Bacterial and Seed Health Issues in Florida Hatcheries and Nurseries

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Sea Grant

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Factors Affecting Clam Health

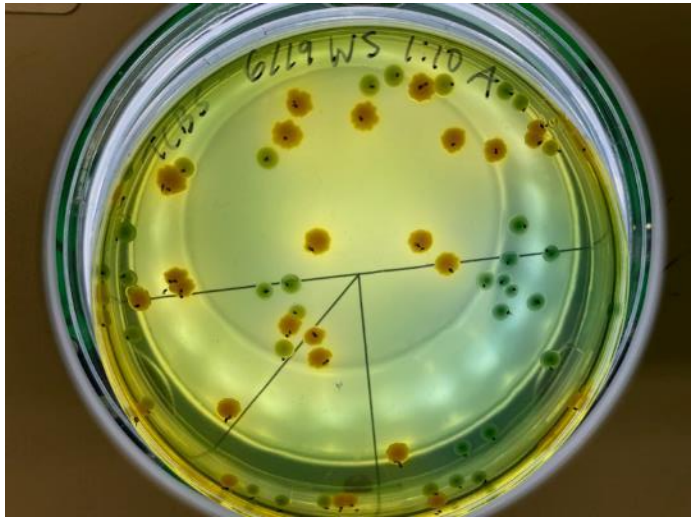
- Bacteria
  - Primary concern in clam hatcheries
    - Impacts movement and feeding ability
- Parasites
  - An additional concern in clam nurseries
- Water Quality
  - Temperature affects bacterial populations
  - Salinity affects parasitic populations
  - High ammonia and low dissolved oxygen are stressors
    - Affects ability to fight infection

Bacterial Monitoring

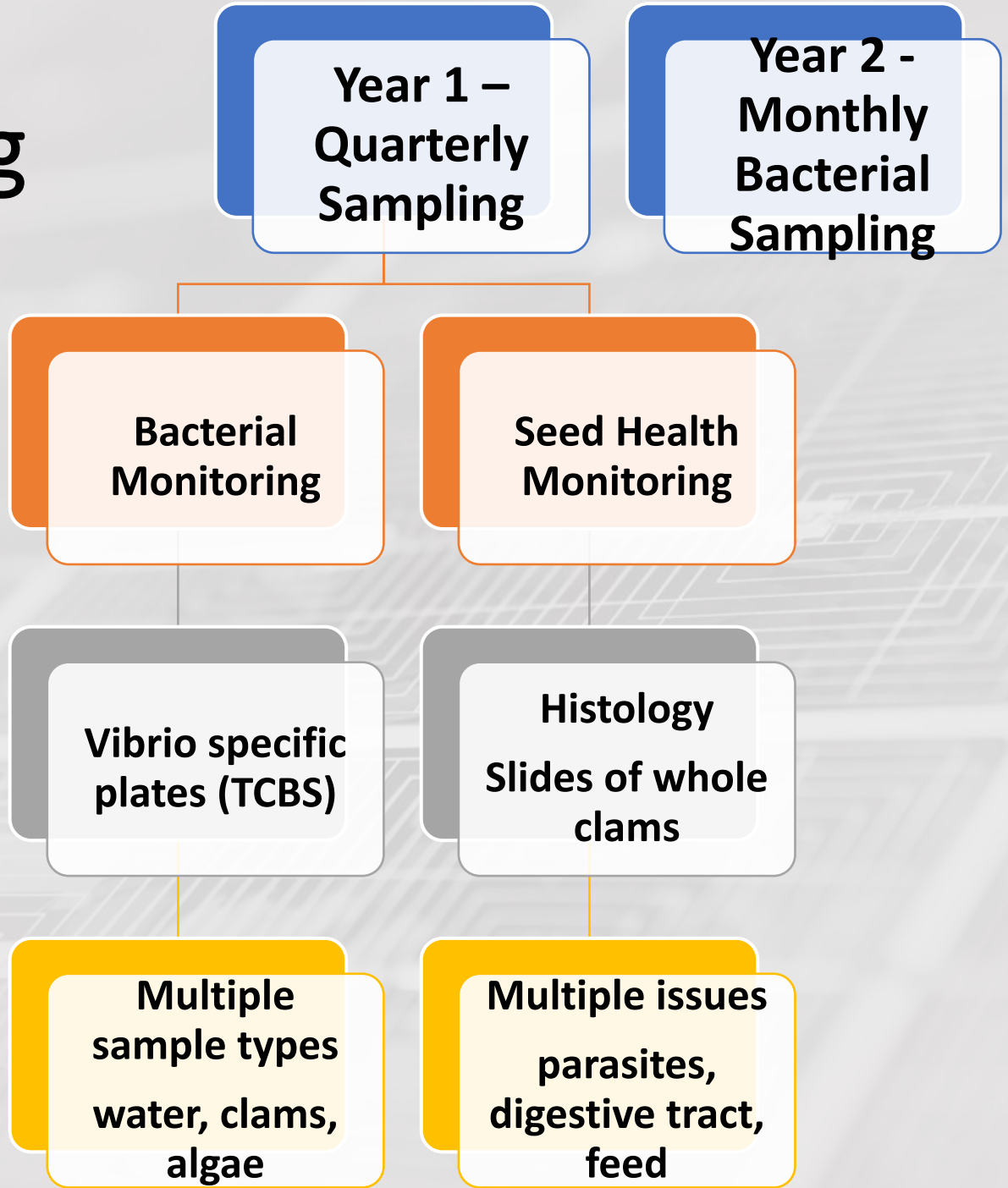
- **Bacteria are always present**
  - Most are necessary or helpful
  - Some are opportunistic and cause disease when present in high numbers
  - Vibrio = bacteria of highest concern in marine aquaculture

- **TCBS media is Vibrio specific**
  - Pathogens appear as blue/green colonies
  - Non-pathogens appear as yellow colonies

- **Routine monitoring of “hotspots”**
  - Areas that should be Vibrio free
    - algae cultures, source water



Monitoring


Year 1 – Quarterly Sampling
Year 2 - Monthly Bacterial Sampling
Bacterial Monitoring
Seed Health Monitoring
Vibrio specific plates (TCBS)
Histology
Slides of whole clams
Multiple sample types
water, clams, algae
Multiple issues
parasites, digestive tract, feed

Bacterial Monitoring: Samples Collected

Year	Total	Water	Clams	Algae
Year 1	162	89	40	33
Hatchery	136	78	25	33
Nursery	26	17	15	0
Year 2	185	107	61	17
Hatchery	178	105	56	17
Nursery	7	2	5	0

Locations with high Vibrio counts in Florida Hatcheries

Year 1	
Larvae	**70%**
Larval Tanks	**60%**
Broodstock Tanks	**100%**
Algae	**45%**
Source/Reservoir*	**30%**

Year 2**	
Larvae	**40%**
Larval Tanks	**100%**
Broodstock Tanks	**ND**
Algae	**ND**
Source/Reservoir*	**60%**

** Generally higher levels seen in Surface compared to Well Water*

***Too few samples were submitted from brood tank water or algae cultures to determine patterns*

Seasonal Changes in Hatchery Vibrio Populations

Year 1 – Winter to Spring		
Location	**% Increase**	**% Decrease**
Larvae*	**60%**	**-**
Larval Tanks*	**60%**	**-**
Brood Tanks	**45%**	**30%**
Algae	**15%**	**15%**
Source/Reservoir**	**15%**	**-**

Year 1 - Spring to Summer		
Location	**% Increase**	**% Decrease**
Larvae	**-**	**30%**
Larval Tanks	**-**	**30%**
Brood Tanks	**-**	**15%**
Algae	**45%**	**-**
Source/Reservoir	**30%**	**15%**

Low Vibrio concentrations were seen in samples collected in Winter

** A corresponding increase in pathogenic Vibrios was noted at some locations*

Seasonal Changes in Hatchery Vibrio Populations

Year 2 – Winter to Spring

Location	% Increase	% Decrease
Larvae**	40% (60%)	20% (-)
Larval Tanks**	80% (60%)	20% (-)
Source/Reservoir**	20% (30%)	- (-)

Year 2 – Spring to Summer

Location	% Increase	% Decrease
Larvae**	- (-)	75% (30%)
Larval Tanks	25% (-)	50% (30%)
Source/Reservoir**	25% (30%)	50% (15%)

Low Vibrio concentrations were generally seen in samples collected in Winter

** A corresponding increase in pathogenic Vibrios was noted at some locations

For Source water, this was only seen with surface water

Purple = Y1 for comparison

Seasonal Changes for Vibrio in Nurseries (Year 1 only)

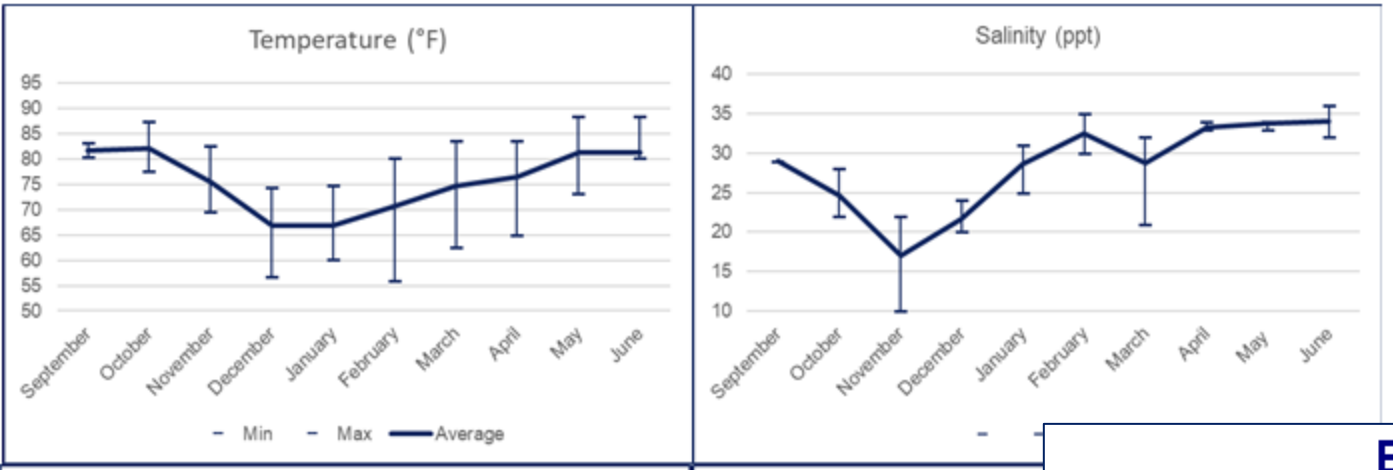
Winter to Spring		
Location	**Increase**	**Decrease**
Post-set	**67% (60%)**	**- (-)**
Wellers	**33% (60%)**	**- (-)**

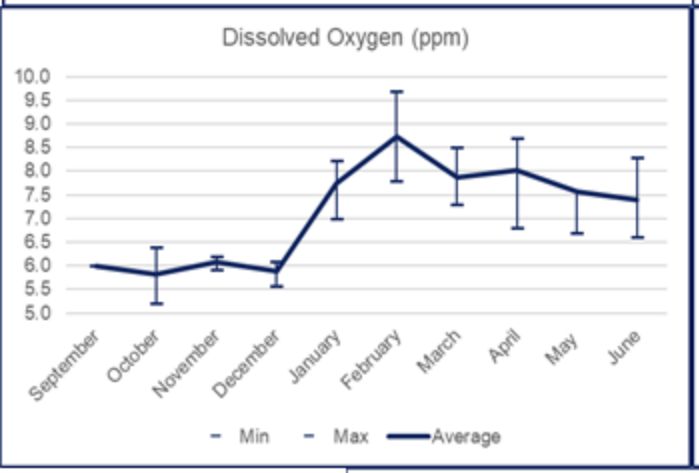
Spring to Summer*		
Location	**Increase**	**Decrease**
Post-set	**- (-)**	**50% (30%)**
Wellers	**75% (-)**	**- (30%)**

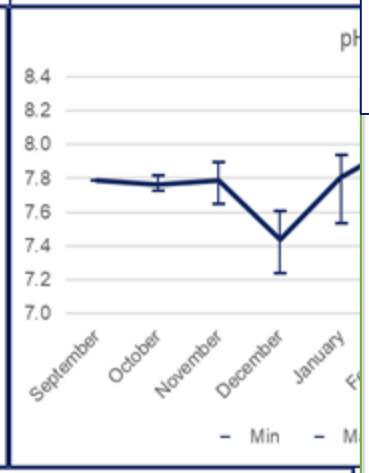
Low Vibrio concentrations were seen in samples collected in Winter

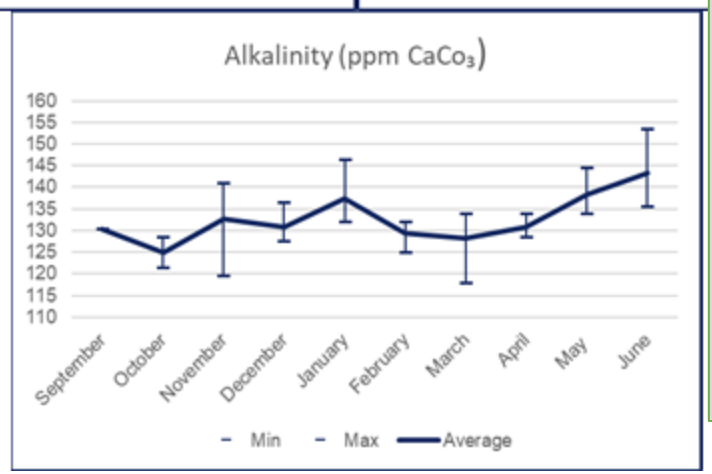
* *Increase in pathogenic Vibrios in one location*

Purple = Hatchery's in Y1

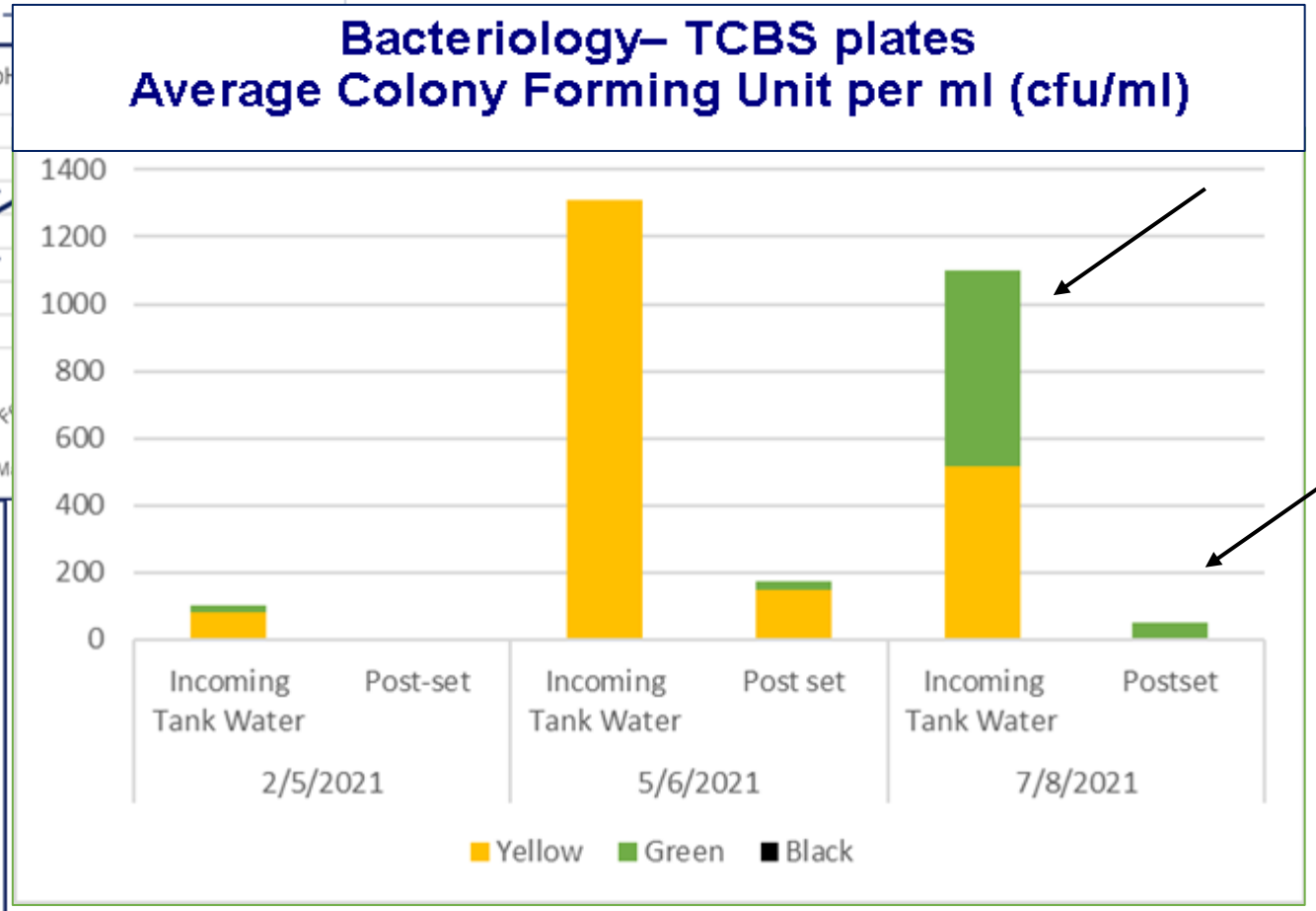








- **Increased bacterial growth concurrent with increased temperature rise in spring.**
- **Note increased number of pathogenic Vibrios in summer.**



Clam Seed Heath Pathology Report

Facility ID__G109_____

Date sampled __11/18/2020_____

Age/size __ Post-set__

Routine_____X_____ **Other**_____

Histopathology

Condition	**Prevalence**	**Location**	**Degree (rank or %)**
***Digestive Diverticula** _____	___NA_____	_____	___0-1____
Hemocyte accumulation _____	_____	_____	_____
Necrotic lesions _____	_____	_____	_____
Granulomas_____	_____	_____	_____
Tissue degradation_____	_____	_____	_____

Bacteria, parasites, etc.	**Prevalence**	**Location**	**Degree**
Bacteria _____	_____	_____	_____
Fungi_____	_____	_____	_____
****Protozoa**_____	_____	_____	_____
****Metazoa**_____	_____	_____	_____
Other_____	_____	_____	_____

Summary Comments

Healthy seed, no pathogens, or pathologies present; a few dead clams present but those that were alive look healthy.

Clam Seed Heath Pathology Report

Facility ID__G109_____

Date sampled __06/09/2021_____

Age/size __ +2 Post-set (tank 2), 5/25/ spawn__

Routine_____X_____ **Other**_____

Histopathology

Condition	**Prevalence**	**Location**	**Degree (rank or %)**
***Digestive Diverticula** _____	___NA_____	_____	___1____
Hemocyte accumulation _____	_____	_____	_____
Necrotic lesions _____	_____	_____	_____
Granulomas_____	____8%_____	____conn tissue__	___min (1)_
Tissue degradation_____	____16%_____	__dig div_____	___min____

Bacteria, parasites, etc.	**Prevalence**	**Location**	**Degree**
Bacteria _____	____8%_____	_____dig tract____	__min_____
Fungi_____	_____	_____	_____
****Protozoa**_____	_____	_____	_____
****Metazoa**_____	_____	_____	_____
Other_____	_____	_____	_____

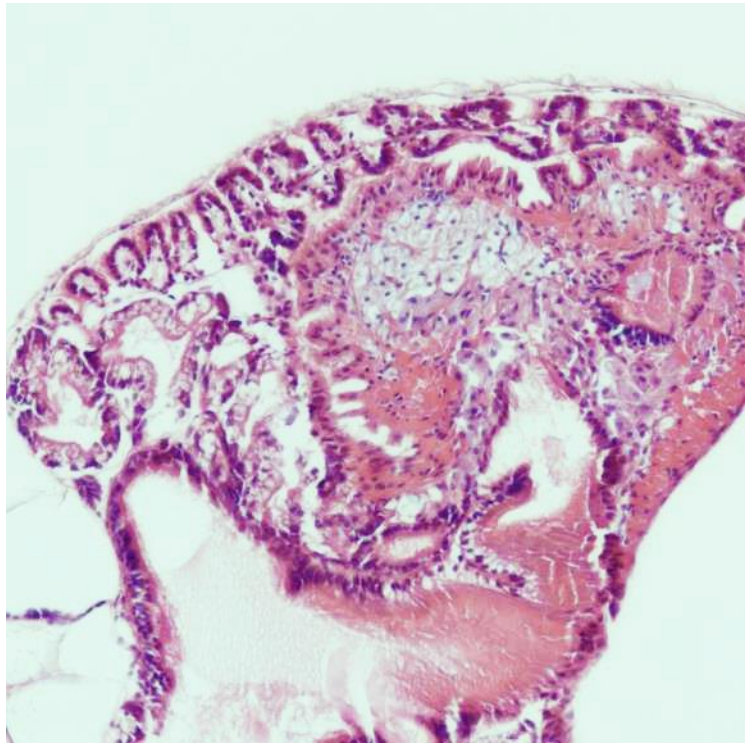
Summary Comments

Healthy seed, >75% feeding. One clam had a granuloma (foreign substance, not always pathogen) surrounded by hemocytes. A fifth of the samples had **concretions** in the digestive diverticula, and a small number had some tissue disintegration. Two clams had some digestive tract disintegration, one missing cells and 1 with some bacteria.

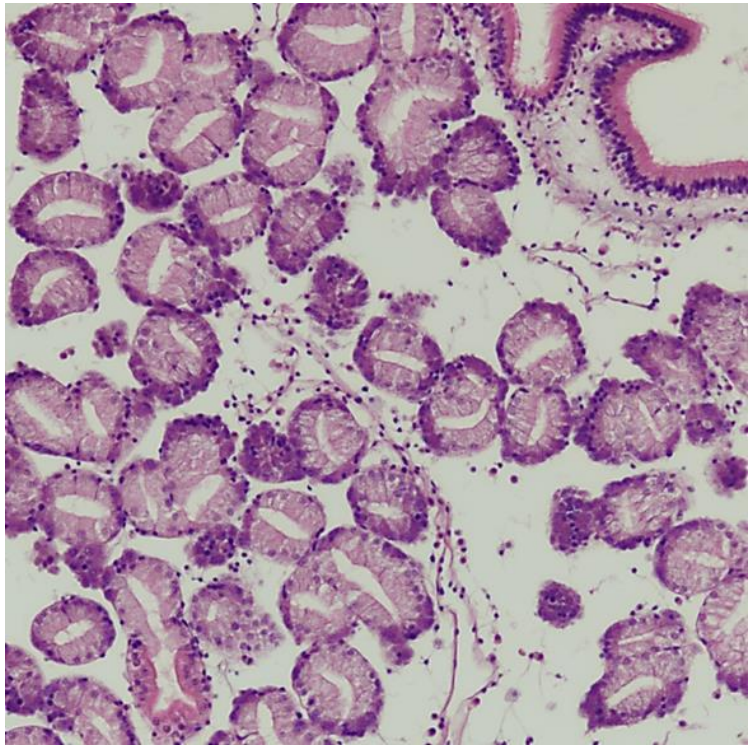
Clam Seed Health Report Example:

- Note increase in negative condition in summer

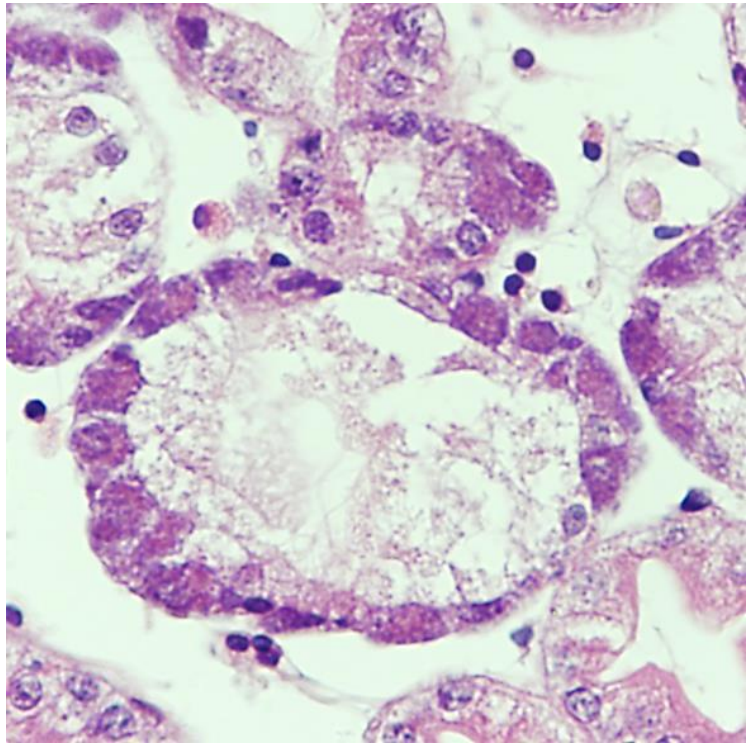
Histopathology: Condition



800-micron clam

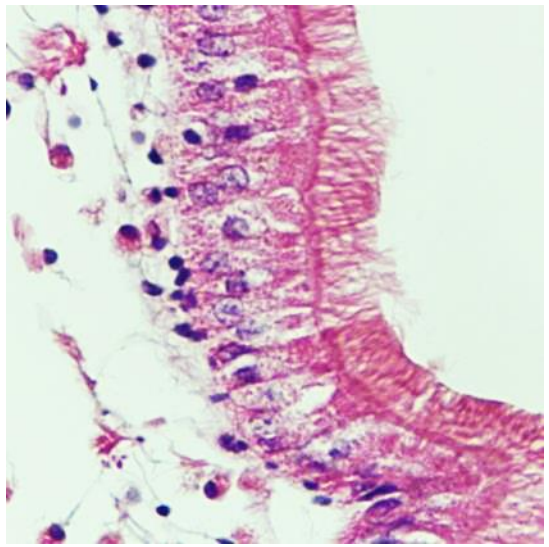


Digestive Diverticula - normal



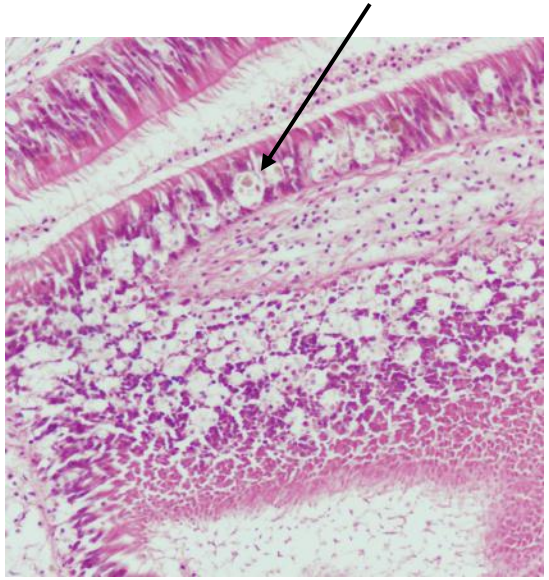
Digestive gland tubule disintegration

Histopathology: Condition

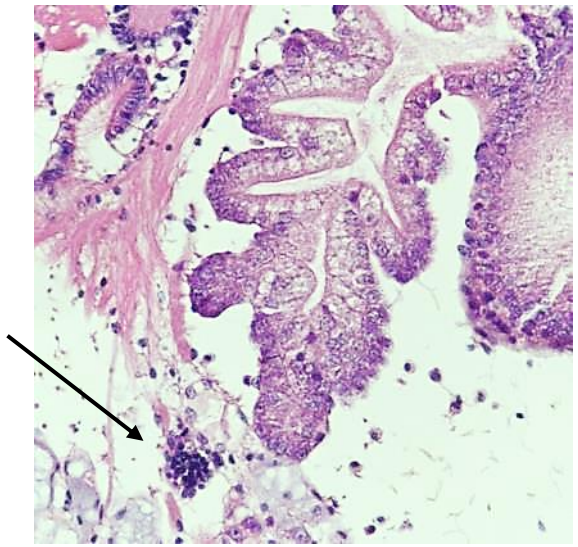


Normal digestive tract

Hemocyte accumulation

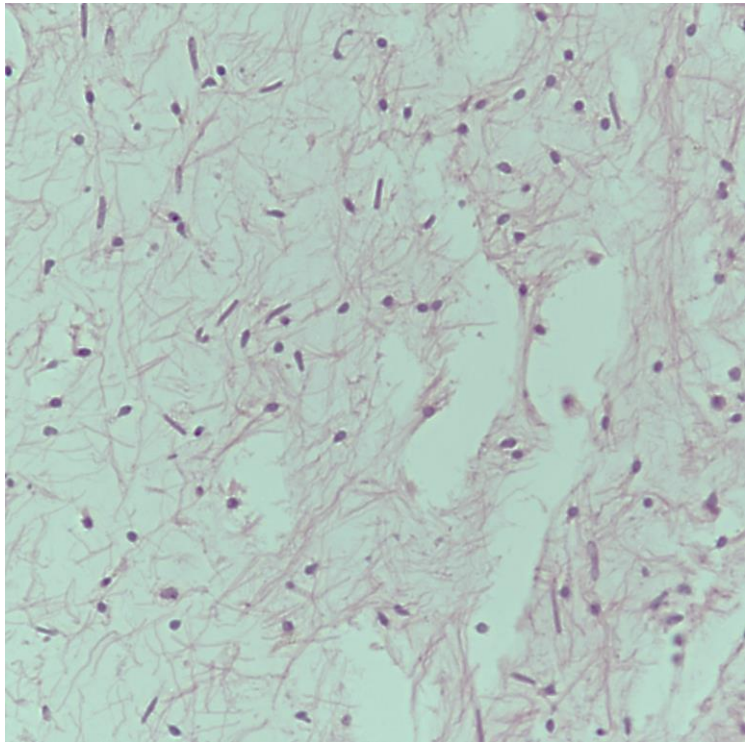


Concretions

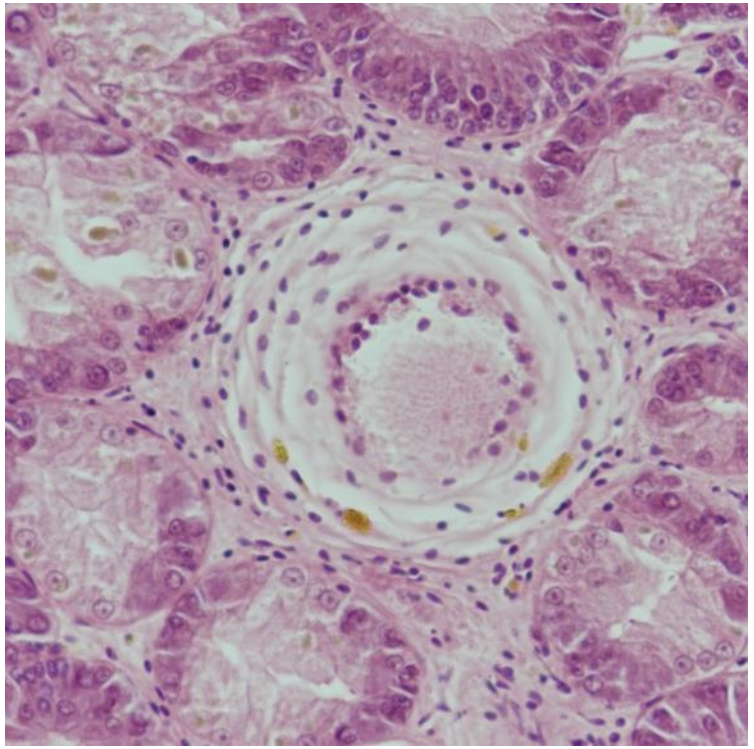


Developing granuloma
Hemocytes attacking bacteria

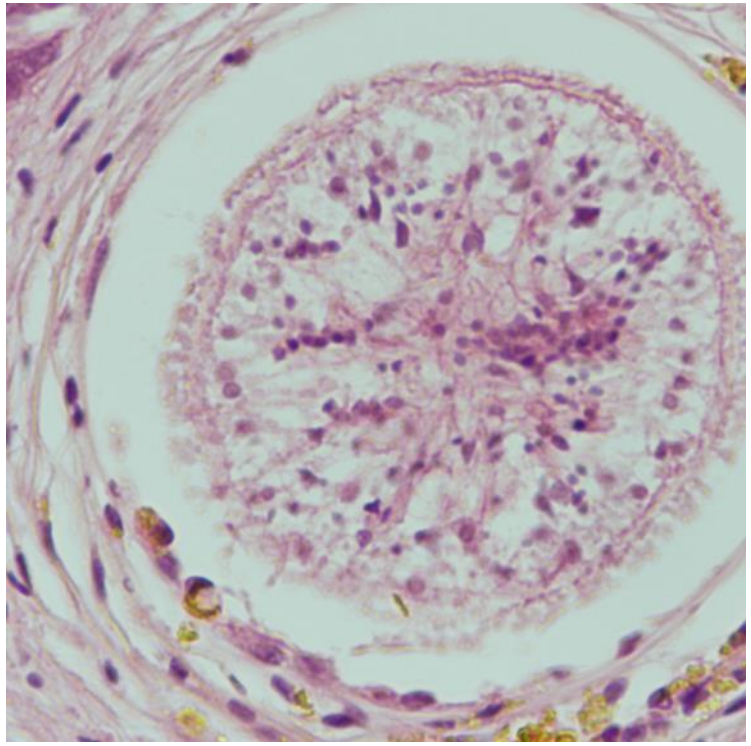
Histopathology: Bacteria and Parasites



Filamentous bacteria, hemocytes



Granuloma, hemocytes



Metazoan parasite

Hatchery Seed Health

Year 1 – Winter to Spring

Condition	Increase	Decrease
Hemocytes	60%	-
Necrosis/ Granulomas	20%	-
Tissue Degradation	80%	-
Pathogens		
Bacteria	60%	-
Protozoans	-	-
Metazoans	20%	-

- *Winter – 70% had no issues*

Year 1 – Spring to Summer

Condition	Increase	Decrease
Hemocytes	-	20%
Necrosis/ Granulomas	-	20%
Tissue Degradation	-	40%
Pathogens		
Bacteria	40%	20%
Protozoans	20%	-
Metazoans	40%	20%

- *Spring – 20% had no issues*
- *Summer – 40% had no issues*

Hatcheries that used surface water tended to have higher levels of clam digestive tubule destruction

Concretions = 20% winter, 33% spring and summer

Hatchery Seed Health

Year 2 – Winter to Spring

Condition	Increase	Decrease
Hemocytes	- (60%)	20% (-)
Necrosis/ Granulomas	50% (20%)	- (-)
Tissue Degradation	- (80%)	20% (-)
Pathogens		
Bacteria	33% (60%)	- (-)
Protozoans	33% (-)	- (-)
Metazoans	- (20%)	- (-)

- *Winter – 40% (70%) had no issues*

Year 2 – Spring to Summer

Condition	Increase	Decrease
Hemocytes	- (-)	- (20%)
Necrosis/ Granulomas	- (-)	33% (20%)
Tissue Degradation	- (-)	- (40%)
Pathogens		
Bacteria	33% (40%)	- (20%)
Protozoans	- (20%)	33% (-)
Metazoans	33% (40%)	- (20%)

- *Spring – 20% (20%) had no issues*
- *Summer – 40% (40%) had no issues*

Concretions = 67% Spring & Summer

Purple = Y1 for comparison

Nursery Seed Health

Year 1 – Winter to Spring

Condition	Increase	Decrease
Hemocytes	75% (60%)	25% (-)
Necrosis/ Granulomas	25% (20%)	25% (-)
Tissue Degradation	50% (80%)	- (-)
Pathogens		
Bacteria	100% (60%)	- (-)
Protozoans	- (-)	- (-)
Metazoans	25% (20%)	- (-)

- *Winter – 60% (70%) had no issues*

Year 1 – Spring to Summer

Condition	Increase	Decrease
Hemocytes	60% (-)	20% (20%)
Necrosis/ Granulomas	20% (-)	20% (20%)
Tissue Degradation	20% (-)	- (40%)
Pathogens		
Bacteria	40% (40%)	20% (20%)
Protozoans	40% (20%)	- (-)
Metazoans	40% (40%)	- (20%)

- *Spring – 20% (20%) had no issues*
- *Summer – 40% (40%) had no issues*

No concretions in any samples!

Purple = Hatchery's in Y1

Nursery Seed Health

Year 2 – Winter to Spring

Condition	Increase	Decrease
Hemocytes	- (75%)	25% (25%)
Necrosis/ Granulomas	25% (25%)	- (25%)
Tissue Degradation	25% (50%)	50% (-)
Pathogens		
Bacteria	75% (100%)	- (-)
Protozoans	50% (-)	- (-)
Metazoans	20% (25%)	- (-)

- *Winter – 40% (60%) had no issues*

Year 2 – Spring to Summer

Condition	Increase	Decrease
Hemocytes	40% (60%)	- (20%)
Necrosis/ Granulomas	- (20%)	- (20%)
Tissue Degradation	40% (20%)	- (-)
Pathogens		
Bacteria	20% (40%)	20% (20%)
Protozoans	20% (40%)	20% (-)
Metazoans	- (40%)	20% (-)

- *Spring – 40% (20%) had no issues*
- *Summer – 20% (40%) had no issues*

Concretions in 25% of spring samples

Purple = Y1 for comparison

Recommendations for Hatcheries

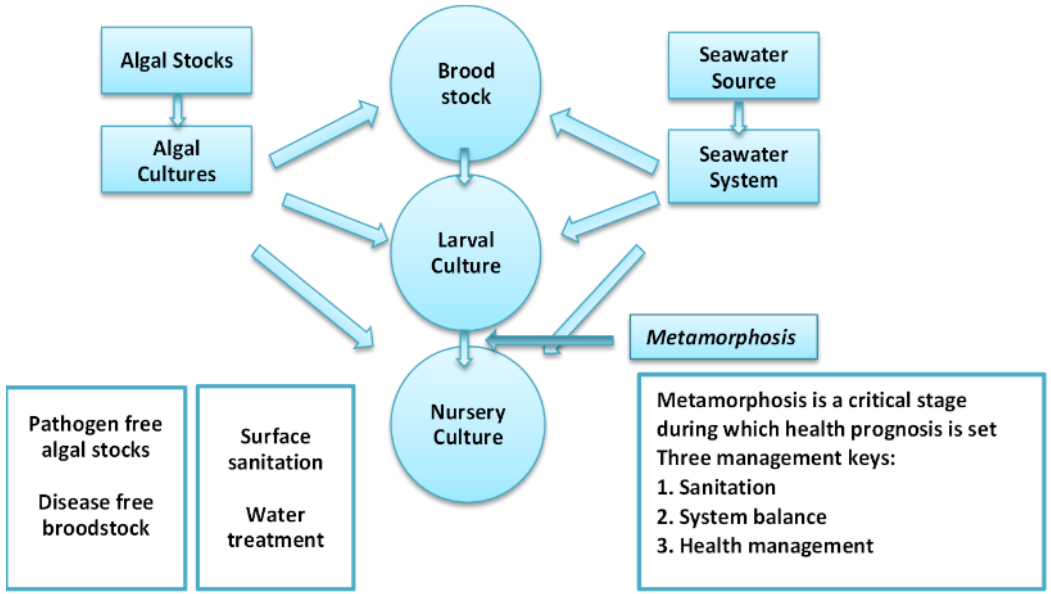
- **Where to test for Vibrio**
- **When to test**
- **What's acceptable**

Upper and lower acceptable Vibrio ranges

Location	Total Vibrios CFU/mL	Non-pathogenic CFU/mL (yellow)	Pathogenic CFU/mL (green)
Algae cultures	<10	<10	0
Incoming water	<10	<10	0
Tank Culture Water	1000 - 10,000	1000 - 10,000	10 - 100
Larvae/Post-set	10 - 100	10 – 100	0

**Expect to see highest number in broodstock tank water*

Identify bacteriological problems and how to locate them by process of elimination and systematic sampling:



Schematic diagram of intensive hatchery and nursery production of molluscan shellfish with notes regarding health management. *Adapted from Elston & War (2003).*

Emergency Samples

- **Additional samples submitted during mortality events**
- **Y1 = 5 emergency samples**
  - **Jan = 1, Feb = 1, Mar = 3**
  - **All samples showed high levels of *Vibrio*, including pathogenic *Vibrios***
    - **Histological confirmation**
  - ***Vibrio* populations in tank water were substantially lower than in clams**
  - **Both facilities used surface water**
    - **Algae and incoming water were tested to locate source in one facility**
    - **Incoming water was the culprit**
- **Y2 = 3 emergency samples**
  - **Mar = 1, Apr = 1, Sept = 1**
  - **Vibrio bacteria was NOT the cause of mortalities in 2 of the 3 cases!**

Vibrio Counts (CFU/mL)			
Source	**Total**	**Yellow**	**Green**
Larvae 1	93200	31200	62000
Tank water 1	1425	365	1060

Histopathology

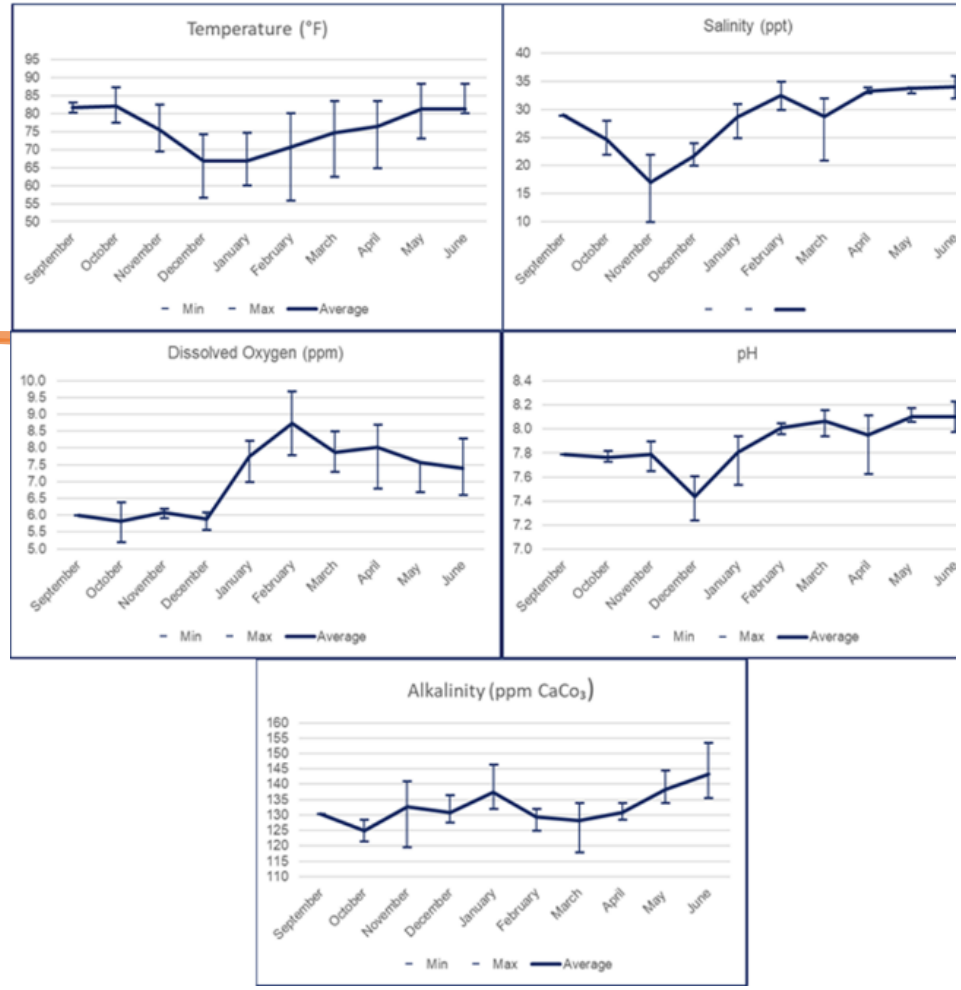
Condition	**Prevalence**	**Location**	**Degree (rank or %)**
***Digestive Diverticula** _____	___NA_____	_____	___3-4_____
Hemocyte accumulation _____	_____	_____	_____
Necrotic lesions _____	_____	_____	_____
Granulomas_____	_____	_____	_____
Tissue degradation____yes_____	__>75%_____	_Digestive tubules_	___>7%_____

Bacteria, parasites, etc.	**Prevalence**	**Location**	**Degree**
Bacteria _____	_____26%_____	__*CT, DT, DD_____	__Mod____
Fungi_____	_____	_____	_____
****Protozoa**_____	_____	_____	_____
****Metazoa**_____	_____	_____	_____
Other_____	_____	_____	_____

*CT=connective tissue, DT= digestive tract, DD= digestive diverticula

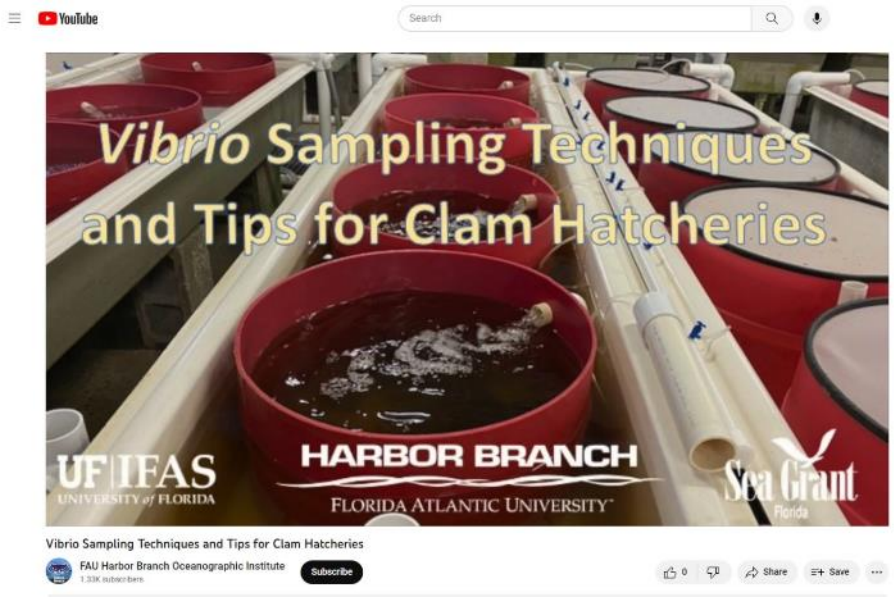
Summary and Recommendations

- Vibrio load increases with higher temperatures
- Parasite load increases with higher salinities
- Low DO & high ammonia causes additional stress
  - *No associations were noted in this study*
- Bacterial and parasitic problems ↓ winter and ↑ in spring
- Hatchery operators
  - Lower stress
  - Temperature control spring & summer
  - Bacterial testing of “hot spots” spring & summer
- Nursery operators
  - Prayer, meditation, a cold beverage?



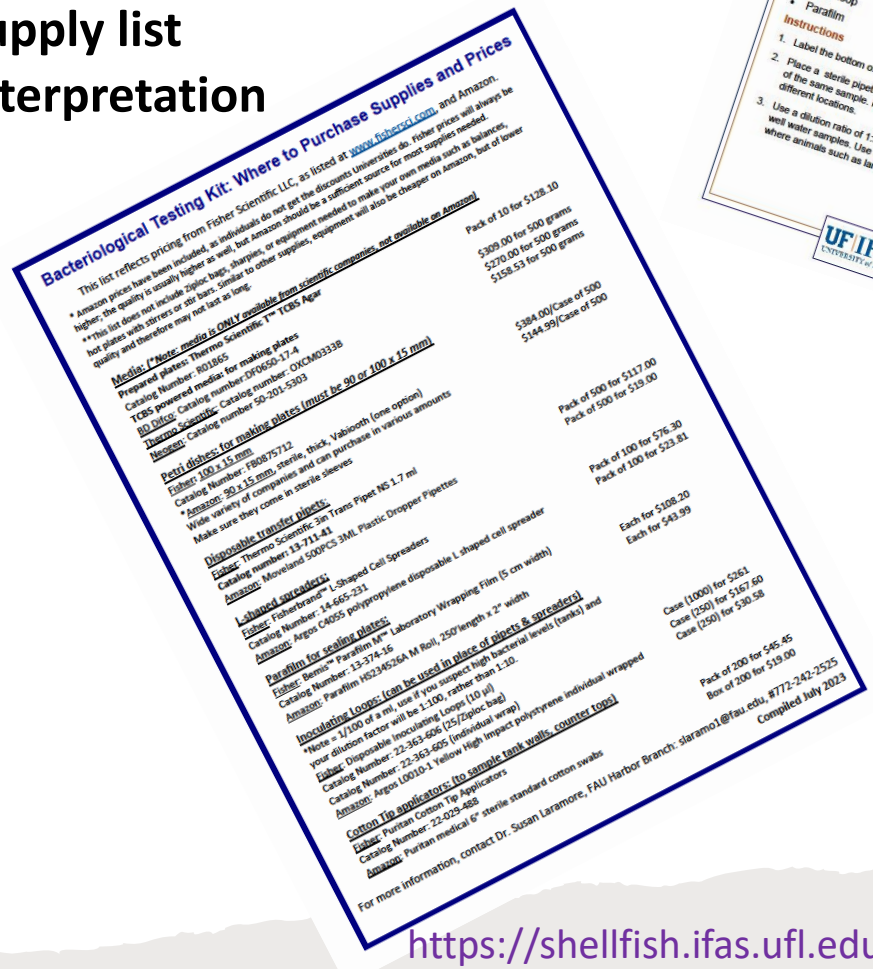
Additional Resources

- **Video**
  - Sampling
  - Media prep
  - Plating
  - Interpretation



https://youtu.be/SUQ1vSvfg10

- **Handouts**
  - Prevention and Management
  - Plating Instructions
  - Supply list
  - Interpretation



https://shellfish.ifas.ufl.edu/clam-seed-project-2020-22/