

Managing Diseases In Bivalve Hatcheries Through Bacterial Monitoring

Shellfish Workshop Series
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Adapted from Laramore & Laramore FL Clam Industry Workshop 2015 & Ralph Elston, FL Clam Ind Wksp, 2008

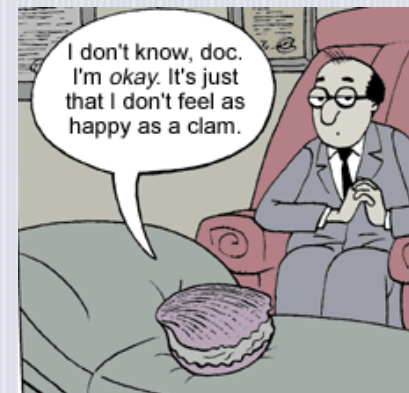
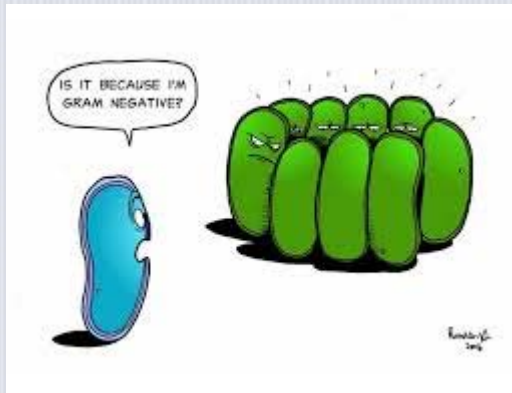


Health Management Goals.....

- **Predictable production**
- **High survival to and after sale**
- **High growth, health and condition**
- **Efficient, profitable production**
- **Minimization of waste**
- **Compliance with regulatory requirements**

Health Management Topics

1. Water quality monitoring and management
2. Bacterial contamination



Hatchery & Nursery Health Management

- **Water source(s), quality and management**
- **Brood stock source, condition and management**
- **Larval and juvenile handling and management**
- **Micro-algal food culture management**
- **Bacterial monitoring and management**

Water Quality Monitoring

Measured parameter	Approximate recommended range
Rearing water temperature	Depends on species reared.
pH	7.8 to 8.4 units
Salinity	Depends on species reared
Dissolved oxygen	> 5.0 mg/L, < 5% over saturation
Oxidation reduction potential (ORP)	150-250
Nitrogen cycle	<i>Ammonia</i> : 0.1 ppm generally safe. <i>Nitrite</i> : 0.2 ppm generally safe. <i>Nitrate</i> : 16 ppm in SW
Hypochlorite	None detectible
Alkalinity	110-140; few adverse consequences if higher than 200 ppmCaCO ₃ .
Total dissolved gas saturation	< 5% greater than saturation

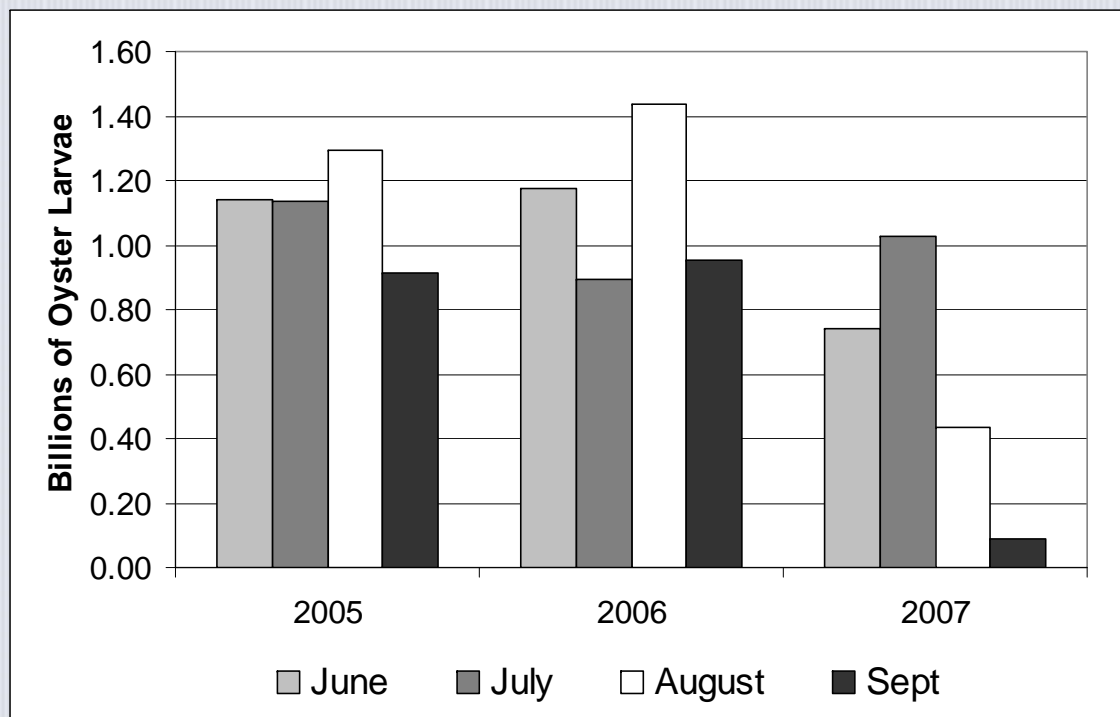


Water Treatment.....

- **Multimedia filters**
 - Reduction of suspended solids
 - Removes bacteria that stick to the filter
 - Back flush with filtered water, not raw seawater
- **Cartridge filters**
- **Charcoal filtration**
- **UV filtration**
- **Protein skimmers**
- **Bioreactors**
- **Addition of conditioning agents for alkalinity and pH control**
- **Probiotics**



Consequence of Vibriosis

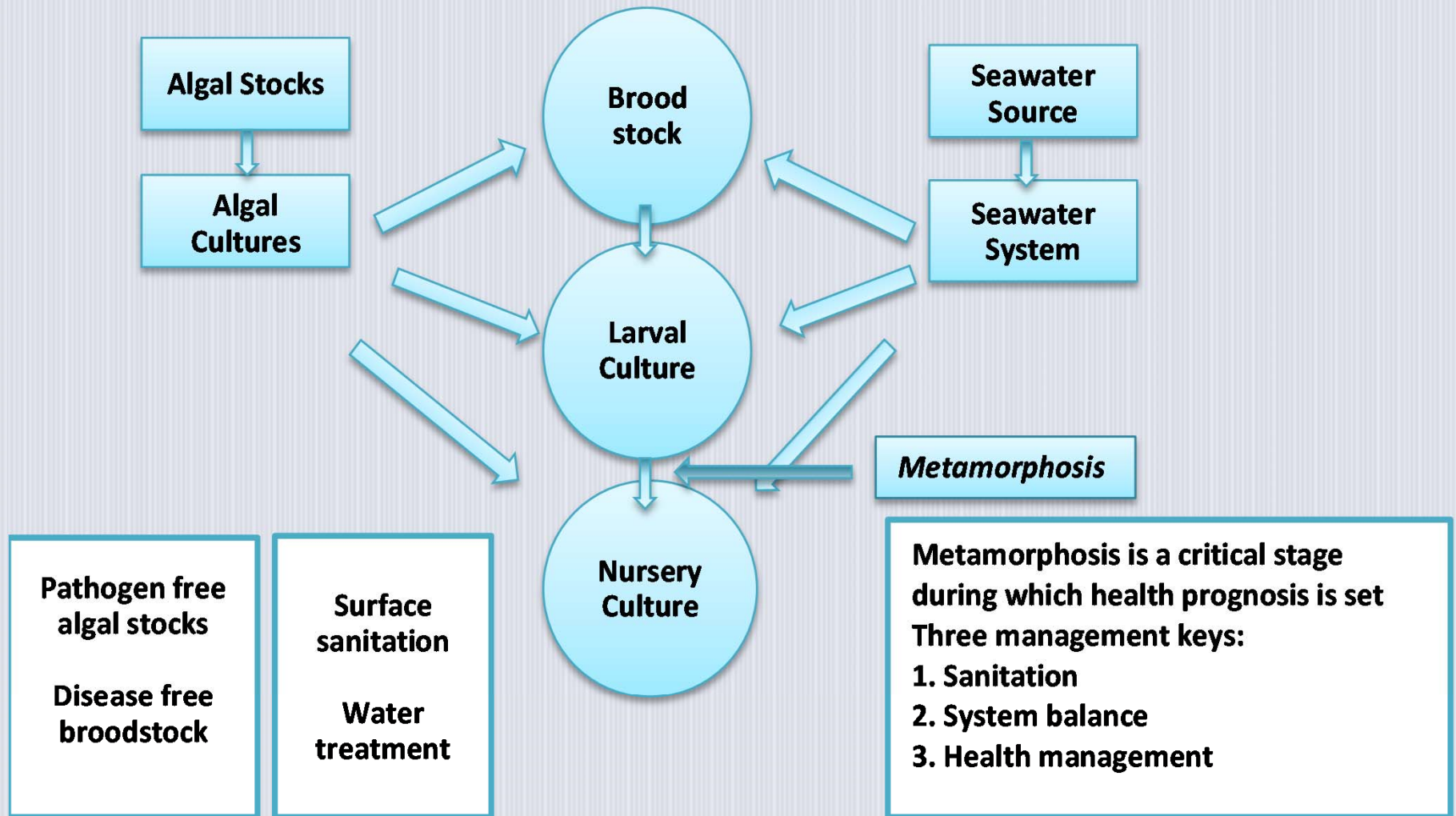


West coast shellfish hatchery...**Production drop of 51% in one year**

Bacterial Monitoring

- **Where to sample?**
 - *All locations of input...*
- **How often to sample?**
 - Routine monitoring
 - More often during warm weather
 - More often if continual problems
- **Sampling techniques**
 - Sterile technique
 - Proper equipment
 - Steady hand 😊
- **Interpretation**
 - What do my results mean?
- **Remediation**
 - Details provided in handout and during laboratory session*

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



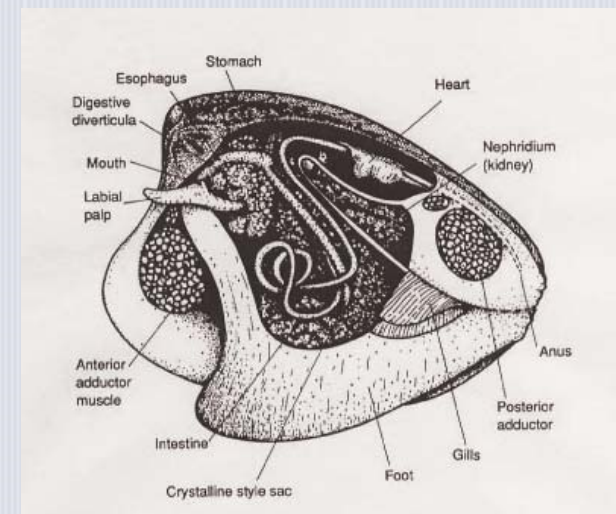
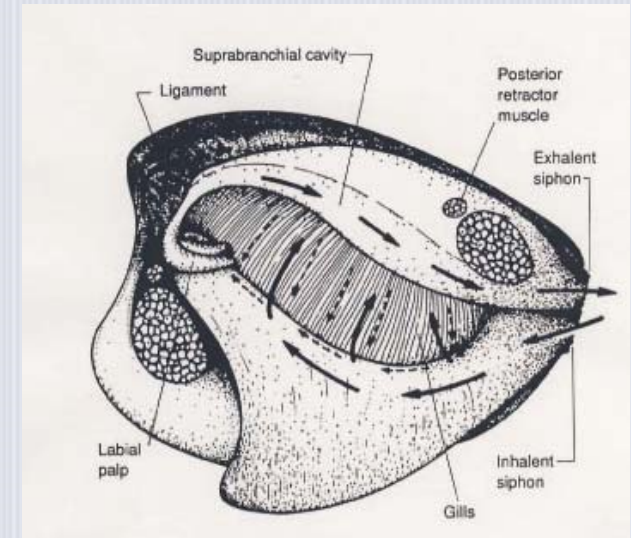
Schematic diagram of intensive hatchery and nursery production of molluscan shellfish with notes regarding health management. *Adapted from Elston & War (2003).*

High Risk Locations

- **Larval tank bottoms**
- **Brood stock conditioning systems**
- **Areas with high humidity**
- **Wet areas that have high air flow**
 - air coolers and condensation
- **Sodium thiosulfate stock solutions**
 - Contamination increases with age
 - Refrigerate

Broodstock...

- **Free from reportable diseases**
 - Need for a Shellfish High Health Program for every shellfish farm
- **Condition factor**
 - If naturally conditioned, condition could be variable
 - Possible bacterial or parasitic contamination
- **Hatchery conditioning tanks**
 - Potential source of bacterial contamination
- **Contamination *can be* transferred to eggs & larvae**
 - But possibility is low
 - high degree of dilution



Algae Culture

- **Bacteria often co-exist with algae**
 - If they are pathogenic species and are fed to larvae
- **Start with:**
 - Clean stock cultures
 - Sterile water
 - Sterile equipment
- **Sterile technique during transfer**
- **Minimize contamination during expansion of culture**
 - (Flask to carboy, etc.)



Example of a West coast hatchery with *Vibrio tubiashii* contamination*

Sample Type	Number of Samples	<i>Vibrio spp.</i> as % composition of total 48 hour plate counts (average)	Average concentration of <i>Vibrio spp.</i>	Maximum observed concentration of <i>Vibrio spp.</i>
Microalgal stock cultures	12	85%	5.44×10^5	2.01×10^6
Microalgal carboy cultures	6	83%	3.52×10^5	6.72×10^5
Microalgal continuous flow bag cultures (vertical)	38	49%	2.60×10^4	1.32×10^6
Microalgal continuous flow bag cultures (horizontal)	13	66%	3.60×10^4	6.00×10^5
Microalgal static tank cultures (20L to 25,000 L volume)	31	34%	7.20×10^3	3.92×10^5
Larval tank water	22	35%	1.06×10^3	3.28×10^4

*Data from R. Elston et al. 2008

***V. tubiashii* contamination in hatchery air supplies and algal culture rooms***

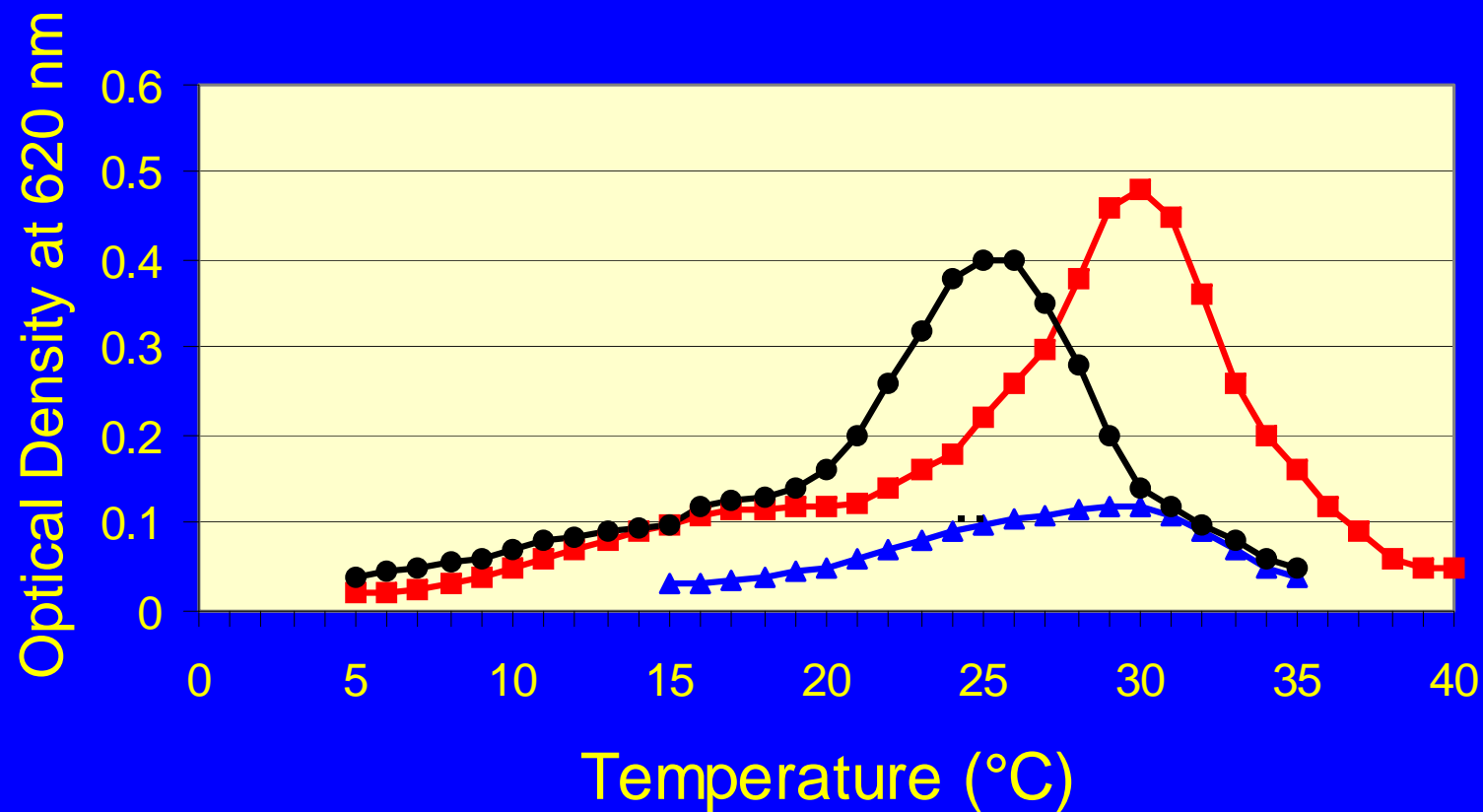
Sample Type	<i>V. tubiashii</i> (cfu/minute)	Average Relative % Humidity	Average Temperature (°C)
Algae stock transfer room air, static plate	up to 0.3	65	23
Algae carboy and small tank culture room, static plate	6.7	65	23
Air conditioner air flow in tank culture room	36	77	23
Tank room carboy air system, air flow	234	77	20
Tank room tank air system, air flow	> 2,000	77	20
Larvae airline, air flow	1500		

*Data from R. Elston et al. 2008

Wetter air = more bacteria

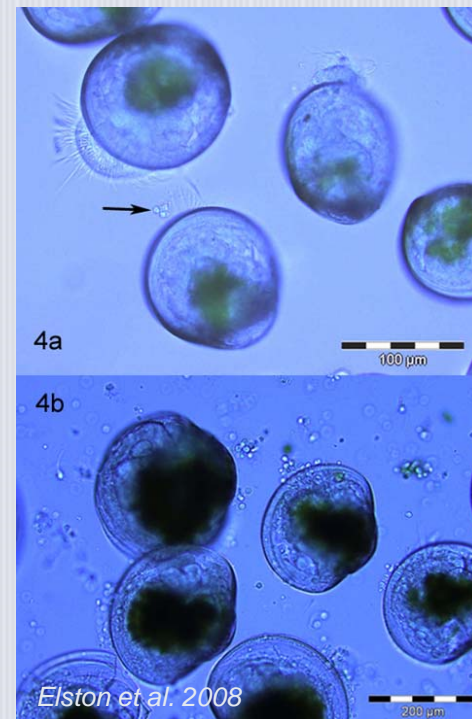
Solutions: drier air and/or air disinfection systems

Temperature Effects: Growth Response of various bacteria associated with juvenile shellfish mortality



Vibriosis can be “acute” (fast acting) or “chronic” (slow & debilitating):

- Poor larval survival
- Slow Growth
- Shell deformations
- Poor nursery & out plant survival & growth



Vibrio Pathogenicity of larvae and seed depends on...

- **Age and species**
- **Concentration of pathogenic *Vibrios***
- **Temperature**
- **Growth phase of pathogenic *Vibrios***
- **Degree of toxin production**
- **Other stress factors**
 - Water quality, nutrition

Pathogenic *Vibrio* "carry over"....

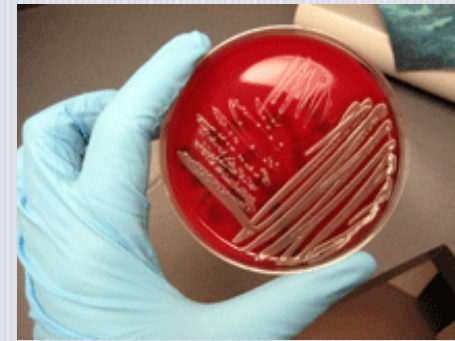
- Larval contamination can carry over to nursery seed....
 - Seed are too dense
 - Water flow is poor
 - Floating upwellers
- Non-larval associated infections may also occur
 - Result in loss of large amount of seed

Summary

- Test for and eliminate (reduce), bacterial load starting with highest risk areas
- Requires sustained effort and constant management
- Water filtration:
 - Filters need to be cleaned of particulate and large debris and disinfected during periods of high *Vibrio* load
 - Removal of particulates aids in the removal of many bacterial cells
 - Sterilization removes majority of bacteria

Bacterial Sampling and Culture

- Media preparation
- Sample collection
- Bacterial techniques
- Interpretation of results



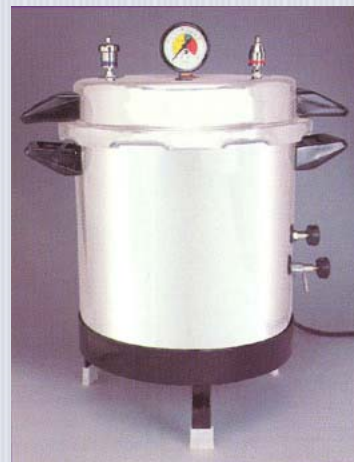
Growth Media

- Provides a suitable environment for growth.
- Contains a mixture of nutrients that microbes need to live and grow.
 - Provides a surface & the necessary moisture & pH to support microbial growth.



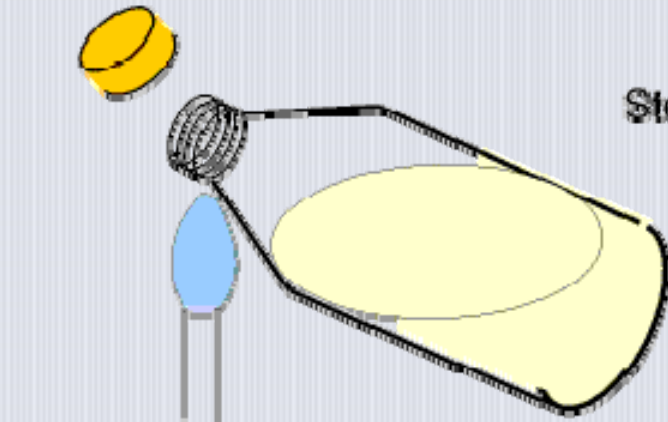
How is Media Made?

- Measure out a quantity of dry powdered media, add distilled water, mix well (stir bar) & heat to boiling
- **Cover top of flask and autoclave.**
 - The autoclave exposes the media to high temperature (121°C) and pressure (15 psi) for 20 minutes.
 - Once the media is autoclaved (*or pressure cooked*) it is considered sterile (*all life forms killed*) and can be used to make plates.
 - This is similar to home canning techniques in food preservation
- ***Note: not all media requires autoclaving**
 - Example: TCBS (*Vibrio* selective media)



"Pouring a Plate"

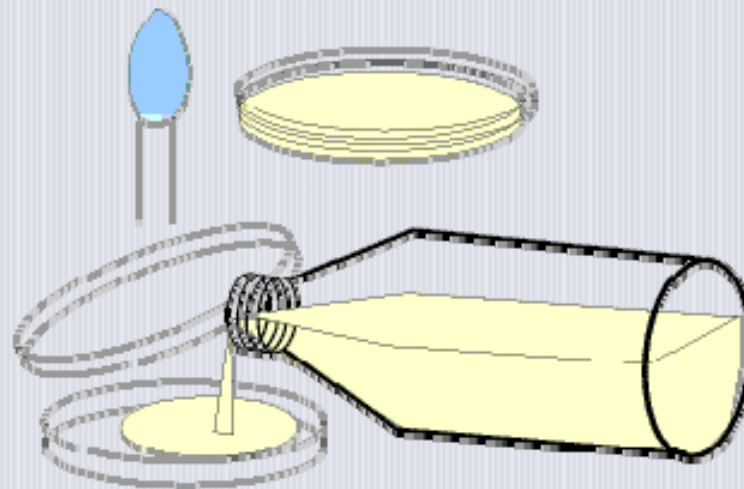
Sterilised molten agar is poured in and left to set.



Neck of agar bottle is passed through flame



Petri dish lid is opened as little as possible, angled and kept over the base.

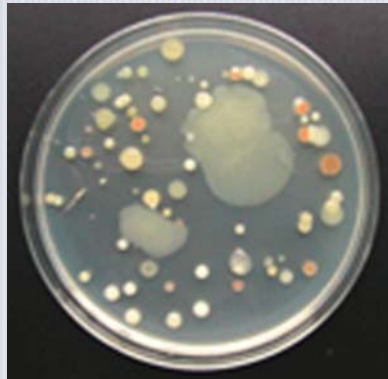


Each Petri dish hold about 20 ml, so 200ml will do for 10.

Media & Aquaculture

Marine Agar

- Non selective for marine bacteria
- Used to obtain total bacterial counts
- Grow a variety of bacteria



TCBS

- Selective for *Vibrio spp.*
- Yellow colonies
 - May be pathogenic
- Blue-Green colonies
 - Are pathogenic



Plating Procedure: Spread Plates

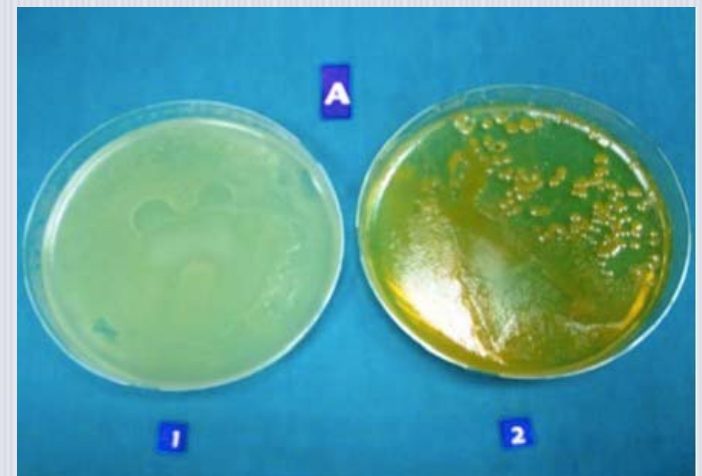
- **Labeling the plate**
 - With a sharpie label the bottom half of the plate (media half)
 - Date, initials, collection site, dilutions
- **Adding the sample:**
- **To determine presence/absence**
 - Swab
- **To quantify the amount**
 - Add 1/10 or 1/4 of a ml to plate and spread
 - Or directly streak sample with a loop
 - Loop (10 and 1 μ l)



Bacterial Plating Procedure

- **Storing the Plate**

- Seal plate with parafilm or tape
 - Keeps plate moist, keeps bugs out
- Place upside down (media side up) in incubator or plastic tub
 - Prevents "spreaders"
- Observe in 24 and/or 48 hours



Numbers and Interpretation

- **Counting colonies**
 - If you have made a dilution multiply # of colonies by dilution factor
 - Dilutions that give 30-300 colonies are preferred
 - If too many, divide plate with a marker & count a portion
 - Don't forget to multiply!
- **Interpretation**
 - How much is too much?
 - Depends on sampling source
 - Total counts (Marine Agar)
 - Vibrio counts (TCBS)
 - Yellow colonies
 - Blue-green colonies

