bacteria	$1.9x10^3 \pm 7.6x10^2$	$7.4x10^2 \pm 9.2x10^1$	$7.1x10^4 \pm 8.9x10^3$	$2{,}1x10^4 \pm 3.4x10^3$
Bacteria + chitin	$1.1x10^3 \pm 8.1x10^2$	$5.3x10^2 \pm 8.4x10^1$	$5.2x10^4 \pm 8.2x10^3$	$1.1x10^4 \pm 8.9x10^3$
Uninfected control	0	0	$2.1x10^4 \pm 7.2x10^3$	$3.1x10^3 \pm 8.4x10^2$

Chitin fragments (size <100µm)



Analysis of hemocyte LMS of the tested samples (treated and untreated with bacteria and chitin both alone and in combination) showed no statistically significant differences in NRRT (not shown).

NOT SIGNIFICANT

EXPERIMENTAL INFECTIONS (PHYTOPLANKTON)

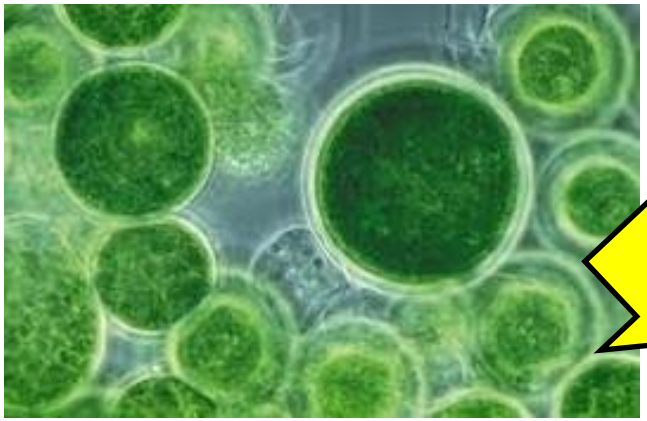
INFECTION EXPERIMENTS

RESULTS

V. aestuarianus 02/041 and *Vibrio*-like bacteria concentration in hemolymph of oysters infected with either *V. aestuarianus* 02/041 bacteria alone, or *N. gaditana* cells alone, or mixture of bacteria plus microalgae

Oyster infection with	*V. aestuarianus* bacteria/ml	*V. aestuarianus* CFU/ml	*Vibrio spp* bacteria/ml	*Vibrio*-like CFU/ml
Only microalgae	0	0	$2.7 \times 10^4 \pm 5.4 \times 10^3$	$9.6 \times 10^3 \pm 9.4 \times 10^2$
Only bacteria	$5.2 \times 10^3 \pm 7.8 \times 10^2$	$2.1 \times 10^3 \pm 9.2 \times 10^2$	$3.5 \times 10^4 \pm 6.3 \times 10^3$	$1.5 \times 10^4 \pm 4.3 \times 10^3$
Bacteria + microalgae	$1.2 \times 10^4 \pm 8.1 \times 10^3$	$6.1 \times 10^3 \pm 8.3 \times 10^2$	$8.4 \times 10^4 \pm 7.2 \times 10^3$	$3.2 \times 10^4 \pm 5.1 \times 10^3$
Uninfected control	0	0	$1.5 \times 10^4 \pm 3.4 \times 10^3$	$8 \times 10^3 \pm 6.6 \times 10^2$

Nannochloropsis gaditana



Effects of low salinity stressed oyster challenge with either *V. aestuarianus* 02/041 bacteria alone, or *N. gaditana* cells alone, or mixture of bacteria plus microalgae on hemocyte LMS

Oyster infection with	NRRT (% of control)
Only microalgae	102±3
Only bacteria	99±8
Bacteria + microalgae	50±8

SIGNIFICANT

EXPERIMENTAL INFECTIONS (MARINE SNOW)

INFECTION EXPERIMENTS

RESULTS

V. aestuarianus 02/041 and *Vibrio*-like bacteria concentration in hemolymph of oysters infected with either *V. aestuarianus* 02/041 bacteria alone, or marine snow alone, or mixture of bacteria plus marine snow

Oyster infection with	*V. aestuarianus* bacteria/ml	*V. aestuarianus* CFU/ml	*Vibrio spp* bacteria/ml	*Vibrio*-like CFU/ml
Only marine snow	0	0	$1.1 \times 10^4 \pm 8.4 \times 10^2$	$8.8 \times 10^3 \pm 7.3 \times 10^2$
Only bacteria	$4.1 \times 10^3 \pm 2.3 \times 10^2$	$1.8 \times 10^3 \pm 5.3 \times 10^2$	$2.6 \times 10^4 \pm 3.3 \times 10^3$	$7.8 \times 10^4 \pm 2.9 \times 10^3$
Bacteria + marine snow	$6.2 \times 10^4 \pm 4.1 \times 10^3$	$2.4 \times 10^3 \pm 3.2 \times 10^2$	$3.1 \times 10^4 \pm 6.4 \times 10^3$	$2.2 \times 10^4 \pm 1.9 \times 10^3$
Uninfected control	0	0	$1.4 \times 10^4 \pm 6.6 \times 10^3$	$9.2 \times 10^3 \pm 4.7 \times 10^2$

Marine snow particles *(size <150µm)*

Effects of low salinity stressed oyster challenge with either *Vibrio aestuarianus* 02/041 bacteria alone, or marine snow alone, or mixture of bacteria plus marine snow on hemocyte LMS

Oyster infection with	NRRT (% of control)
Only marine snow	108±2
Only bacteria	90±7
Bacteria + marine snow	70±6

SIGNIFICANT

Conclusions IV

PLANKTONIC MATRICES (MARINE SNOW AND PHYTOPLANKTON) FAVOR *V. AESTUARIANUS* TRANSMISSION TO THE C. GIGAS HOST

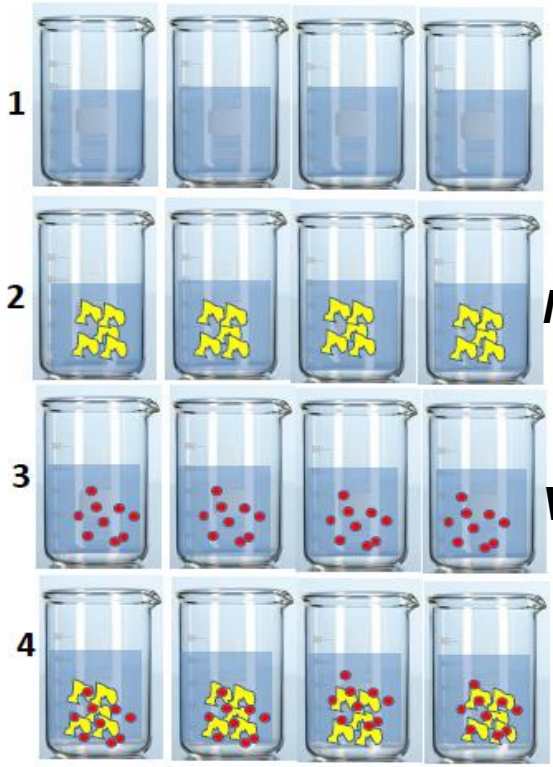
EFFECT OF THE INTERACTION Va/PLANKTON ON THE VIBRIO VIRULENCE

1. 350ml ASW, 15ppt, 18°C
2. **Tested vectors** [1x10^5 aggr/ml]
3. *Vibrio aestuarianus* 02/041 [1x10^8 cell/ml]
4. **Tested vectors** [1x10^5 aggr/ml]+ *Vibrio aestuarianus* [1x10^8 cell/ml]

INTERACTION EXPERIMENTS

EXPERIMENTAL SET-UP

Marine snow particles
(size <150µm)



ASW

MARINE SNOW PARTICLES (SIZE <150µM)

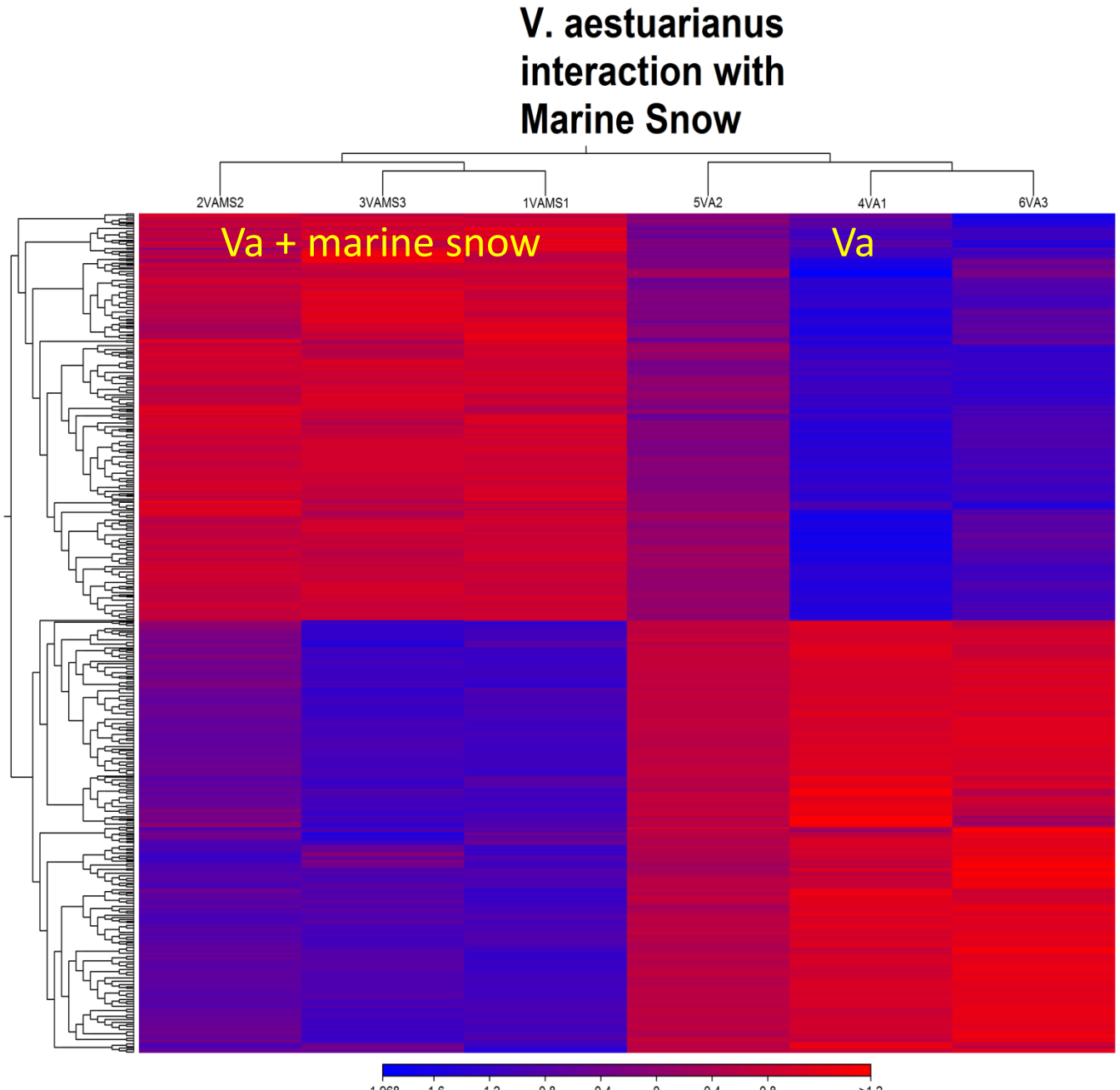
***V. AESTUARIANUS* 02/041**

***MARINE SNOW PARTICLES (SIZE <150µM) + V. AESTUARIANUS* 02/041**

After 2h

Transcriptomics analysis and extracellular protease acivities

Va TRANSCRIPTOMICS ANALYSIS



TRANSCRIPTOME ANALYSIS (HEAT MAP)

2787 CDS

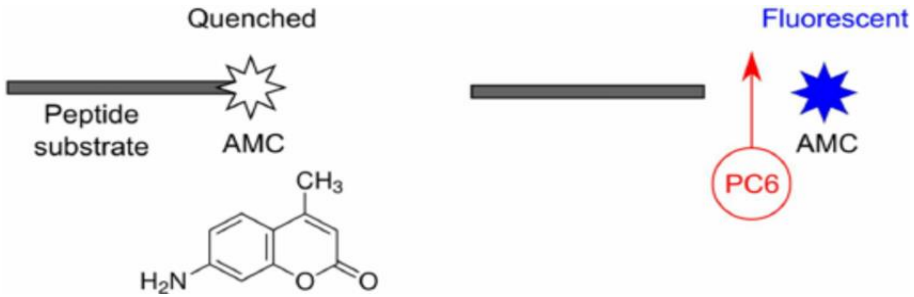
Respiration and protein synthesis

Virulence (aminopeptidase, protease, ompU, mshA)

Motility

Va EXTRACELLULAR PROTEASE ACTIVITY

extracellular enzymatic aminipoetpidase activity using fluorogenic assays

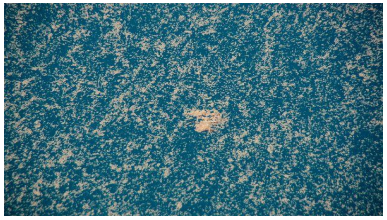


INTERACTION EXPERIMENTS

RESULTS

Marine snow particles *(size <150µm)*

Chitin fragments *(size <100µm)*



Effect of the presence of either chitin fragments or marine snow particles on extracellular aminopeptidase activity produced by different *Vibrio* strains (* p<0.05)

Experimental conditions	Aminopeptidase activity (µmol/10^8cells/h) by strain				
	V. astuarianus 02/041	*V. tasmaniensis* LGP32	*V. coralliilyticus* ATCC BAA450	*V. harveyi* VH2	*V. tapetis* CECT 4600
ASW	24.6±0.7	7.1±0.2	26.8±0.9	24.7±0.6	23.6±0.4
ASW + chitin	22.6±0.2	6.6±0.2	28.2±o.8	21.9±0.8	21.7±0.4
ASW+marine snow	37.0±0.2*	7.0±0.2	42.4±0.8*	37.8±0.4*	21.6±0.8

Conclusions V

INTERACTION WITH PLANKTONIC MATRICES (MARINE SNOW) MAY INCREASE VIRULENCE POTENTIAL OF V. AESTUARIANUS

General conclusions

Plankton represent an Environmental Reservoir for *V. aestuarianus* 01/32 and *V. splendidus (clade)*

Planktonic matrices (marine snow and phytoplankton) favor *V. aestuarianus* transmission to the bivalve host

Interaction with planktonic matrices (marine snow) may increase virulence potential of *V. aestuarianus* (e.g. extracellular protease activity)

Genova University:

- Carla Pruzzo
- Giovanni tassistro
- Alessio borello
- Elisabetta Pezzati
- Monica Stauder
- Laura Canesi
- Chiara Grande

DISTAV
Dipartimento di Scienze della Terra, dell'Ambiente e della Vita
Università degli Studi di Genova

VIVALDI