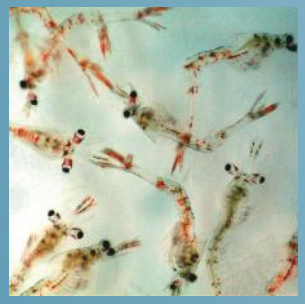
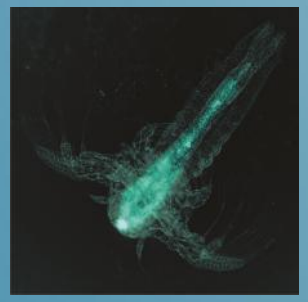
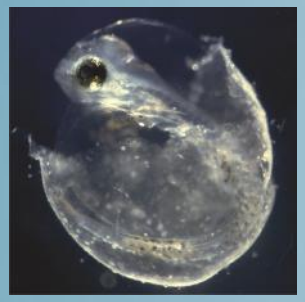
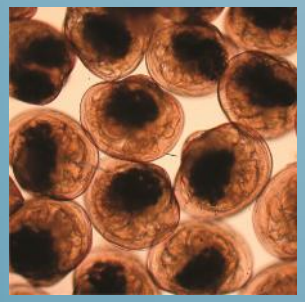




larvi 2013

6th fish & shellfish larviculture symposium

Selection study of potential probiotic bacteria for shrimp hatcheries in New Caledonia

Dominique Pham

ghent university, belgium, 2-5 september 2013

Selection study of potential probiotic bacteria for shrimp hatcheries in New Caledonia

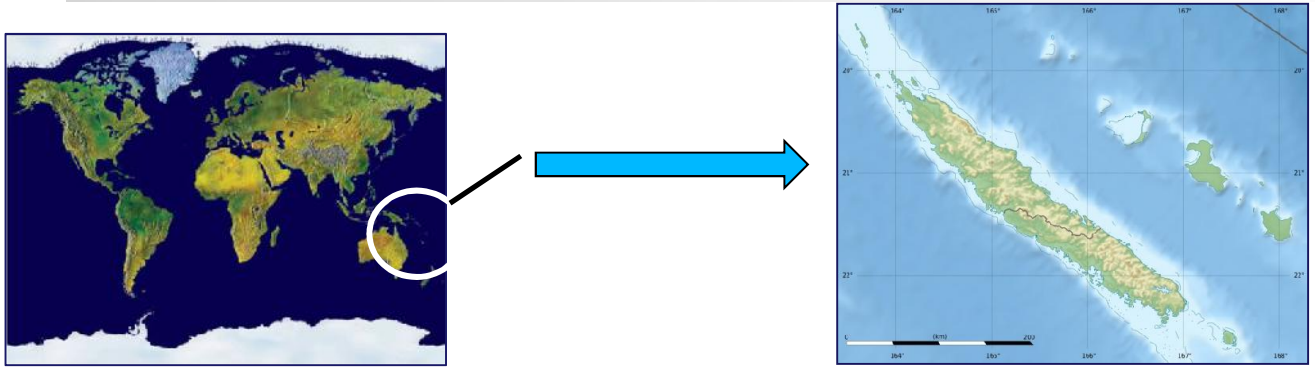
Pham Dominique, Ansquer Dominique, Chevalier Anne, Peyramale Aude, Dauga Clément, Wabete Nelly and Labreuche Yannick.

Unité de Recherche Lagon, Eco-système et Aquaculture Durable,
Ifremer

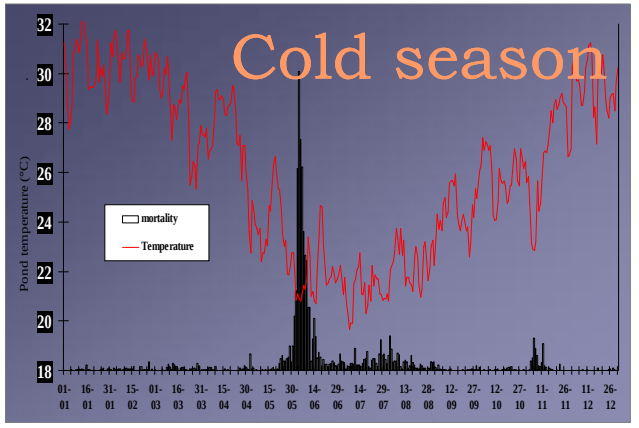
BP 2059, Nouméa - New Caledonia

Larvi 2013, September 2nd-5th, 2013, Ghent, Belgium

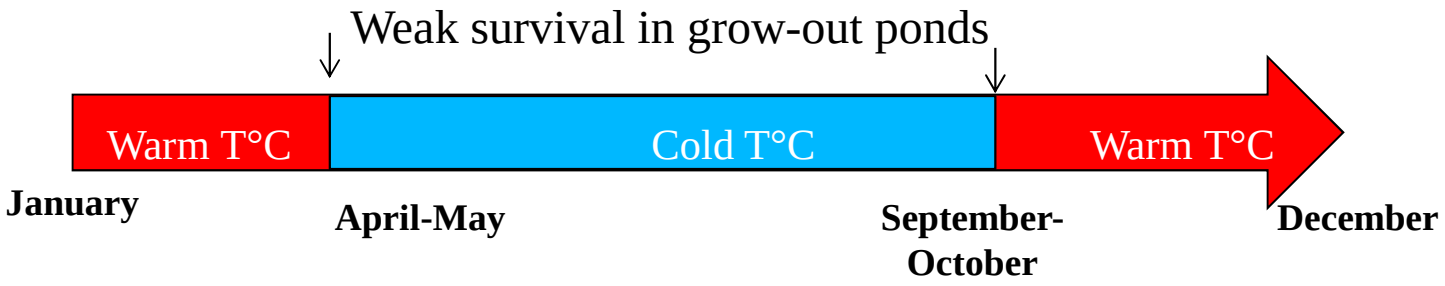
Shrimp farming in New Caledonia



- Commercial production since 1983
- Semi-intensive culture from *Litopenaeus stylirostris* captive broodstock
- 2500 tons in 2005, less than 1200 tons in 2010
- 2 seasonal *Vibrio* pathogens in grow-out ponds (*V. penaeicidae* and *V. nigripulchritudo)*



Hatchery constraints in New Caledonia



- Most of postlarvae demand concentrated in warm season (october to january)

- Increasing use of antibiotics to stabilize production

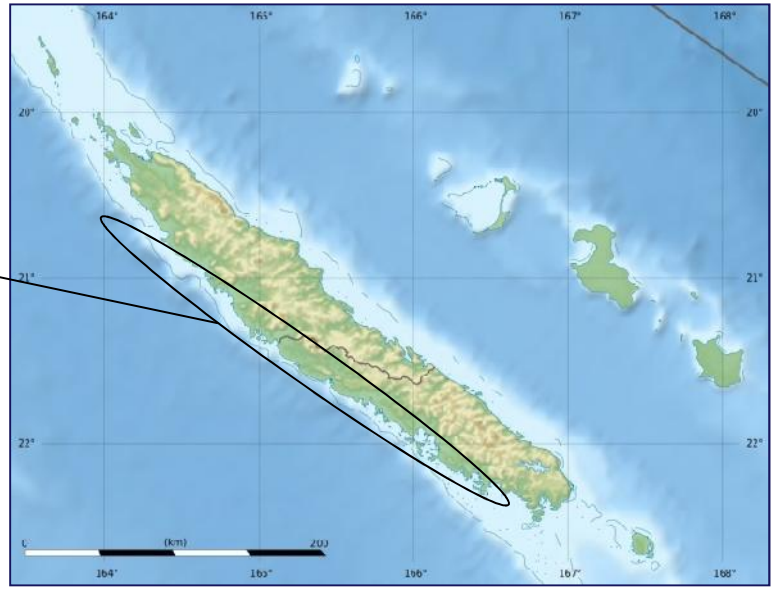
  - Research project for evaluating alternative prophylactic treatments in hatchery such as probiotics

Experimental approach

Potential probionts selection

- **Strains characterization / identification**
- ***In vitro* growth-inhibition test**
- **Innocuousness test**
- **Effectiveness test in larval rearing**
- **Impact on animal status**

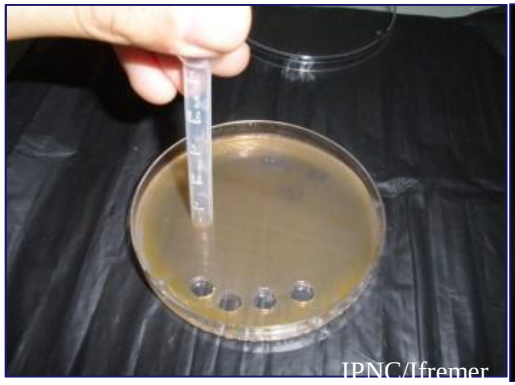
Potential probionts selection

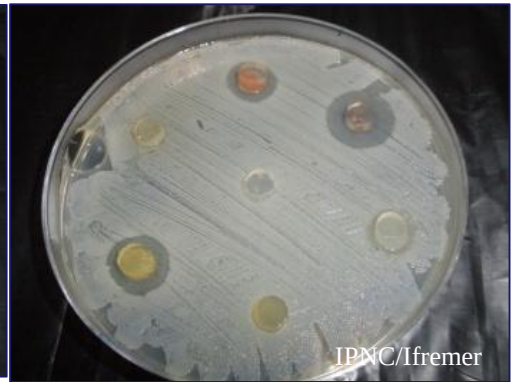


493 isolates sampled in extreme marine environments in New Caledonia

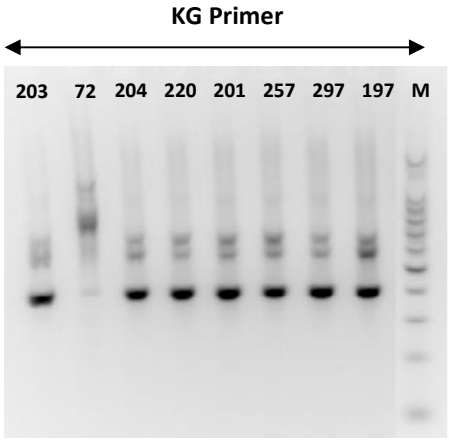
Screening for antagonistic activity against *V. nigripulchritudo.*

Selection of 7 isolates :
NC72, NC197, NC201, NC203, NC204, NC257 and NC297



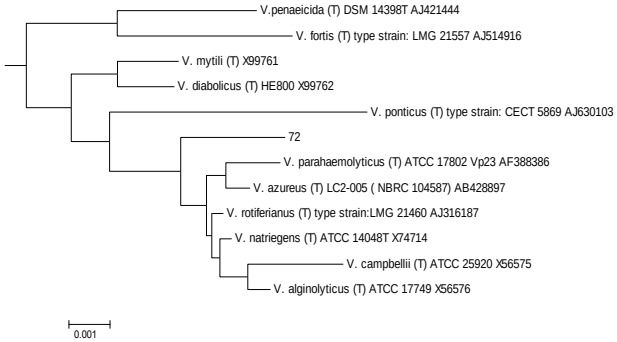


Strains characterization / identification



AP-PCR

API 20E Gallery Test



Phylogenetic analysis

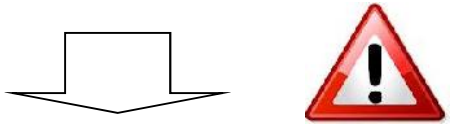
1 isolate : ***Vibrio*** genus

- NC72 belonging to *Harveyi* clade

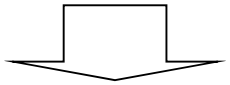
5 isolates : ***Pseudoalteromonas*** genus

- NC201 close to *P. maricaloris*
- NC257,NC297, NC203, NC204,

NC197 close to *P. piscicida*

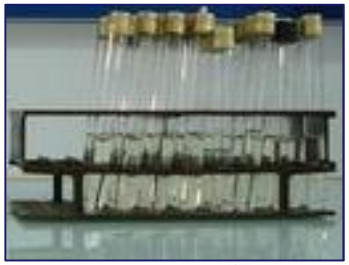


Bacteria group including pathogens and probiotic strains for marine invertebrates



Several species with antibacterial, antifungal and antialgal properties

Inhibitory test on *V. harveyi* ORM4-GFP growth



1 — x 3 — 2

10^5 CFU.mL^{-1}

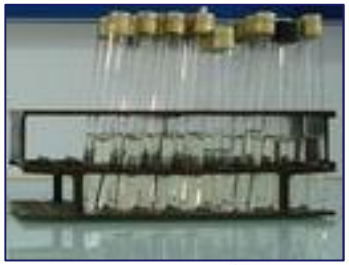
29°C

V. harveyi GFP

V. harveyi GFP + Isolate

Fluorescence reading after 48h

Inhibitory test on *V. harveyi* ORM4-GFP growth



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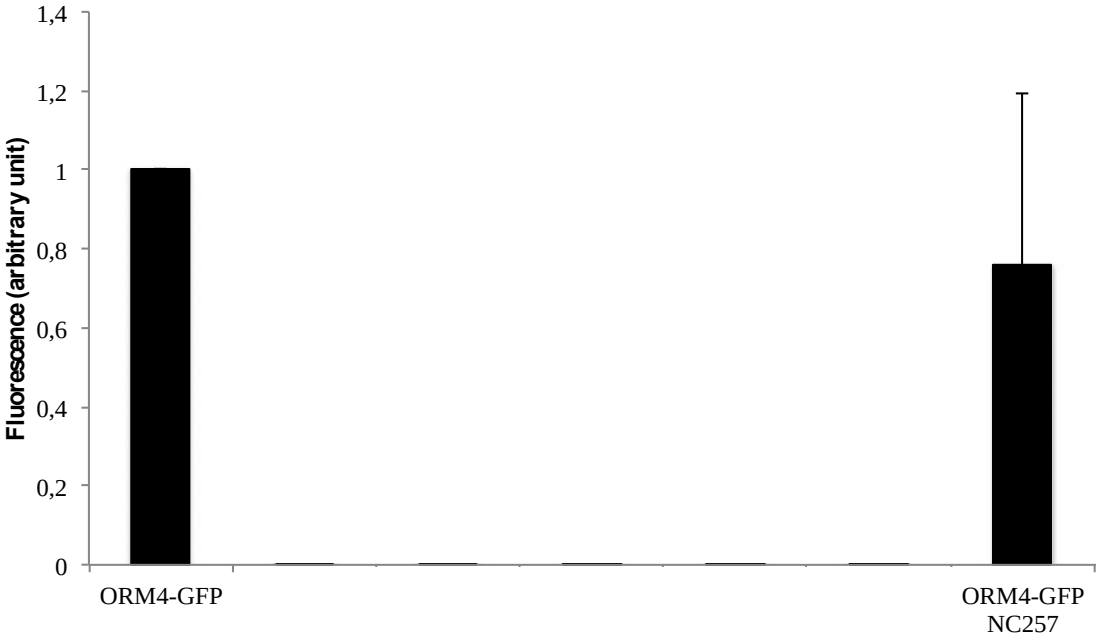
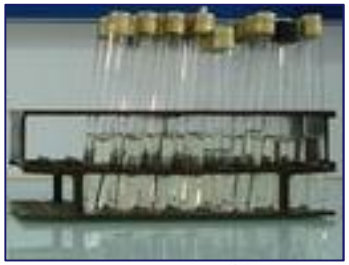
V. harveyi GFP + Isolate

Fluorescence reading after 48h



- Weak inhibition activity from NC257

Inhibitory test on *V. harveyi* ORM4-GFP growth



1 — x 3 — 2

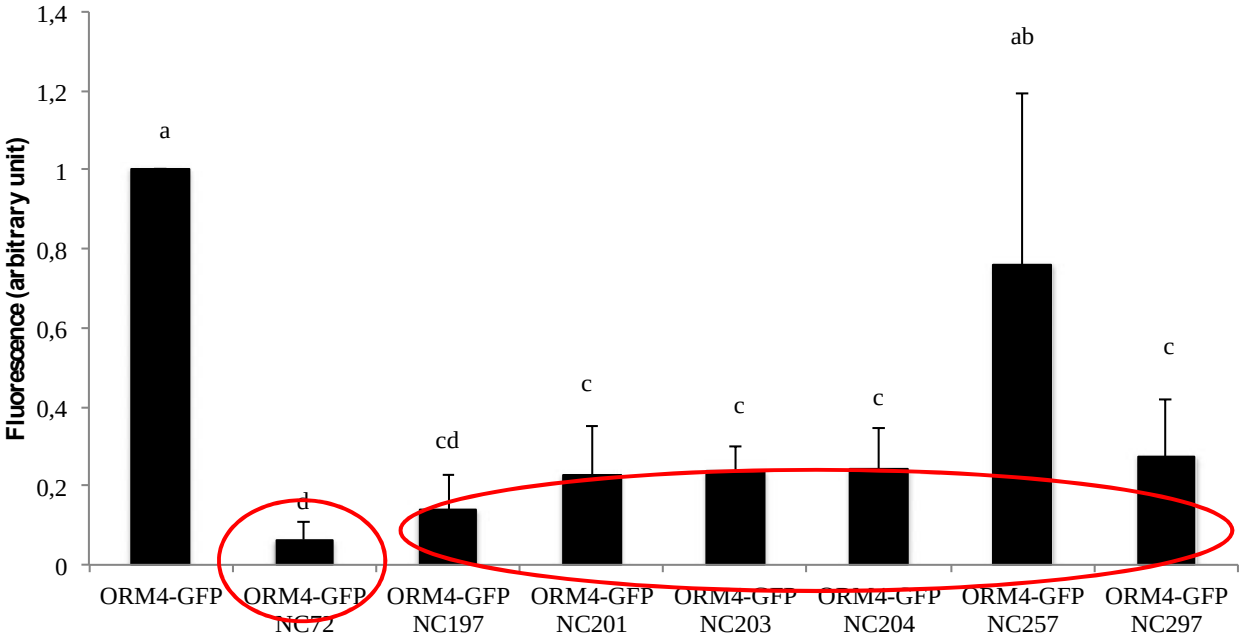
10^5 CFU.mL^{-1}

29°C

V. harveyi GFP

V. harveyi GFP + Isolate

Fluorescence reading after 48h



- Very strong inhibition activity from NC72

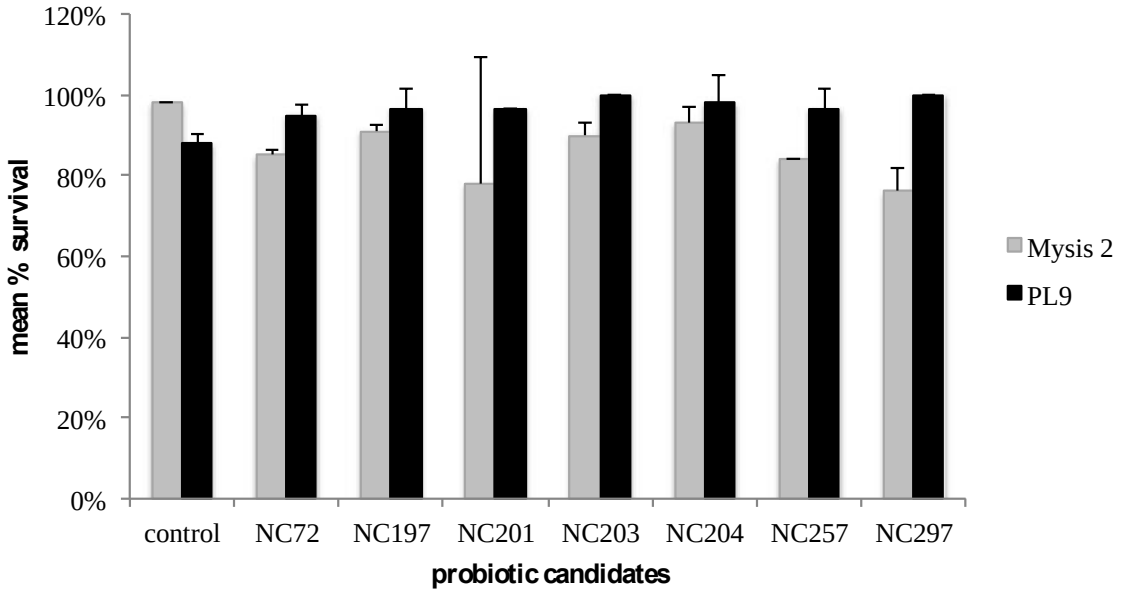
- Strong inhibition from NC197,NC201, NC203, NC204, NC297

Excellent antagonistic activity from 6 out of the 7 isolates towards *V. harveyi* ORM4-GFP

Harmfulness of probiotic candidates

Pathogenicity test towards Mysis 2 and PL9

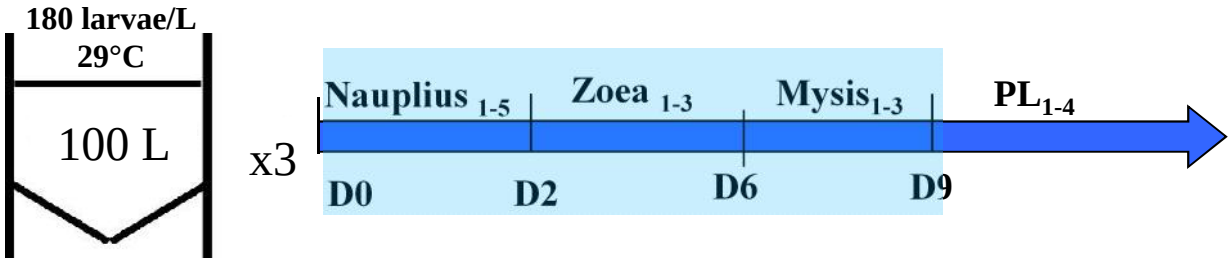
- Mysis 2 in duplicates for 48 hours
- PL9 in triplicates for 72 hours
- Isolates final concentration of 10^5 CFU/mL



- No survival alteration from any isolates in both stages
- No abnormality in larvae and postlarvae behaviour with all isolates

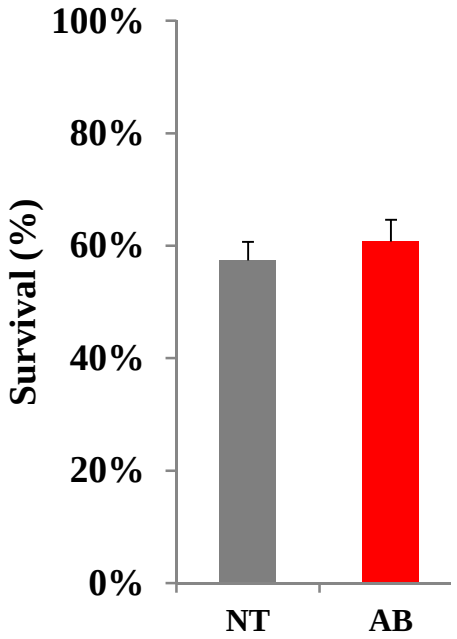
No pathogenicity detected for any isolates

Probiotic effectiveness in larval rearing : from *nauplius* to *mysis*



Survival and growth

- No treatment (NT)
- Antibiotic treatment (AB) at D3, D5, D7 and D9 at 2.5g/m^3
- 5 probiotic treatments: *Vibrio* NC72 and *Pseudoalteromonas* NC197, NC201, NC203, NC297 every day administration at 10^5 CFU/ml



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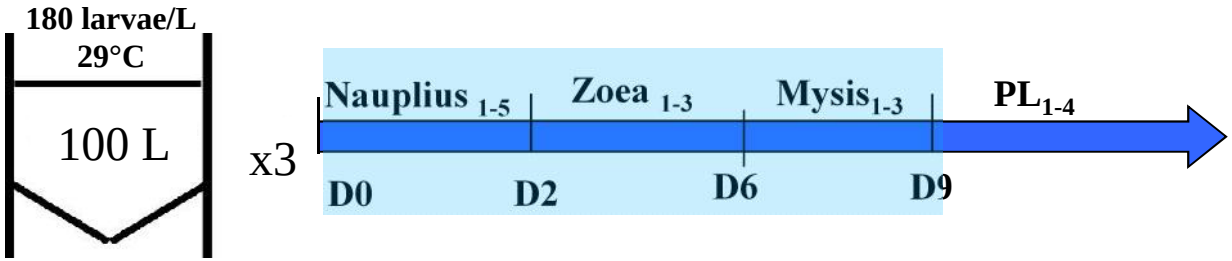

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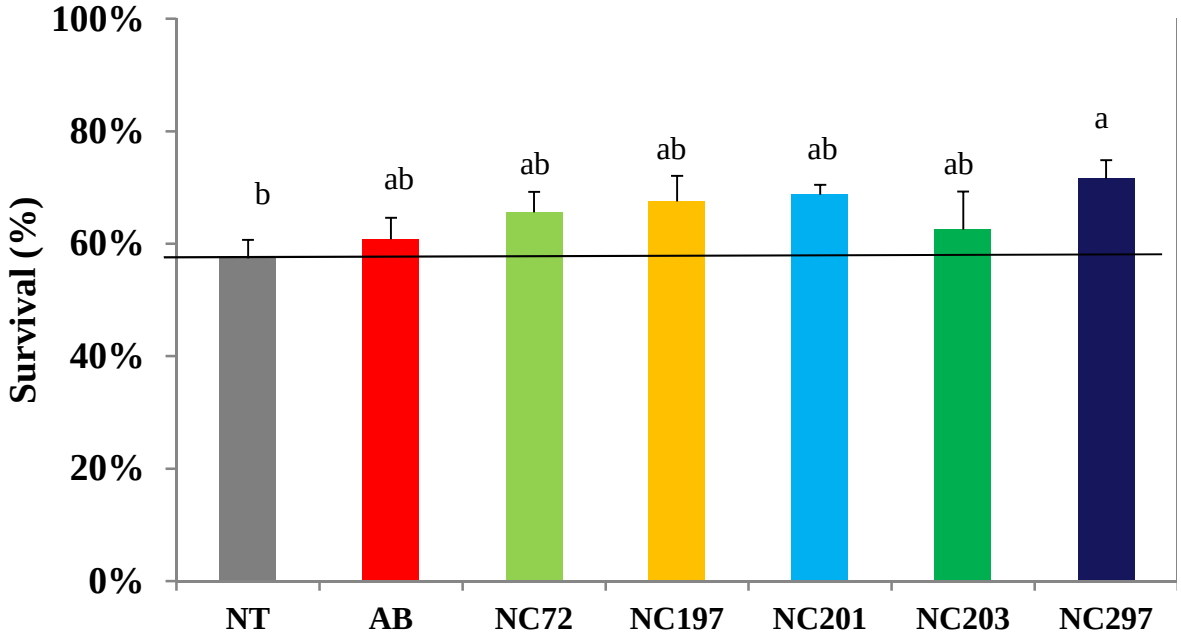
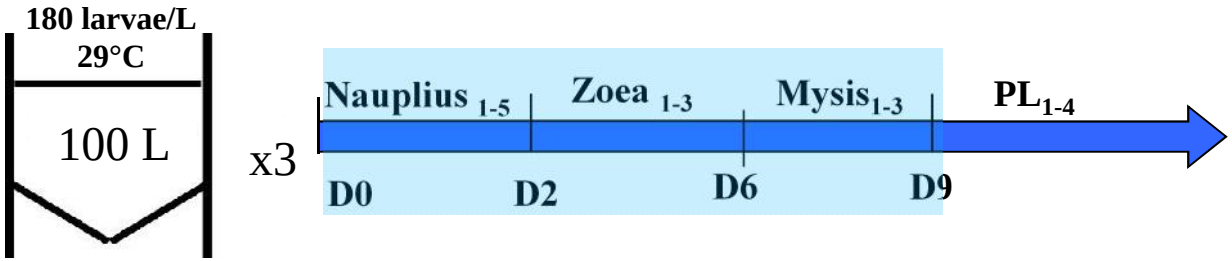


Similar or better survival rates with probiotic treatments

Probiotic effectiveness in larval rearing : from *nauplius* to *mysis*



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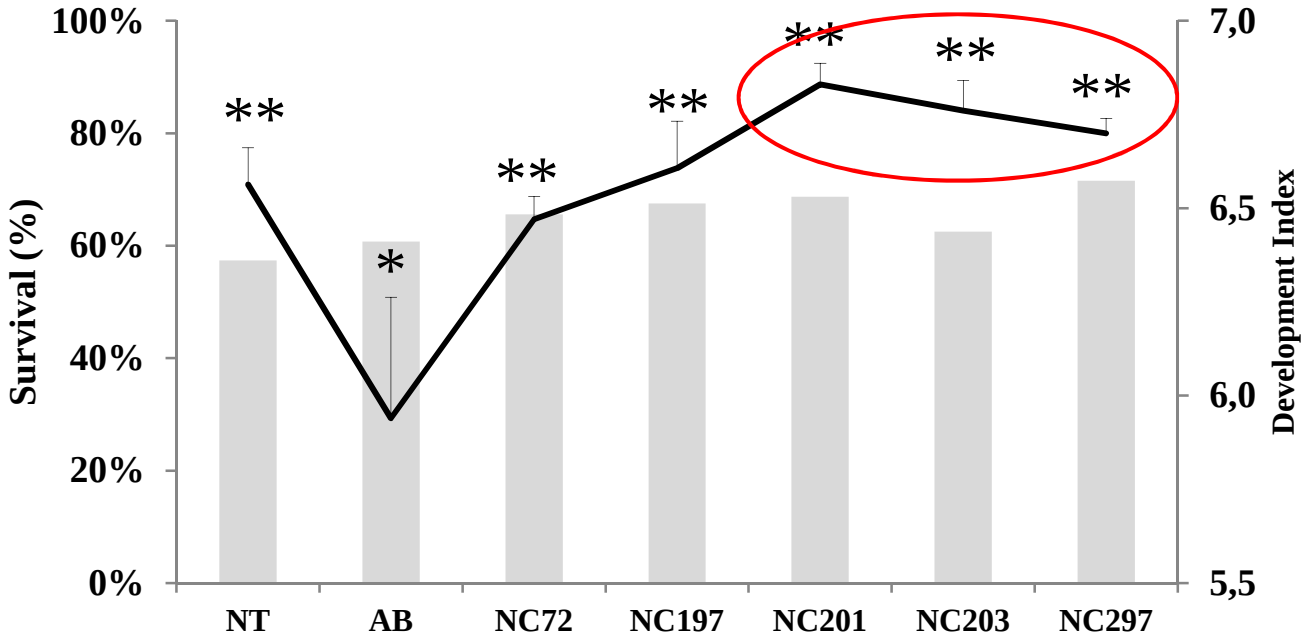

Better growth with probiotics compared to antibiotic

Probiotic effectiveness in larval rearing : from *nauplius* to *mysis*

- No treatment (NT)
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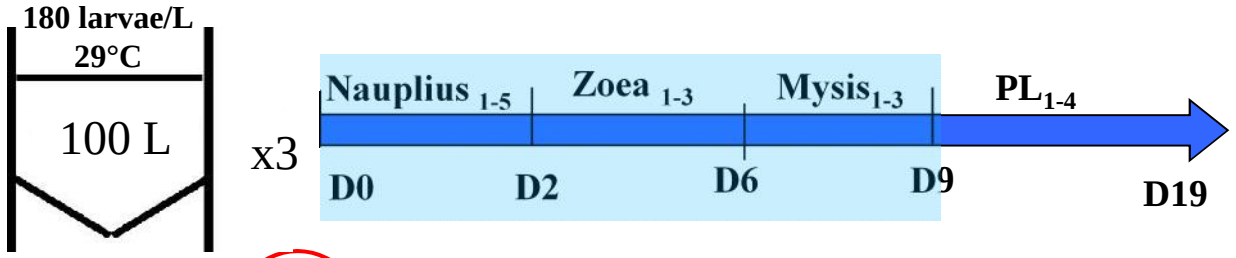
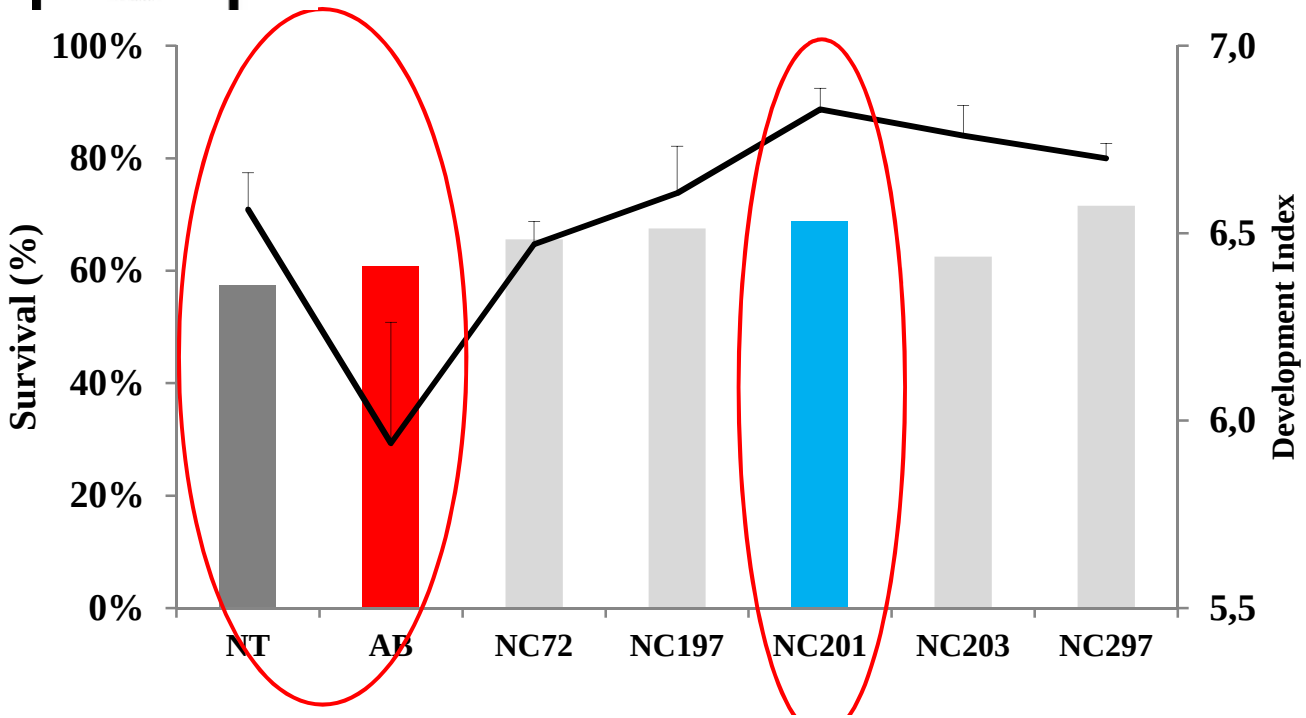


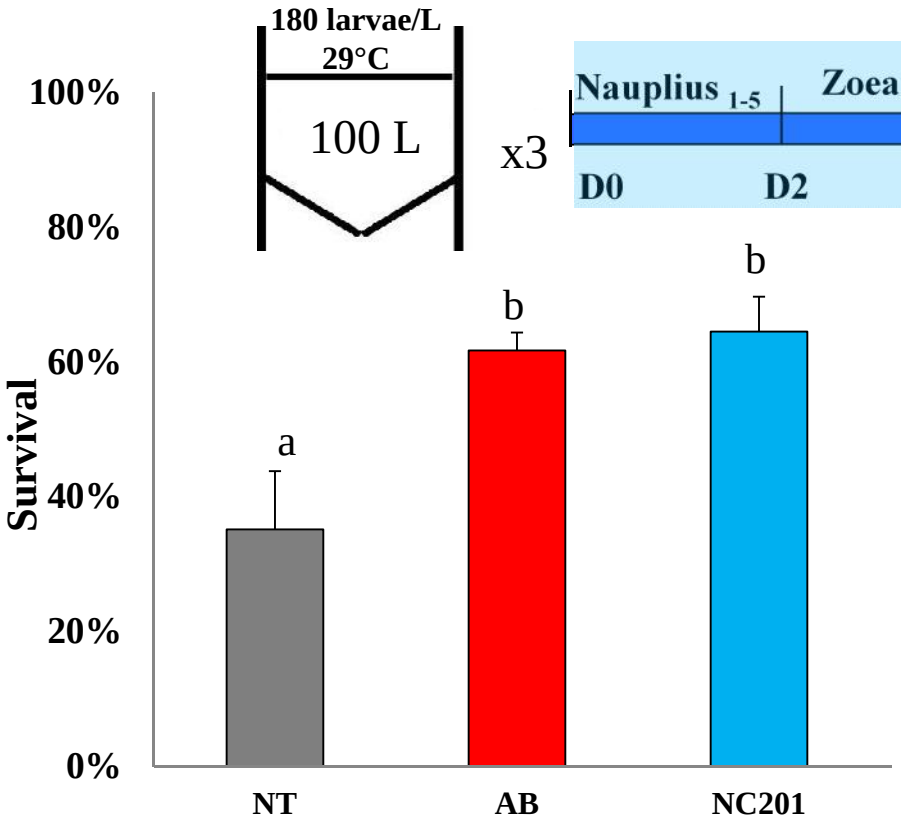
Survival and growth

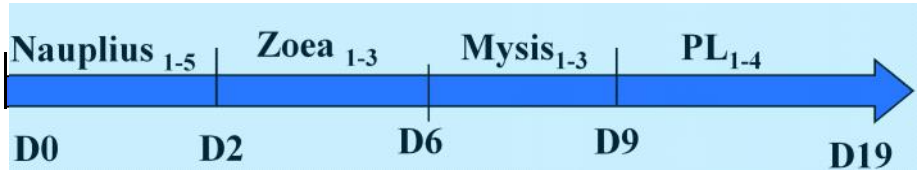


Selection of NC201 for further trials

NC201 effectiveness in larval rearing : from *nauplius* to P9

Posology : every day (NC201) *vs* each other day ($NC201^{1/2}$)





- **Survival**
- **Growth**

NC201 effectiveness in larval rearing : from *nauplius* to P9

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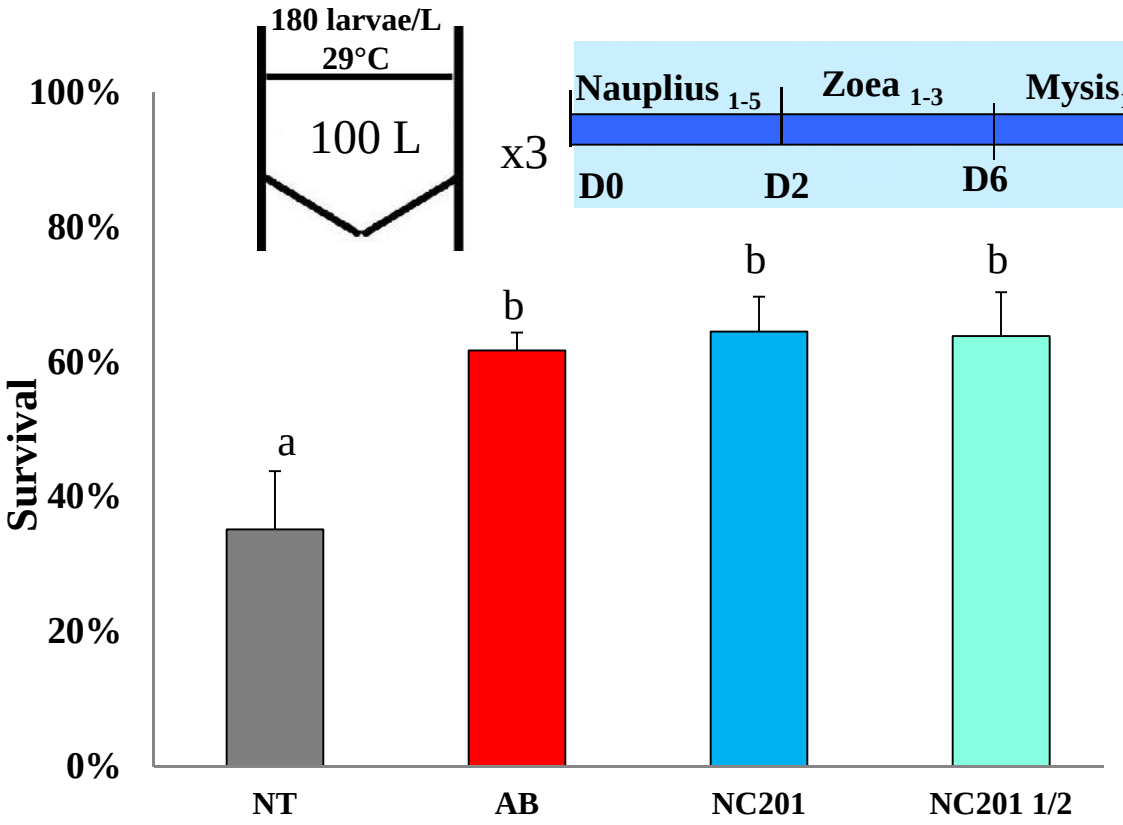
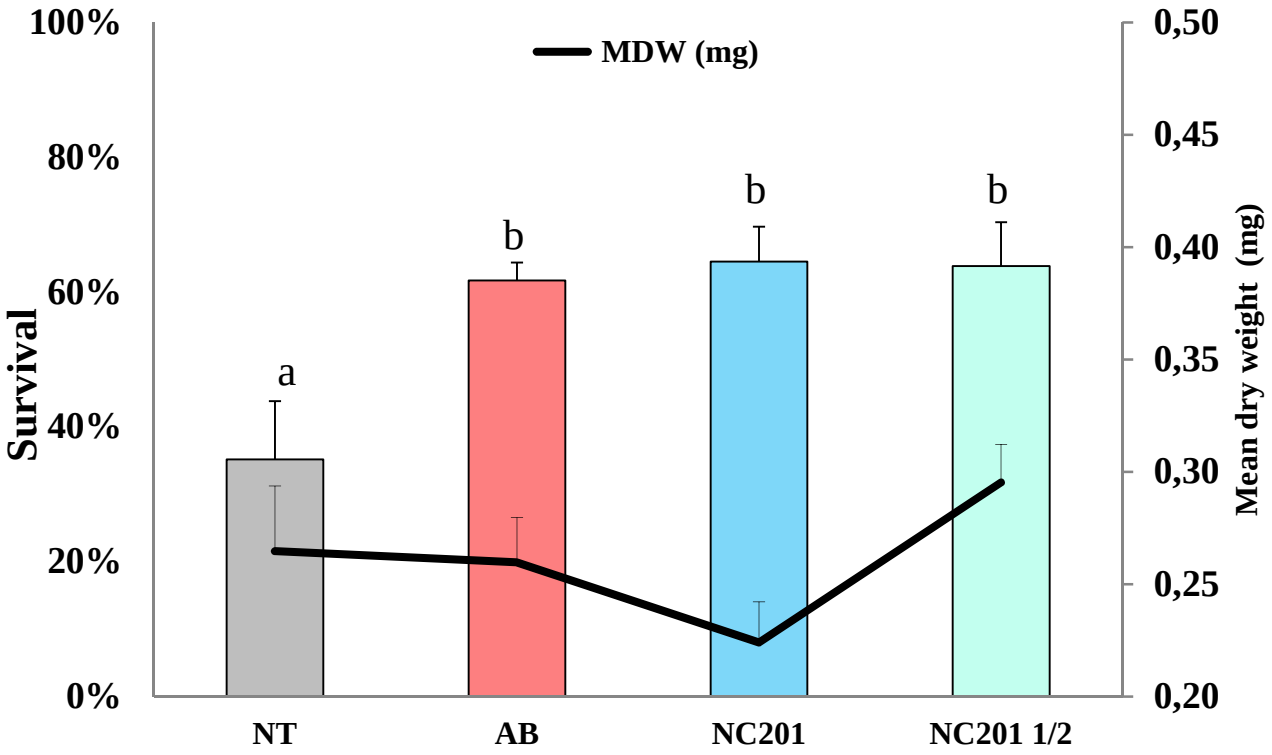


Same survival enhancement of NC201 each other day compared to every day supply

NC201 effectiveness in larval rearing : from *nauplius* to P9

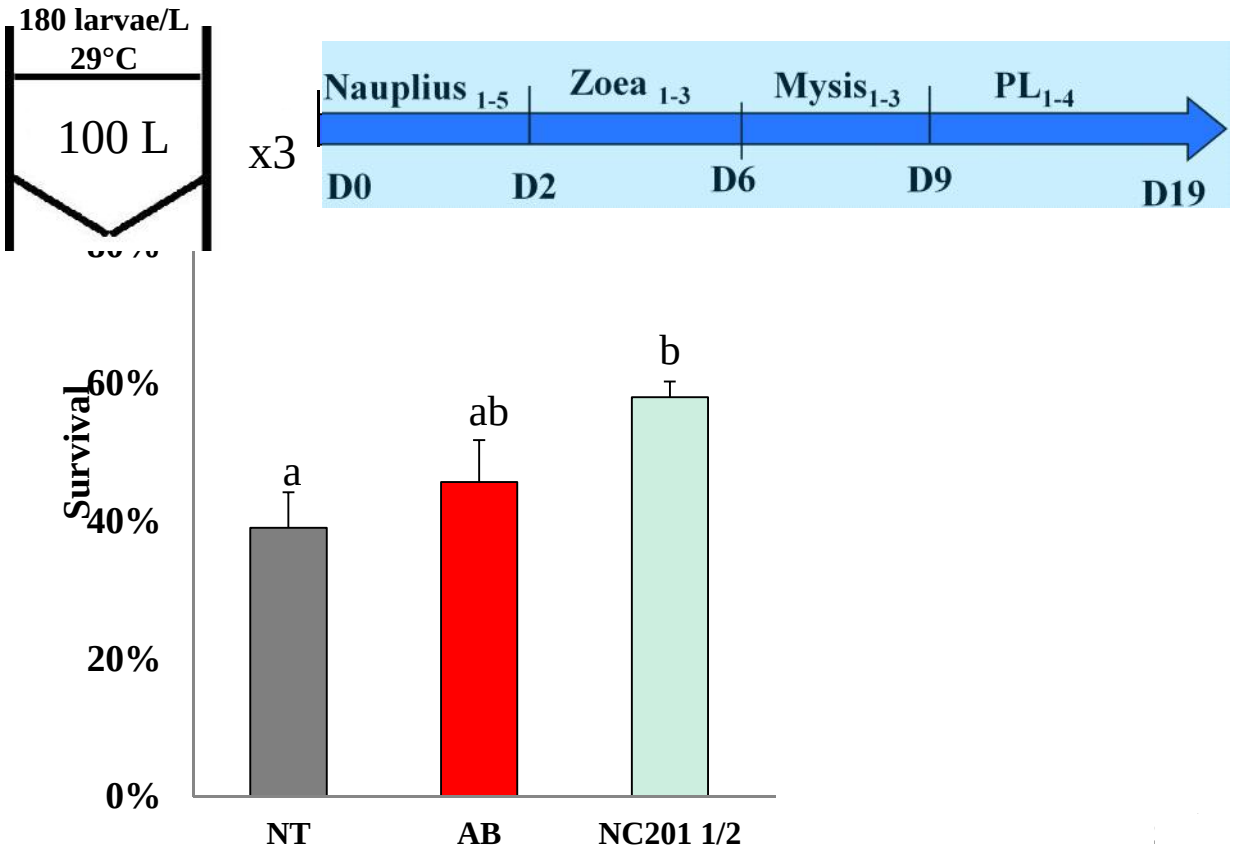
Posology : every day (NC201) *vs* each other day (NC201$^{1/2}$)



NC201 each other day as effective as NC201 every day

NC201 effectiveness in larval rearing : from *nauplius* to P9

Single administration *vs* combination of two probiotics



- **Survival**
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NC201 effectiveness in larval rearing : from *nauplius* to P9

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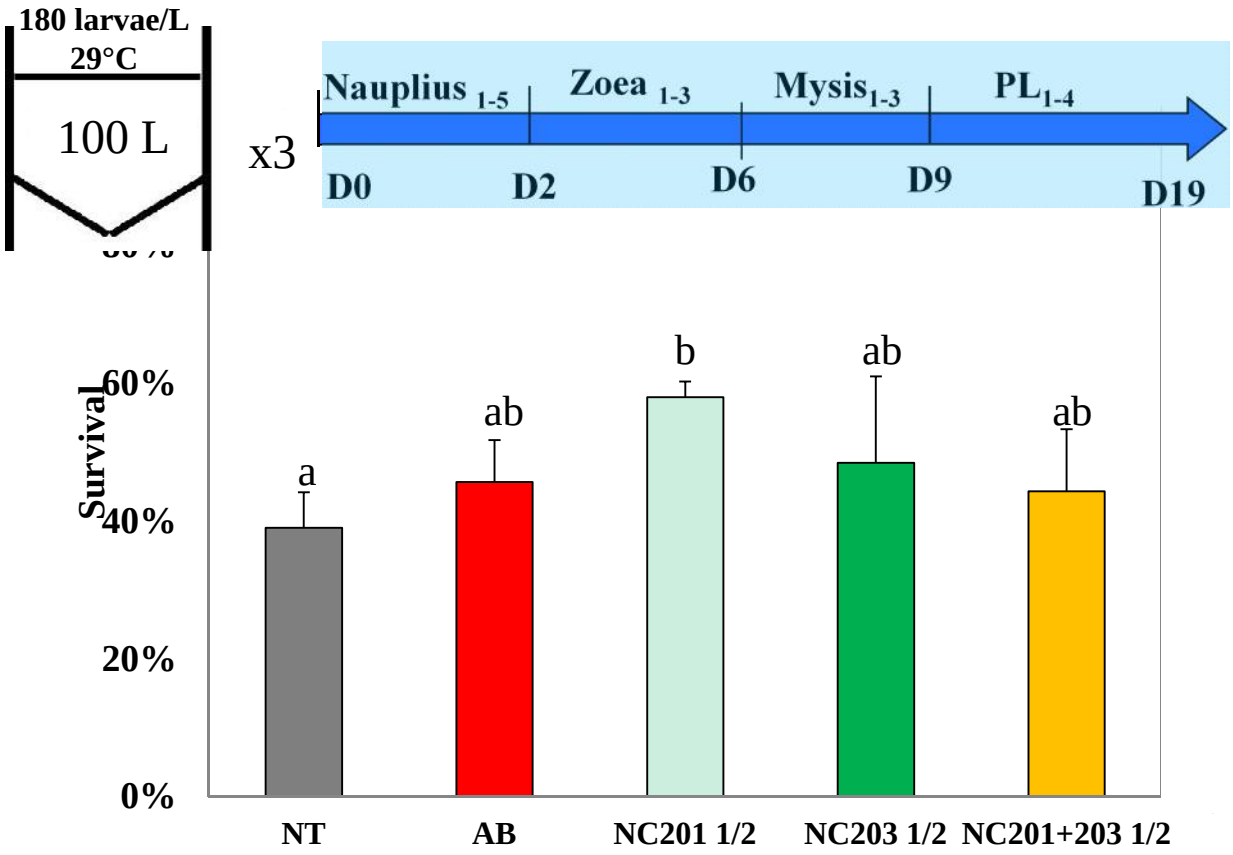
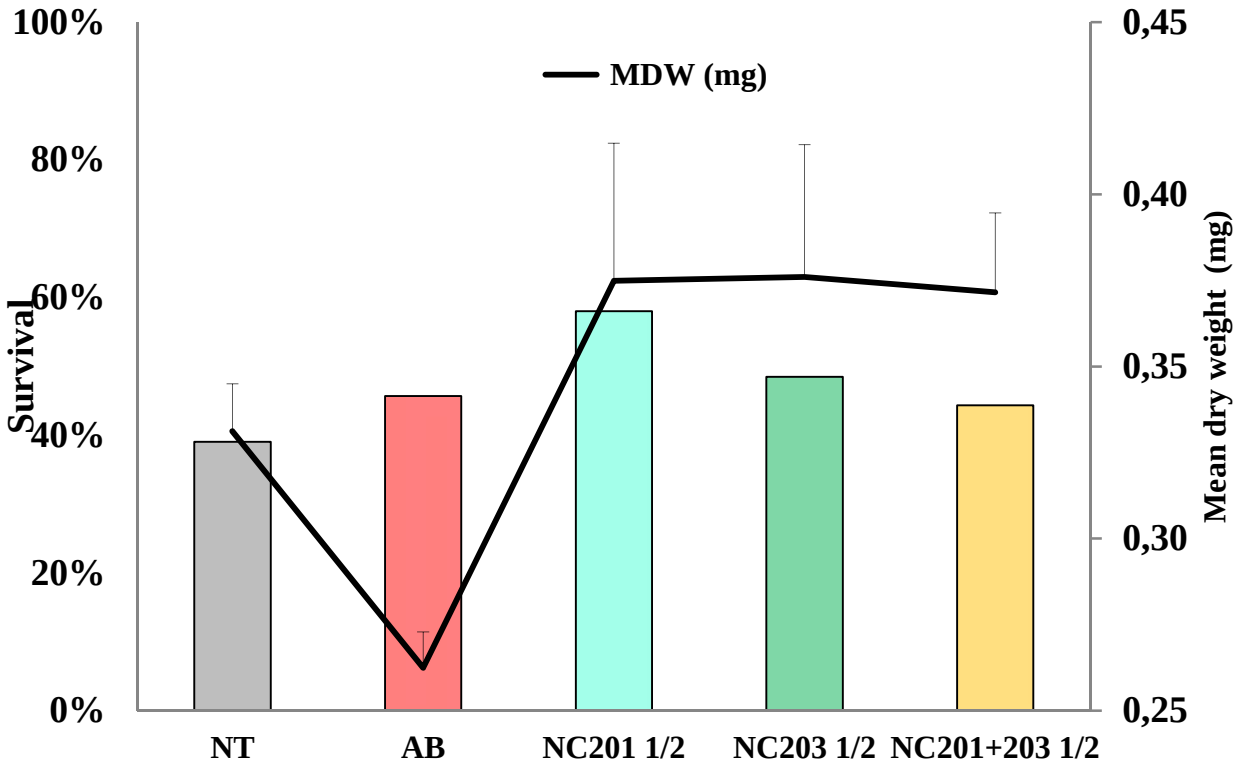


No survival improvement with probiotics combination

NC201 effectiveness in larval rearing : from *nauplius* to P9

Single administration *vs* combination of two probiotics



No improvement with probiotic combination compared to single administration

Probiotic impact on animal status

Three parameters were evaluated :

- Vibrio load in animal
- AMP gene expression
- Resistance to salinity stress test

Probiotic impact on animal status

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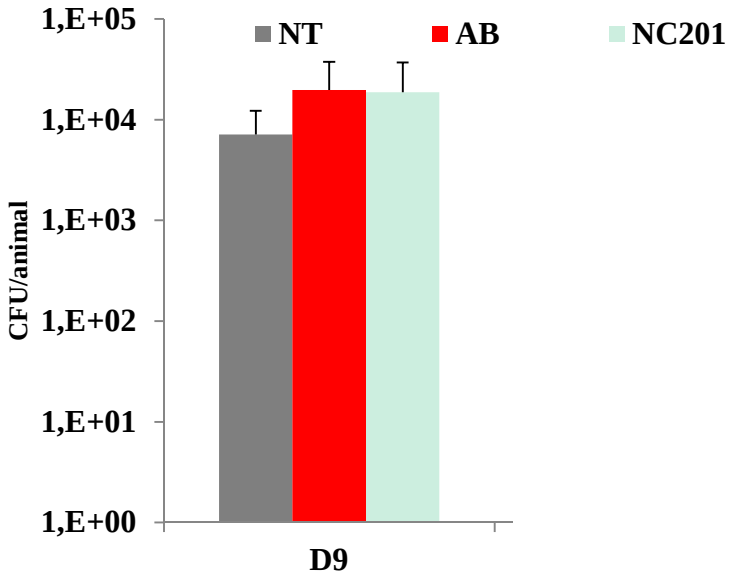
Estimation of *Vibrio* concentration at D9 and D19 in animals on TCBS media.



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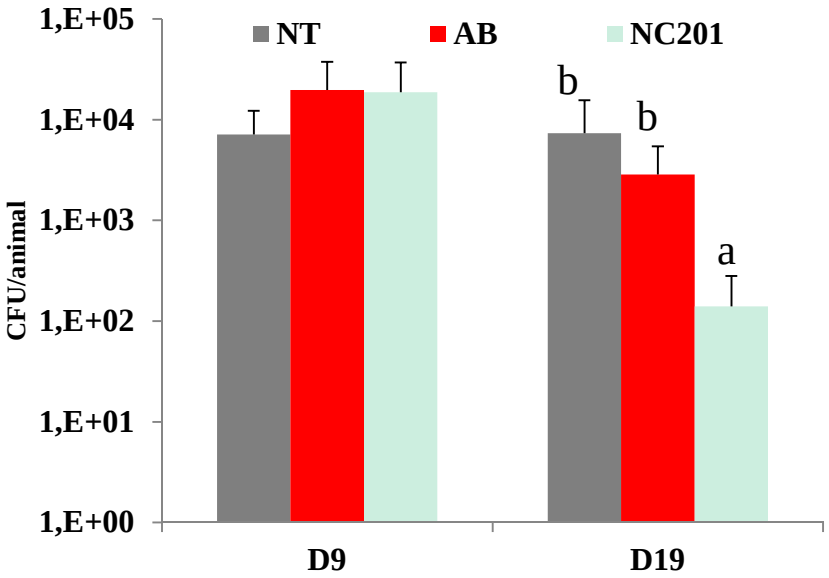


Could this load drop be due to higher antimicrobial peptide action ?

Lower *Vibrio* concentration in animal at D19 with probiotics

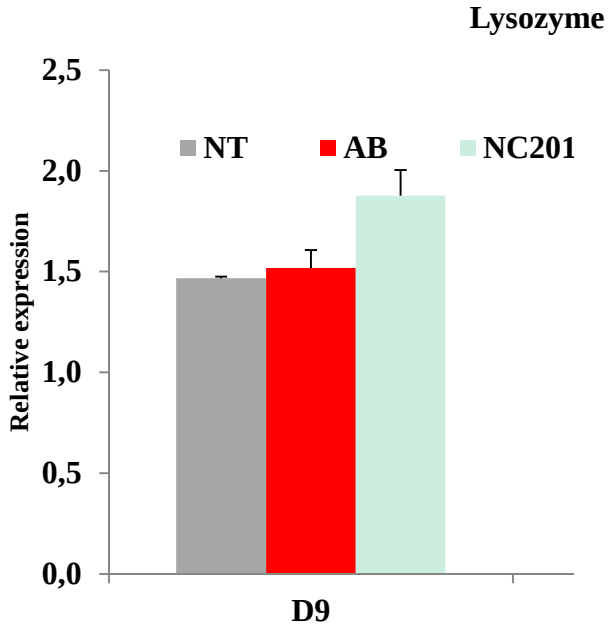
Probiotic impact on animal status

Three parameters were evaluated :

- Vibrio load in animal at D9 and D19
- Lyzozyme gene expression at D9 and D19
- Resistance to salinity stress test

Sampling of pooled 20 animals at D9 and pooled 10 animals at D19 for each triplicate

Gene expression profile analysed by quantitative RT-PCR, using EF1 as housekeeping gene.



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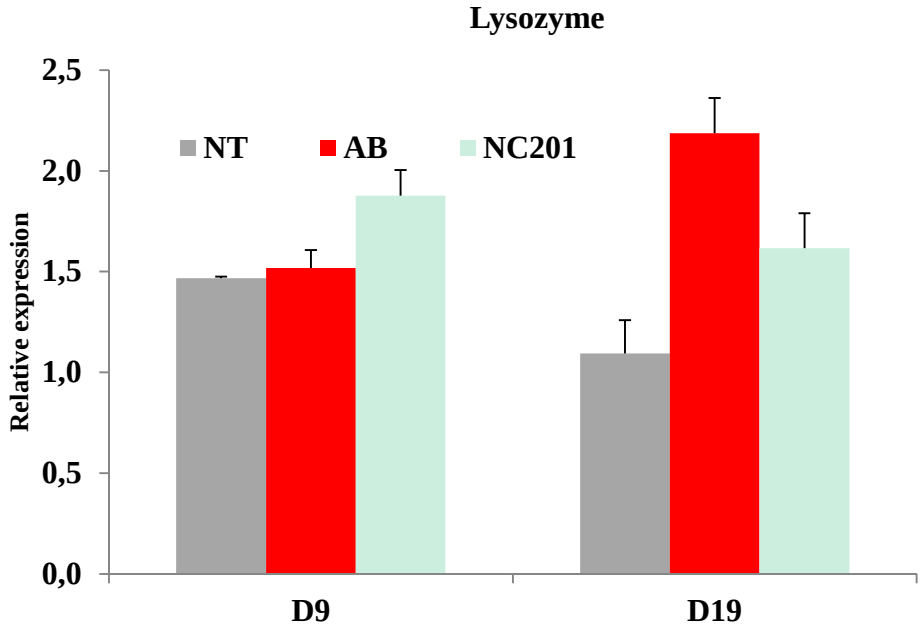

No detection of significant modulation of Lysozyme expression with probiotics

Probiotic impact on animal status

Three parameters were evaluated :

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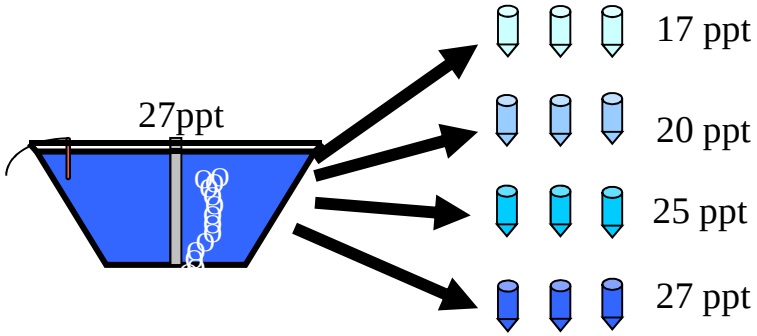
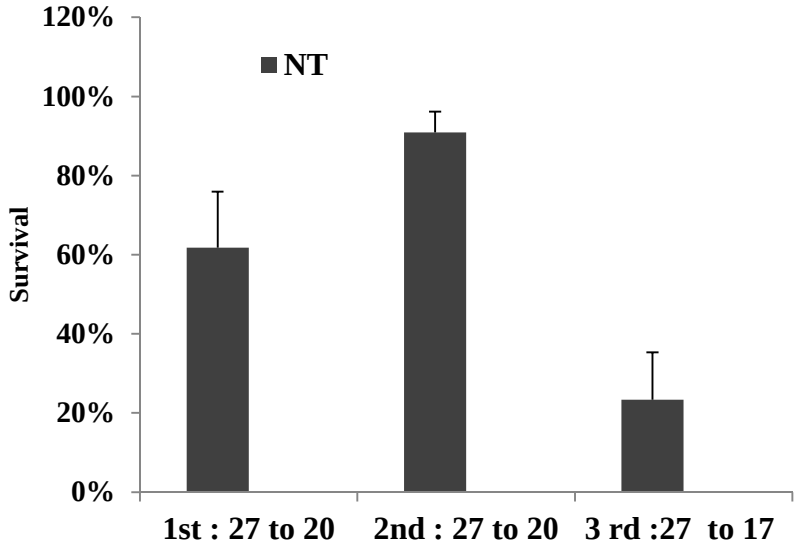
- Resistance to salinity stress test

Direct transfer from 27 ppt to 17-25 ppt in 5 days-old post-larvae from three different batches reared with or without probiotics-> survival after 24h





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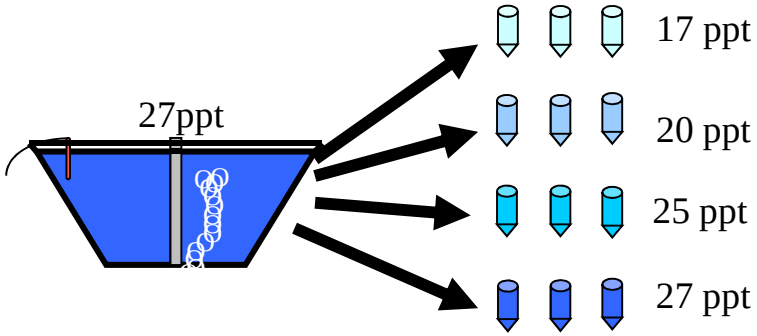

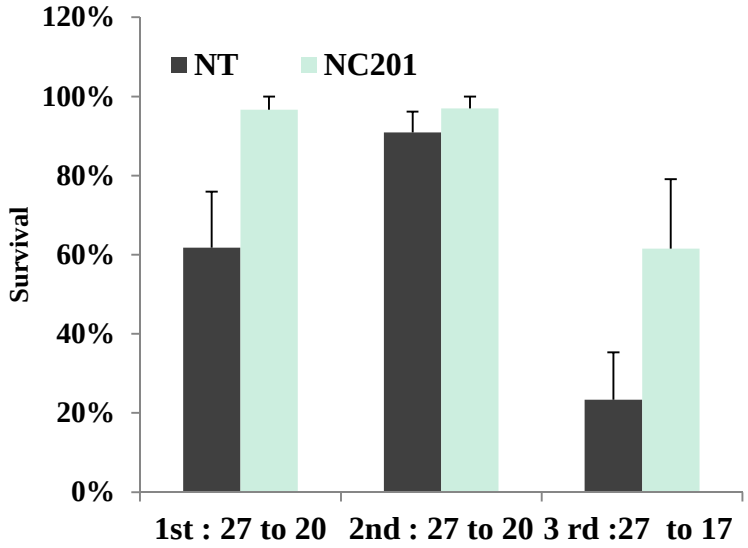

Resistance enhancement of postlarvae with probiotics

- Possible alternativ… …n hatcheries
- *Pseudoalterom…*
- NC 201 i… …s zootechnical perform…
- Prob…

- More stud… …rval rearing :
  -

- Better knowledge by experimenting :
  - bacterial challenge in larval and postlarval phases
  - probiotic effect on juveniles and adults

Thank you for your attention

- This research was financially supported by Ifremer, the Provincial Institutions and the Government of New Caledonia. Thanks to :
  - Hatchery staff in Saint-Vincent (F. Broutoi, S. Collet, S. Girard, J.R. Mailliez and J.M. Peignon) for rearing animals
  - Institut Pasteur in New Caledonia for collaboration in bioprospection
  - “Plate-forme de Recherche pour les Sciences du Vivant” de NC (PFV-NC) for allowing access to RT-PCR facility.