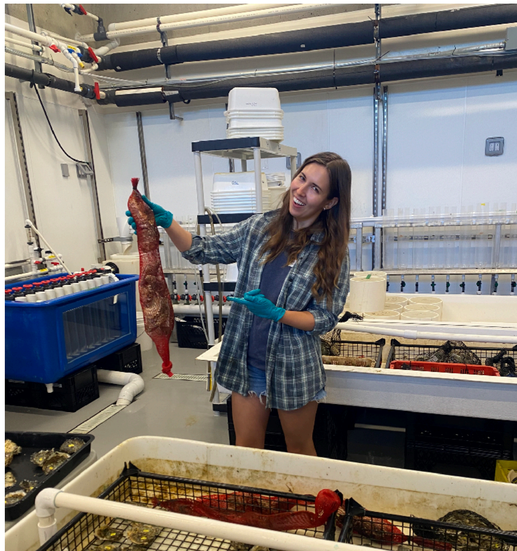
Using probiotics to improve oyster disease resilience

Marissa Wright-La Greca

Contact: marissawrightlagreca@uvic.ca

Supervisors: Dr. Amanda Bates & Dr. Timothy Green



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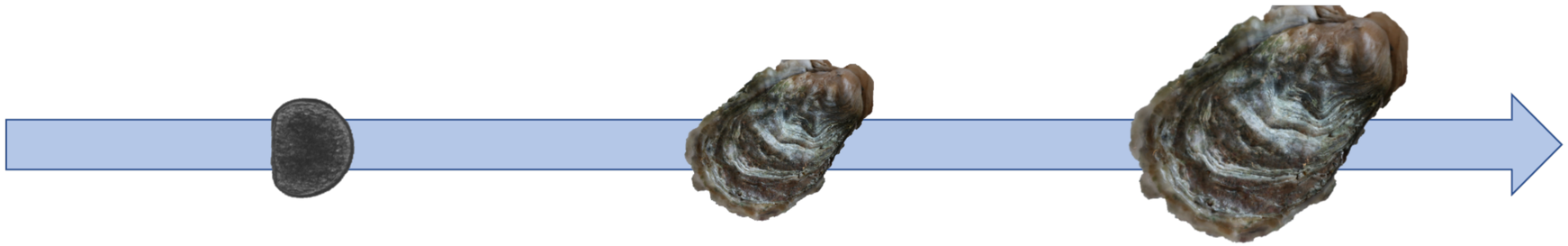
Vibrio pathogens & oyster aquaculture

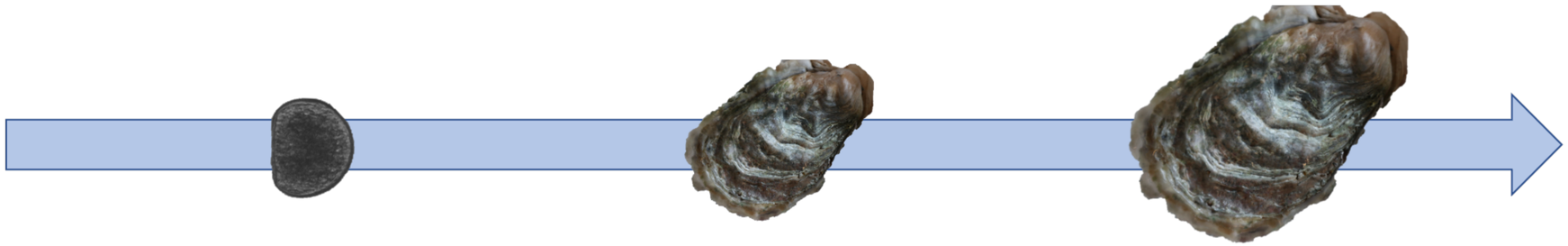
- **Summer mortality**
  - *Vibrio aestuarianus* in Baynes Sound - Adult and juvenile (spat) life-stages



Larval stage Spat stage Adult stage

Vibrio pathogens & oyster aquaculture

- Summer mortality
  - *Vibrio aestuarianus* in Baynes Sound - Adult and juvenile (spat) life-stages
  - **What can we do at the larval stage to improve long-term disease resilience?**



Larval stage Spat stage Adult stage

Probiotics early in life

- **Major parts of the immune system are shaped early in life by the external environment**
  - Immune system defends against pathogens and determines what bacteria can colonize the microbiome (extra protection)

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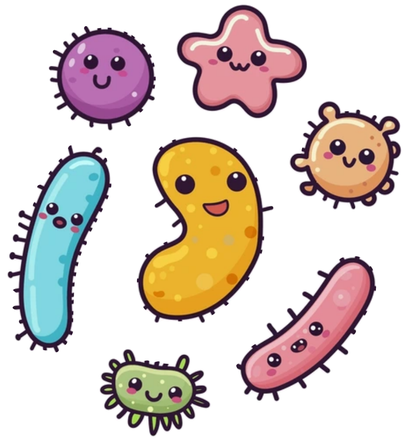
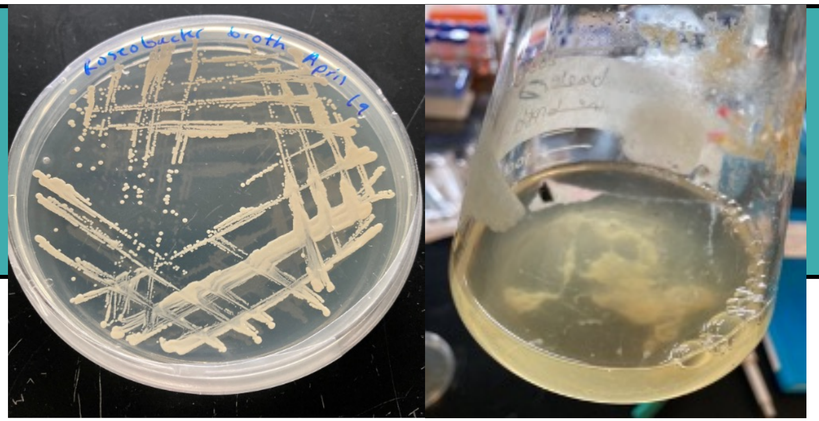
  - Addition of beneficial bacteria (probiotics) early in life may train the immune system to:
    - Better defend against pathogens
    - Sequester beneficial bacteria



Experiment #1



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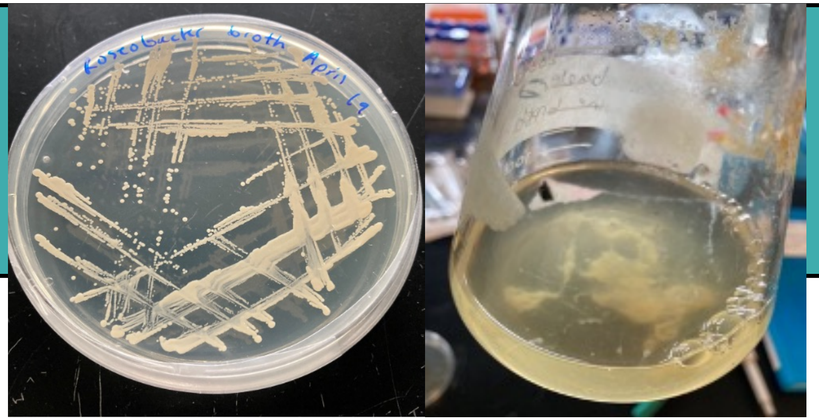
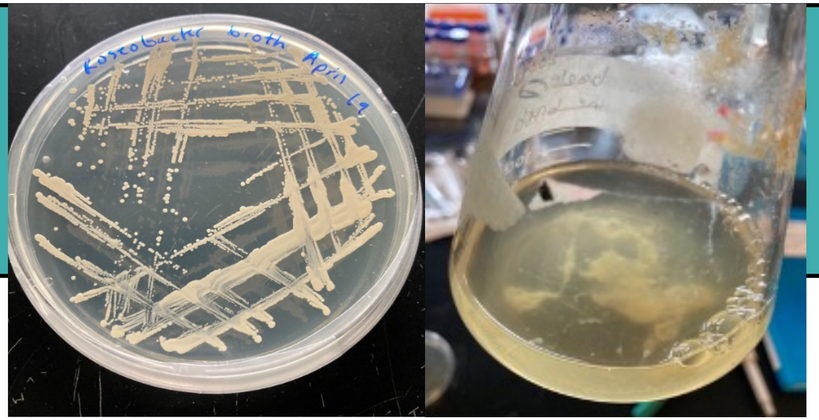


Deep Bay Marine Field Station

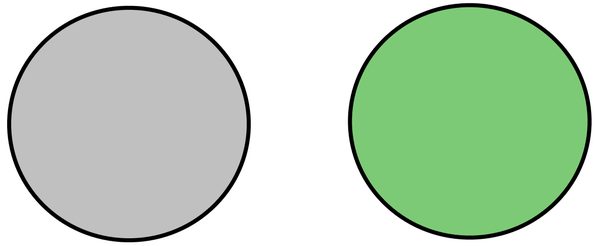
Experiment #1



- Added a probiotic (*Roseobacter* bacteria) during the first 24 hours of life.
  - Probiotic concentration: 1.8 x 10^2 cells/ml
  - When probiotics were added, oysters were in 20L buckets (not tanks) filled with seawater for the 1st 24 hours.
    - After 24hrs, larvae were moved to 250L tanks.

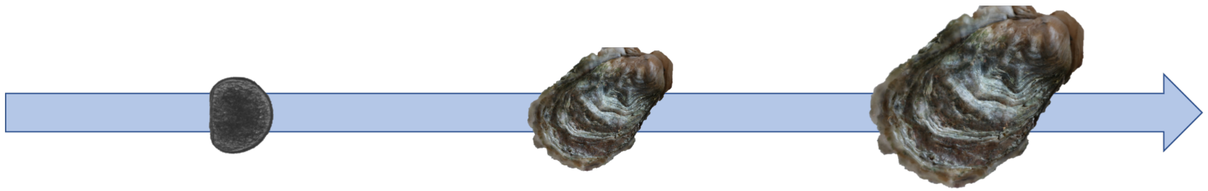
<u>Tank treatments:</u>

Control **Probiotics**

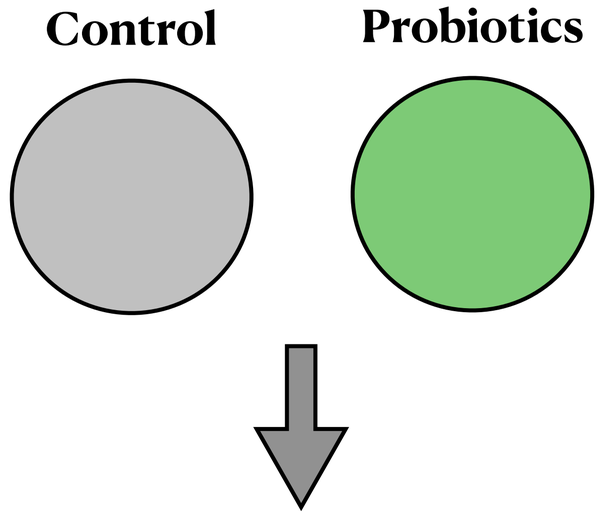


Experiment #1

- Added a probiotic (*Roseobacter* bacteria) during the first 24 hours of life.
  - Tested survival against *Vibrio aestuarianus* at the larval stage & spat stage



Larval stage **Spat stage** Adult stage



Larval *Vibrio* laboratory challenge

Spat *Vibrio* laboratory challenge

Experiment #1

- Added a probiotic (*Roseobacter* bacteria) during the first 24 hours of life.
- Qs. Does adding probiotics impact:
  - 1. Larval survival in the hatchery?

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  - 3. How does it change survival?
    - Microbiome changes?

Experiment #1: Results

- Qs. Does adding probiotics impact:
  - **1. Larval survival in the hatchery? ⟶ Did not negatively impact survival**
  - 2. Survival against *Vibrio aestuarianus* pathogen?
  - 3. How does it change survival?
    - Microbiome changes?

Experiment #1: Results

- Qs. Does adding probiotics impact:
  - 1. Larval survival in the hatchery?
  - **2. Survival against *Vibrio aestuarianus* pathogen?**
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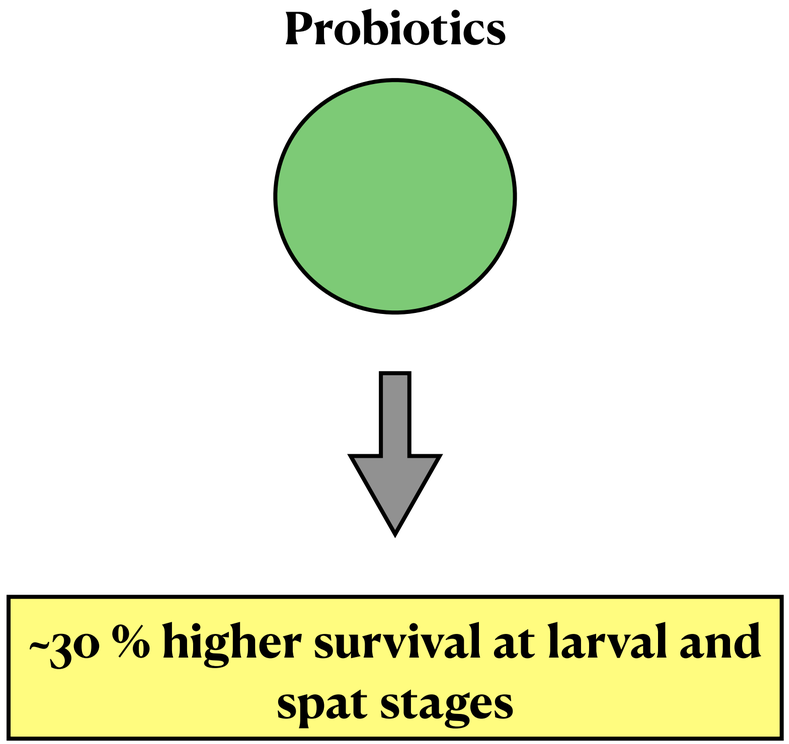

*note: variability in survival between oyster “families” (different genetic lines) in the larval disease challenge; however, when family is statistically controlled for, overall positive effects of probiotics

Experiment #1: Results

- Qs. Does adding probiotics impact:
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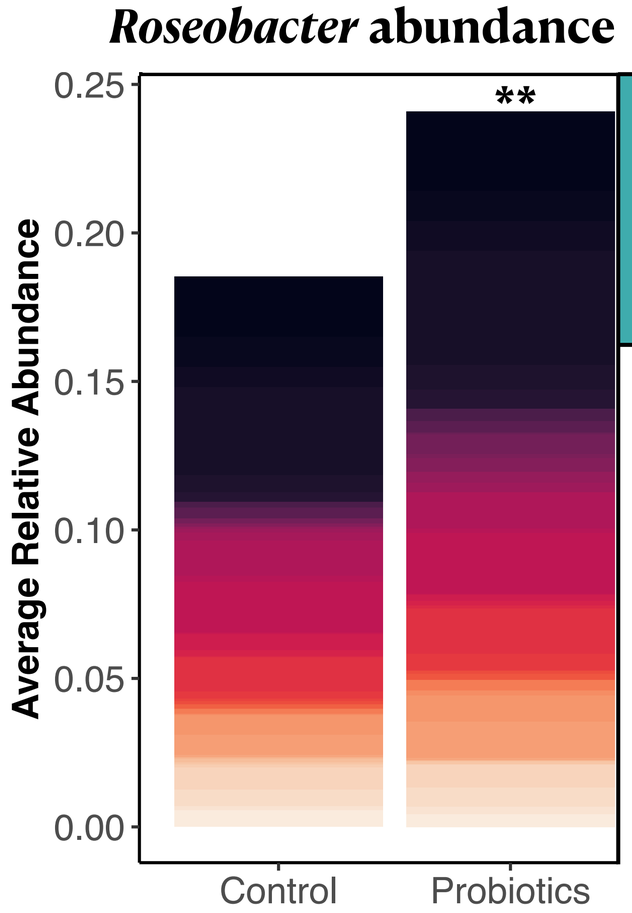
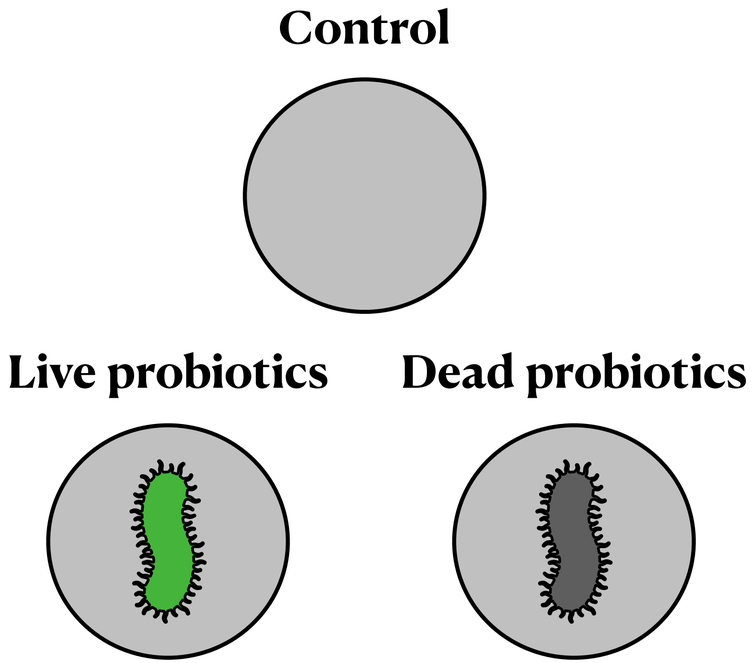
  - 2. Survival against *Vibrio aestuarianus* pathogen?
  - **3. How does it change survival?**
    - **Microbiome changes? ⟶ Increased abundance of probiotic bacteria (*Roseobacter* bacteria)**



Experiment #2

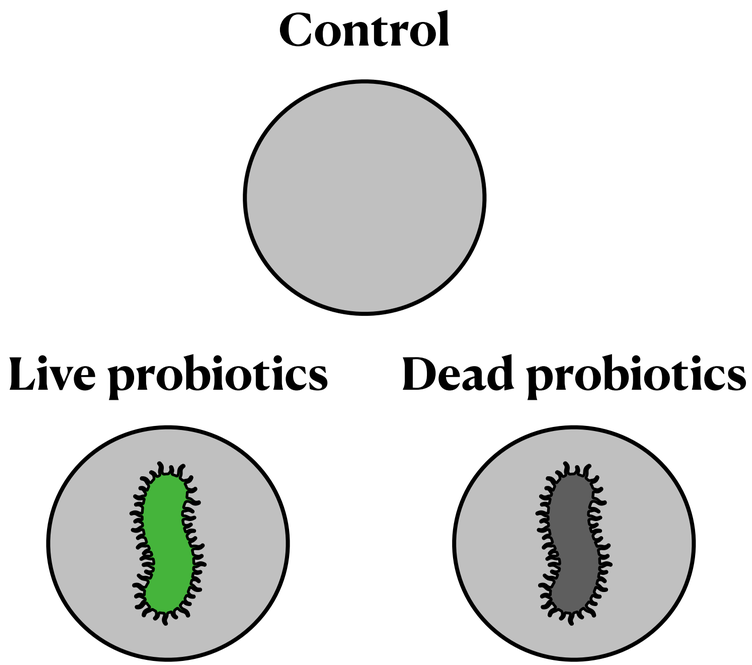
- Added a different probiotic bacterium (*Staphylococcus xylosus*) **live or dead** during the first 24 hours of life.
  - **Probiotic concentration:** 1.5 x 10^5 cells/ml in 200L of seawater (250L tanks)

*Note: More concentrated dose of probiotics compared to the 1st experiment. *Staphylococcus xylosus* is fast-growing so I was able to add more bacteria to the tanks compared to the 1st experiment



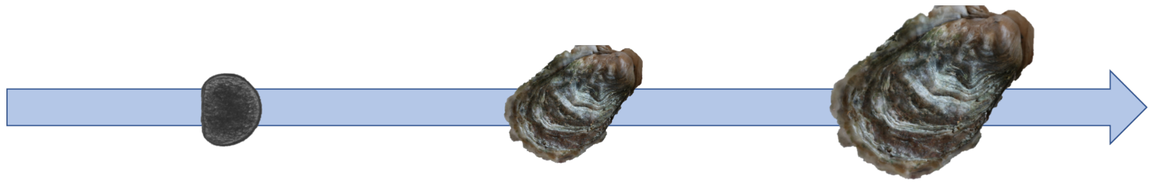
Experiment #2

- Added a different probiotic bacterium (*Staphylococcus xylosus*) **live or dead** during the first 24 hours of life.
- **Qs:**
  - 1. Type of probiotic matter?

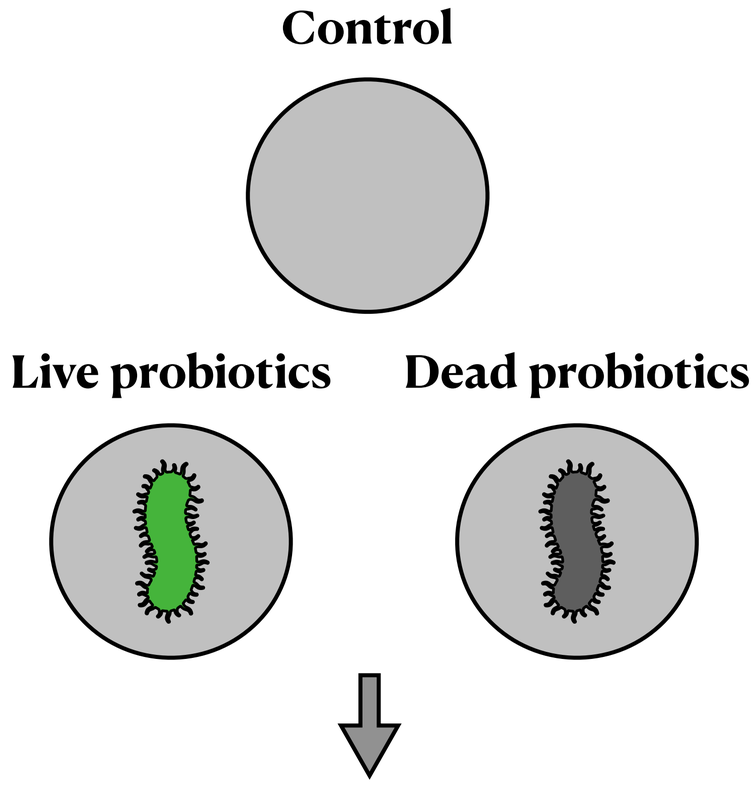


Experiment #2

- Added a different probiotic bacterium (*Staphylococcus xylosus*) live or dead during the first 24 hours of life.
- **Qs:**
  - 1. Type of probiotic matter?
  - **2. Improved survival last until the adult stage?**



Larval stage | **Spat stage** | **Adult stage**



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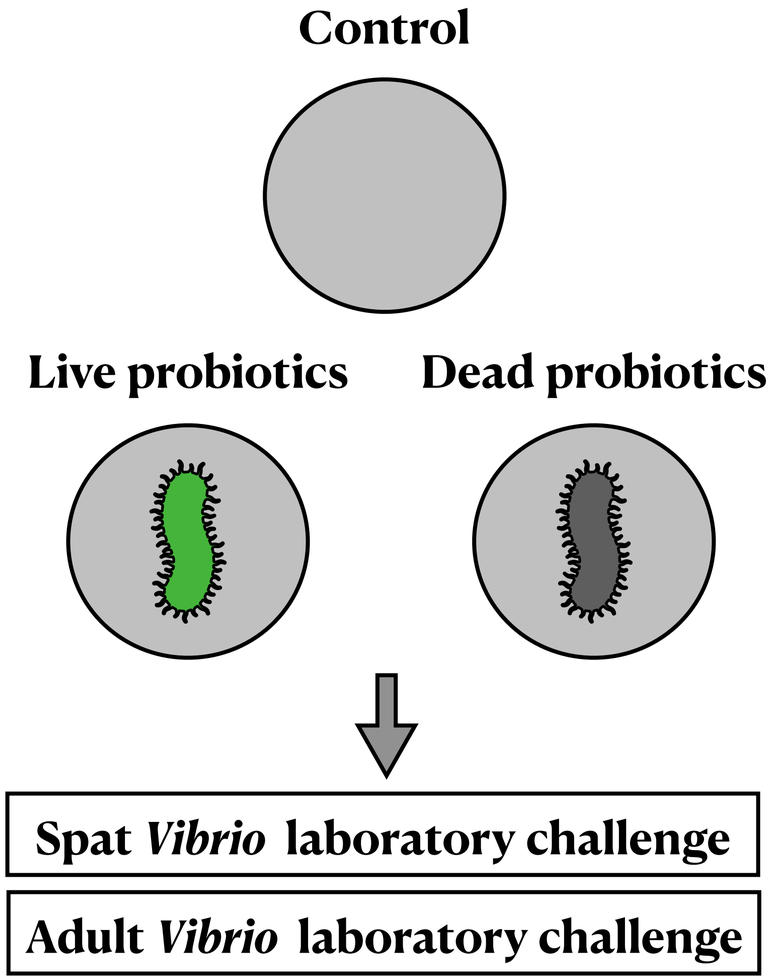
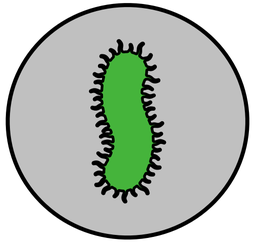
- Added a different probiotic bacterium (*Staphylococcus xylosus*) **live or dead** during the first 24 hours of life.
- **Qs:**
  - 1. Type of probiotic matter?
  - 2. Improved survival last until the adult stage?
  - **3. Same microbiome changes as experiment #1?**
    - Increase in Roseobacter bacteria?



Experiment #2: Results - *Vibrio aest.* challenges

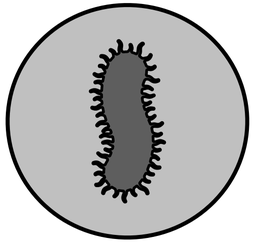
Spat stage (3 months later)

Live probiotics



↑ survival by 45%

Dead probiotics

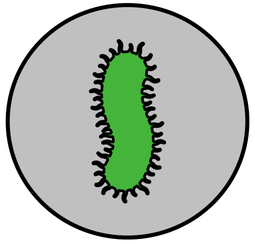


↑ survival by 30%

Experiment #2: Results - Microbiome changes

Spat stage (3 months later)

Live probiotics



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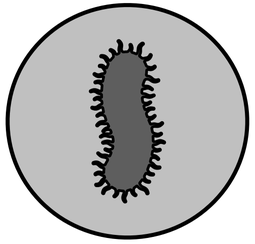
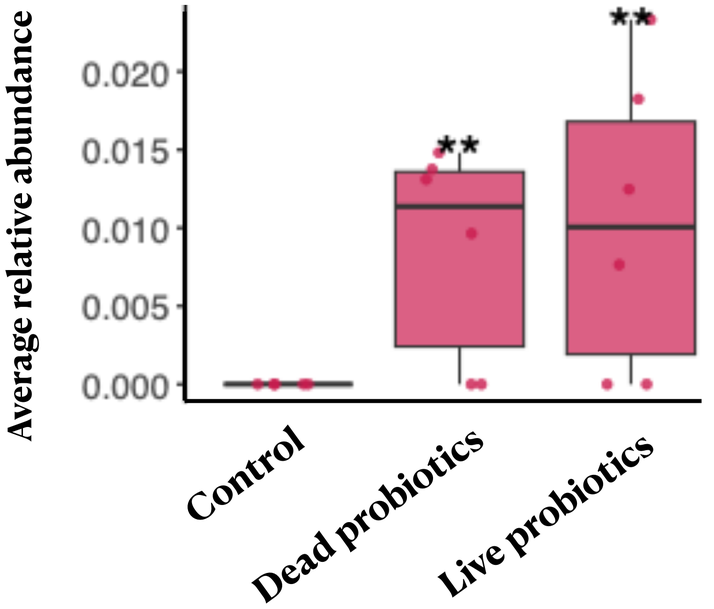
Dead probiotics



↑ survival by 30%

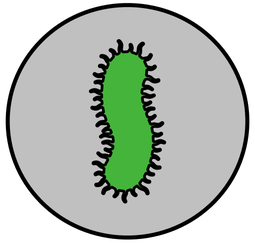
Increased abundance of Roseobacter bacteria



Experiment #2: Results

Spat stage (3 months later)

Live probiotics



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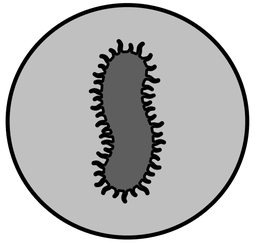
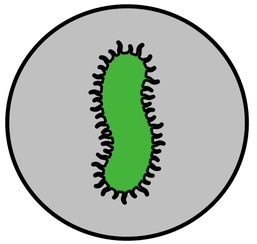


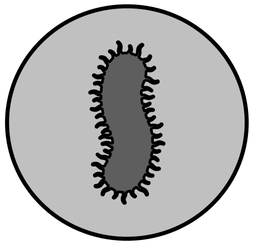
↑ survival by 30%

Adult stage (2 years later)

Live probiotics



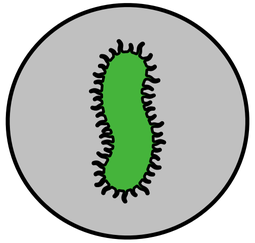
Dead probiotics



Experiment #2: Results - *Vibrio aest.* challenges

Spat stage (3 months later)

Live probiotics



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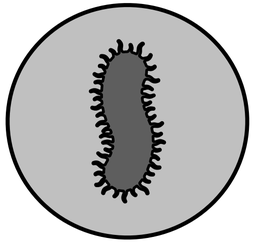

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Adult stage (2 years later)

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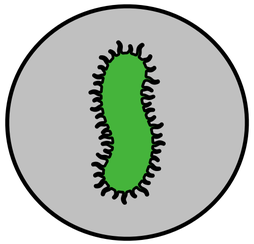
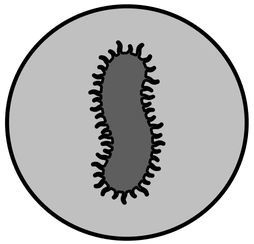


↑ survival by 90%*

Dead probiotics



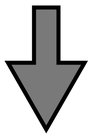
No increase in survival

*There was variability between different oyster genetic lines

Experiment #2: Results - *Vibrio aest*. challenges

Antibiotics

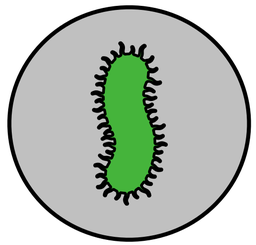
Wipe out microbiome



Vibrio aestuarianus challenge

Adult stage (2 years later)

Live probiotics



↑ survival by ~90%*

Experiment #2: Results - *Vibrio aest.* challenges

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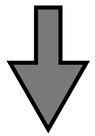
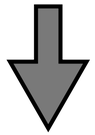


Vibrio aestuarianus challenge

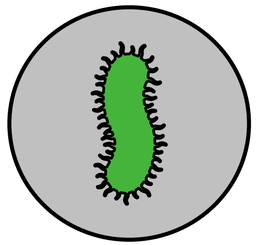


No increase in survival

Microbiome is mediating survival

Adult stage (2 years later)

Live probiotics



↑ survival by ~90%*

Comparing to other studies

- Most probiotic mixtures resulted in moderate to severe larval mortality.
- One of the probiotic mixtures improved survival against *Vibrio aestuarianus* 1 year later by ~25%.

Article | Open access | Published: 06 November 2024

Microbial education plays a crucial role in harnessing the beneficial properties of microbiota for infectious disease protection in *Crassostrea gigas*

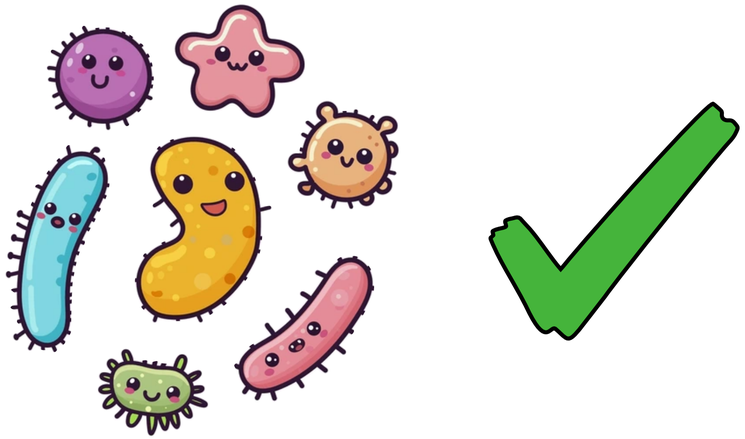
Luc Dantan ✉, Prunelle Carcassonne, Lionel Degrémont, Benjamin Morga, Marie-Agnès Travers, Bruno Petton, Mickael Mege, Elise Maurouard, Jean-François Allienne, Gaëlle Courtay, Océane Romatif, Juliette Pouzadoux, Raphaël Lami, Laurent Intertaglia, Yannick Gueguen, Jeremie Vidal-Dupiol, Eve Toulza & Céline Cosseau ✉

Scientific Reports **14**, Article number: 26914 (2024) | Cite this article

Summary

- **Exposure to probiotics in the first 24hrs can improve survival against *Va* by ~30-90% up to 2 years later**

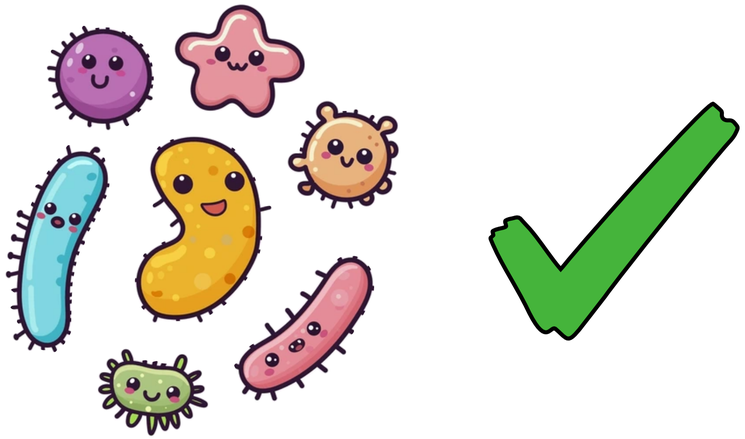
Improved survival mediated by the microbiome



Summary

- Exposure to probiotics in the first 24hrs can improve survival against *Va* by ~30-90% up to 2 years later
  - **No evidence of negative effects on larval development**
  - Sterile hatchery conditions may decrease long-term survival

Improved survival mediated by the microbiome



Summary

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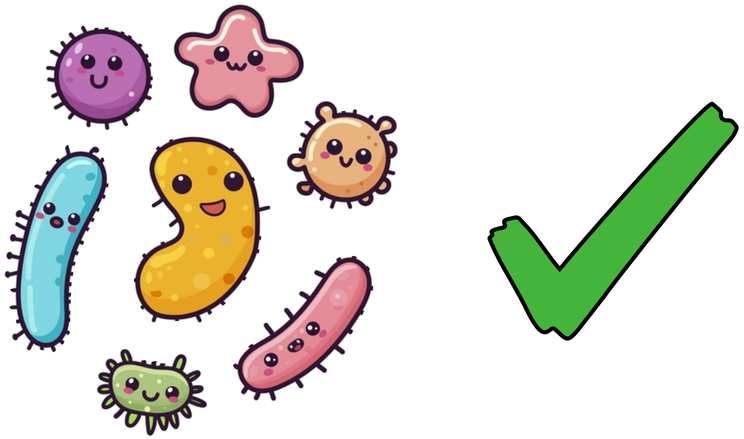
  - **Sterile hatchery conditions may decrease long-term survival**

Improved survival mediated by the microbiome



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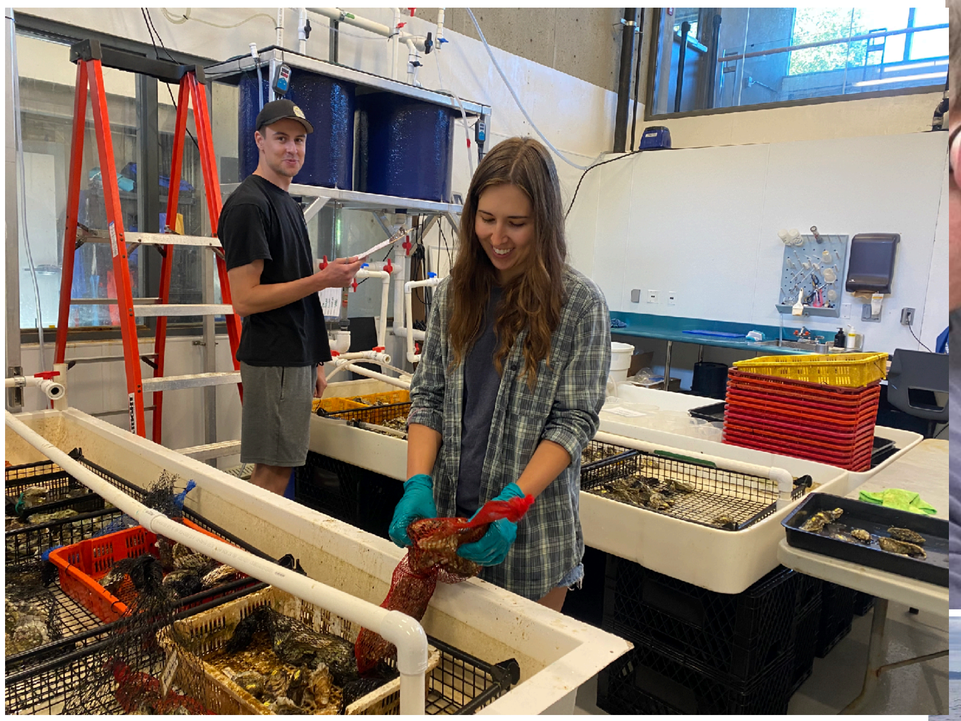
Deep Bay staff - Carl Butterworth, Sarah Leduc, Megan Lebeuf, Daniel Roth, Coreen Forbes, Connor Yamamoto, Emma Brisebois

Bates lab



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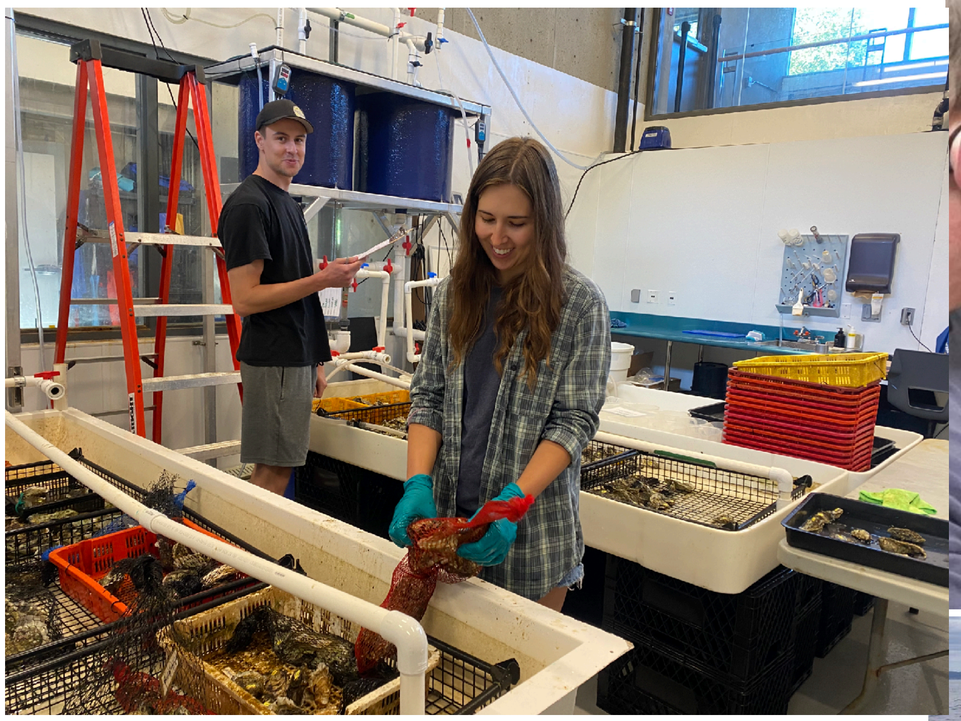
Questions?

Contact:
marissawrightlagreca@uvic.ca



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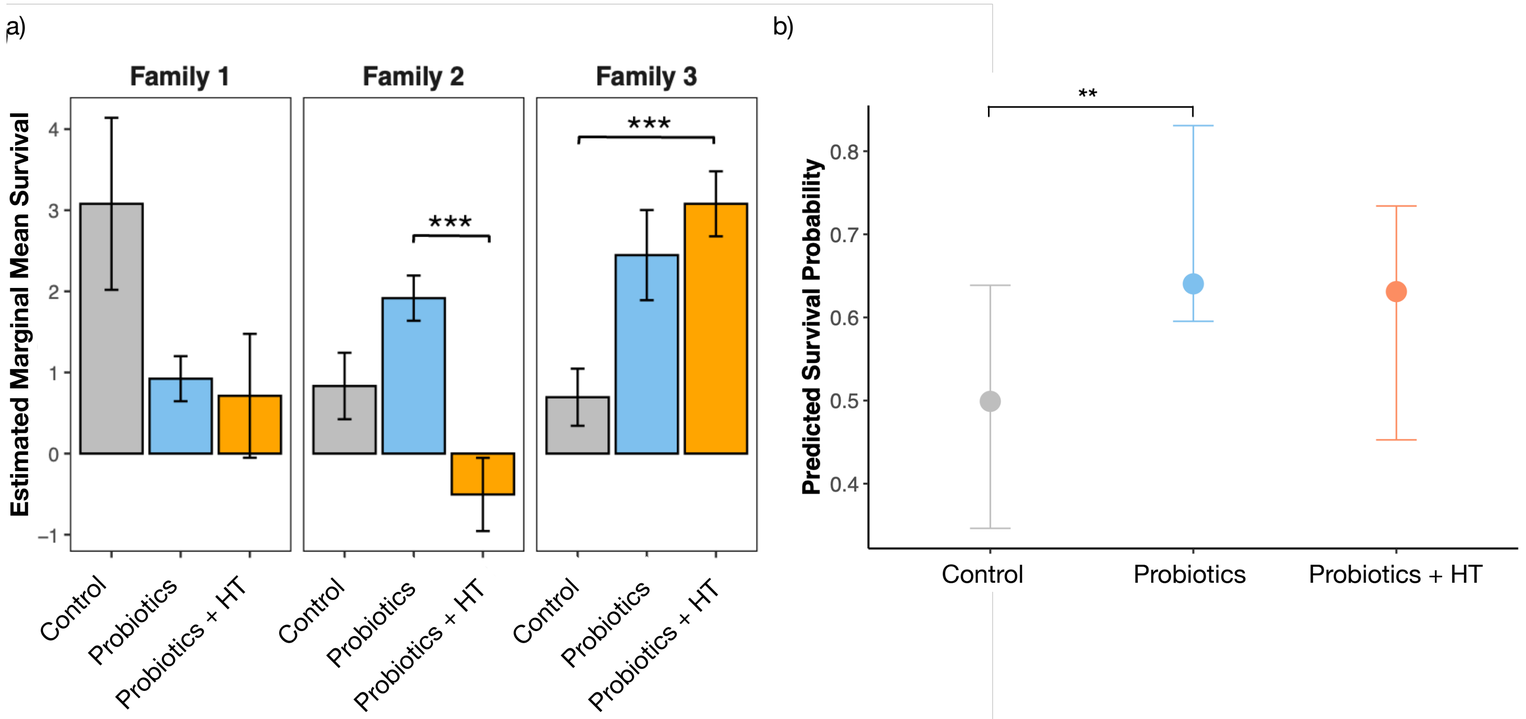
Detailed results

- The next slides show detailed results of disease challenges for those interested.
  - Please note that some of the following are preliminary results.
  - Additionally, not presented on but in my 1st experiment I had another treatment called “Probiotics + high temperature (HT)” that exposed larvae to probiotics and high temperature (26.5C) during the first 24 hours of life. **results: exposure to high temps early in life did not improve long-term disease resilience.
- The results of my 1st experiment will be available in a publication shortly.
- Unfortunately, field survival following early probiotic exposure was not able to be measured and is likely the next step for this research.

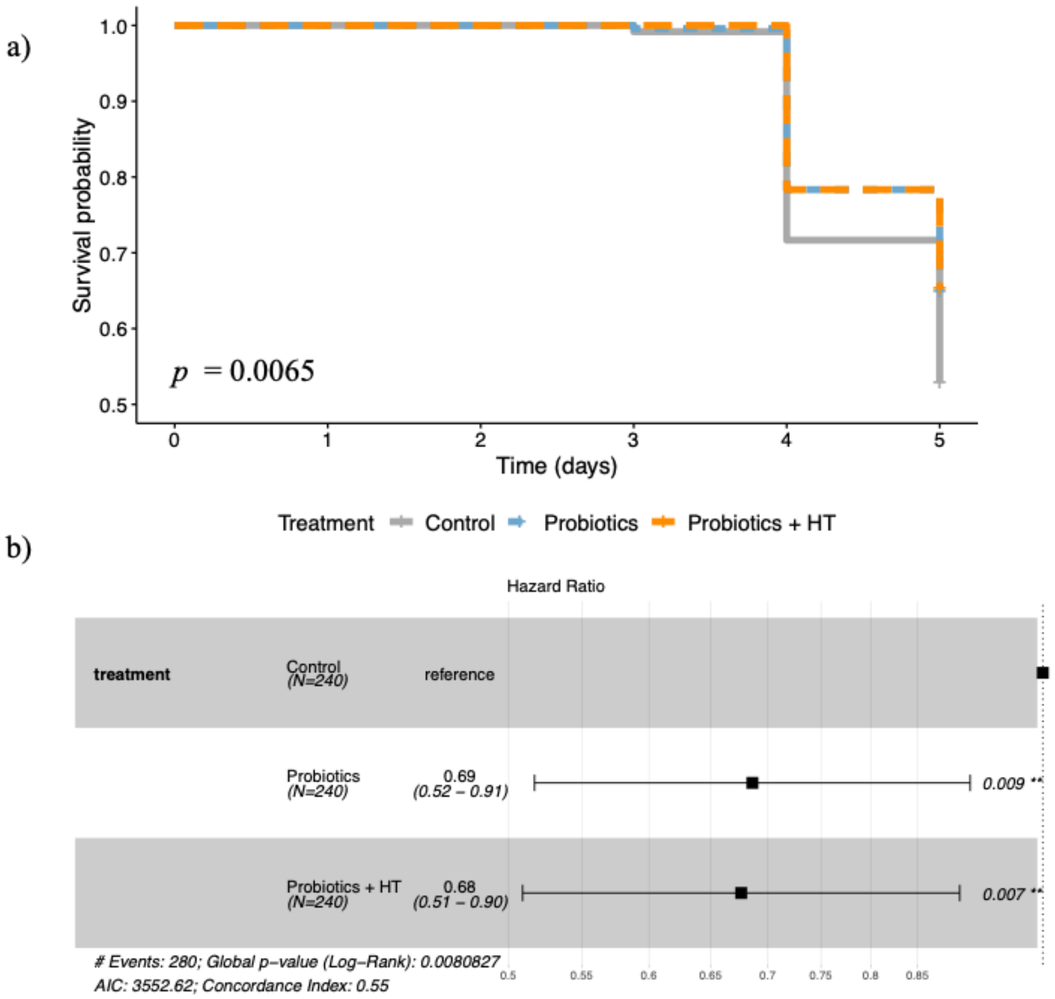
1st experiment - Larval (13 days) Va + heat challenge



*note: variability in survival between oyster “families” (different genetic lines); however, when family is statistically controlled for, overall positive effects of probiotics

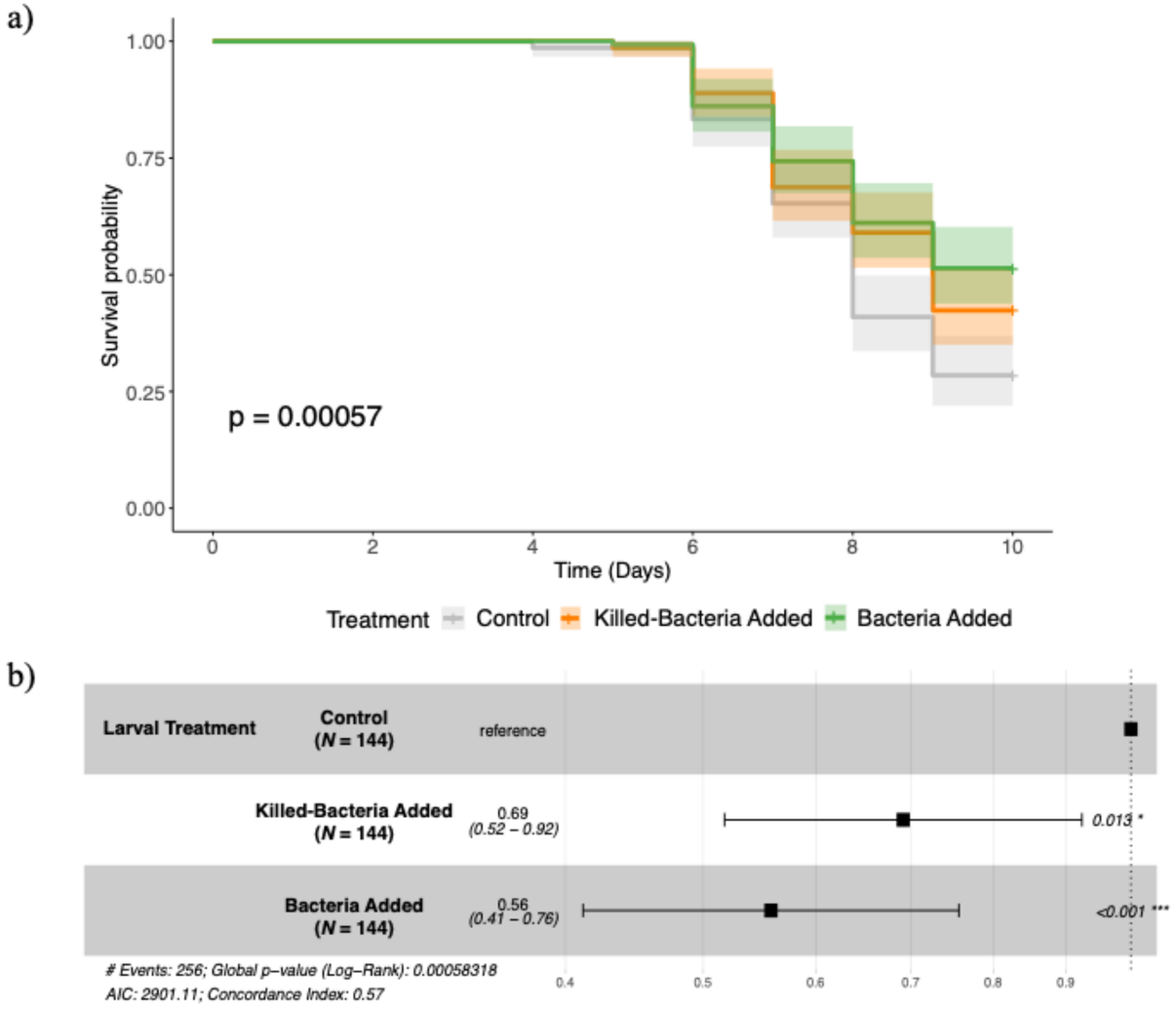
Left figure caption: a) Oyster larval survival between early-life treatments (HT = High temperature) within different oyster families. b) Predicted survival probability was generated using a binomial generalized mixed model with oyster family as a random factor

1st experiment - Spat (3 months) Va + heat challenge



- Left figure caption: a) Survival probability over time (days following exposure to *Vibrio aestuarianus* pathogen) of Pacific oysters (90 days post-fertilization) originating from Control (grey), Probiotics (blue), or Probiotics + HT (orange) treatments during the first 24 hours of life. b) Hazard ratio plot using survival data from the *Vibrio aestuarianus* + high temperature challenge. Oysters originating from the Control treatment are set as the reference (hazard ratio = 1). ** $p < 0.01$.

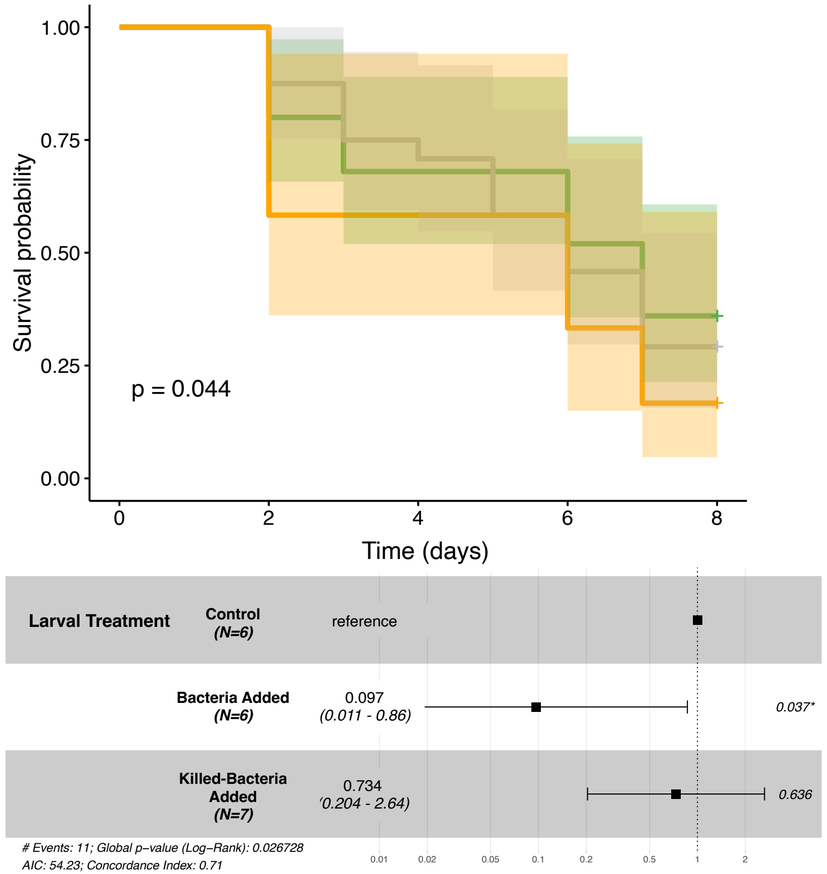
2nd experiment - Spat (3 months) Va + heat challenge



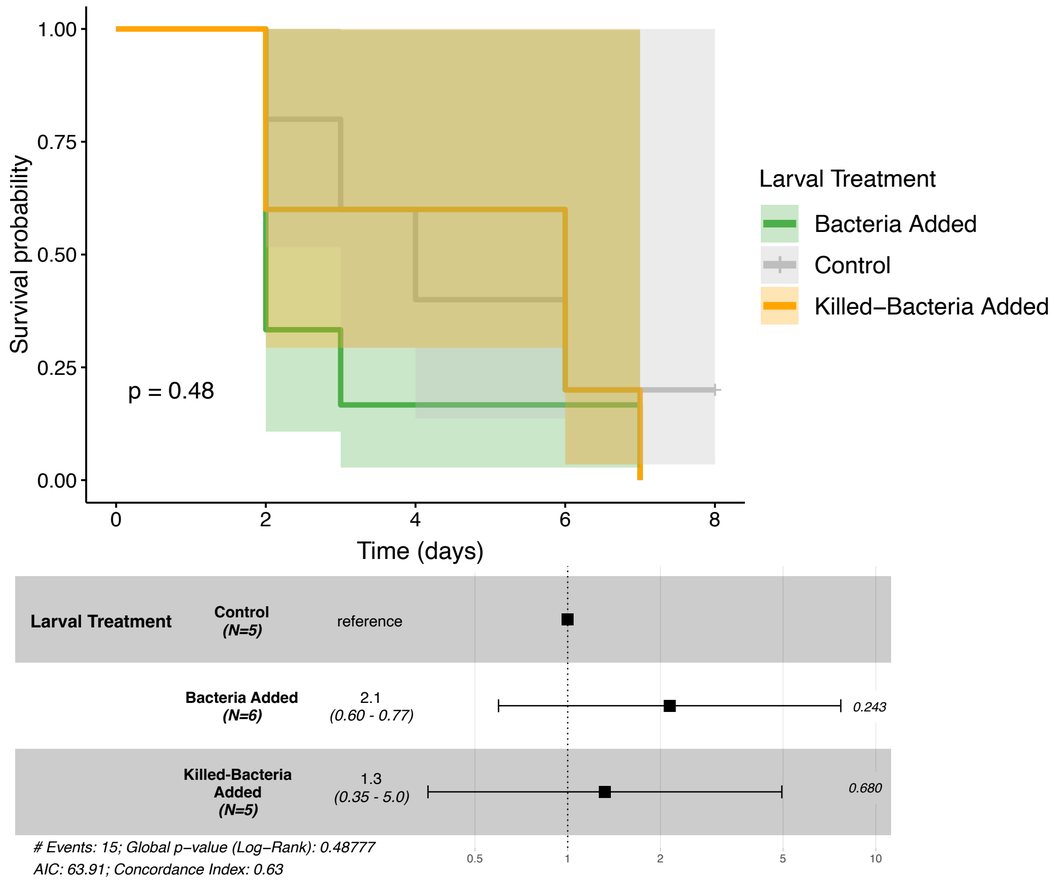
- Left figure caption: a) Survival probability curves of the Control (grey), Killed-bacteria Added (orange), and Bacteria Added (green) early-life treatments following exposure to *Vibrio aestuarianus* pathogen and heat stress (24 °C). b) Hazard ratios of the Killed-Bacteria Added and Bacteria Added treatments in reference to the Control treatment (hazard ratio set as 1). * $p < 0.05$, ** $p < 0.01$.

2nd experiment - Adult (2 years) Va + heat challenge

a)



b)



- Left figure caption: Adult *Vibrio aestuarianus* + heat (24 °C) challenge where oysters where exposed to a) seawater ("Seawater-Exposed") or b) antibiotics ("Antibiotic-Exposed") prior to the disease challenge. The top figures indicate survival probability curves between early-life treatments (Control = grey, Bacteria Added = green, Killed-Bacteria Added = orange) and the bottom figures indicate hazard ratios relative to the Control treatment (reference).