The Impacts of Probiotics and Prebiotics in RAS Pompano Aquaculture

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Fish Health

Stress and Disease

Effects Production and Profitability

Disease can be reduced by good management

But not eliminated

Diseases are often combated by

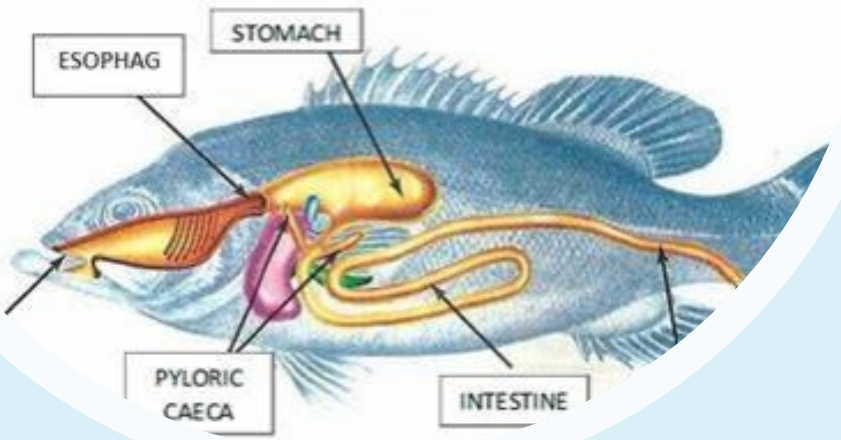
Chemicals and antibiotics, but….

- Potential risks to consumers and the environment

Alternatives

Probiotics, prebiotics, immunostimulants

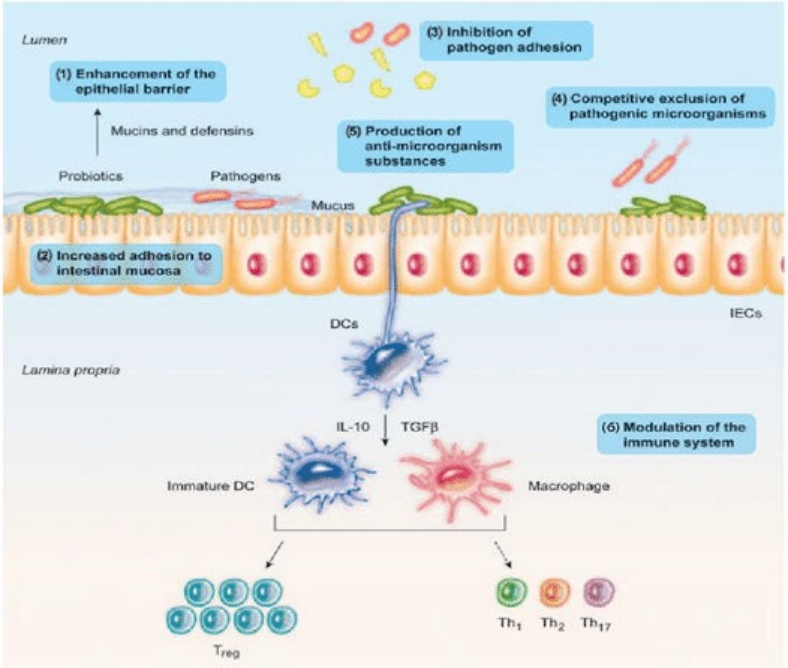
Fish Gastrointestinal Tract

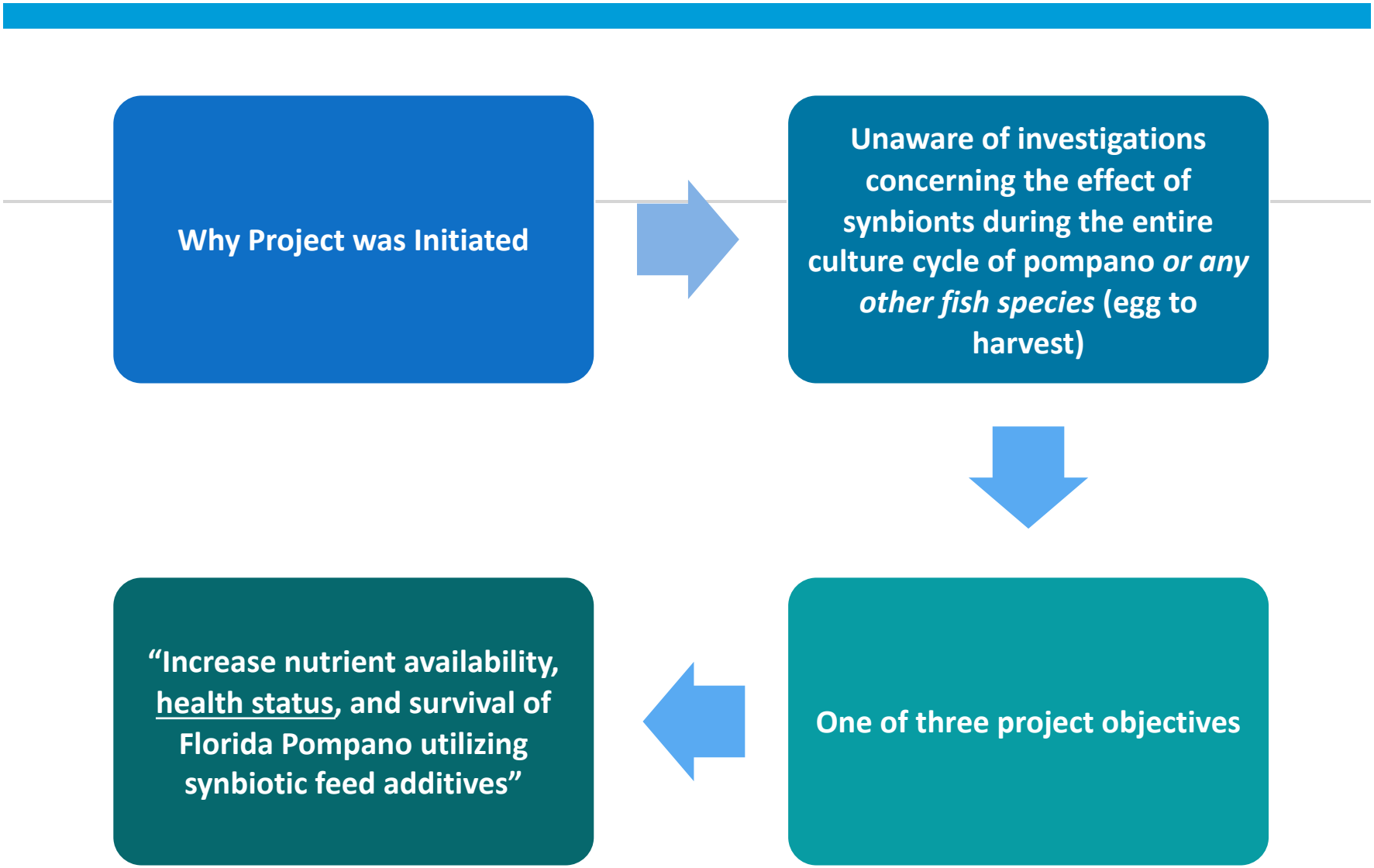


- **Gut microbiome consists of trillions of microbes**
- **Develops throughout a fishes lifetime**
- **Impacted by bacteria from the water and feed**
- **Balanced GIT – 85% good guys**
  - **Produce antimicrobial agents that promote an immune response**

Probiotics, Prebiotics, Synbionts

- **Probiotics = live beneficial bacteria that improve intestinal microbial balance**
  - **LABs and *Bacillus* sp.**
- **Prebiotics = non-digestible feed ingredients that selectively stimulate growth or activity of probiotics**
  - **fructooligosaccharides (FOS), mannanooliogsaccharides, inulin and β-glucan**
- **Synbionts = Probiotics + Prebiotics**
- **Modulate the non-specific immune system**
  - **First line of defense**

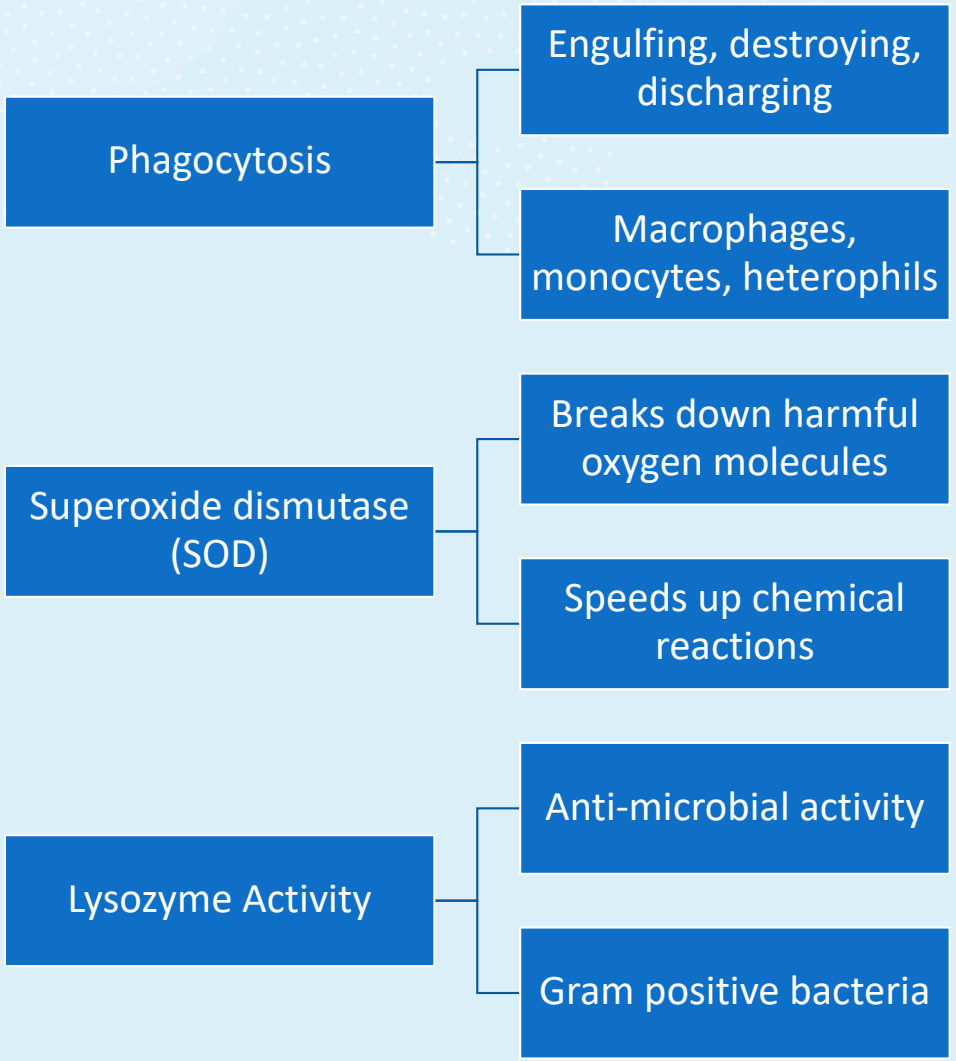



Why Project was Initiated
Unaware of investigations concerning the effect of synbionts during the entire culture cycle of pompano *or any other fish species* (egg to harvest)
One of three project objectives
"Increase nutrient availability, health status, and survival of Florida Pompano utilizing synbiotic feed additives"

Hematological Indices

- **Red blood cells (RBCs, Erythrocytes)**
- **White blood cells (WBCs, Leukocytes)**
- **Total counts**
- **Differential leukocyte counts**
  - **Lymphocytes, thrombocytes, heterophils, monocytes, eosinophils**
  - **Lymphocytes – produce B (antibodies) and T (killer cells)**
  - **Granulocytes – produce enzymes**
    - **eosinophils, heterophils**
  - **Phagocytes – heterophils, monocytes**

Immune System Function



Digestive Enzymes

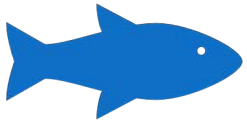
- **Amylase**
  - converts starch into simple sugars
- **Lipase**
  - breaks down fats
- **Protease**
  - breaks proteins into peptides and amino acids
- **Alkaline Phosphatase**
  - breaks down proteins, protects GIT from bacteria and aids in digestion
- **Proteins**

Experiment 1 – Synbiont Screening

Ten treatments

Control, Probiotic, Probiotic + Prebiotic 1 (4 levels), Probiotic + Prebiotic 2 (4 levels)

- ***Bacillus* sp blend + *Pedicoccus acidilactici* (10^6 CFU g^{-1})**
- **FOS or β-glucan (0.5, 1.0, 2.0 and 4.0 g kg^{-1})**



Three months

5 to 50 g larval pompano

8 - 16 Fish/Treatment

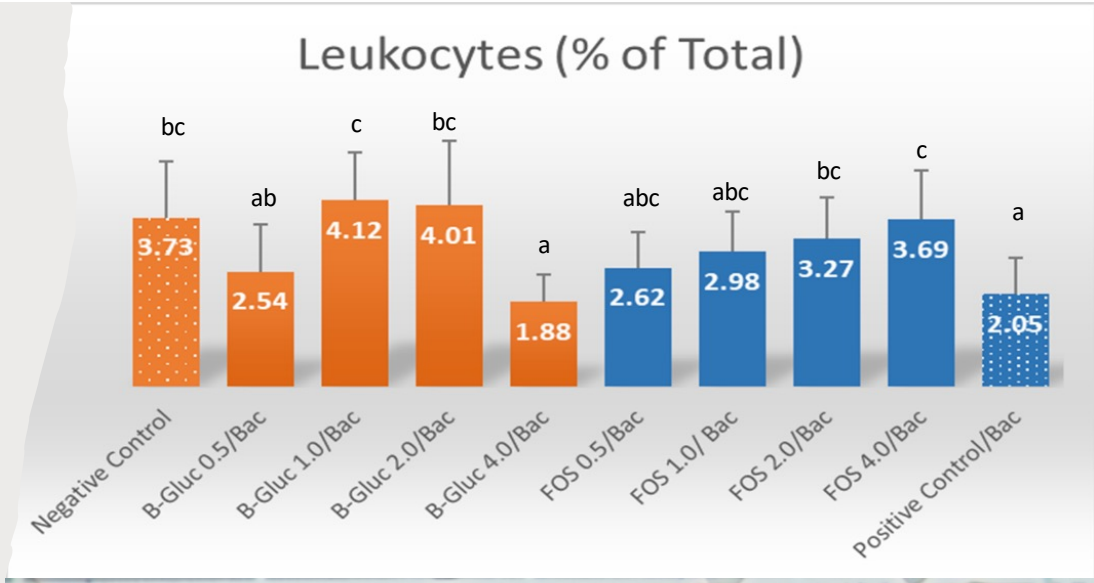
Hematological Indices

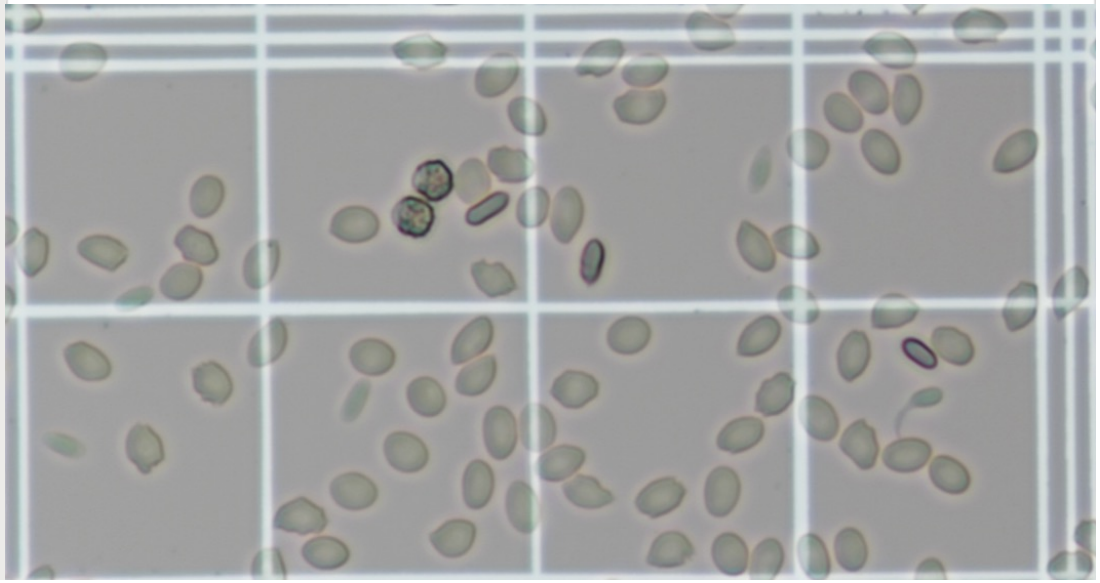
Proportion of RBC's or WBC's

- **(P<0.001)**

Differential Leukocyte Counts

- **Lymphocytes (P=0.1733)**
- **Thrombocytes (P=0.3341)**
- **Monocytes (P=0.0142)**
  - ↑ β-Glu 2.0, FOS 4.0
- **Granulocytes**
  - **Heterophils (P=0.0511)**
    - ↑ β-Glu 1.0 & 2.0, FOS 4.0
  - **Eosinophils (P=0.2912)**





Immune Function

Phagocytosis

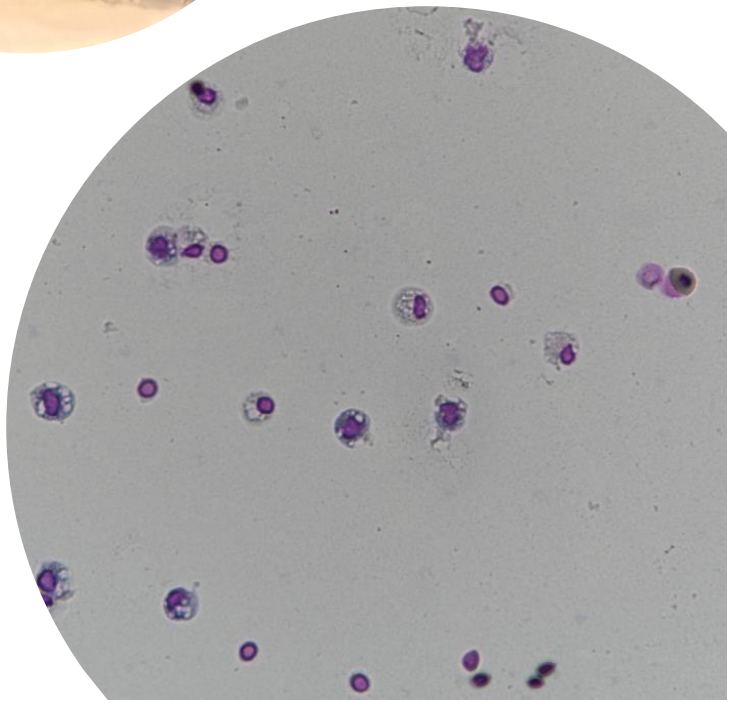
- NS (P=0.857)
  - β-Gluc 1.0/Bac, FOS 2.0/Bac

SOD

- NS (P=0.872)
  - β-Gluc 1.0/Bac, FOS 4.0/Bac

Lysozyme

- NS (P=0.344)
  - β-Gluc 2.0/Bac, FOS 1.0/Bac



Digestive Enzymes

- **Protein**
  - **NS (P=0.69)**
- **Alkaline Phosphatase**
  - **NS (Ppro=0.72, Ptiss=0.76)**
- **Amylase**
  - **NS (Ppro=0.42, Ptiss=0.47)**
- **Lipase**
  - **Sig (Ppro=0.011, Ptiss=0.013)**
  - **Highest in FOS 4.0/Bac**
- **Protease**
  - **NS (Ppro=0.260, Ptiss=0.241)**

Take Away

- **No clear-cut winner**
  - **0.5% prebiotic addition didn't perform well**
  - **4% prebiotic addition did not increase benefit in β-Gluc**
    - **Cost prohibitive for FOS**
- **Best performance in…**
  - **β-Gluc 1.0 and 2.0/*Bacillus sp + P. acidilactici***
  - **FOS 2.0 and 4.0/*Bacillus sp + P. acidilactici***

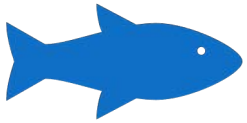
Experiment 2 – Pellet Type ± Synbiont

Four treatments

Hard pellet ± Synbiont

Soft pellet ± Synbiont

- ***Pedicoccus acidilactici*** **(10^6 CFU g^{-1})**
- **β-glucan (1.0 g kg^{-1})**



Three months

250 g pompano

12 Fish/Treatment

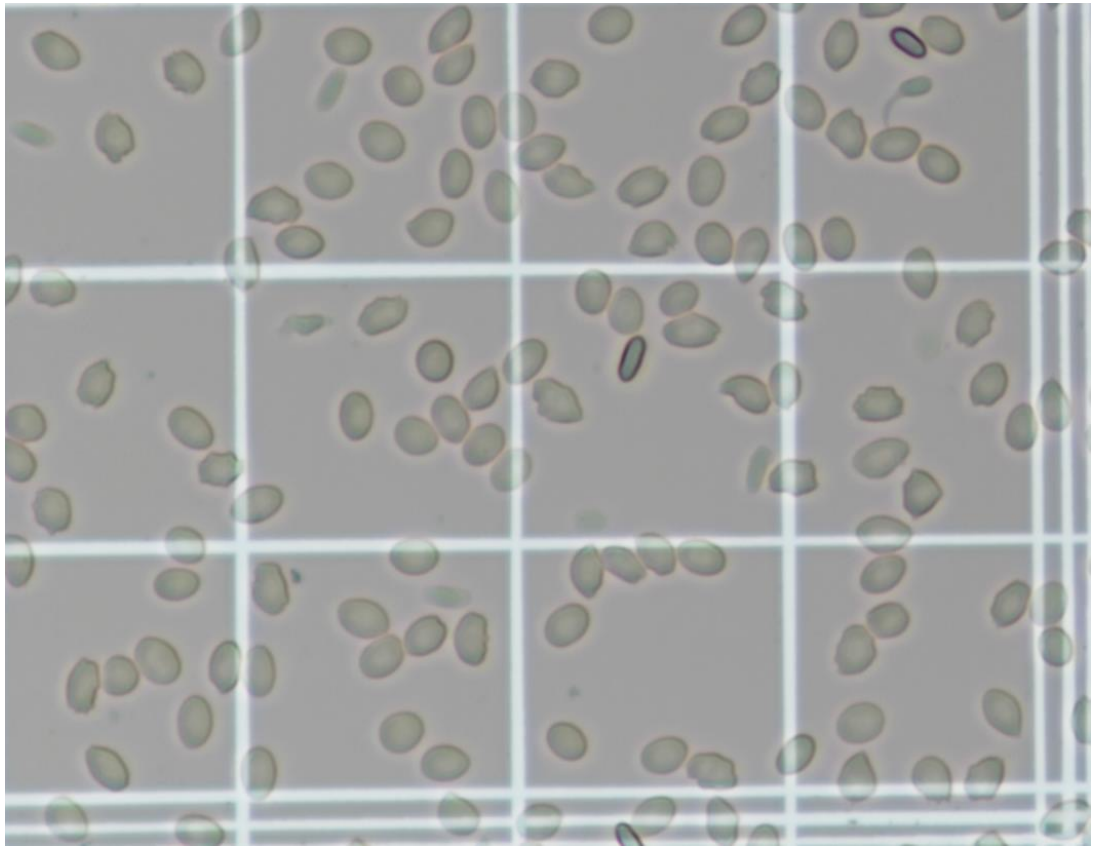
Hematological Indices

Proportion of RBC's or WBC's

- NS (P=0.277)

Differential Leukocyte Counts

- Thrombocytes (P=0.378)
- Lymphocytes (P=0.056)
  - ↑ Hard vs ↓ Hard β-glucan (P=0.0188)
- Monocytes (P=0.806)
- Granulocytes
  - Heterophils (P=0.549)
  - Eosinophils (P=0.749)



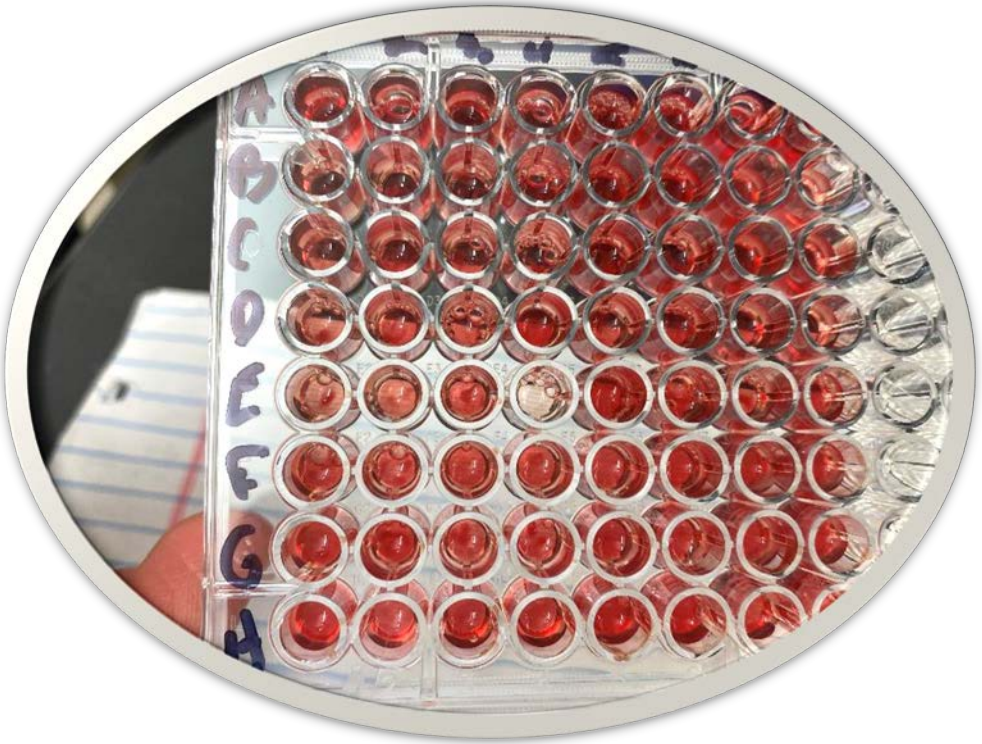
Immune Function

- **Phagocytosis**
  - **Sig (P=0.023)**
- **SOD**
  - **NS (P=0.743)**
- **Lysozyme**
  - **NS (P=0.590)**

OBJ 2 Phagocytosis Activity		
Macrophage Engulfing (% Positive)		
Treatment	Mean	SD
Hard Pellet	20	6.3
Hard Pellet (β-glucan)	29.8	12.6
Soft Pellet	36.2	8.9
Soft Pellet (β-glucan)	36.7	9.4
ANOVA Omnibus p =	0.023*	
A Priori Orthogonal Contrast		
Macrophage Engulfing (% Positive)		
Hard vs Soft	0.0076*	
Hard vs Hard β-glucan	0.0998	
Soft vs Soft β-glucan	0.9471	
No β-glucan vs -glucan	0.2195	

Digestive Enzymes

- **Protein (mg/L)**
  - **NS (P=0.199)**
- **Alkaline Phosphatase (U/mg)**
  - **NS (Ppro=0.862)**
- **<u>Amylase</u> (U/mg)**
  - **Sig (Ppro=0.030)**
    Hard vs Soft (P=0.005)
- **Lipase (U/mg)**
  - **NS (Ppro=0.308)**
- **Protease (mg/L)**
  - **NS (Ppro=0.259)**



More Questions Than Answers!

- **The synbiontic provided no apparent health benefits**
  - **Differences were only seen between pellet type**

Was the sample size too small to detect differences?

Did we choose the right levels and synbiont combination?

Was addition at 250 g too late in life cycle for any benefit?

Were the fish too healthy to detect any benefit?

Experiment 4 – Early Synbiont Application

Phase 1	Phase 2	Phase 3
1 month • 2 Treat • 16 fish/Trt	1 month • 4 Treat • 8 fish/Trt	8 months • + Pellet type • 16 Treat • 8 fish/Trt • Stats for 4: • Hard, Soft, Non, Syn • 24 fish/Trt

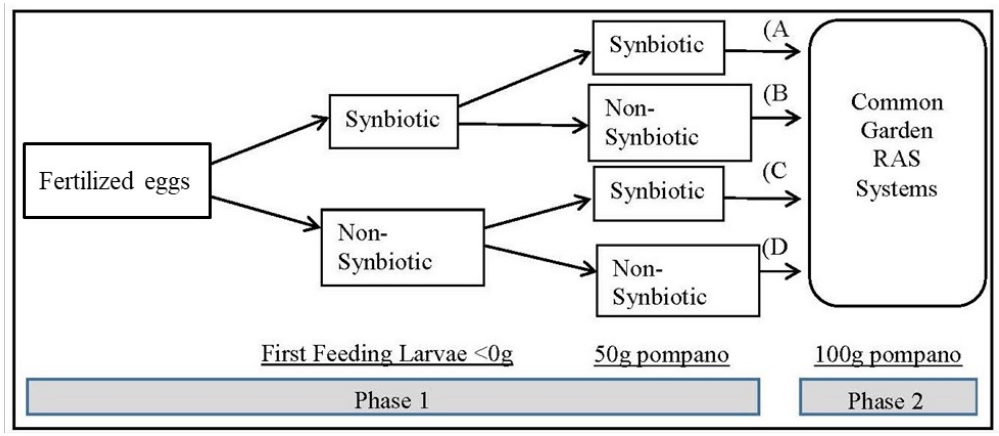
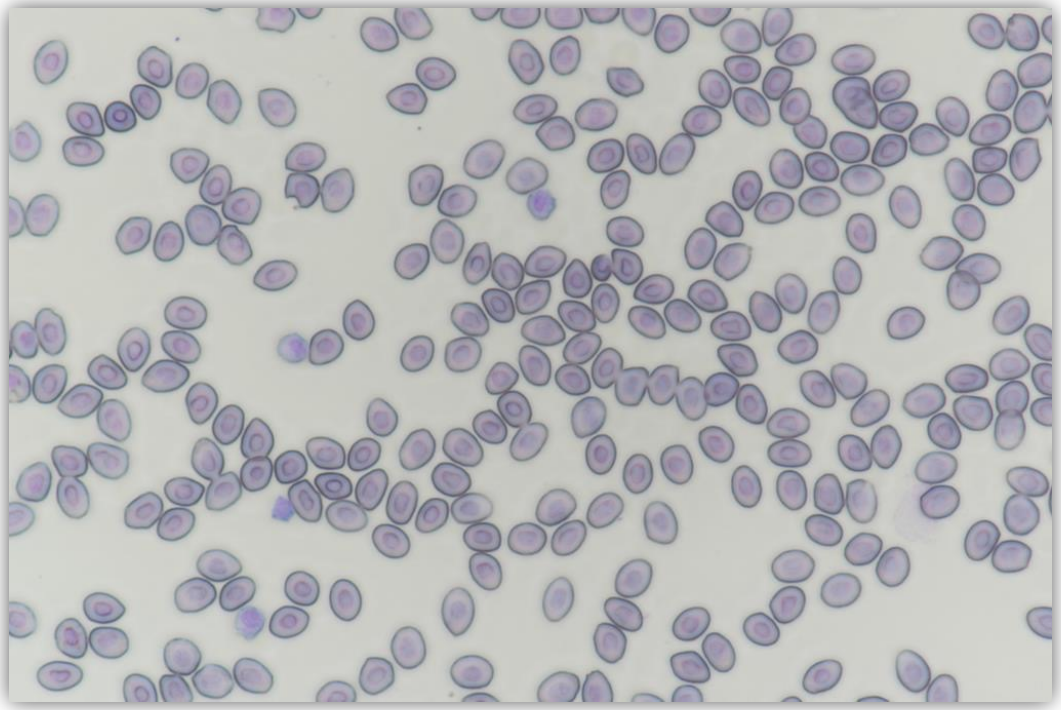


Figure 1. Dietary treatments during experiment 4 with Florida pompano from first feeding through mean weight of 100g.

Hematological Indices

- **Only significant differences were seen in Phase 1:**
- **Proportion of RBC's or WBC's**
  - ⬆ **RBCs in Non Synbiont (P=0.019)**
- **Differential Leukocyte Counts**
  - **Heterophils (P=0.0167)**
    - ⬆ **Non Synbiont**
  - **Thrombocytes (P=0.058)**
    - ⬆ **Synbiont**



Immune Function

Phagocytosis (Phase 3 only)

- Sig (P=<0.001)
  - ↑ Non Synbiont
  - Synbiont*pellet interaction; ↑ Soft

SOD

- Sig Phase 3 (P=0.033)
  - Pellet type (P=0.0226), Syn (P=0.0659)
    - ↑ Soft Non vs ↓ Hard Syn

Lysozyme

- NS all phases (P=0.940, 0.344, 0.086)

Digestive Enzymes

- **Phase 1 – NS**
- **Phase 2 – NS**
- **Phase 3 - Sig**
  - **Alkaline Phosphatase (P=0.005 – 0.05)**
    - **Pellet*synbiont interaction (P=0.002-0.004)**
    - **Hard non = Soft syn > Soft non, Hard syn**
  - **Amylase (P=0.0339 – 0.05)**
    - **Pellet*synbiont interaction (P=0.004-0.007)**
    - **Hard syn = Soft non > Hard non, Soft syn**

More to Tease out!

- **Phase 1 - early response (larvae – 50 g) to synbionts**
  - **Hematological only**
    - **Syn = deceased RBCs, decreased heterophils, increased platelets**
- **Phase 2 – addition or removal of synbionts (50 – 100 g)**
  - **No difference was seen in tested health parameters**
- **Phase 3 – Pellet type + addition or removal of synbionts (100 g to harvest)**
  - **No Hematological responses**
  - **Immune function responses**
    - **Phagocytosis, SOD**
  - **Digestive enzyme responses**
    - **Alkaline phosphatase, Amylase**

***Still to compare phase 3**

– Tease out Hard pellets only

- Affects of the phase 2 “swap”

Are Synbiotics beneficial in RAS Pompano Culture?

- Inconclusive
  - Impacts of Stress, Disease Challenge should be evaluated
- Right combinations, optimal levels
- Statistical power analysis

Usefulness of Health Indices

- Early life stages - Hematological Assays
  - RBCs vs WBCs, heterophils, lymphocytes, thrombocytes
- Later life stages – Immune system Function, Digestive Enzymes
  - Immune = phagocytosis, SOD
  - Digestive = lipase, alkaline phosphatase, amylase

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