# Diet & Microbial Interactions In Palinurid Lobster Larvae

Presented by: Mike Hall





### **Marine Lobsters**



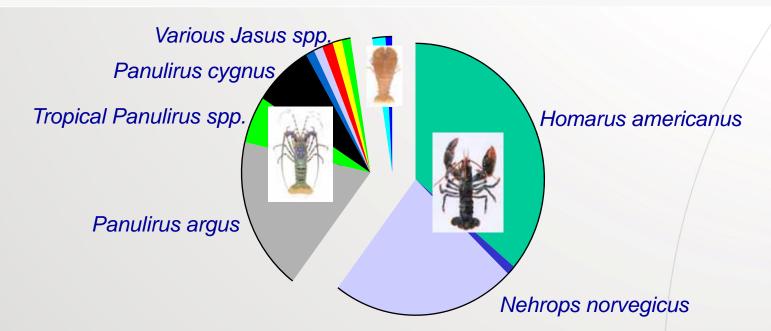
Nephropoidea



Palinuridea



Scyllaridae



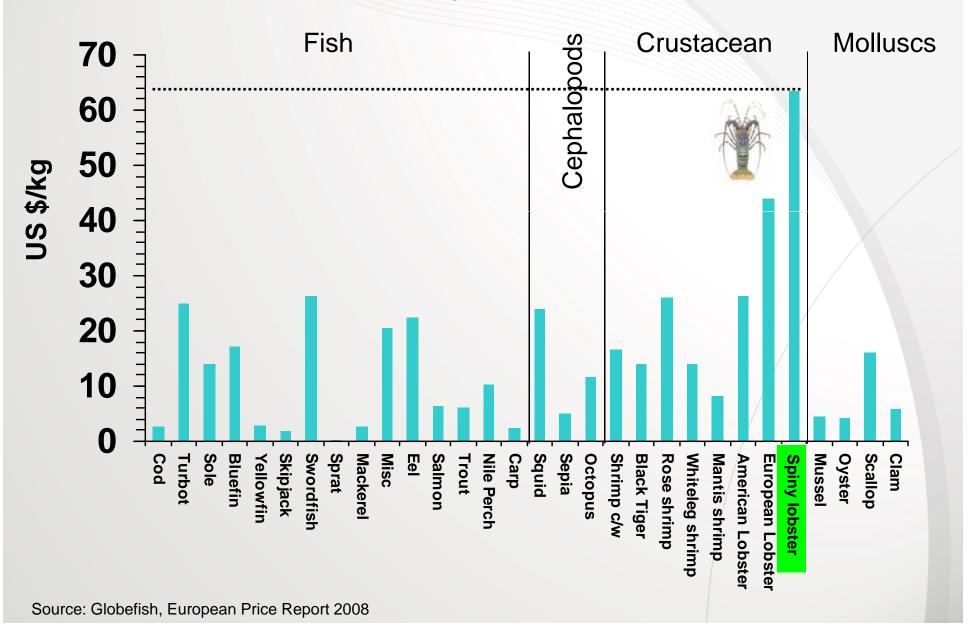
## **Lobster Biodiversity**

Species: Wild Fisheries and Aquaculture Potential

| Group                          | Species | Wild Fishery |            | Aquaculture<br>Potential |          |
|--------------------------------|---------|--------------|------------|--------------------------|----------|
|                                |         | Major        | Minor/     | Seafood                  | Aquarium |
| Family Palinuraidas            | 47      | 31           | None<br>16 |                          |          |
| Family <i>Palinuroidea</i>     | 47      | 31           | 10         | +++                      | ++       |
| Family Scyllaridae             |         |              |            |                          |          |
| Subfamily Arctidinae           | 16      | -            | Some       | ++                       | +        |
| Subfamily <i>Ibacinae</i>      | 13      | -            | Some       | +                        | +        |
| Subfamily Scyllarinae          | +40     | -            | -          | -                        | ++       |
| Subfamily <i>Theninae</i>      | 5       | -            | 5          | +++                      | +        |
| Family Synaxidae               | 3       | -            | -          | -                        | +++      |
| Family Nephropoidea            |         |              |            |                          |          |
| Subfamily <i>Neophoberinae</i> | 2       | -            | -          | - /                      | -        |
| Subfamily <i>Thymopinae</i>    | 20      | -            | -          | +/                       | -        |
| Subfamily Nephropinae          | 26      | 3            | Some       | +(++)                    | +        |
| Family Thaumastochelidae       | 3       | -            | -          | / <del>-</del>           | -        |
| Family Enoplometopoidae        | +11     | -            | -          | -                        | +++      |
| TOTAL                          | +176    | 34           | Some       | Several                  | Some     |

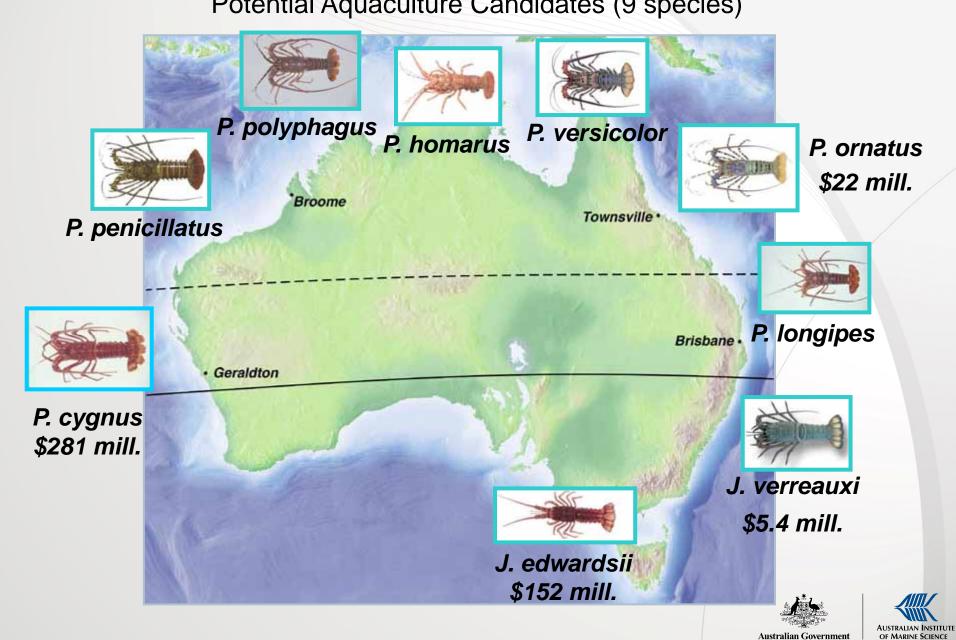
### Seafood Groups – as Production Units

Price per kg per whole animal

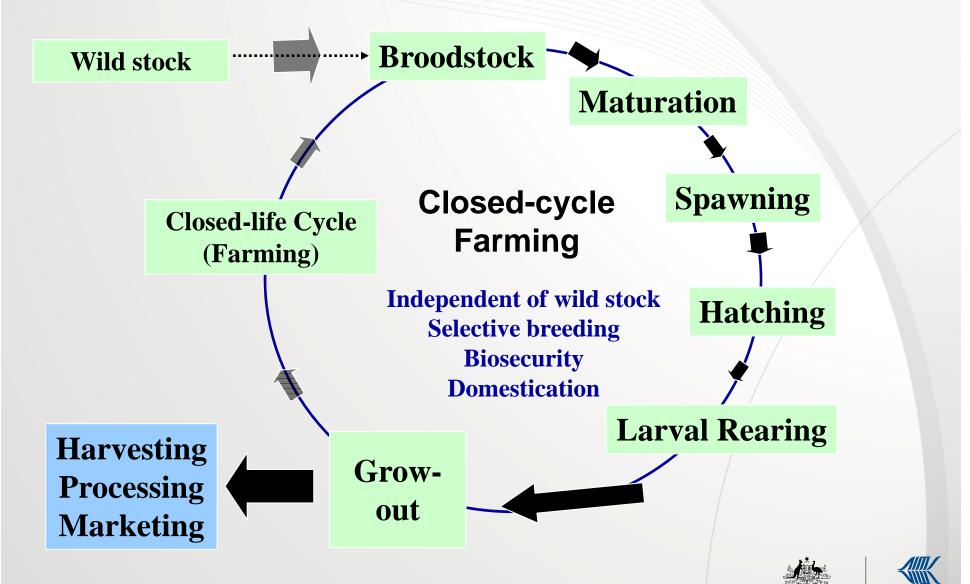


# Australian Rock Lobster Biodiversity

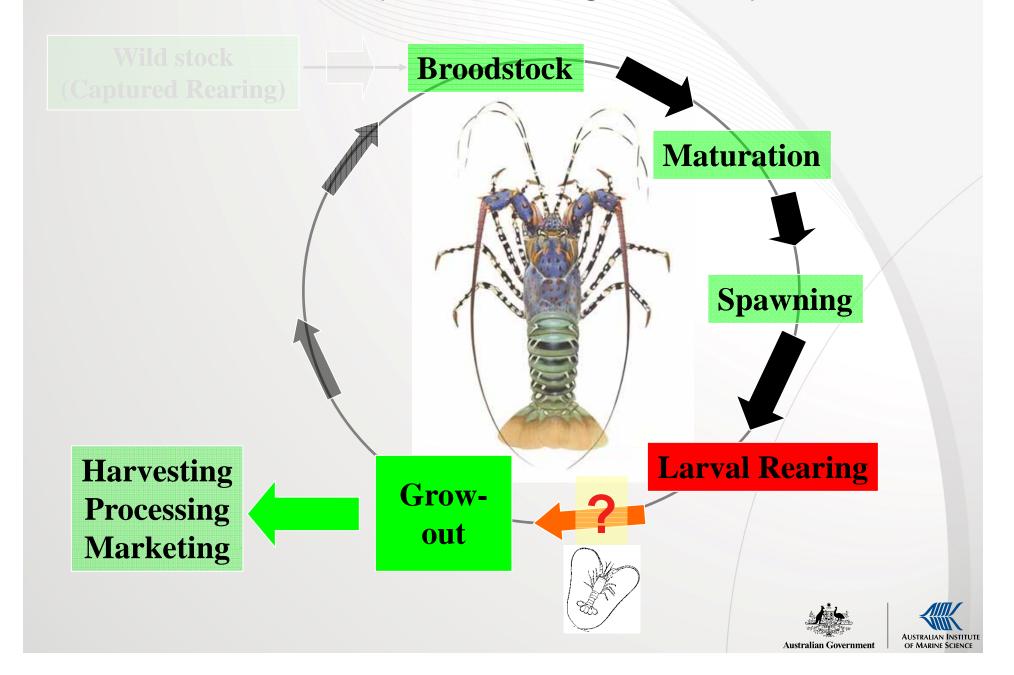
Potential Aquaculture Candidates (9 species)



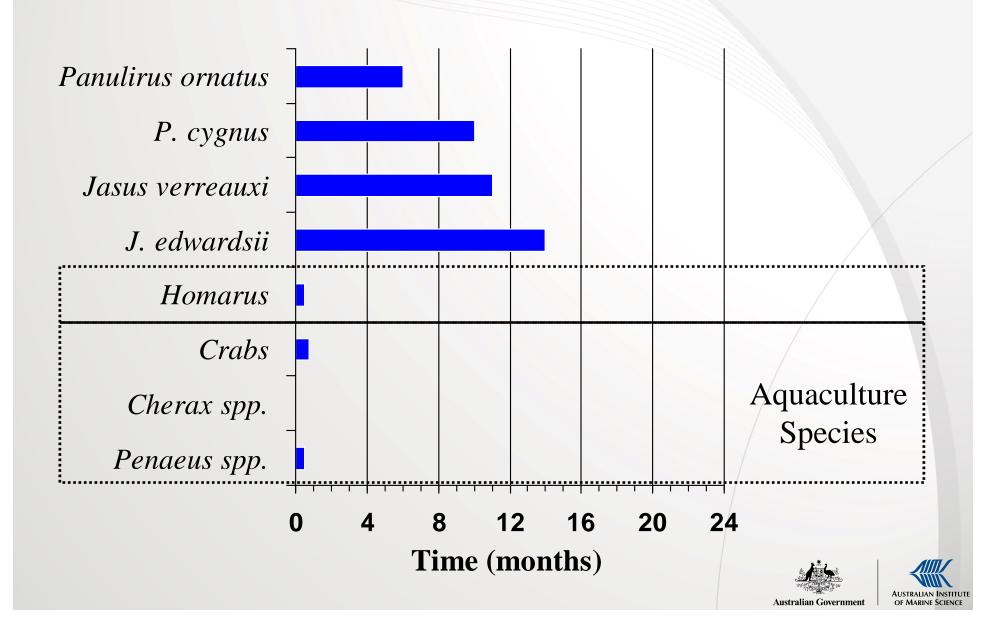
# Model of Aquaculture Propagation System Closed life cycle



# Closed-life cycle Propagation System



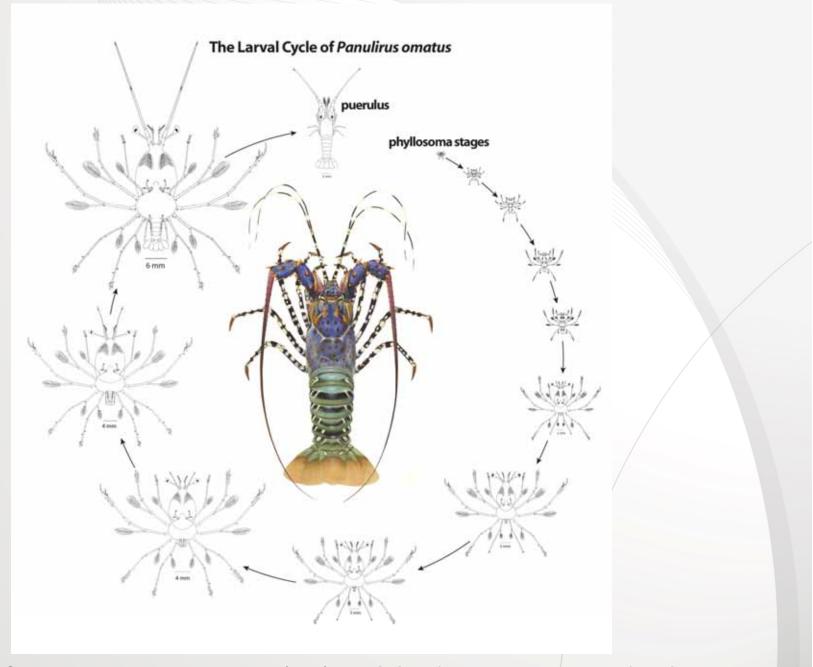
### Larval Periods of Selected Crustacea



# Palinuridae Phyllosoma Larval Rearing

Larval Cycle Completed in > 9 species

| Species               | Reference   |  |  |  |  |
|-----------------------|---|--|--|--|--|
| Panulirus species     |   |  |  |  |  |
| Panulirus argus       | Moe (1991), Goldstein et al. (2008)   |  |  |  |  |
| Panulirus elephas     | Mercer et al. (1997), Kittaka et al. (1988), Kittaka et al. (2000)                                    |  |  |  |  |
| Panulirus homarus     | Radhakrishnan and Vijayakumaran (1993)  |  |  |  |  |
| Panulirus japonicus   | Oshima (1936), Kittaka and Kimura (1989), Yamakawa et al. (1989), Sekine et al. (2000)                |  |  |  |  |
| Panulirus interruptus | Johnson (1956), Dexter (1972)   |  |  |  |  |
| Panulirus lalandii    | Kittaka (1988)  |  |  |  |  |
| Panulirus longipes    | Matsuda and Yamakawa (2000)   |  |  |  |  |
| Panulirus ornatus     | MGKailis (2006), AIMS (2007)  |  |  |  |  |
| Panulirus pencillatus | Matsuda et al. (2006)   |  |  |  |  |
| Panulirus polyphagus  | Saisho (1966), Sin (1967)   |  |  |  |  |
|                       | Jasus species   |  |  |  |  |
| Jasus edwardsii       | Kittaka et al. (1988), Booth (1996), Illingworth et al. (1997), Tong (1997), Moss (2000), TAFI (2005) |  |  |  |  |
| Jasus verreauxi       | Kiattaka et al. (1997), TAFI (2006)   |  |  |  |  |
|                       | Scyllarid species   |  |  |  |  |
| Thenus orientalis     | Mikami and Greenwood (1997)   |  |  |  |  |



Smith, G., Salmon, M., Kenway, M. and Hall, M. (2009) Description of the larval morphology of captive reared *Panulirus ornatus* spiny lobsters, benchmarked against wild caught specimens. Aquaculture 295:76-88.

# Change in Larval Size through Development

11 Stages

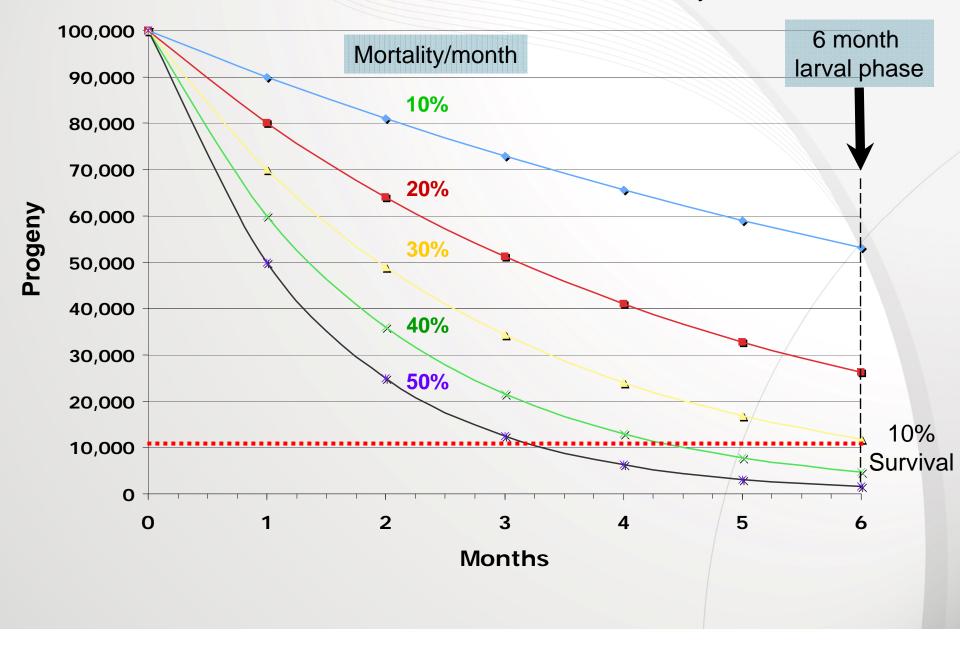






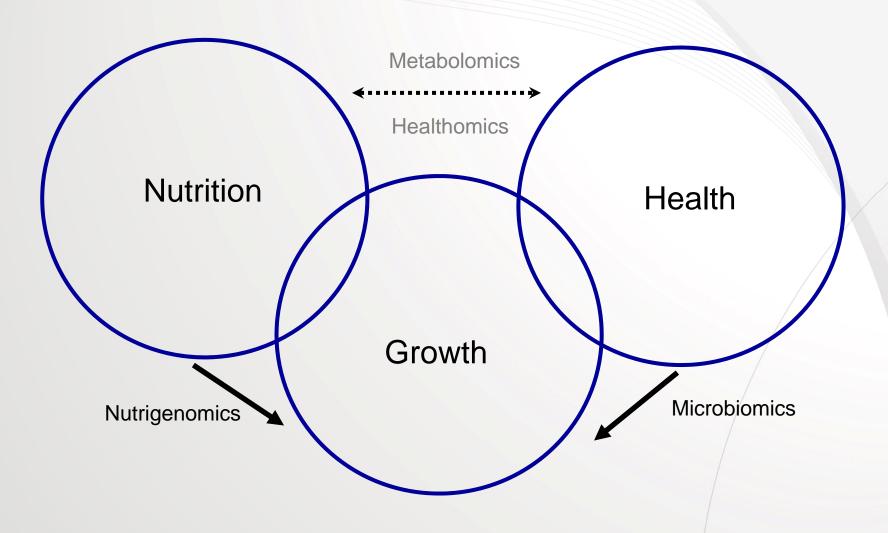
### Model of Larval Survival

Continuous larval attrition and mass mortality events



# Conceptual Model

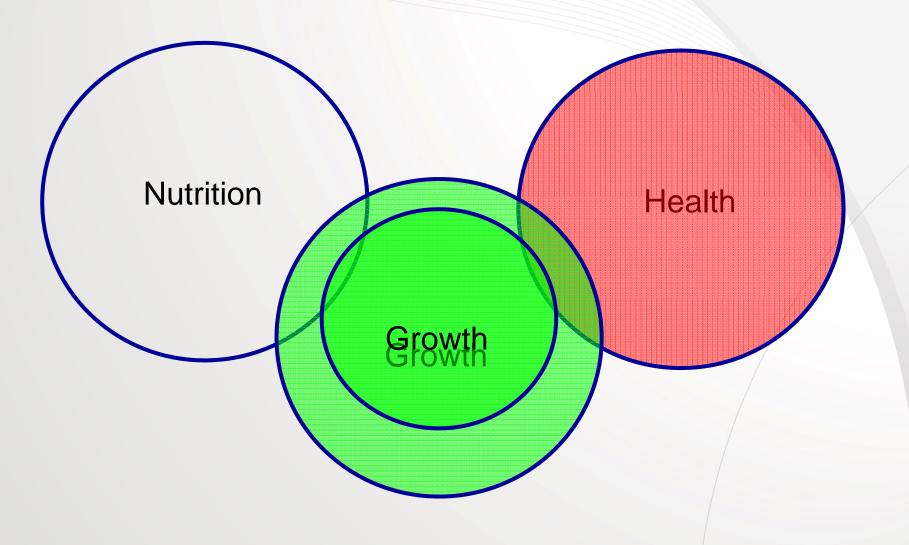
Interactions between Nutrition - Health - Growth







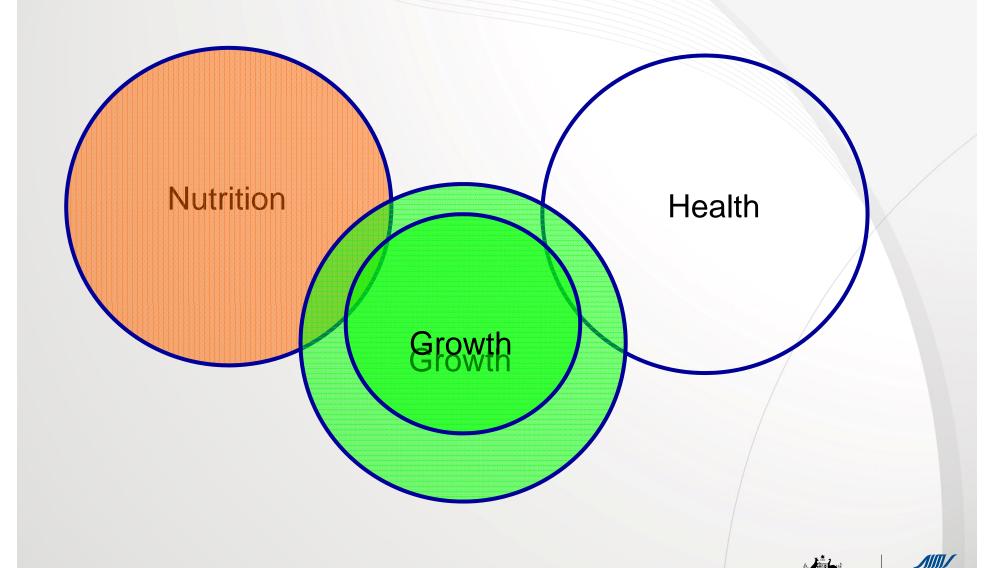
### Nutrition - Disease - Growth



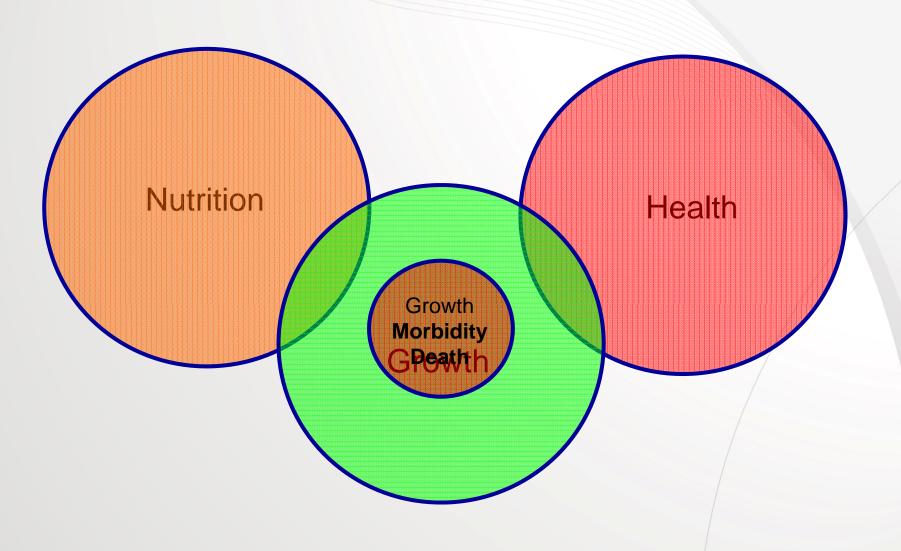




### Poor Nutrition - Health - Growth



### Poor Nutrition - Disease - Growth

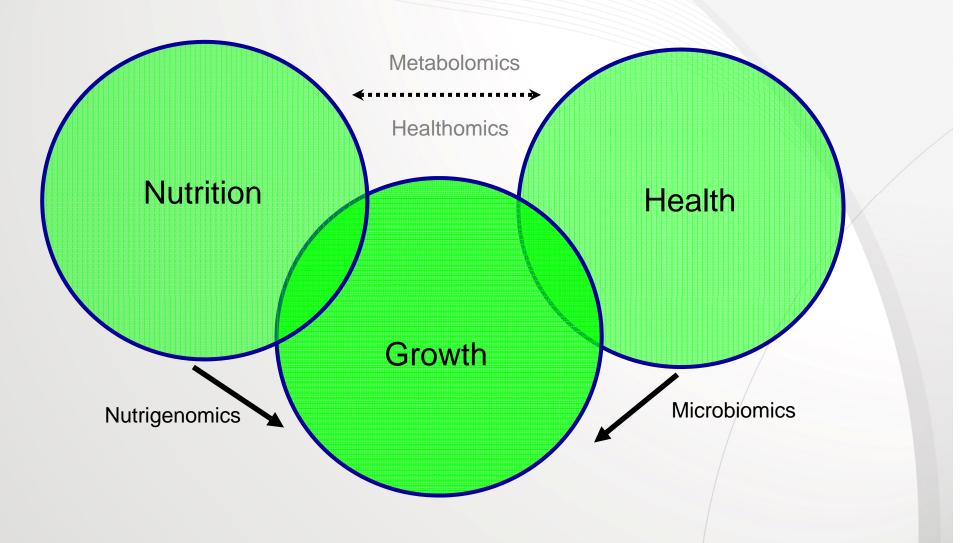






# Optimising the Triad

Interactions between Nutrition - Disease - Growth

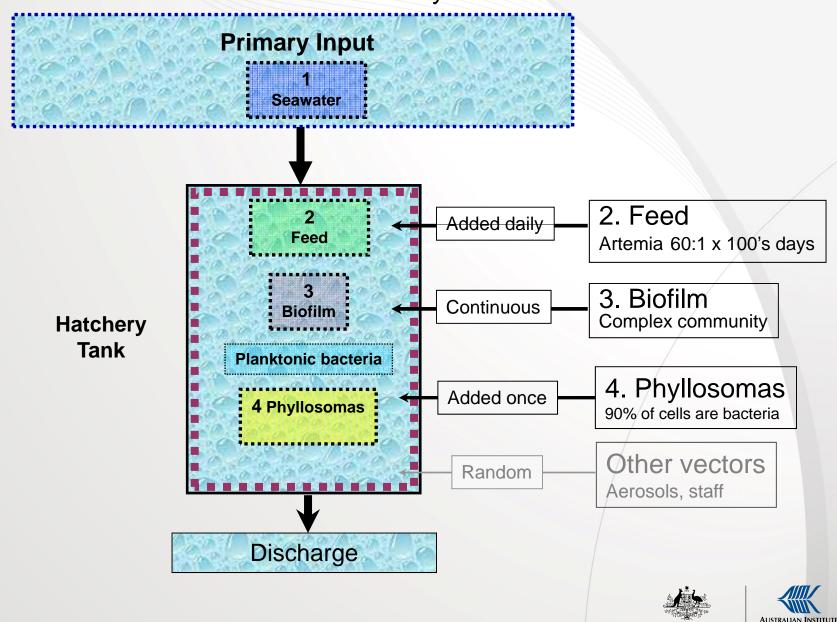






## Microbial Inputs

Microbial Biosecurity





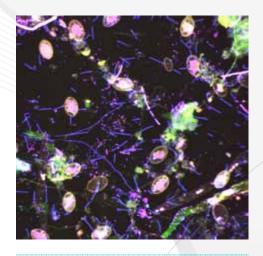


### Microbial Sources and Sinks in Hatchery

Four Compartments



2 13 14 15 16 17 18



1. Seawater Column

2. Larval Feeds

3. Biofilm



4. Phyllosoma



External

Internal

# Biofilm - Complex Bacterial Ecosystem

Diving through the biofilm – Surface to Tank (2 µm steps)







Larval finfish consume feed whole - Feed size related to mouth gape

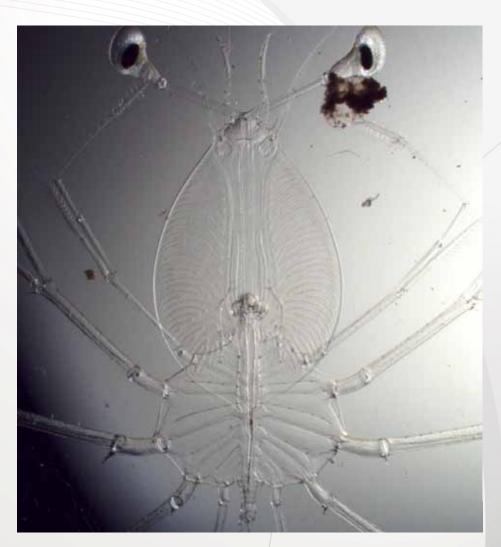


Source: © Artemia World

### Phyllosomas consume feed piecemeal

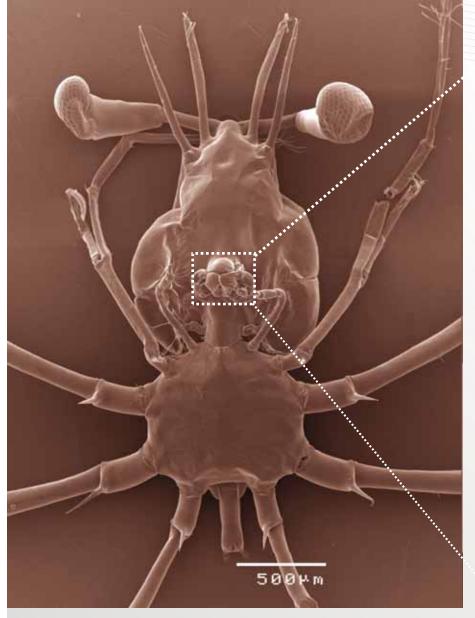


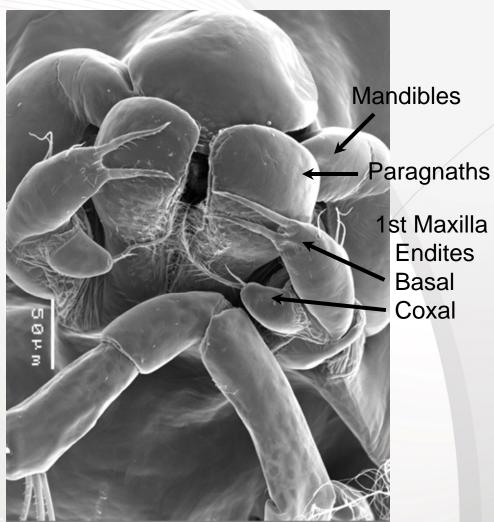
Stage 1



Stage 7

### Health - External Fouling

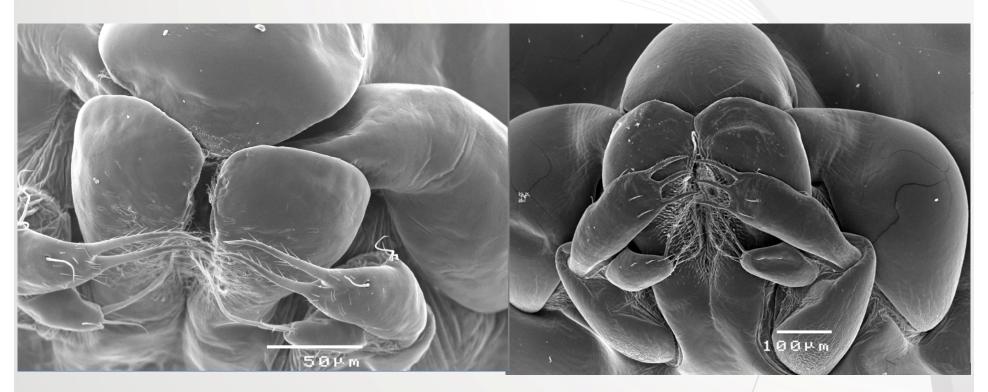








# Changes in Mouthpart through Development Stages 1 to 11



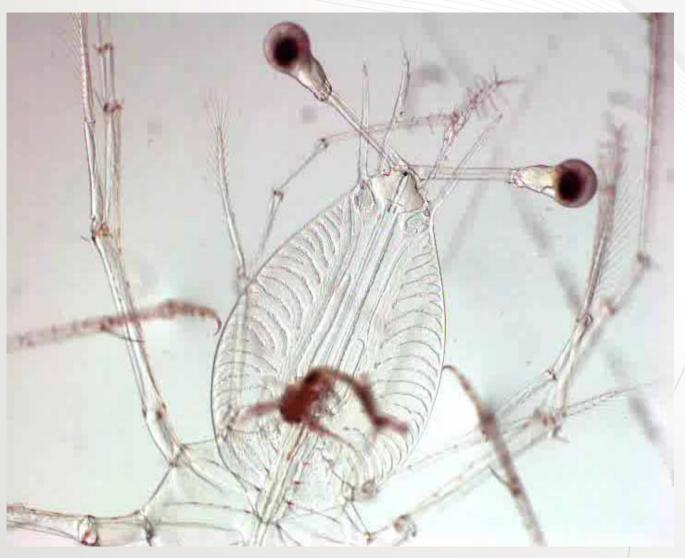
Stage 1 Stage 11





### Larval phyllosomas consume feed piecemeal

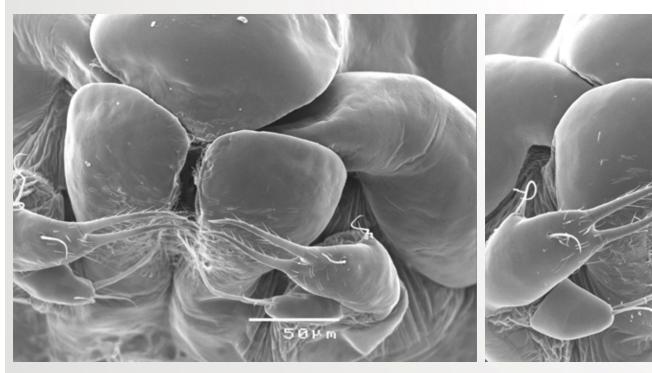
### Feeding video artemia

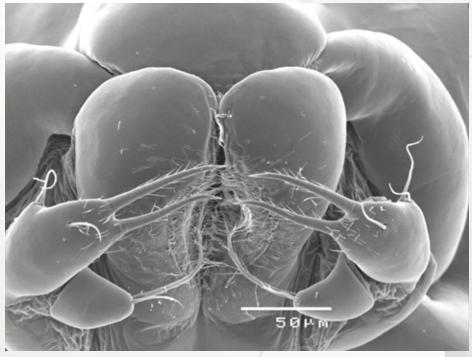






### External Bacterial Fouling of Phyllosoma Mouthparts



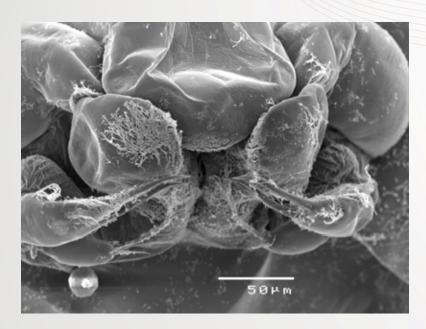


Day 1 Post-molt Day 3
Post-molt

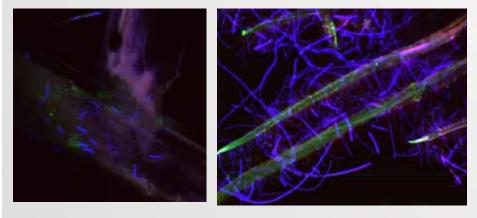




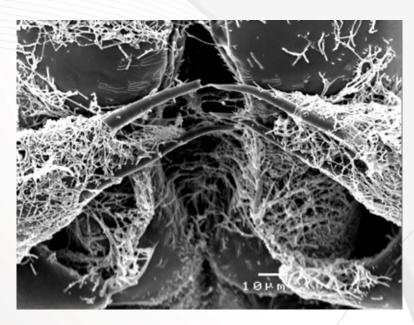
### External Bacterial Fouling of Phyllosoma Mouthparts



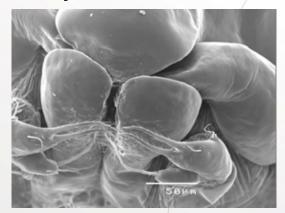
Day 11 Post-molt



FISH probe - Thiothrix

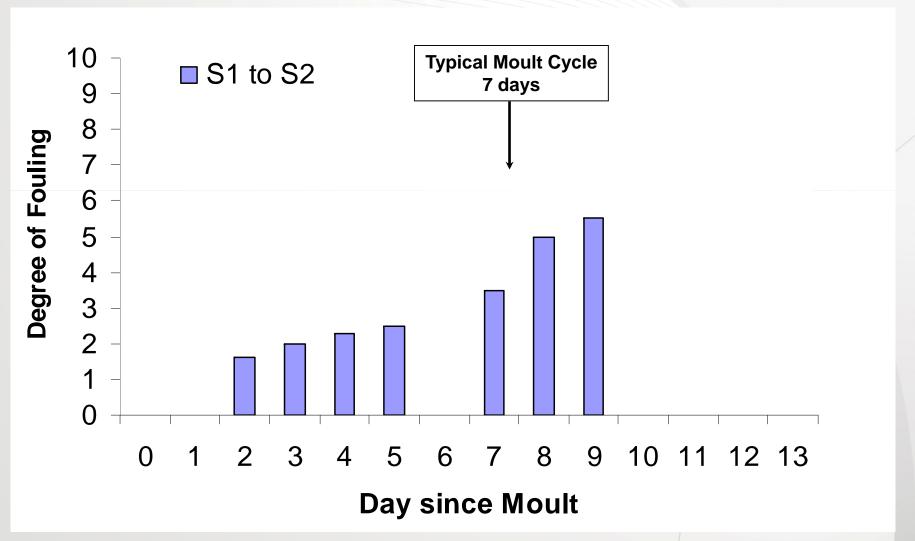


Day 13 Post-molt



Immediate Post-molt

# Fouling (*Thiothrix*) of Phyllosoma Mouthparts Stage 1 to 2

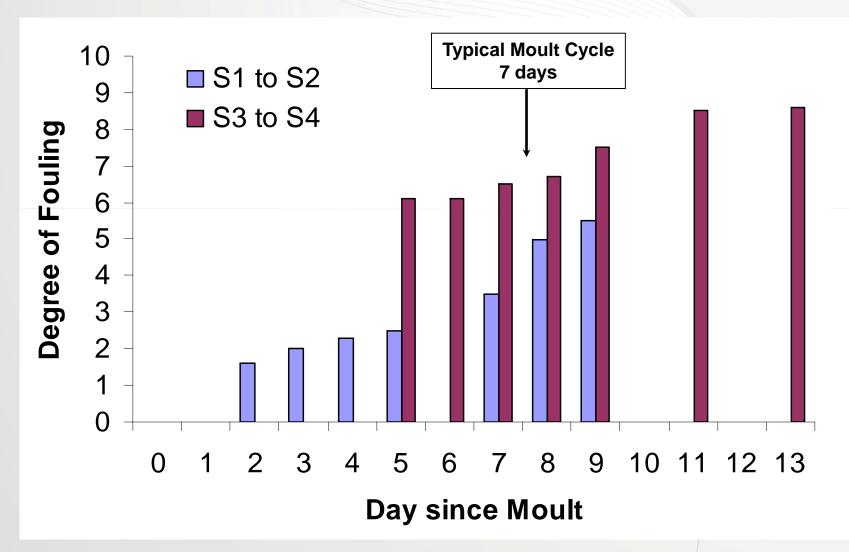






### Fouling (Thiothrix) of Phyllosoma Mouthparts

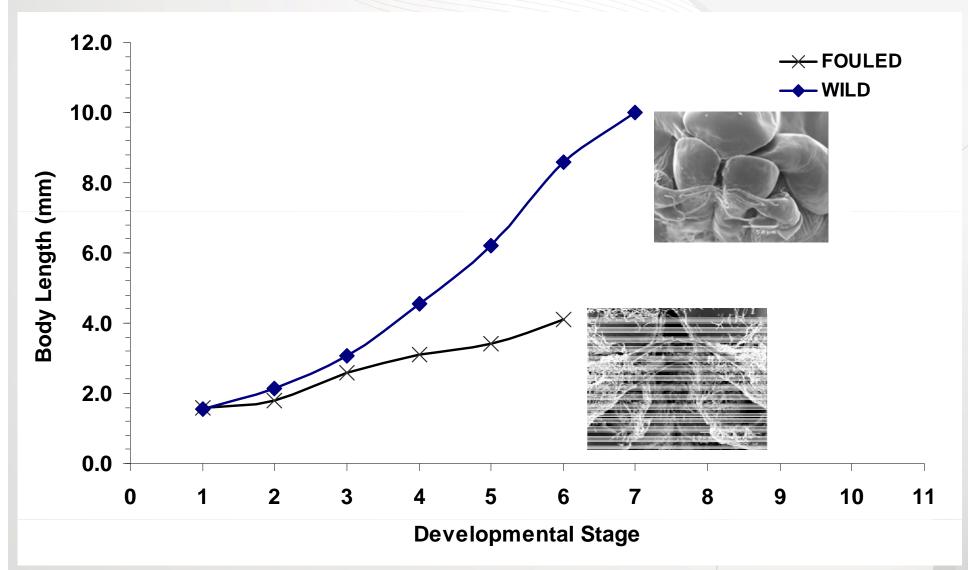
Stage 3 to 4 compared to Stage 1 to 2







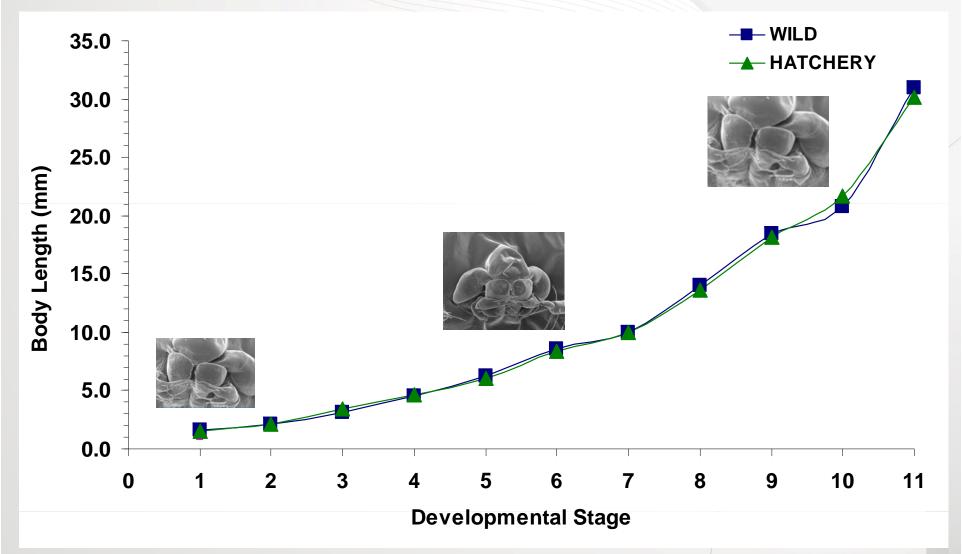
#### **Fouled Hatchery compared to Unfouled**







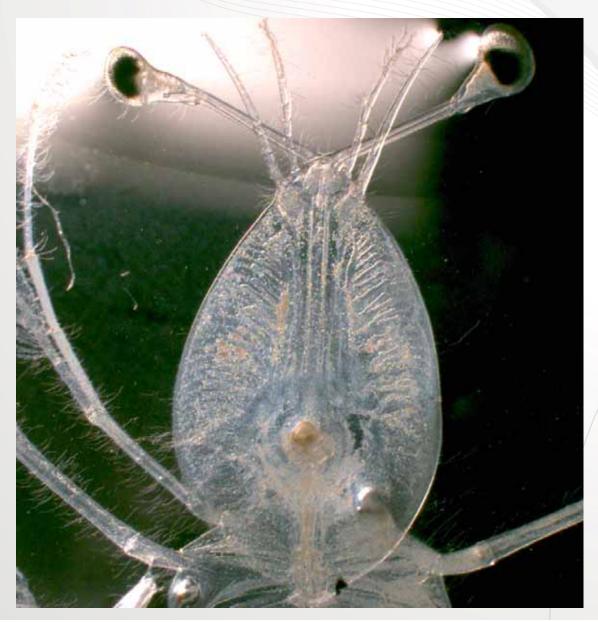
#### **Unfouled Hatchery compared to Wild**







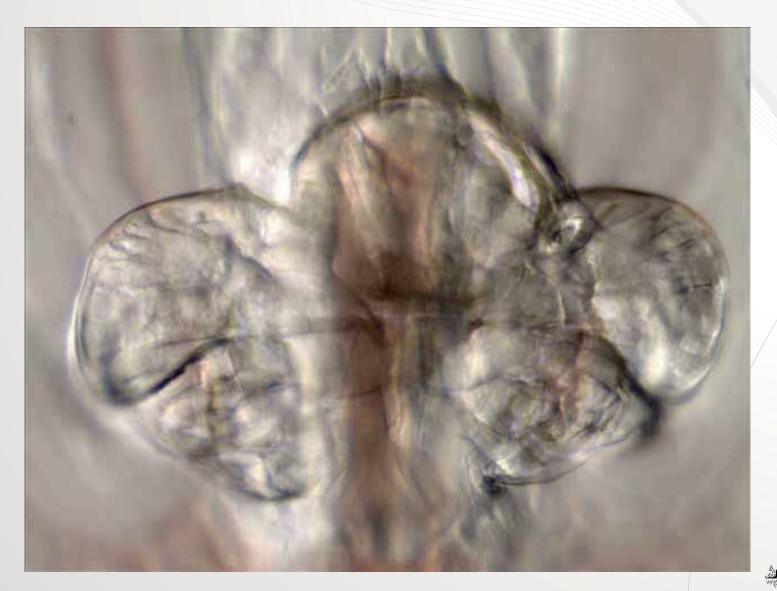
#### **Internal Infection**





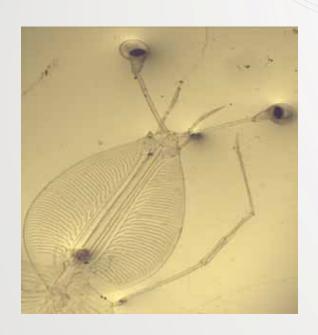


### **Ingestion and Digestion**

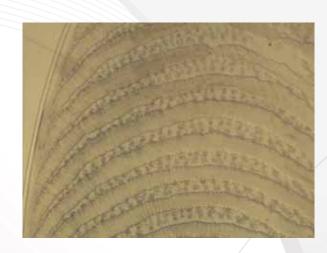




#### **Internal Infection**



Wild-caught larva with intact hepatopancreas





Hatchery larva with 'white-gut syndrome', infected by *Vibrio spp.* 

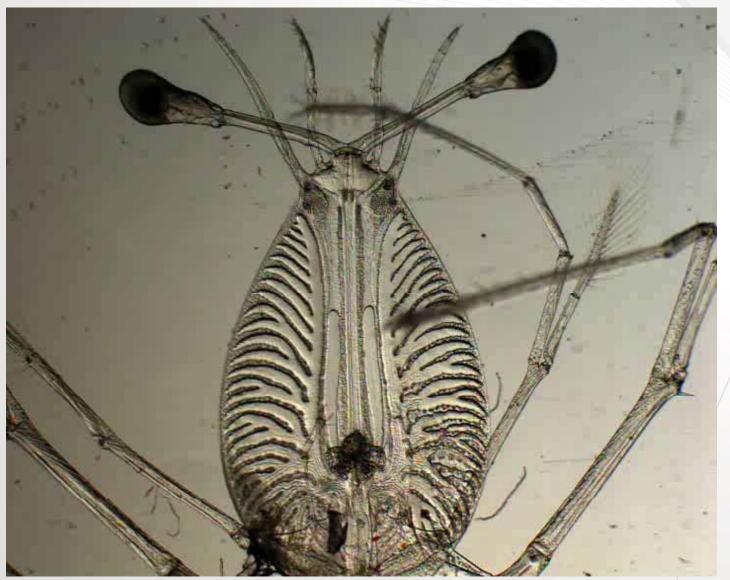






# Phyllosoma gut infection

### Real-time video

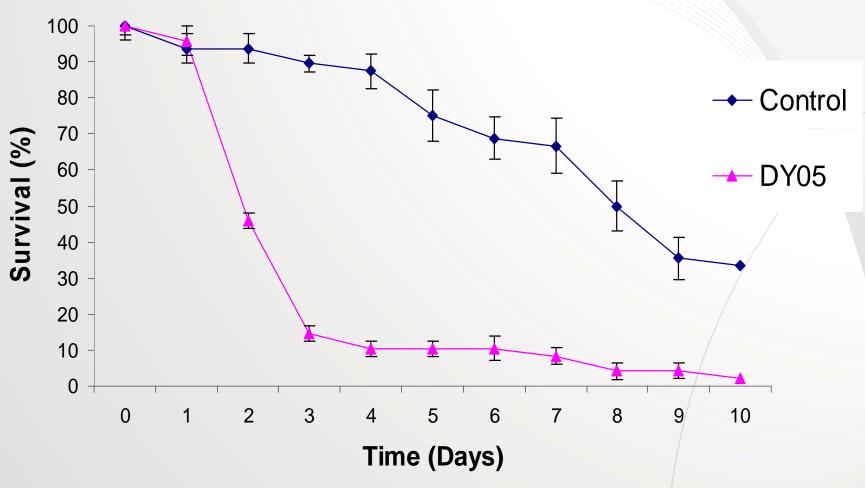






### Health - Internal Infections

Survival of *P. ornatus* phyllosomas (S5) challenged with *Vibrio sp.* (DY05)





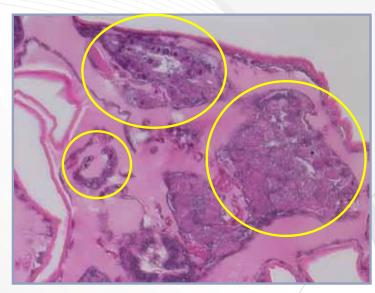


## Phyllosoma gut

#### Internal bacterial colonization



Healthy



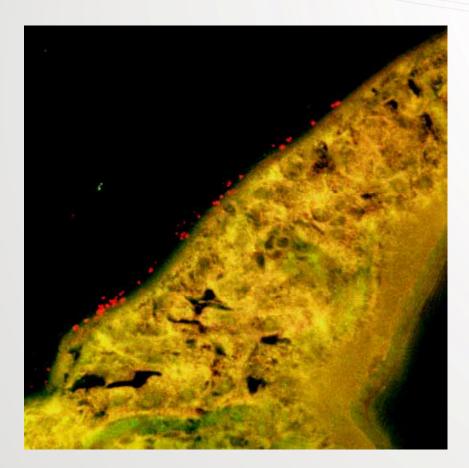
Moribund

- Histopathology of hepatopancreas (H&E)
  - Healthy: caeca open and clear
  - Moribound: caeca are blocked and disrupted

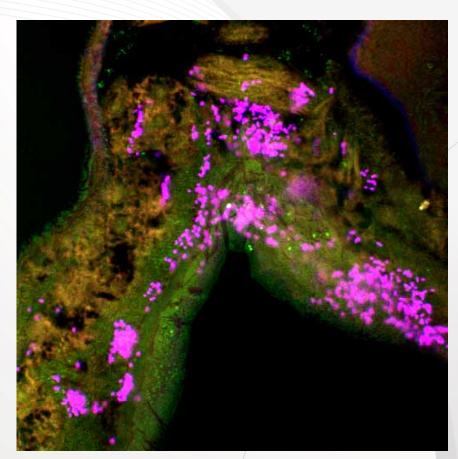




## Fluorescent *in situ* hybridisation (FISH) Bacterial localisation



Healthy



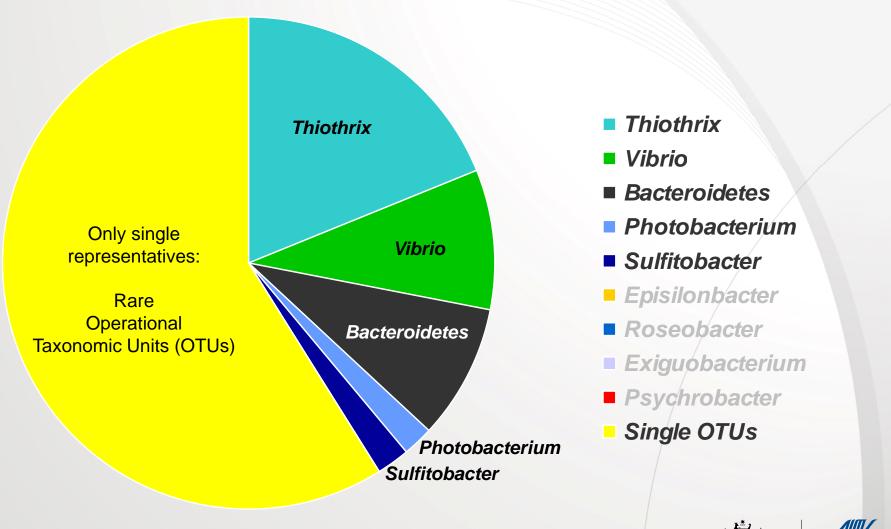
Moribund





### Bacterial Diversity in Hatchery Phyllosoma

#### **Clone Library Analysis**







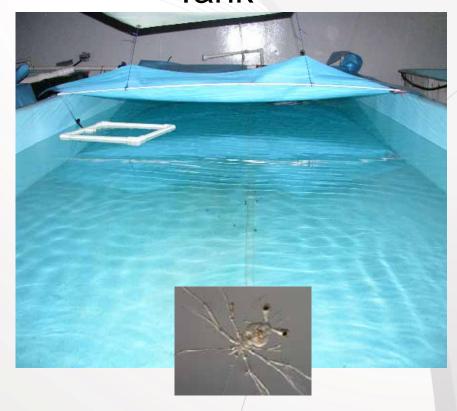
# Interactions between Nutrition – Health - Growth Hunting Probiotics

#### Oceanic



Health – Natural Microbial community

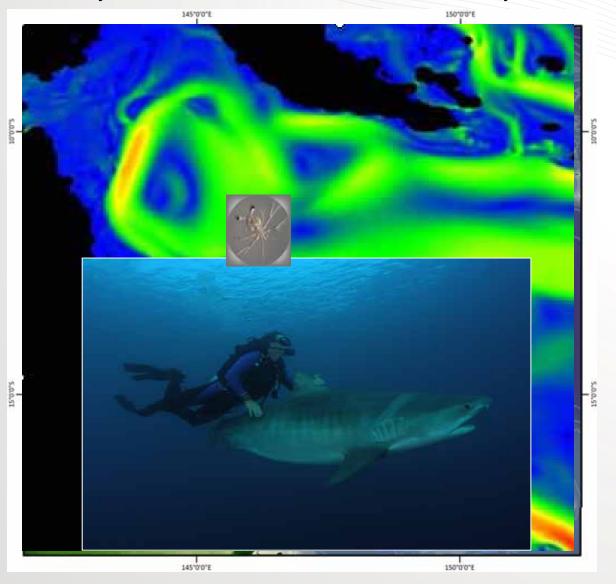
Tank



Health – Probiotic Microbial community

## Larval Ecology of *P.ornatus* in NE Australia

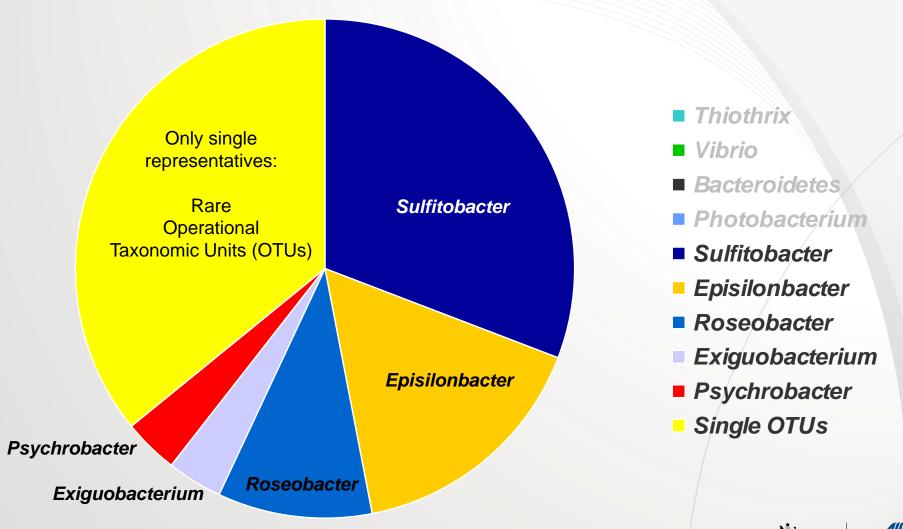
Phyllosoma entrained in the Coral Sea Gyre





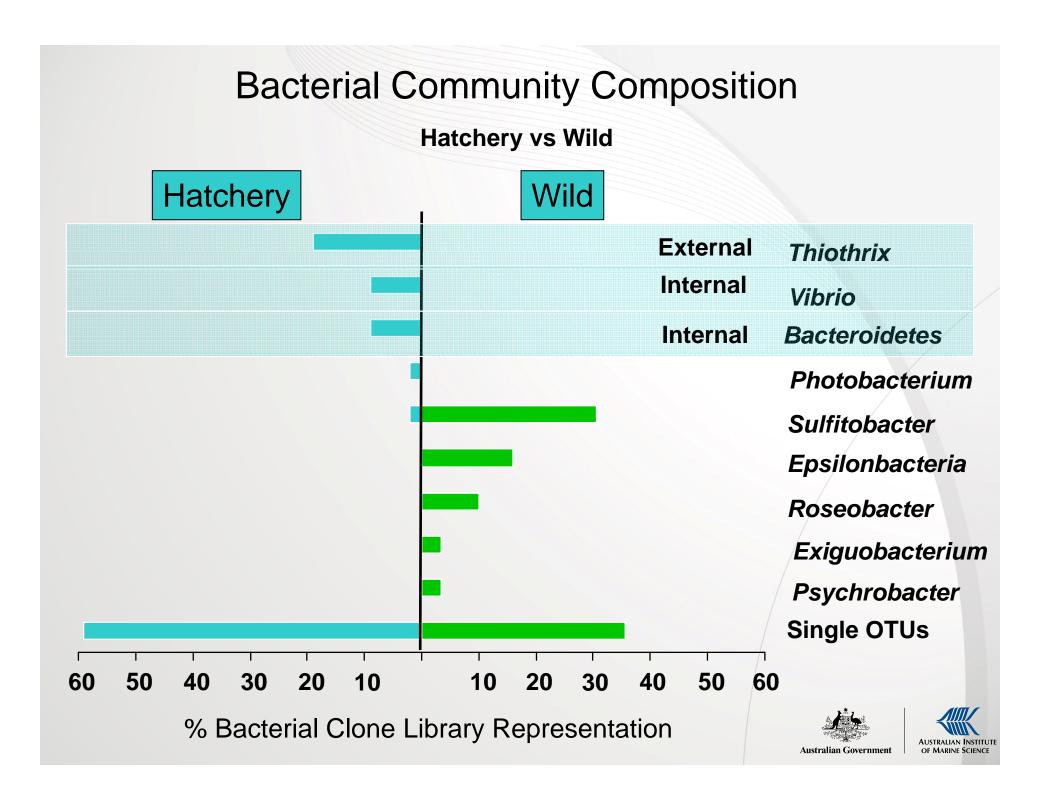
### Bacterial Diversity in Wild Phyllosoma

#### **Clone Library Analysis**



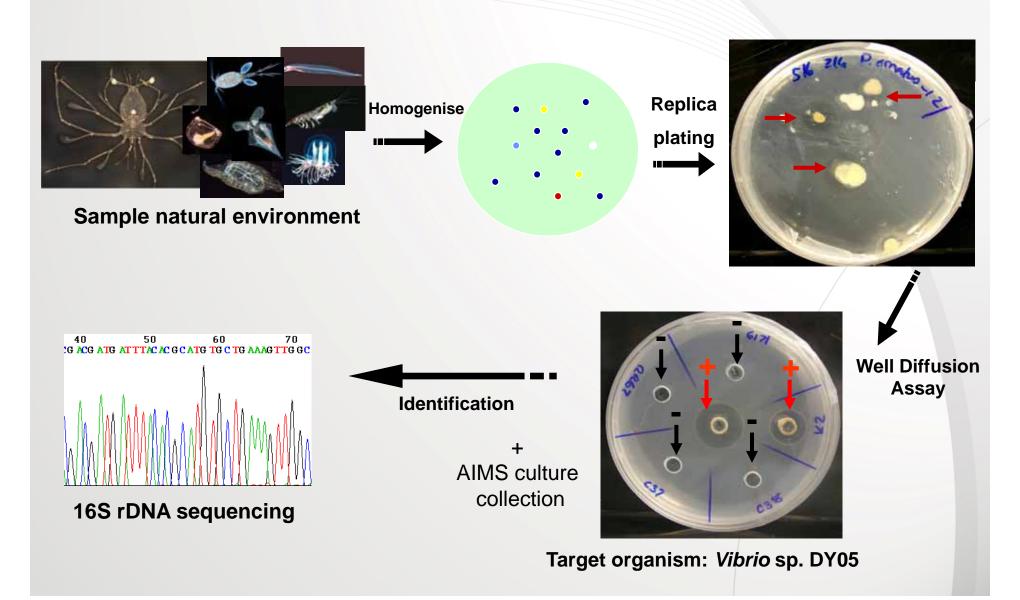






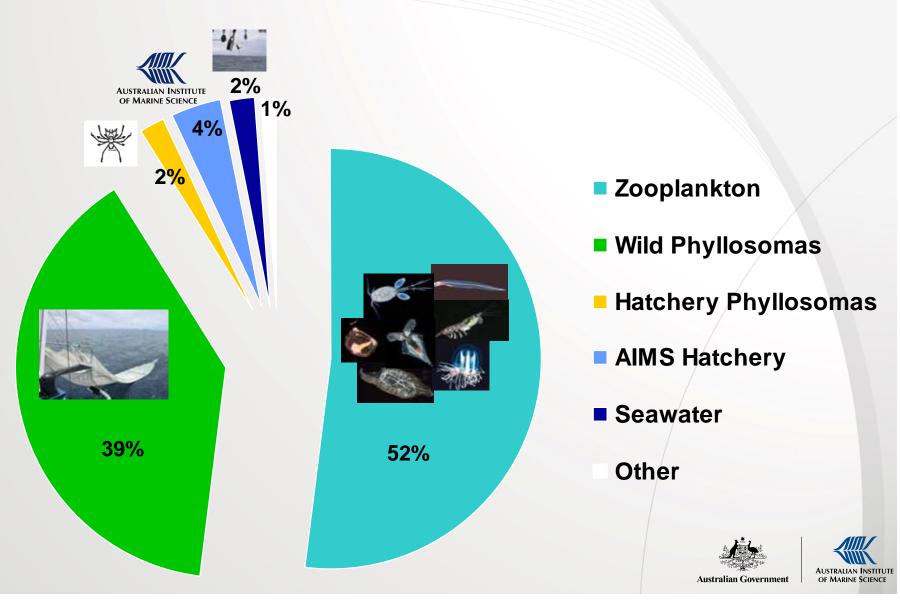
#### The search for Probiotics

Well diffusion assay – agar infused with strain DY05



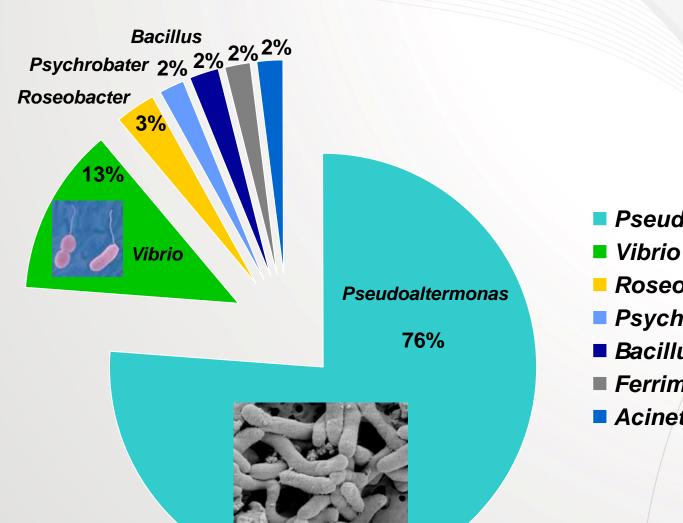
## **Sourcing Probiotic Strains**

18% (92/508) of isolates inhibited growth of known *P. ornatus* pathogens (*Vibrio* strains DY05 & D40) in well-diffusion assays



## Identifying the Probiotic Candidates

16S rDNA sequencing of 60 candidate probionts



- Pseudoaltermonas
- Roseobacter
- Psychrobacter
- Bacillus
- Ferrimonas
- Acinetobacter

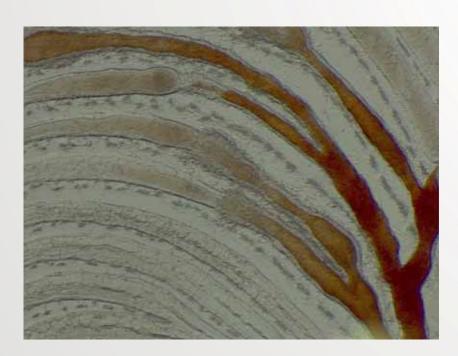




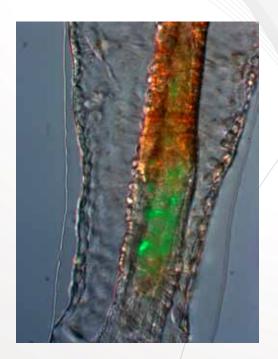
#### Current and future direction

#### Interactions between Nutrition – Health - Growth

Elucidation of pathogen-probiont interactions in vivo



Vibrio (DY05) Infection

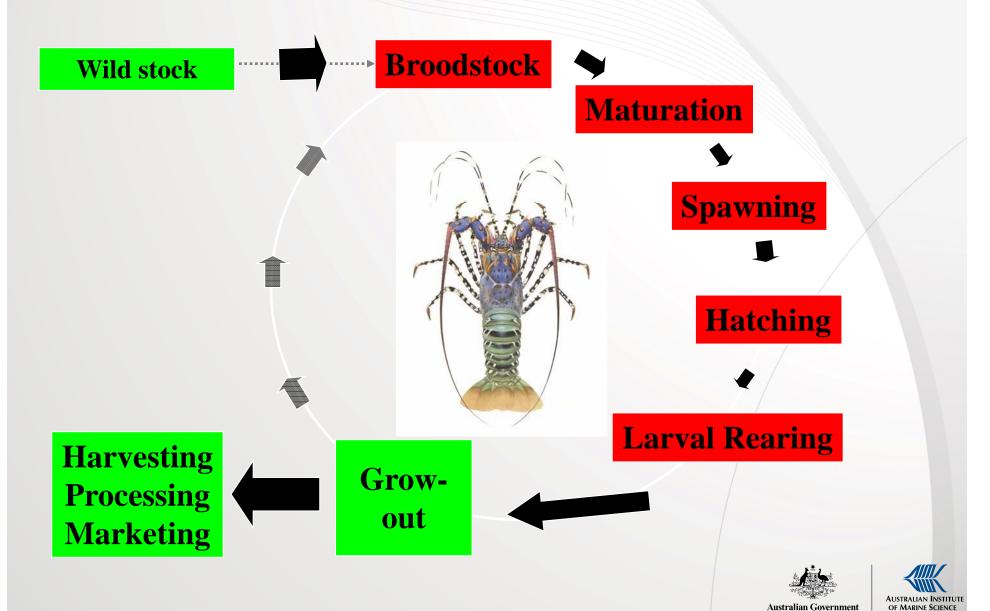


GFP labelled *Vibrio* (DY05) *Artemia* gut





## Closed life cycle Aquaculture Panulirus ornatus



## Closed life cycle Aquaculture Panulirus ornatus Wild stock **Broodstock** (Captured Rearing) Maturation **Closed-life Cycle** (Farming) **Spawning Larval Rearing** Harvesting Grow-**Processing** out **Marketing**

## Staff Investment

| Area  | Staff  |
|---|--|
| Water Quality  Broodstock Facility  Larval Rearing Facility  Live Feed Facility | Matt Kenway Jane Gioffre Michael Clarkson Katie Holroyd Matt Salmon Grant Milton Justin Hochen |
| Health (Microbiology) Research  | Greg Smith  Lone Høj  Mike Hall  |
| Diet (Larval Feeds) Research  | Greg Smith<br>Mike Hall  |
| Cruises – Natural History, Prey,<br>Probiotics, Diet                            | Above staff plus volunteers  |
| Associated Larval Research  | 6 PhDs, 1 MSc<br>Evan Goulden<br>Ana Cano Gomez  |





