UNIVERSITEIT GENT

FACULTY OF BIOSCIENCE ENGINEERING

Host-pathogen interaction: can we steer the conversation among the micro-organisms?

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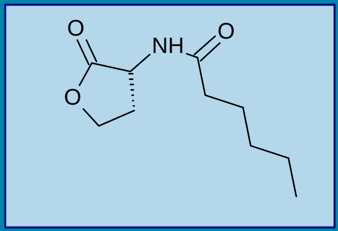
UGent Aquaculture R&D Consortium

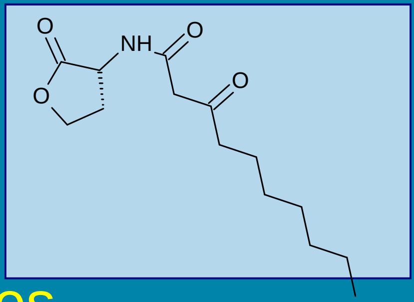
Content

1. Quorum sensing (QS)
2. Communication in vivo
   1. Impact of QS disruption on *Vibrio harveyi* virulence *in vivo*
   2. QS regulation of virulence in other aquatic host systems
3. Steering the conversation
   1. QS-disrupting brominated furanones
   2. Mixed microbial communities degrading QS molecules

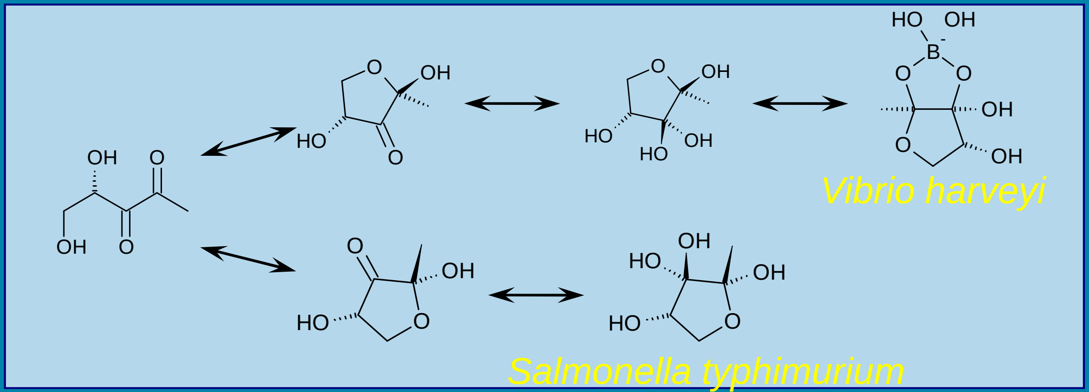
QUORUM SENSING

- Bacterial cell-to-cell communication with signal molecules
  - Gram negative bacteria: N-acylhomoserine lactones





  - Gram positive bacteria: peptides
  - Autoinducer-2 (AI-2): bacterial esperanto (G- and G+)



QUORUM SENSING

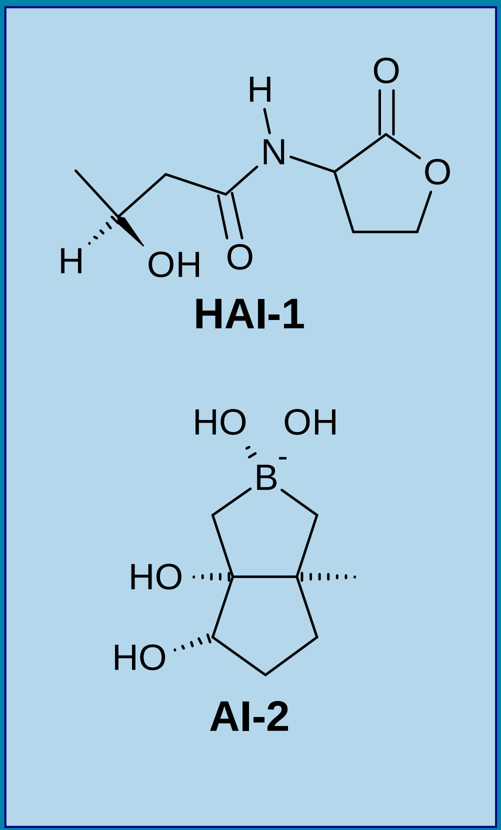
= Mechanism of gene regulation: activity based on presence/absence of signal molecules

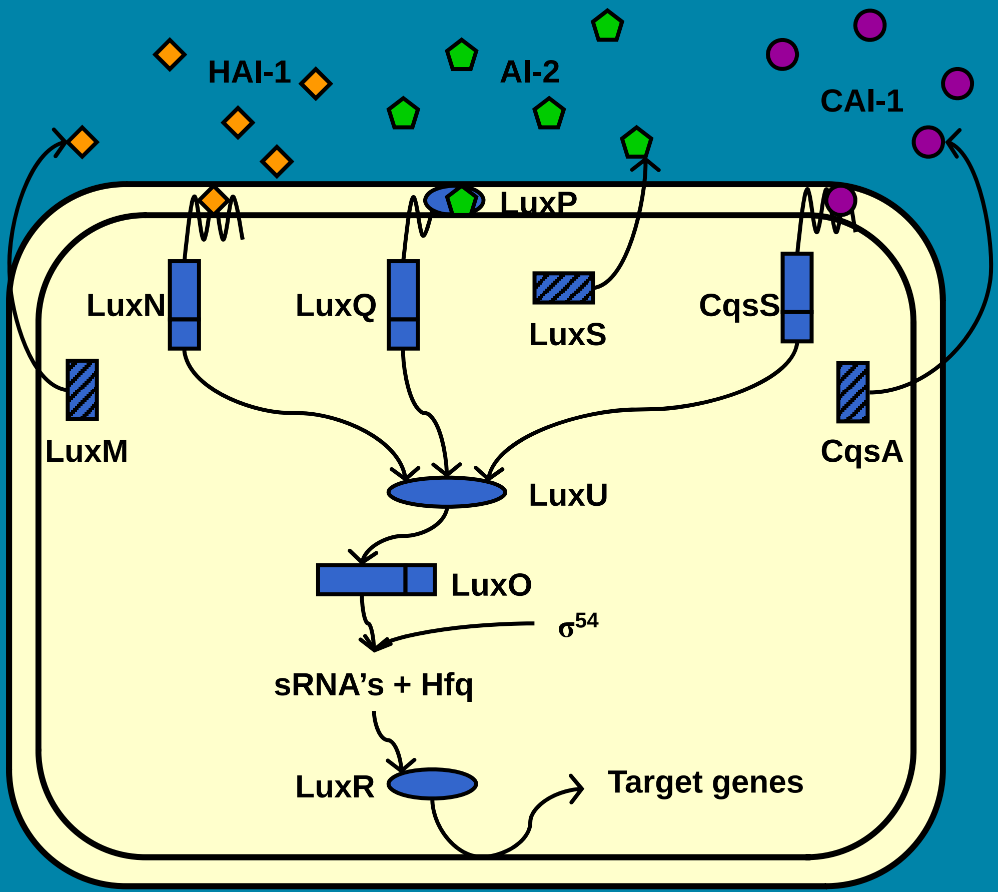
- Swarming, antibiotic production, biocorrosion, conjugation, nodulation,...
- Biofilm formation: *Pseudomonas, Aeromonas, Vibrio,...*
  - functional QS is essential for proper biofilm development
- Production of virulence factors (= gene products involved in pathogenesis)
  - plant pathogens: *Erwinia* spp., *Agrobacterium* spp.,...
  - human & animal pathogens: *C. perfringens, S. aureus, P.aeruginosa, Aeromonas* spp., vibrios

QS disruption as an alternative strategy to control bacterial infections

V. HARVEYI QUORUM SENSING

3-channel quorum sensing system of *Vibrio harveyi*



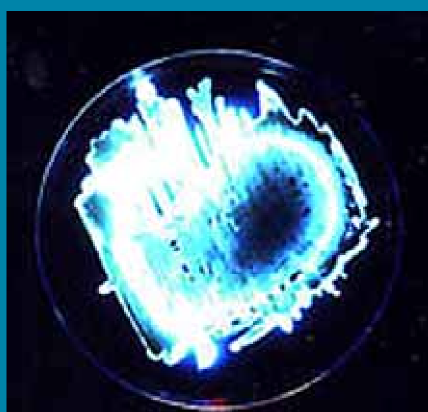


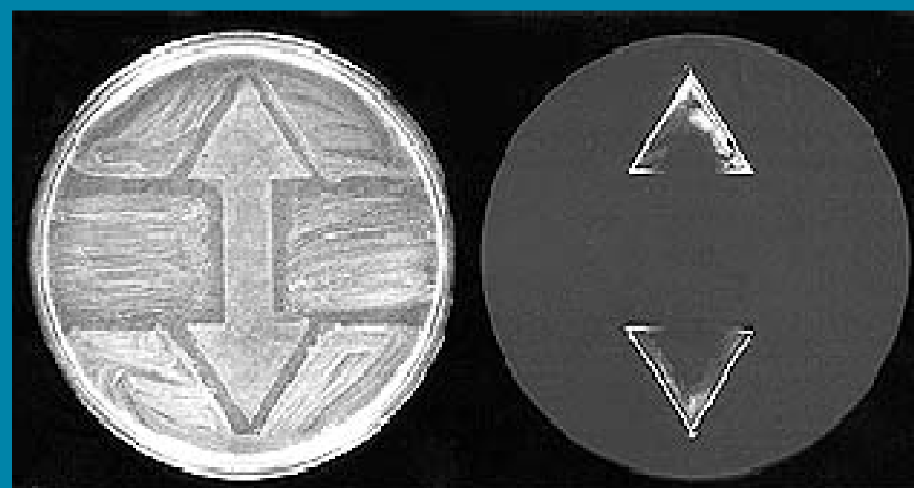
V. HARVEYI QUORUM SENSING

- Quorum sensing (QS) regulates different <u>phenotypes</u> in luminescent vibrios:

  1. <u>Bioluminescence</u>

Quorum sensing
at lab-scale





V. HARVEYI QUORUM SENSING

- Quorum sensing (QS) regulates different <u>phenotypes</u> in luminescent vibrios:

  2. Production of <u>virulence factors</u> *in vitro*

  - extracellular toxin
  - metalloprotease
  - siderophore
  - type III secretion system

<u>Hypothesis</u>: disruption of quorum sensing could be an effective strategy to protect shrimp from luminescent vibriosis.

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THE *IN VIVO* TEST SYSTEM

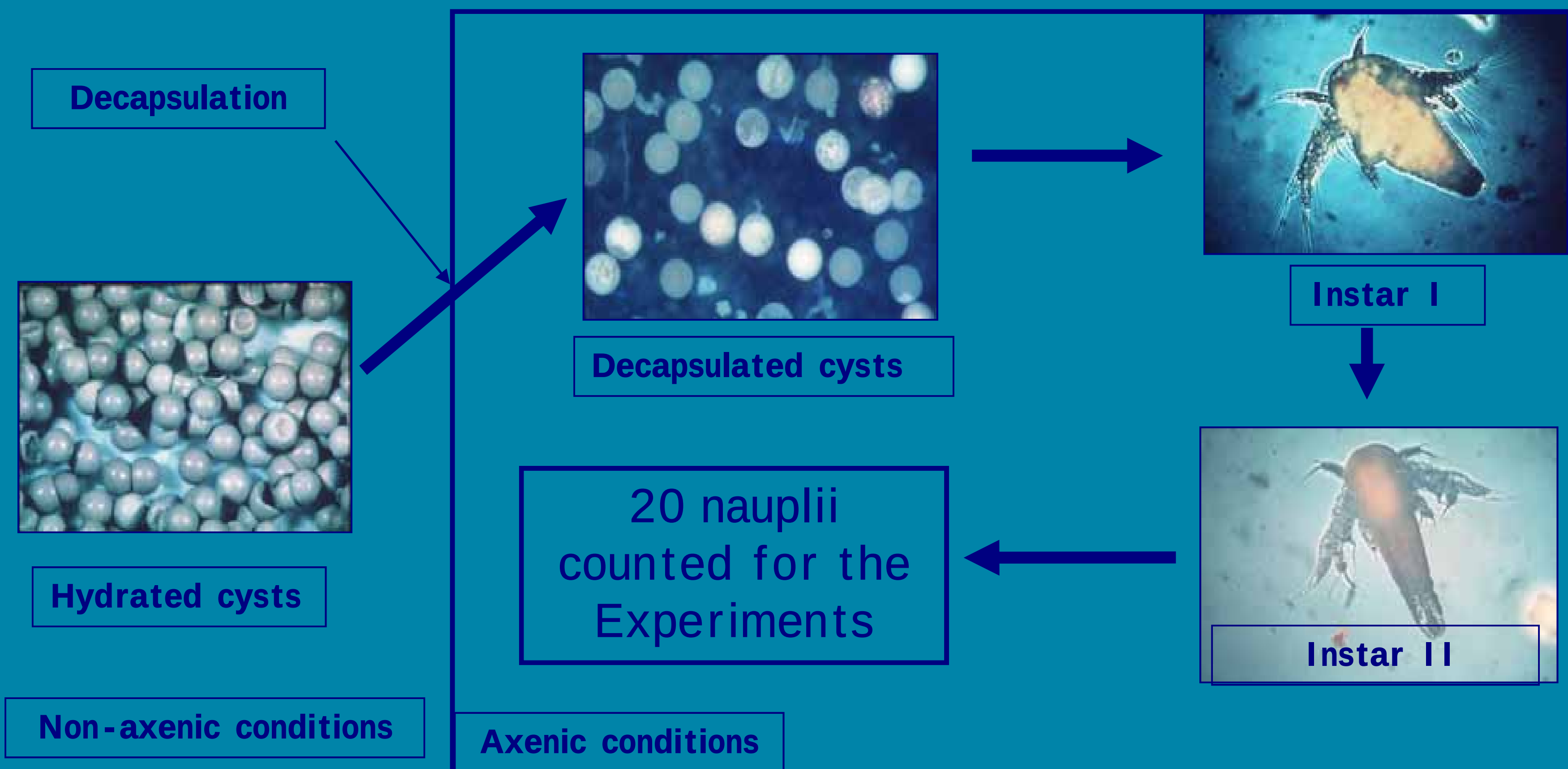
- Brine shrimp (*Artemia*): model organism for shrimp
- Gnotobiotic culture: only micro-organisms added to the system are present

Brine shrimp nauplius

- Challenge tests with brine shrimp nauplii (larvae): survival of nauplii 2 days after addition of luminescent vibrios to culture water

Gnotobiotic Artemia testing system: GART

Creating gnotobiotic condition starting with
AXENIC *Artemia* :



QS IN VIVO EXPERIMENTS

✓ *Artemia*: frequently used as model organism for shrimp

nauplius infected with V.harveyi

✓ *In vivo* challenge tests with gnotobiotic *Artemia*

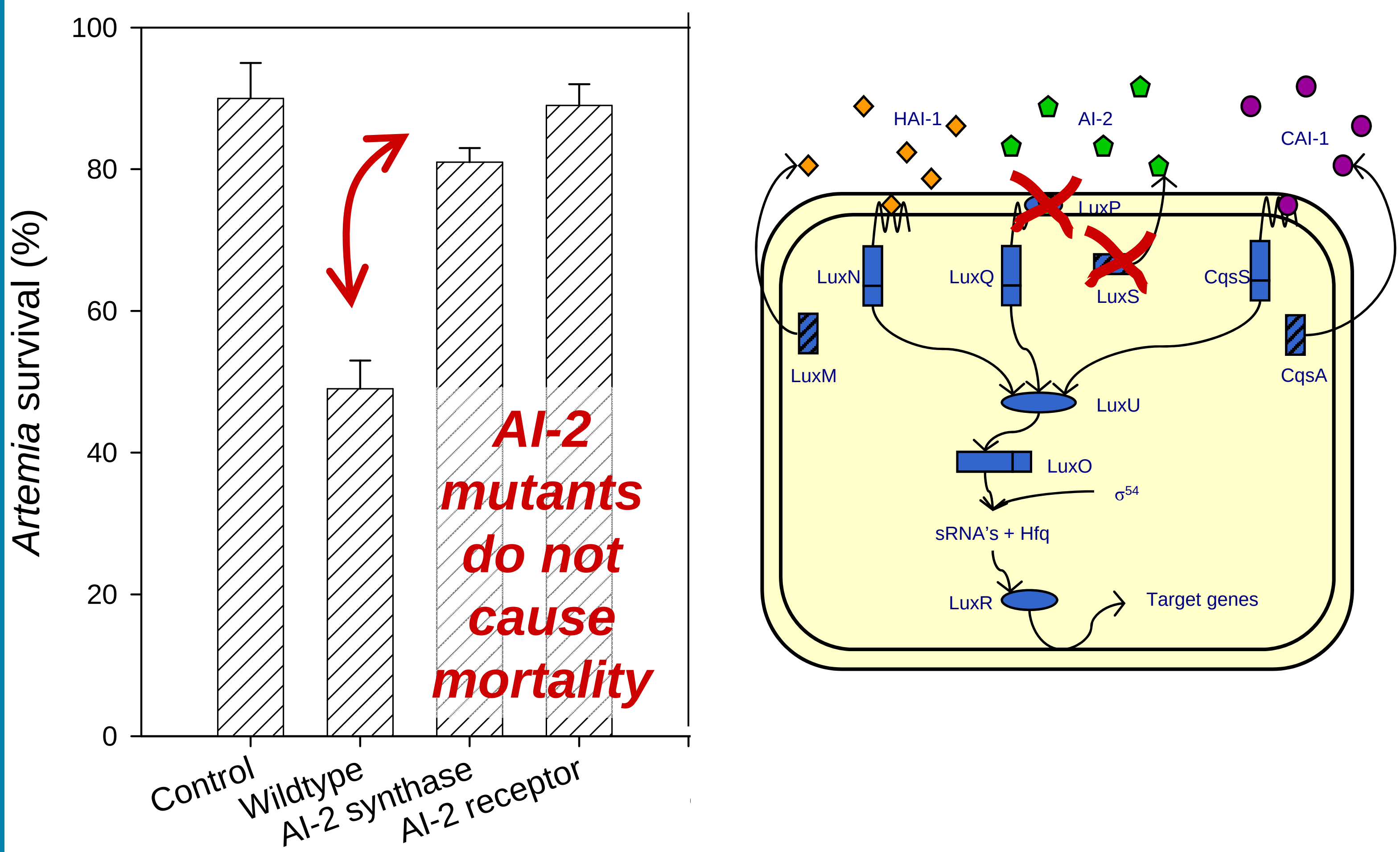
- Fed autoclaved LVS3 bacteria (10^7 /mL)
- Addition of pathogen (10^4 /mL)
- Counting survival after 2 days
- Uninfected nauplii as control
- Each treatment 3x; each experiment 2x

CHALLENGES WITH MUTANTS

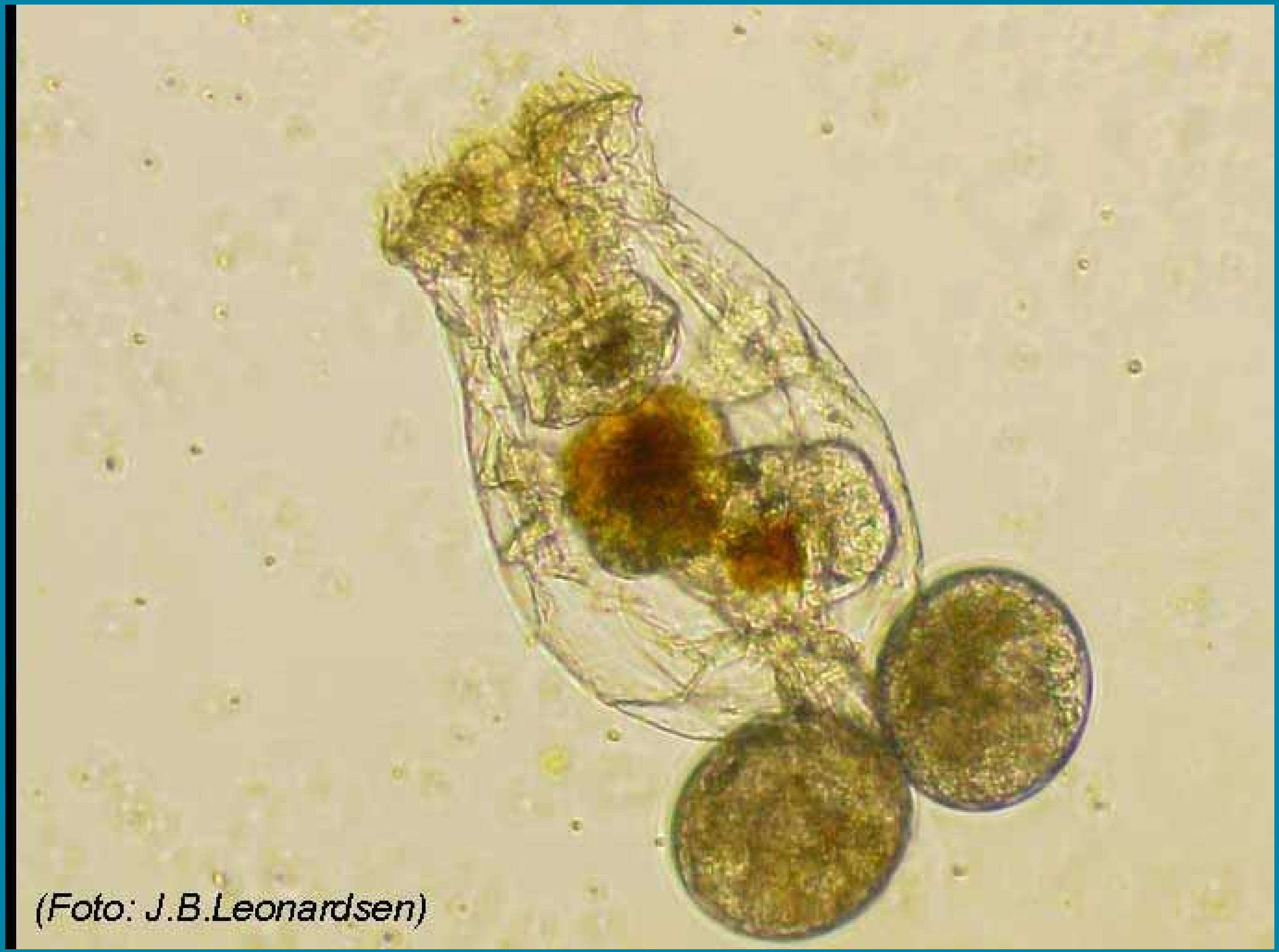
- *Vibrio harveyi* mutants in which different components of the QS system are inactivated have been described in literature

Are quorum sensing mutants less virulent (=do they have lower ‘infective power’) than the wild type *in vivo*?

CHALLENGES WITH MUTANTS



Quorum sensing in *Brachionus*



QS in *Brachionus -Vibrio* interaction (M & M)

- *Brachionus* are disinfected completely with gluteraldehyde
- Surviving axenic amictic eggs are hatched
- *Brachionus* are grown with yeast cells under food limiting conditions
- Culture are counted daily and growth rate determined
- For challenge Vibrio is added at 5 x 10^6 CFU ml^{-1}

Effect of Vibrio QS mutants on Brachionus growth rate

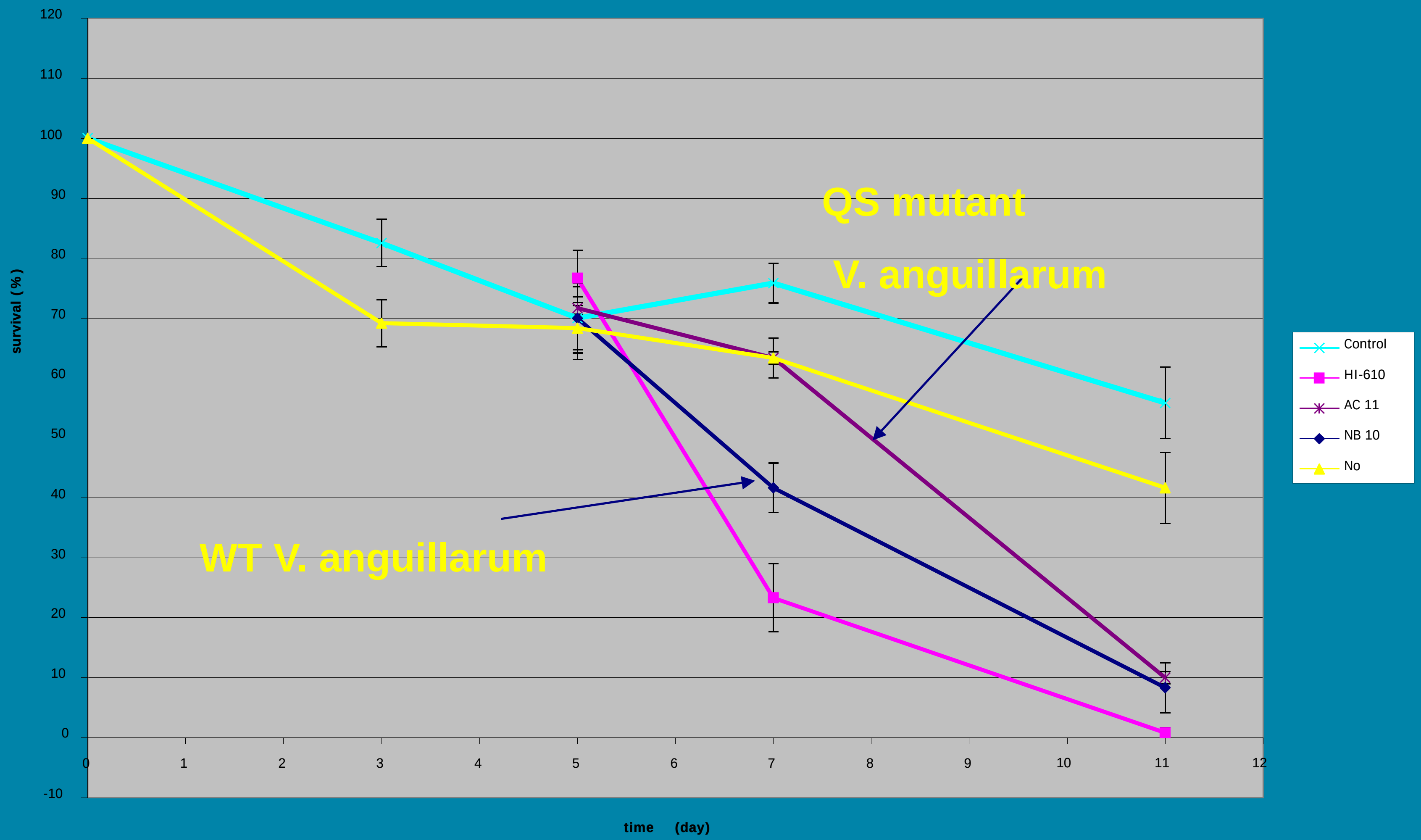
Treatment	Mutation in	Experiment 1	Experiment 2
Control	-	0.28 ± 0.04[c]	0.40 ± 0.09[c]
BB120	-	0.18 ± 0.03[b]	0.22 ± 0.06[b]
MM30	AI-2 synthase	0.13 ± 0.02[ab]	0.15 ± 0.03[a]
BB152	AHL synthase	0.15 ± 0.05[ab]	0.30 ± 0.05[b]
BB886	AI-2 receptor	0.11 ± 0.05[ab]	0.16 ± 0.05[a]
BB170	AHL receptor	0.10 ± 0.04[a]	0.16 ± 0.03[a]
MM77	AHL and AI-2 synthase	0.25 ± 0.08[c]	0.39 ± 0.04[c]

Effect of Vibrio QS mutants on Brachionus growth rate:

effect of exogenous QS molecules

Treatment	Experiment 1	Experiment 2
Control	0.31 ± 0.04[b]	0.54 ± 0.04[b]
MM77	0.27 ± 0.10[b]	0.51 ± 0.05[b]
MM77 + MM30 supernatant	0.15 ± 0.02[a]	0.30 ± 0.06[a]
MM77 + BB152 supernatant	0.12 ± 0.03[a]	0.28 ± 0.08[a]

Effect of QS mutation on survival in gnotobiotic seabass test



CHALLENGES WITH MUTANTS

- Brine shrimp: inactivation of AI-2 QS decreases virulence; HAI-1 inactivation has no effect
- Rotifers (*Brachionus plicatilis*): both AI-2 and HAI-1 need to be inactivated to decrease virulence

➔ Quorum sensing regulates virulence of luminescent vibrios *in vivo*

➔ The importance of the different autoinducers is dependent on the host organism

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Quorum sensing: non-gnotobiotic

A *Brachionus - Turbot* food chain example

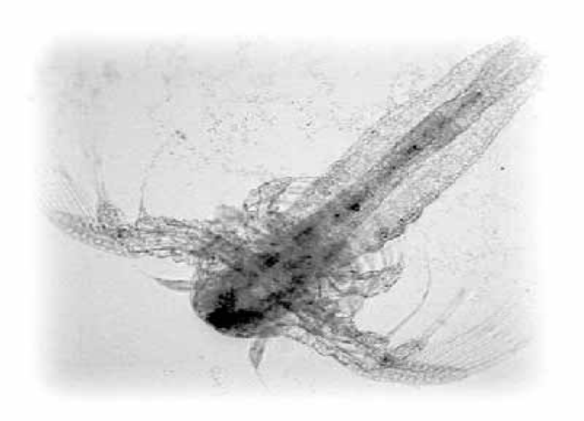
Effect of AHL on Turbot larvae survival in % on DAH5

Factor		AHL addition	
		-	+
		(Treatment 1)	(Treatment 2)
Antibiotic (AB) addition	-	15.5 ± 14.2[b]	1.6 ± 3.5[a]
		(Treatment 3)	(Treatment 4)
	+	98.8 ± 2.6[c]	97.1 ± 3.8[c]

- Survival of turbot larvae on day 5 post-hatch, (mean ± SD, n = 8).
- daily AHL addition of ***1 mg/l***

Quorum sensing: non-gnotobiotic

An *Artemia – Macrobrachium*
food chain example



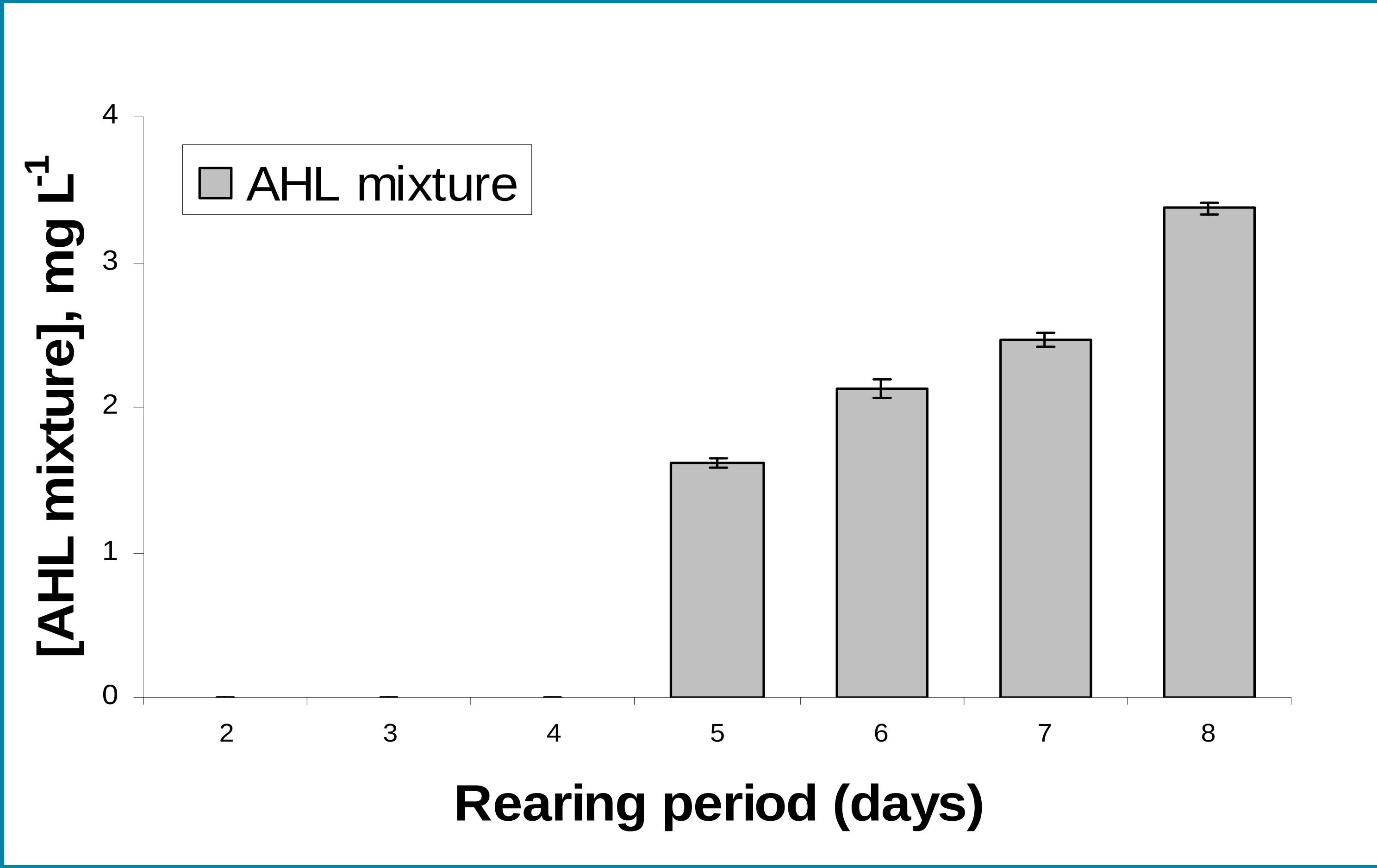
Effect of AHL mixture

Treatments	Survival	LSI
Control	70.0 ± 4.2[b]	5.3 ± 0.4[b]
AHL_{mix1}	49.2 ± 2.6[a]	4.9 ± 0.3[a]

Survival data are means of 6 replicates ± SE.

LSI data are means of 60 larvae ± SE.

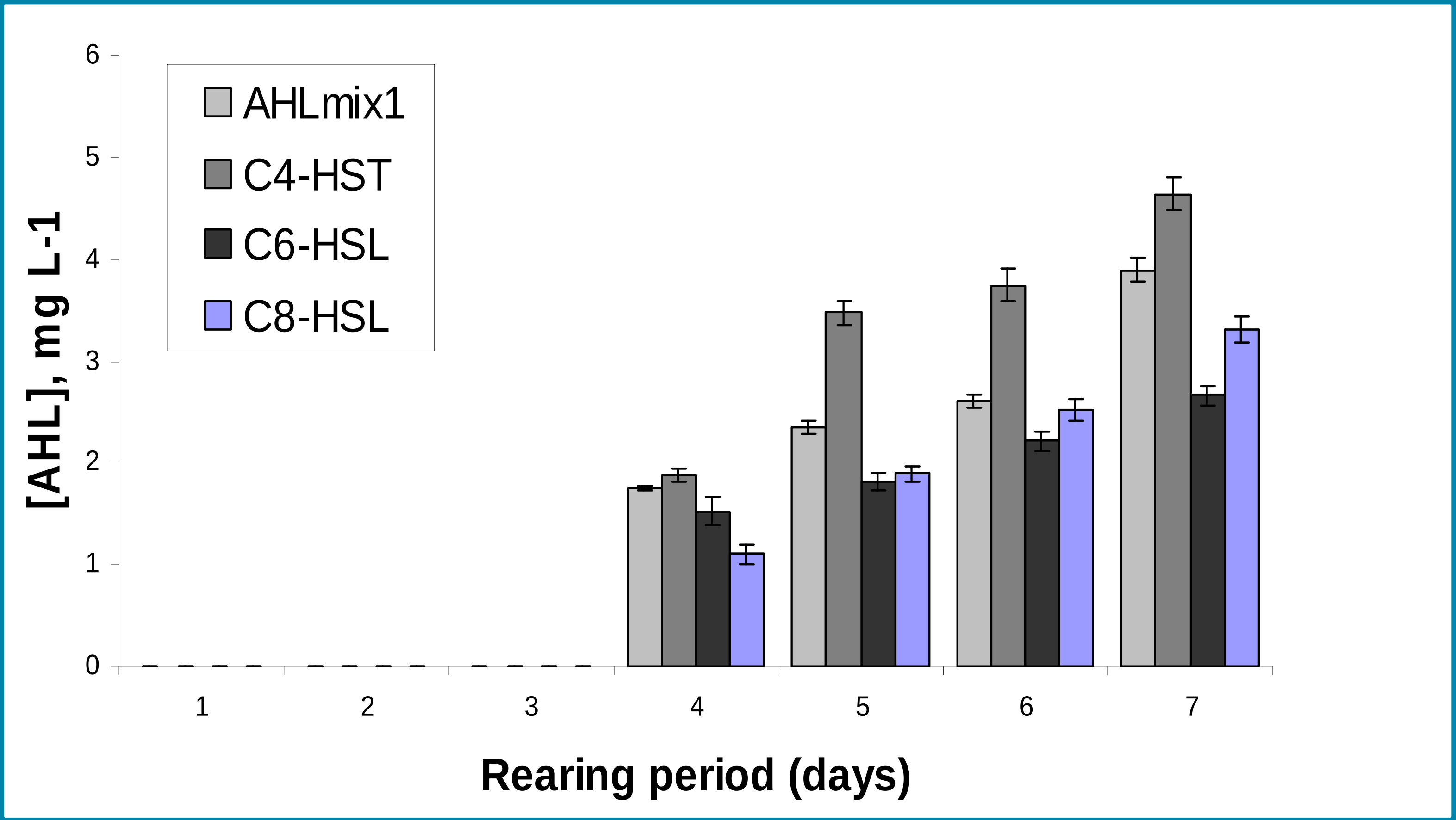
AHL concentration in the water



Effect of single molecules

Treatments	Survival of larvae	LSI
Negative control	92.5 ± 2.6[c]	5.4 ± 0.1[c]
AHL_{mix1} (positive control)	54.7 ± 2.5[a]	4.8 ± 0.1[a]
C4-HSL	88.9 ± 2.5[bc]	5.1 ± 0.1[b]
C4-HST	84.5 ± 1.8[b]	4.9 ± 0.1[ab]
C6-HSL	88.1 ± 1.9[bc]	4.9 ± 0.04[b]
C7-HSL	89.9 ± 2.0[bc]	4.9 ± 0.1[ab]
C8-HSL	89.5 ± 2.7[bc]	5.1 ± 0.1[b]

AHL concentration in the water



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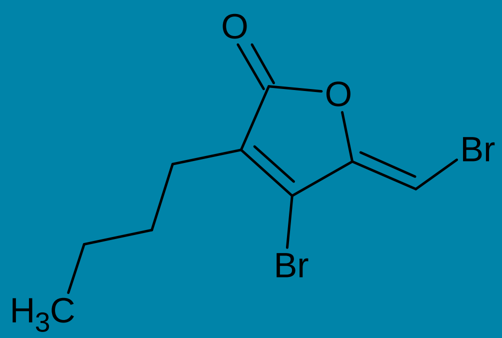
BROMINATED FURANONES

- Brominated furanones produced by the macro alga *Delisea pulchra* were shown before to block AHL and AI-2 QS



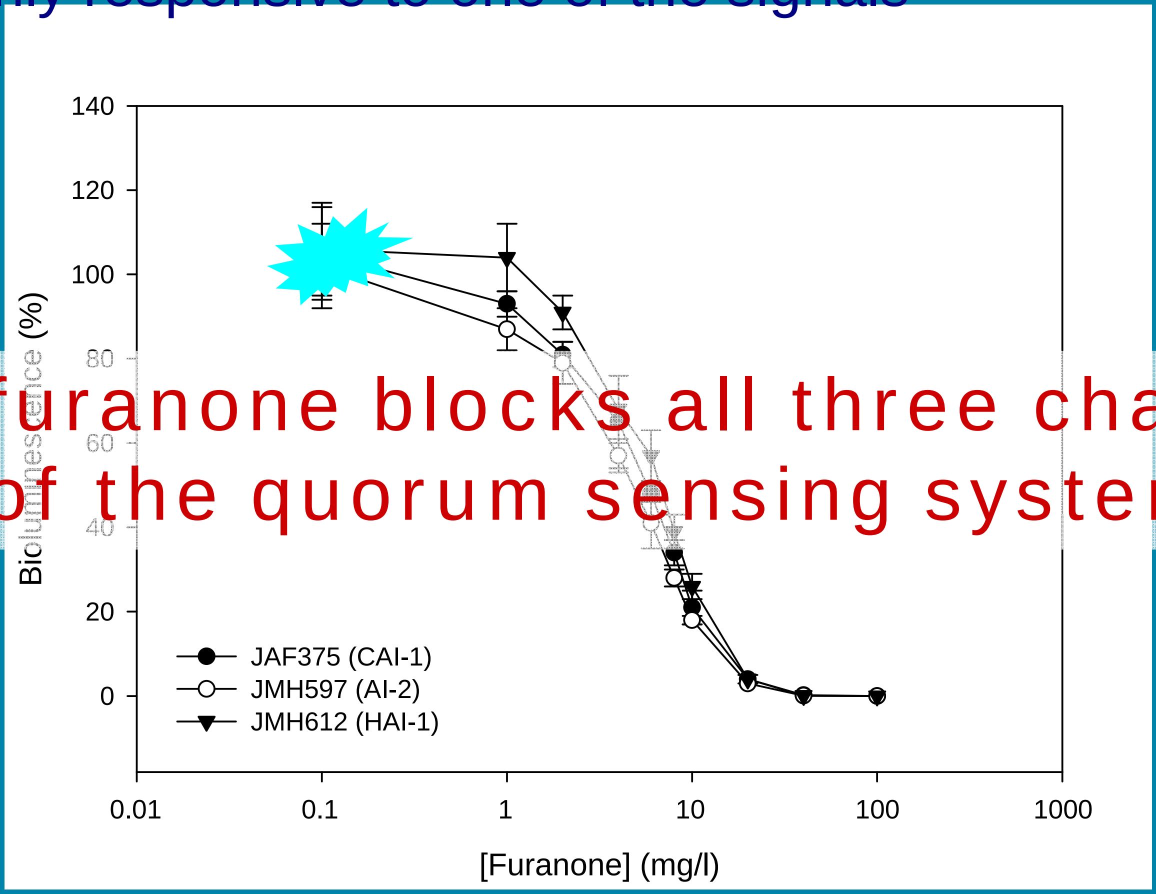
Delisea pulchra

1. Do furanones disrupt quorum sensing in luminescent vibrios?

2. Do furanones protect brine shrimp from the vibrios?

FURANONE *IN VITRO*

- Effect of the furanone on bioluminescence of *V. harveyi* signal molecule receptor double mutants
  - ➔ only responsive to one of the signals



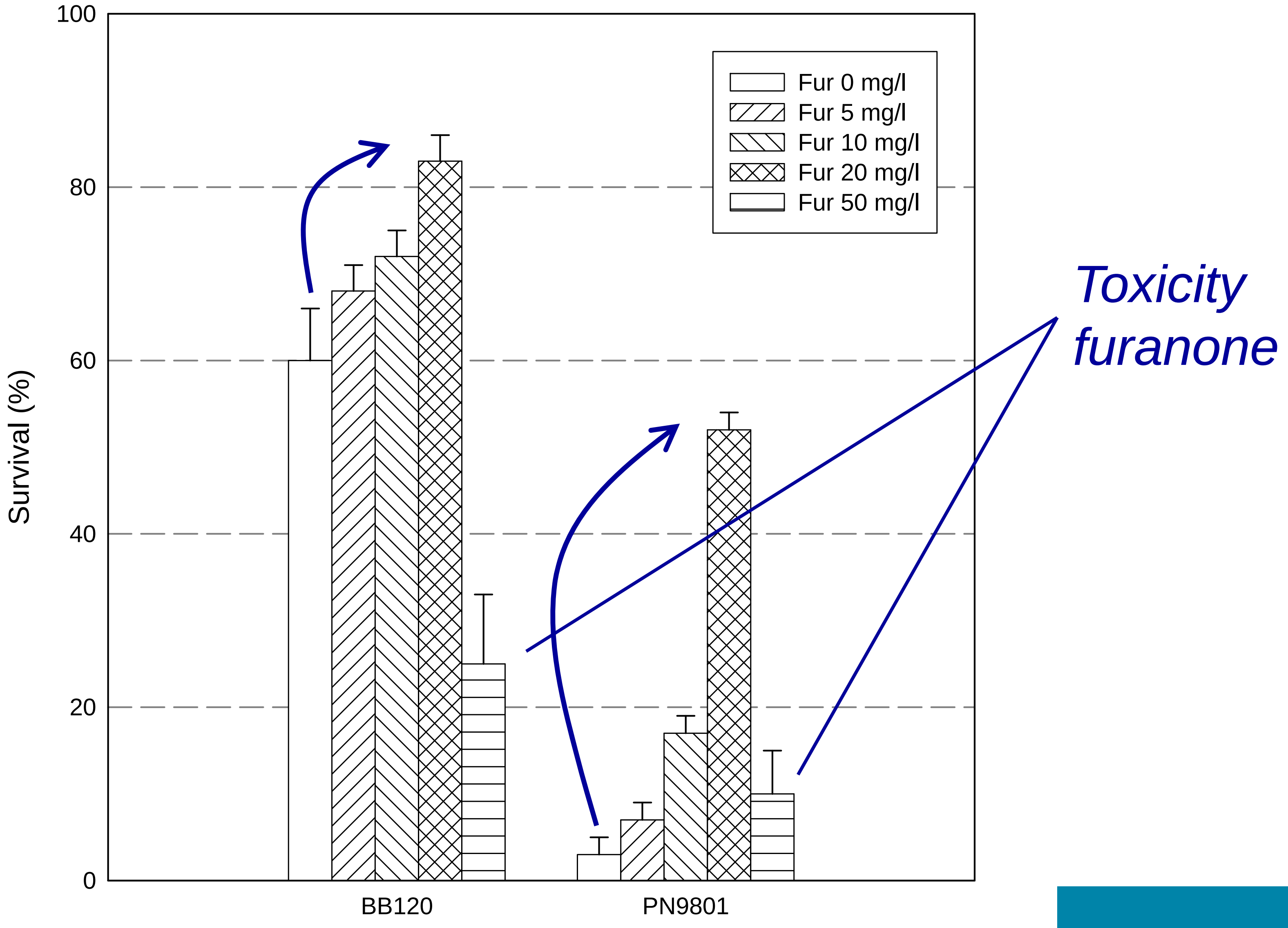
The furanone blocks all three channels of the quorum sensing system

FURANONE *IN VITRO*

- Furanone target is located in the signal transduction cascade

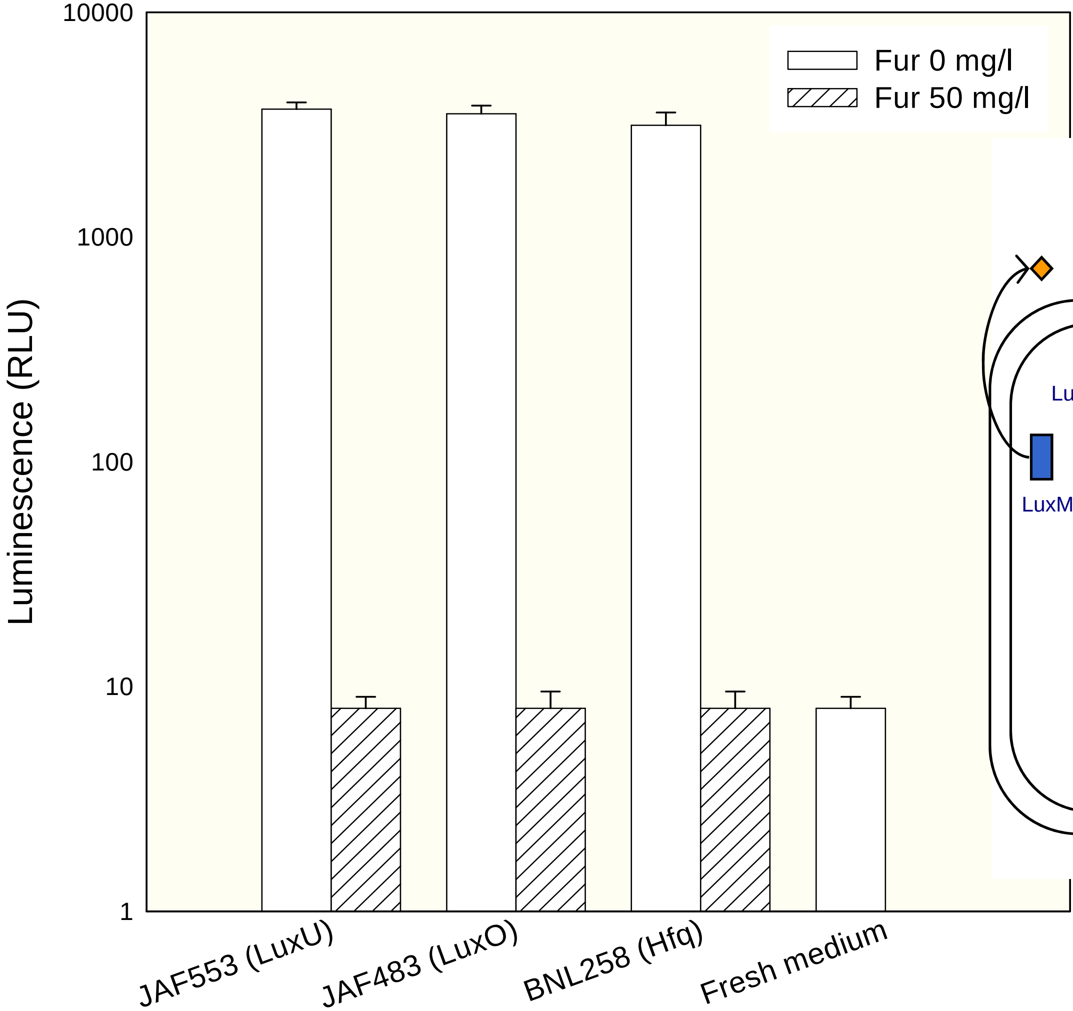
The furanone disrupts quorum sensing in *Vibrio harveyi* by decreasing DNA-binding activity of LuxR

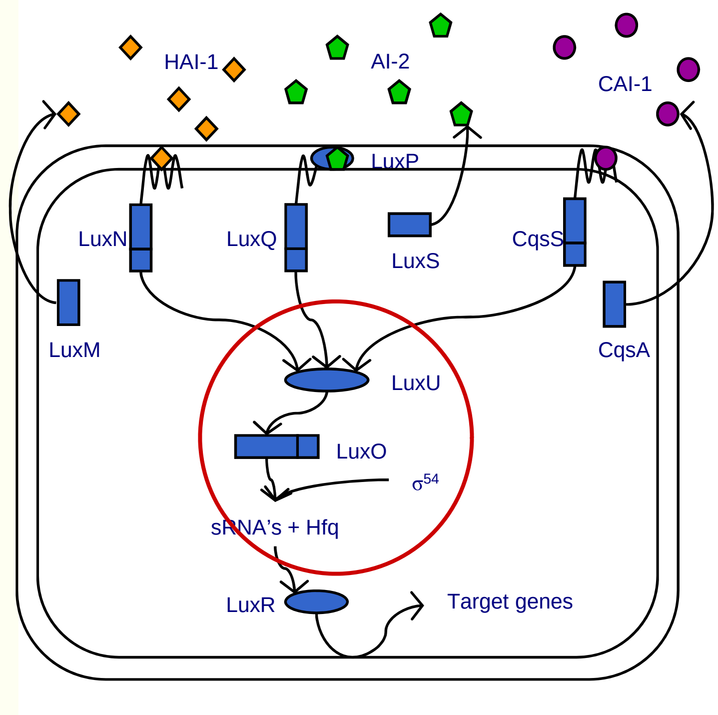
FURANONE *IN VIVO* [1]



QS interference

✓ The furanone acts <u>downstream</u> in the QS signal transduction cascade (LuxR?)





Effect of furanone on Vibrio-Brachionus interaction

Treatment	Experiment 1	Experiment 2
Control	0.51 ± 0.05[d]	0.30 ± 0.05[c]
BB120	0.39 ± 0.07[bc]	0.19 ± 0.03[b]
BB120 + 1 mg l^{-1} furanone	0.48 ± 0.04[cd]	0.25 ± 0.02[bc]
BB120 + 2.5 mg l^{-1} furanone	0.54 ± 0.09[d]	0.32 ± 0.03[c]
BB120 + 5 mg l^{-1} furanone	0.34 ± 0.10[ab]	0.20 ± 0.03[b]
BB120 + 7.5 mg l^{-1} furanone	0.26 ± 0.06[a]	0.05 ± 0.01[a]
BB120 + 10 mg l^{-1} furanone	0	0

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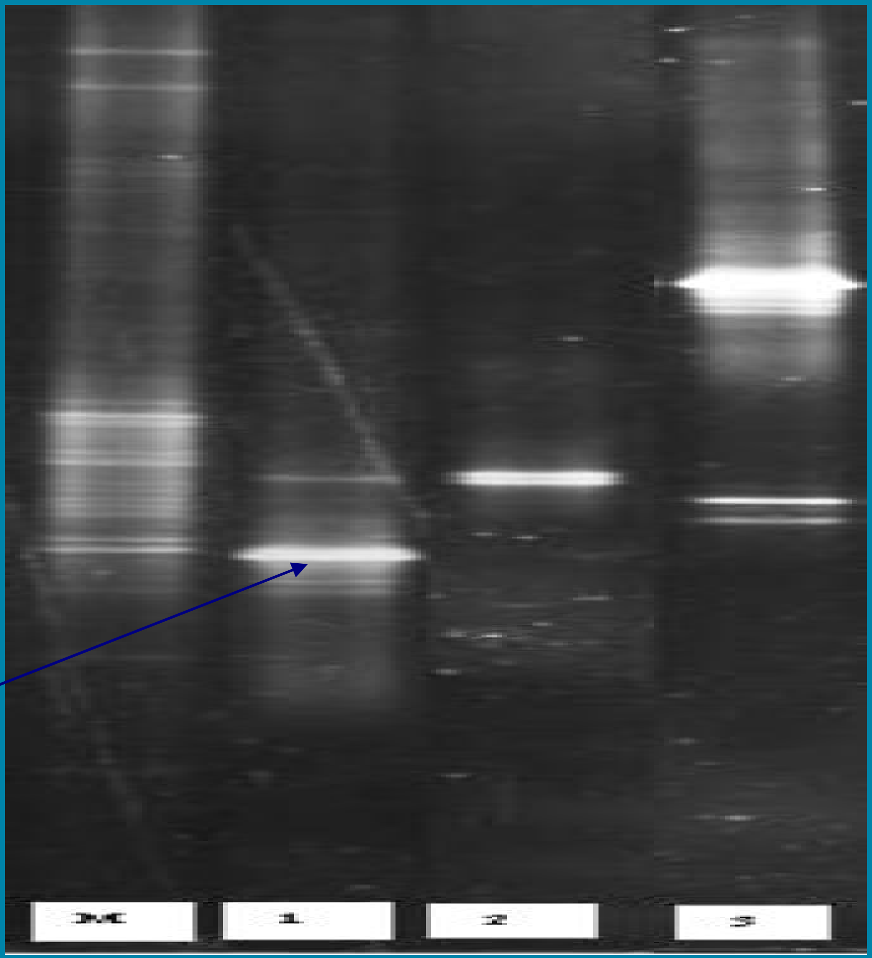
Commercial acyl homoserine lactones

AHL molecule	Abbreviation
N-Butyryl-DL-homocysteine lactone	C_4-AHL
N-Butyryl-DL-homocysteine thiolactone	C_4-AHL
N-Hexanoyl-DL-homoserine lactone	C_6-AHL
N-Heptanoyl-DL-homoserine lactone	C_7-AHL
N-Octanoyl-DL-homoserine lactone	C_8-AHL

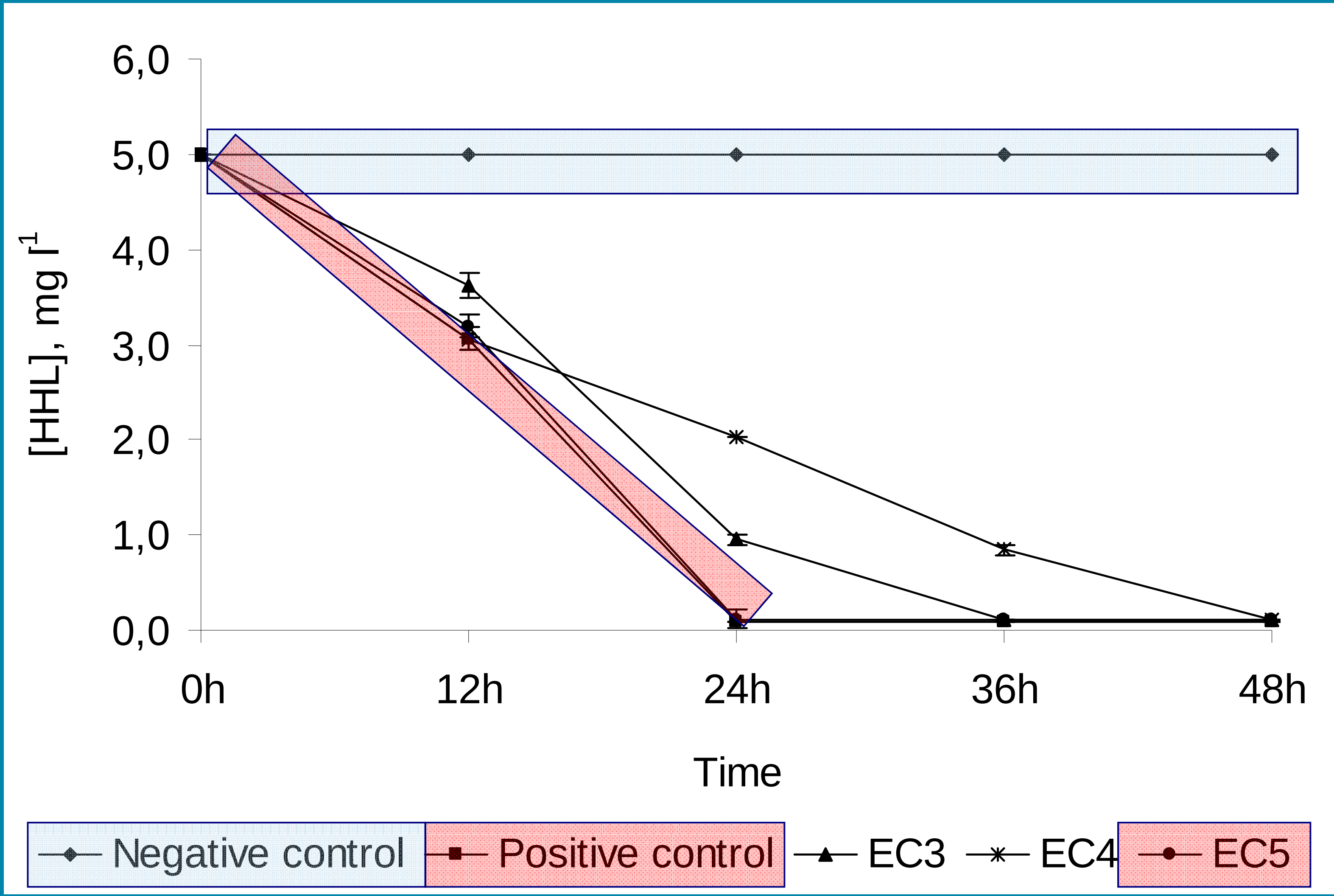
DGGE pattern of the enrichment cultures.



Rhizobium,
Ensifer,
Sinorhizobium,
Aminobacter

M: marker; EC5 EC4 EC3.

Degradation of AHL by MC



Conclusion: EC5 as good as positive control: Pseudomonas with cloned acylase gene

Effect of EC5 on Brachionus-Vibrio interaction

Treatment	Mutation in	Experiment 1
Control	-	0.40 ± 0.096
EC5		0.48 ± 0.02
BB120	-	0.22 ± 0.06
EC5 + BB120		0.25 ± 0.07
MM30	AI-2 synthase	0.15 ± 0.03*
EC5 + MM30		0.34 ± 0.16*
BB152	HAI-1 synthase	0.20 ± 0.08
EC5 + BB152		0.23 ± 0.16
BB886	AI-2 receptor	0.16 ± 0.05*
EC5 + BB886		0.36 ± 0.12*
BB170	HAI-1 receptor	0.13 ± 0.06
EC5 + BB170		0.22 ± 0.08

Effect of AHL on Turbot larvae survival in % on DAH7

Factor		AHL addition	
		-	+
EC addition		(Treatment 1)	(Treatment 2)
	-	92.1 ± 8.3[bc]	10.4 ± 10.0[a]
		(Treatment 3)	(Treatment 4)
	EC3	62.1 ± 24.4[b]	2.1 ± 3.5[a]
		(Treatment 5)	(Treatment 6)
	EC5	96.7 ± 4.0[c]	94.3 ± 7.1[bc]

•daily AHL addition of ***1 mg/l***

Obtained ECs

European sea bass (*Dicentrarchus labrax*)

Asian sea bass (*Lates calcarifer*)

Sea bream (*Sparus aurata*)

Mix. of AHL as N-source →

EC5(D)

EC5(L)

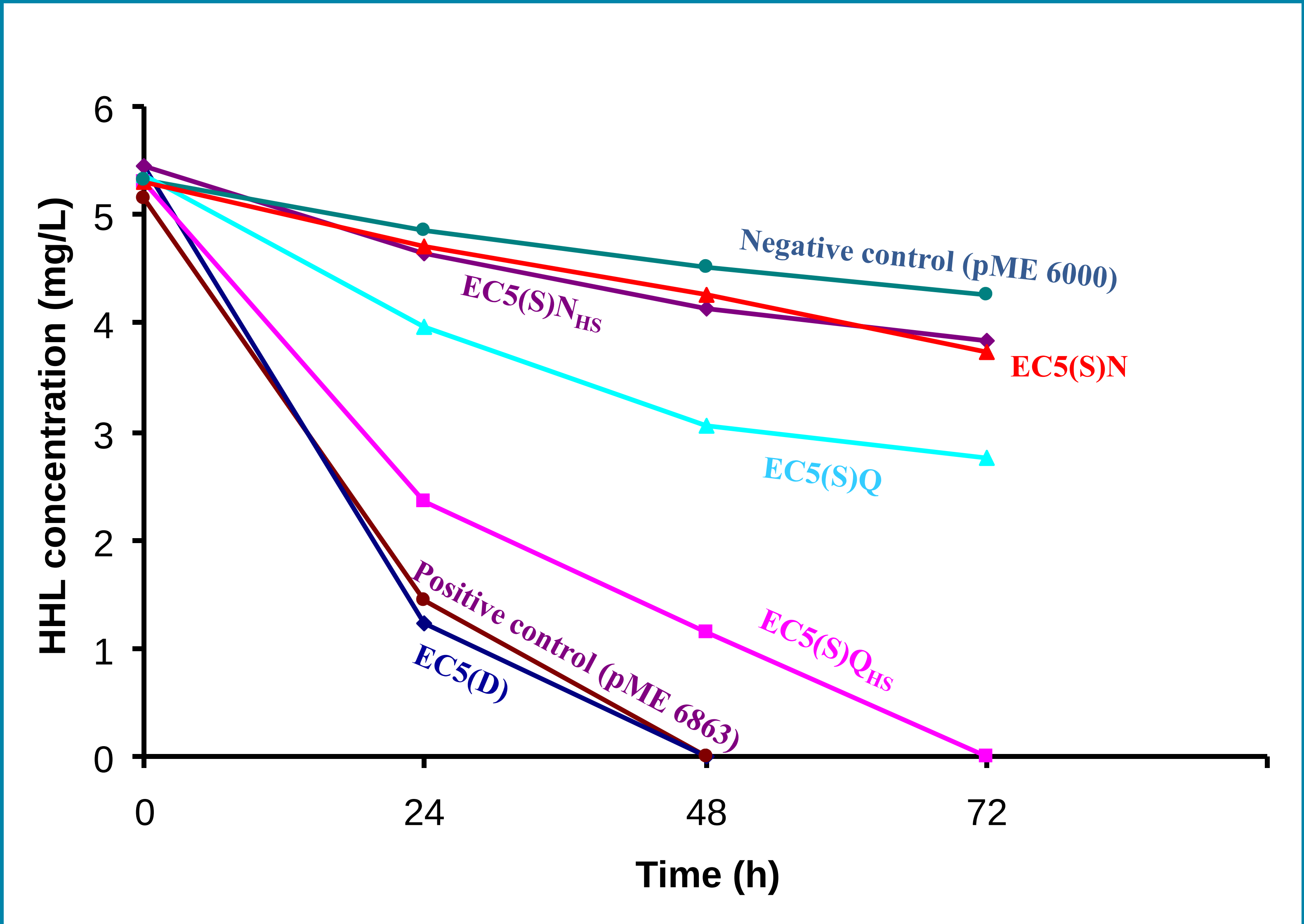
EC5(S)Q_{HS} & EC5(S)Q

Sea bream (*Sparus aurata*)

NH_4Cl as N-source →

EC5(S)N_{HS} & EC5(S)N

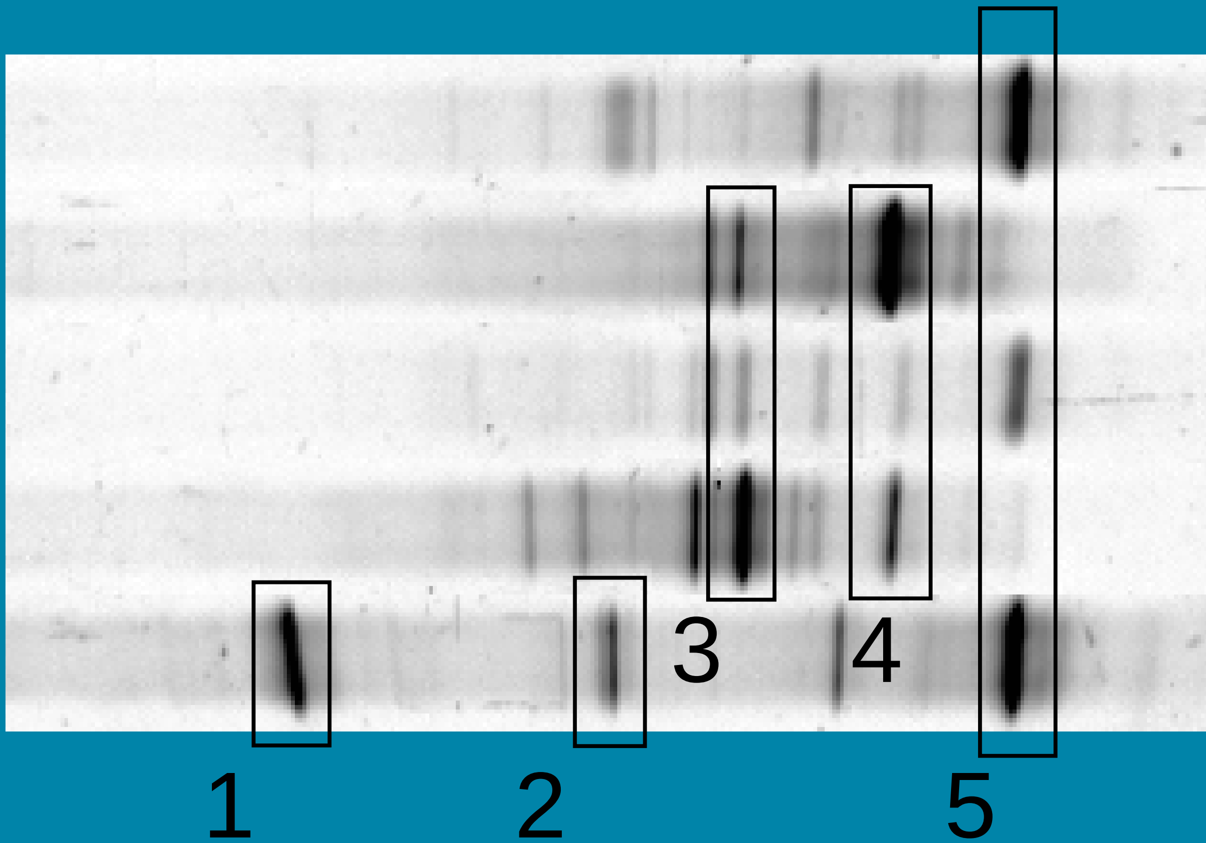
HHL degradation rate of different ECs



PHB content of ECs

EC's	Produced PHB amount (% of the cell dry weight)
EC5(D)	9.4 ± 1.5
EC5(L)	1.6 ± 1.3
EC5(S)Q_{HS}	2.1 ± 0.3
EC5(S)Q	1.6 ± 0.1
EC5(S)N_{HS}	1.7 ± 0.3
EC5(S)N	0.9 ± 0.1
LVS3 (negative control)	0.0

DGGE profile



Lane 1: EC5(S)N

Lane 2: EC5(S)N$_{HS}$

Lane 3: EC5(S)Q

Lane 4: EC5(S)Q$_{HS}$

Lane 5: EC5(D)

Band 1: *Bacillus circulans*

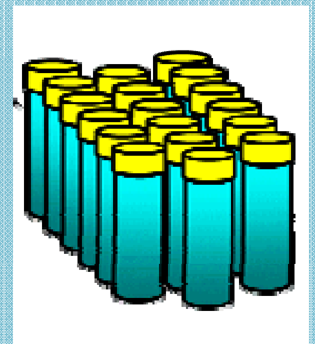
Band 2: *Bacillus* sp.

Band 5: *Vibrio* sp.

Band 3: *Halomonas* sp.

Band 4: Enterobacteriaceae

ECs: source & preparation

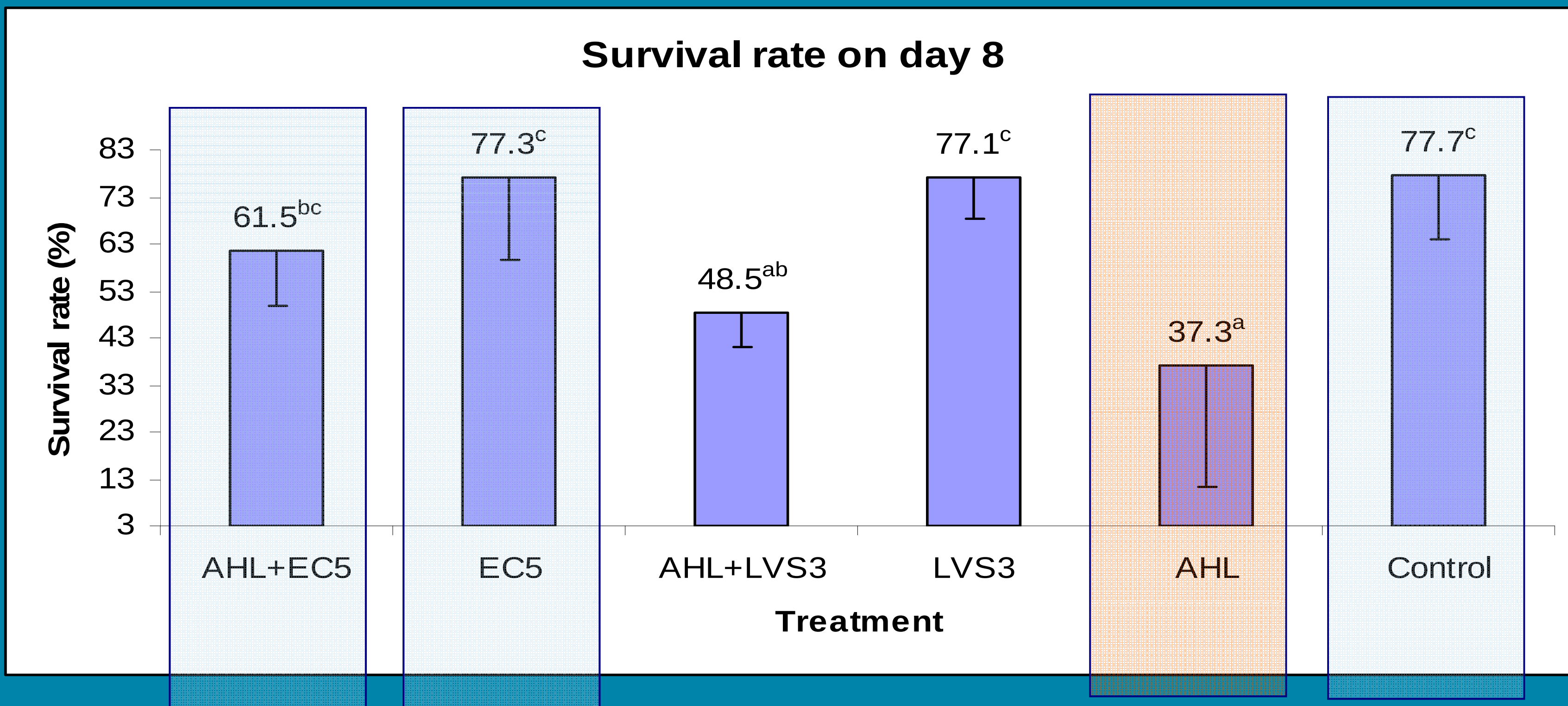


Stock cultures
EC5(D) & EC5(L)
in deep freezer -80ºC

Axenic *Artemia* hatching
with ECs (10^5 CFU/mL)

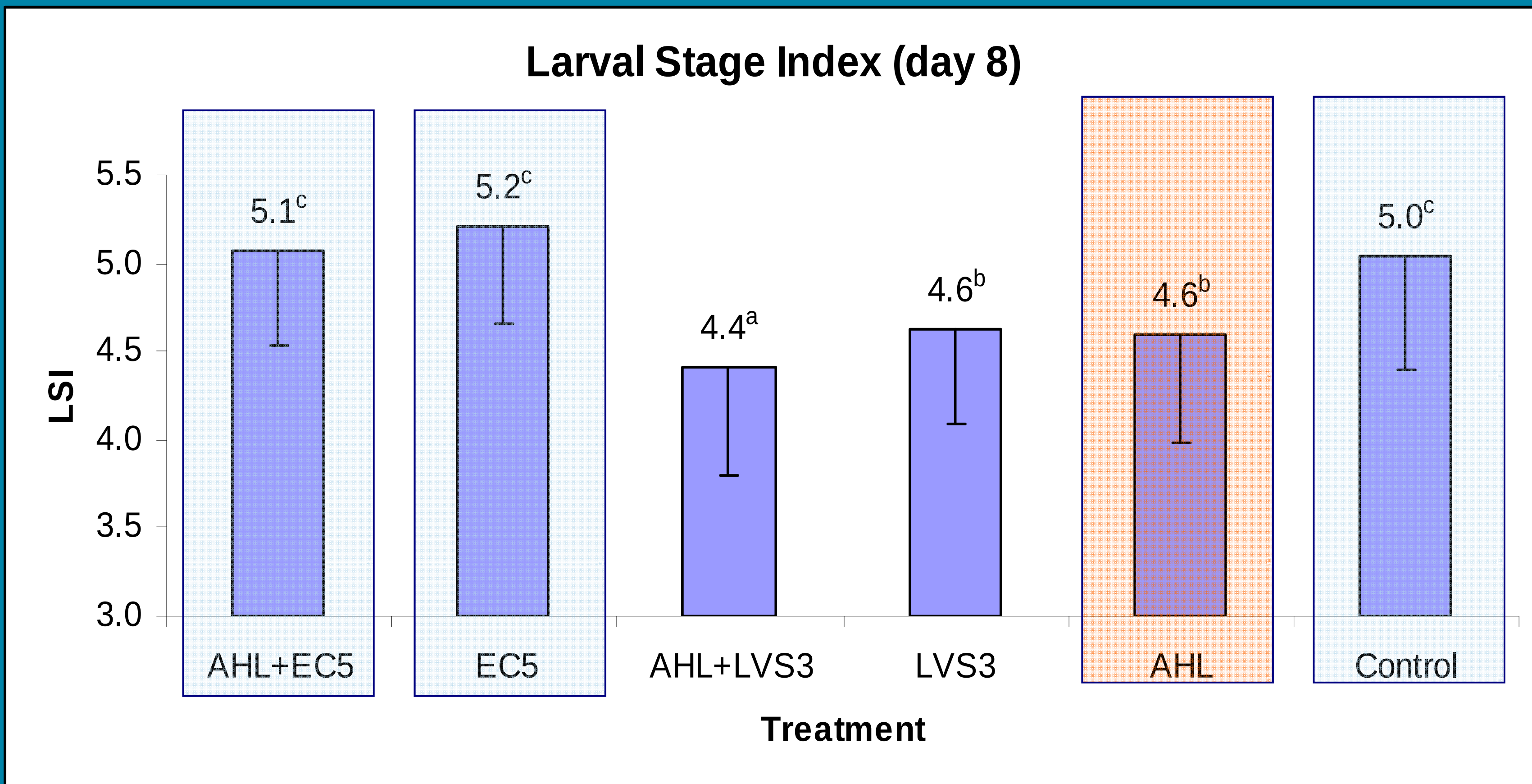
Hatching medium
ECs (0.3×10^8 CFU/mL)

Effect of AHL and EC5D on *Macrobrachium* larviculture: survival



•daily AHL addition of ***1 mg/l***

Effect of AHL and EC5D on *Macrobrachium* larviculture: LSI

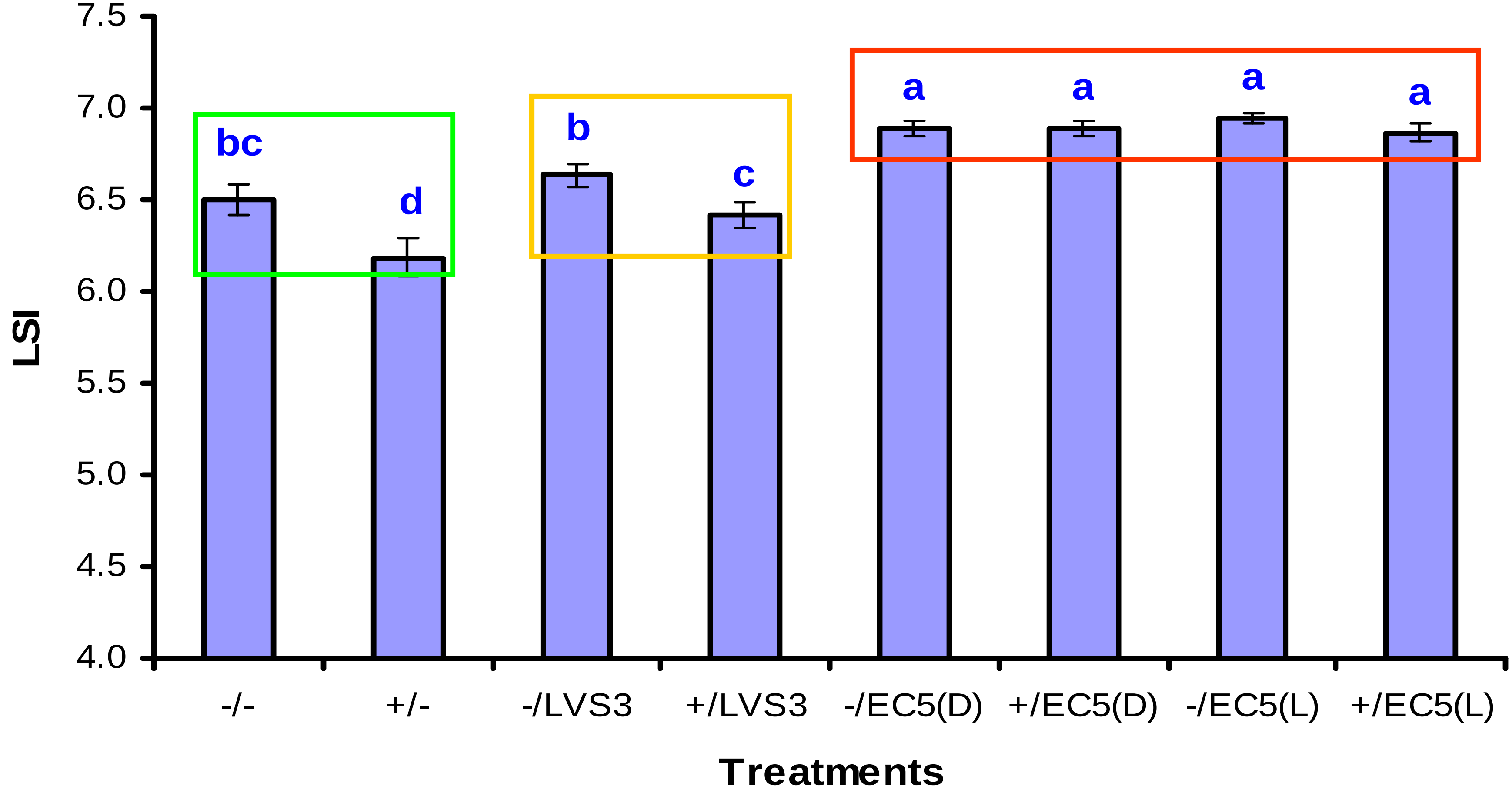


•daily AHL addition of ***1 mg/l***

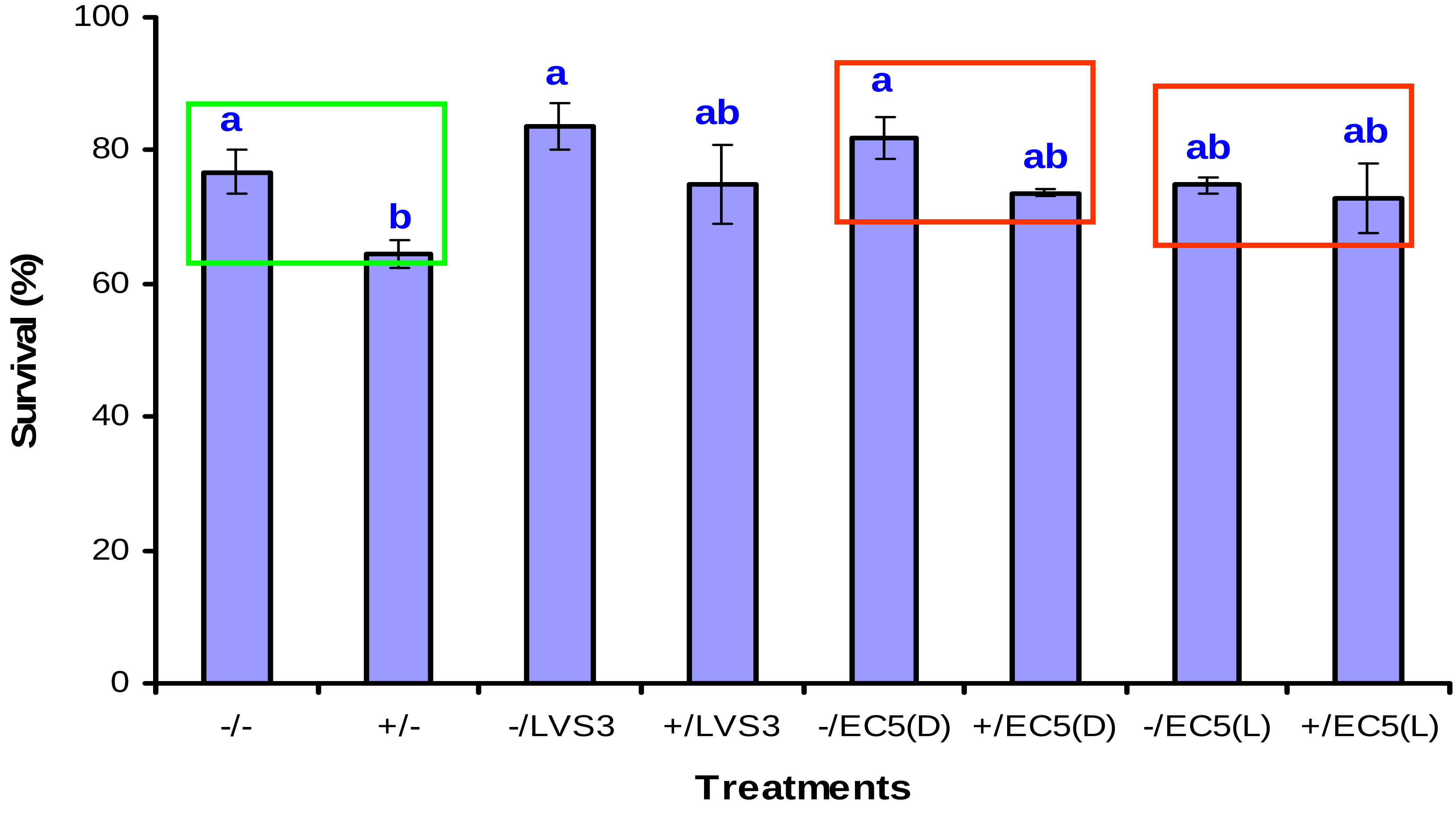
Experimental design

	No bacteria addition	LVS3 addition	EC5(D) addition	EC5(L) addition
No AHL addition	– / –	– / LVS3	– / EC5(D)	– / EC5(L)
AHL addition	+ / –	+ / LVS3	+ / EC5(D)	+ / EC5(L)

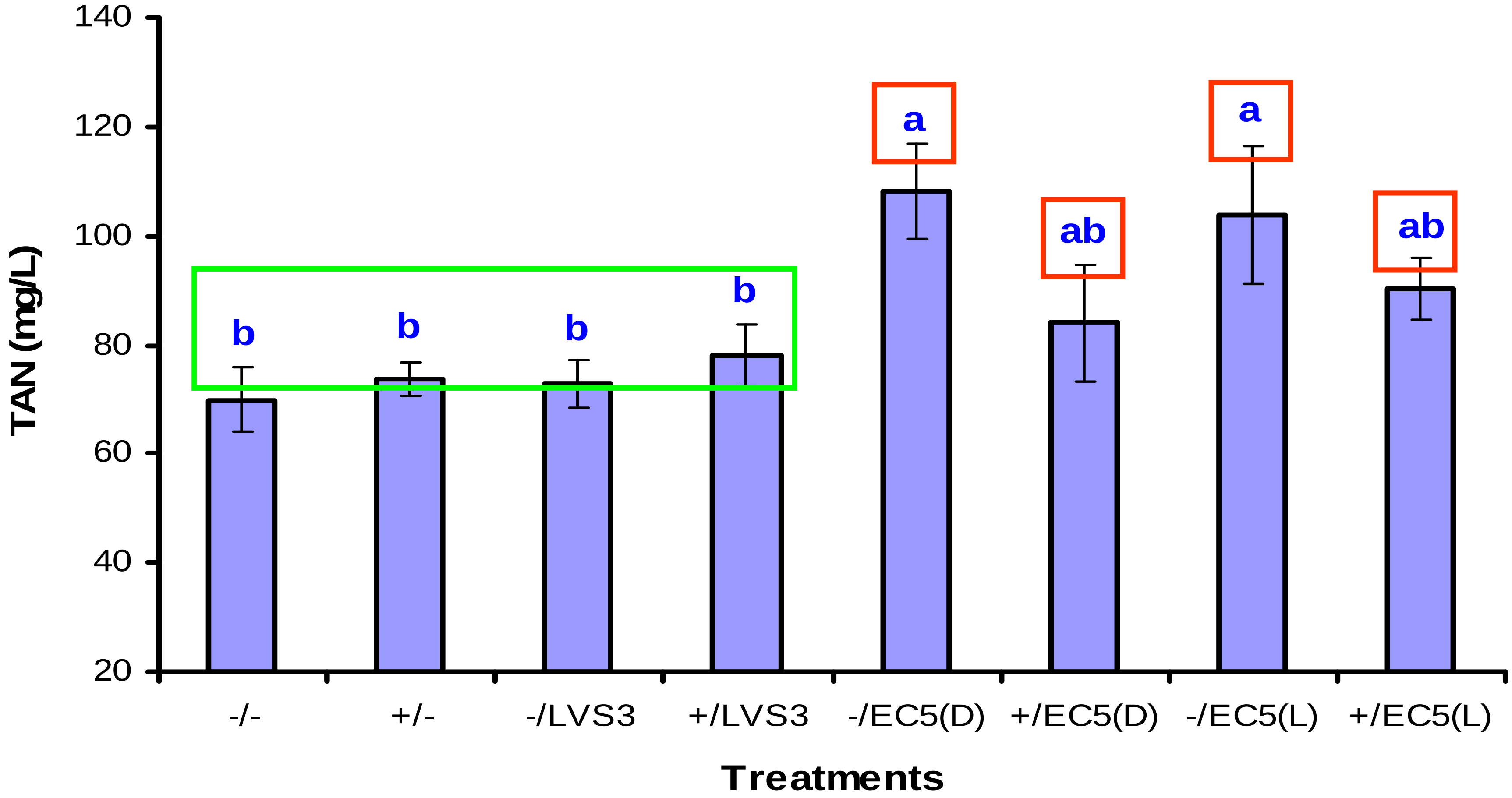
LSI of prawn larvae on day 10



Survival of prawn larvae on day 10



Ammonia tolerance (LC_{50}-24h) of prawn larvae on day 10



General conclusions

- Importance of QS demonstrated *in vivo* for
  - Artemia
  - Brachionus
  - Seabass
  - Macrobrachium

- QS communication can be steered favoroubly by
  - QS interference (furanones; but toxic)
  - QS degrading microbial communities

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THAT YOU