Increased Bivalve Hatchery and Nursery Production Through Improved Management

Florida Clam Industry Workshop
Fau-HBOI
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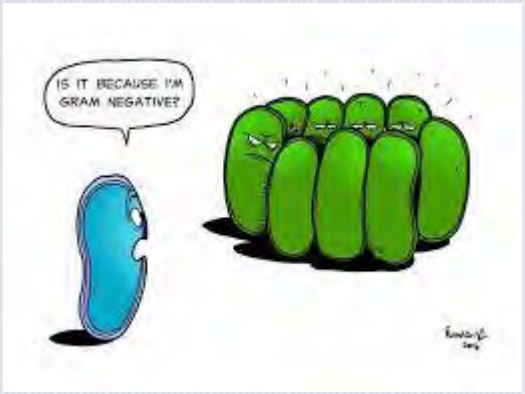
Adapted from Ralph Elston, FL Clam Ind Wksp, 2008

Health Management Goals……

- **Predictable production**
- **High survival to sale 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. **Pathogen contamination, usually bacterial**
3. **Animal condition assessment**



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**

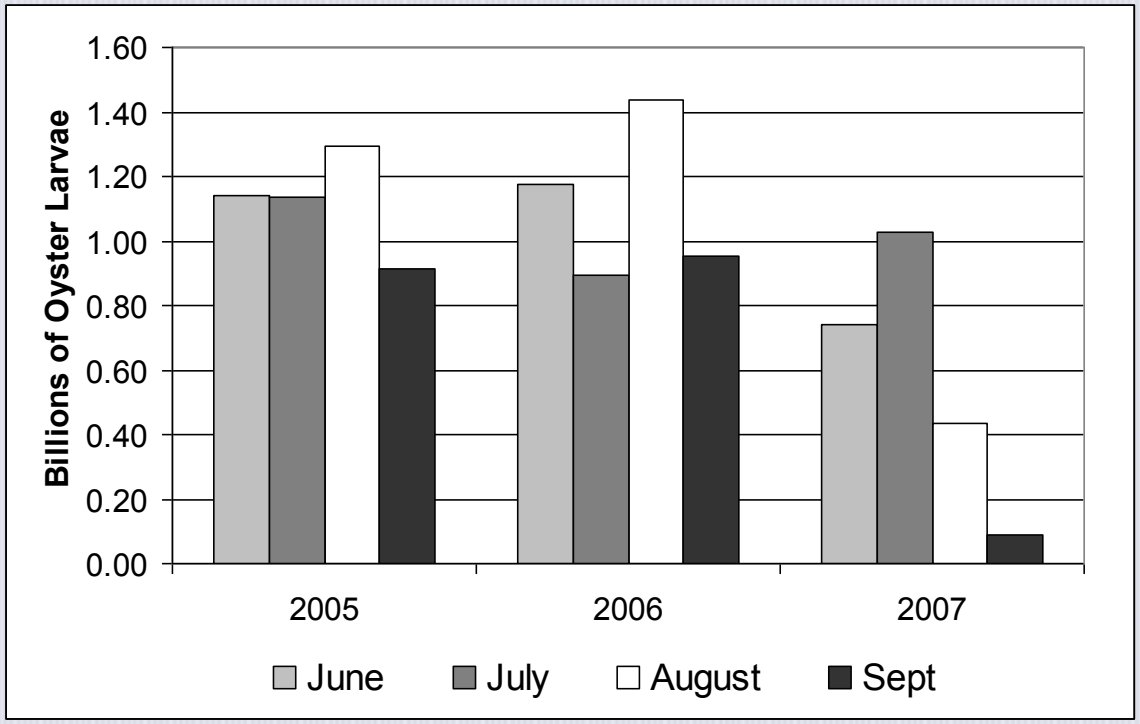
Water Quality Monitoring

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	*Ammonia:* 0.1 ppm generally safe. *Nitrite:* 0.2 ppm generally safe. *Nitrate:* 16 ppm in SW
Hypochlorite	None detectible
Alkalinity	110-140; few adverse consequences if higher than 200 ppm$CaCO_3$.
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
- Probiotics

Consequence of vibiosis, in a West coast shellfish hatchery……



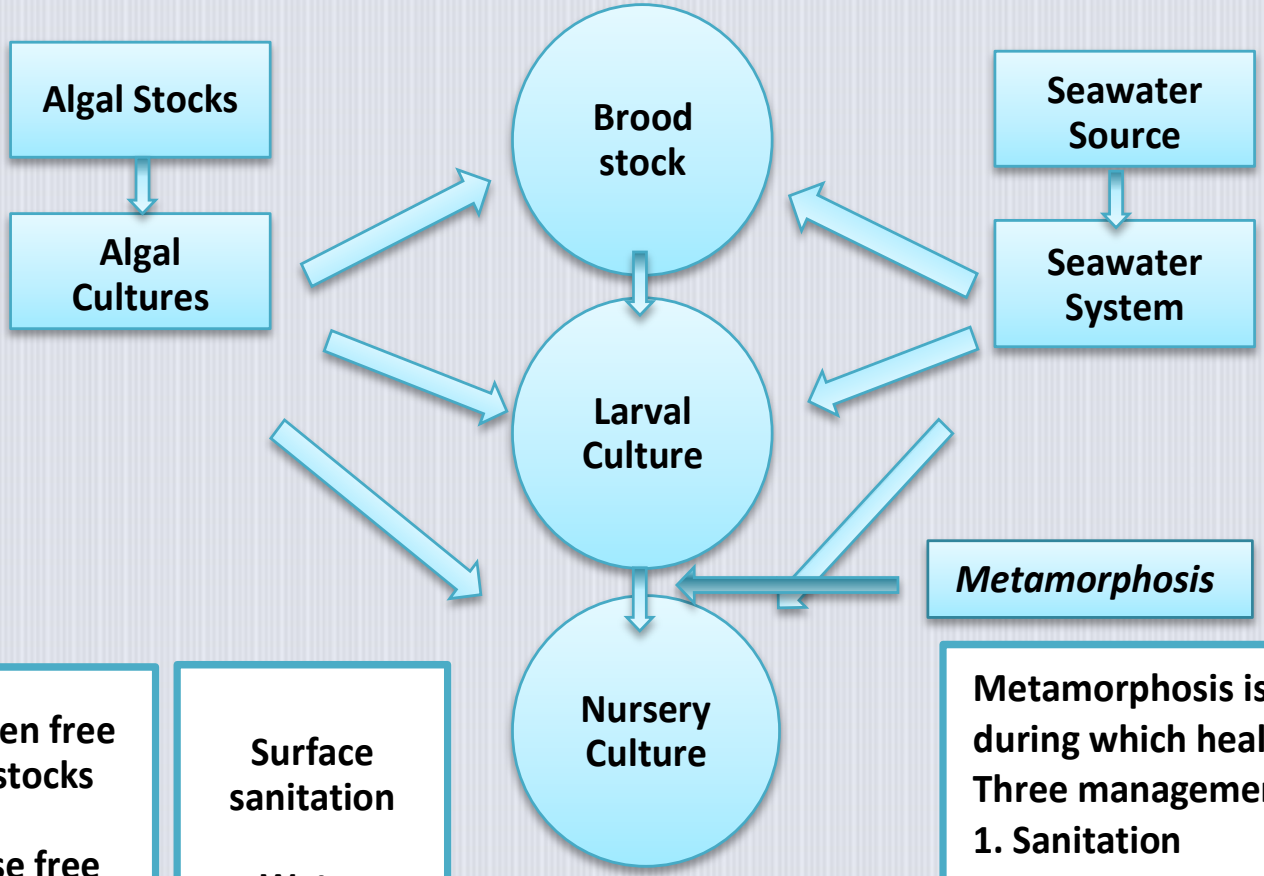
Production drop of 51% plus in 2007…

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:



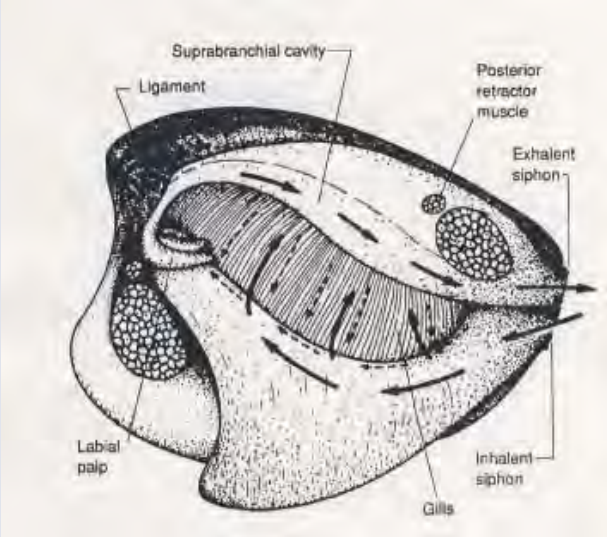
Schematic diagram of intensive hatchery and nursery production of molluscan shellfish with notes regarding health management. *Adapted from Elston & War (2003).*

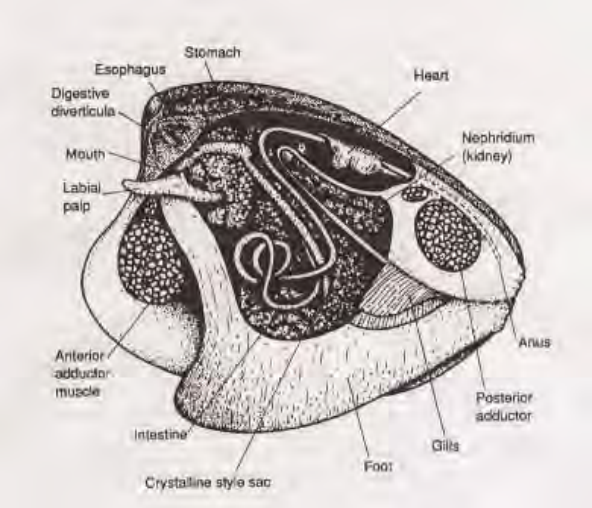
High Risk Locations

- **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…generally there is a 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**
- **Minimization of contamination during expansion of culture**

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

Sample Type	Number of Samples	*Vibrio spp.* as % composition of total 48 hour plate counts (average)[a]	Median concentration of *Vibrio spp.*	Maximum observed concentration of *Vibrio spp.*
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*.....

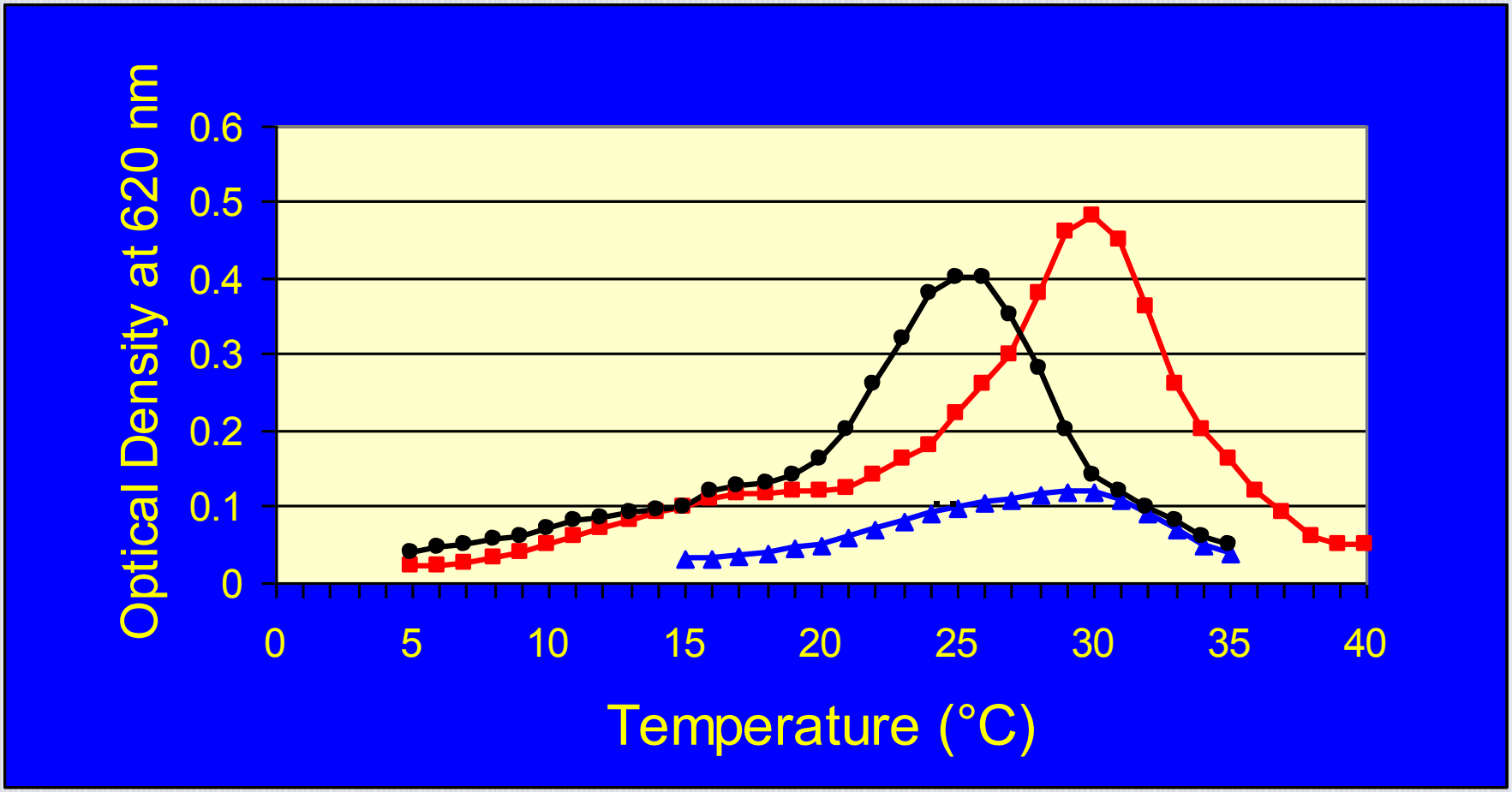
Sample Type	*V. tubiashii* (cfu/minute)	Average Relative % Humidity	Average Temperature (°C)
Algae stock transfer room air, static plate	up to 0.3	65	23
Algae carboy and small tank culture room, static plate	6.7	65	23
Air conditioner air flow in tank culture room	36	77	23
Tank room carboy air system,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

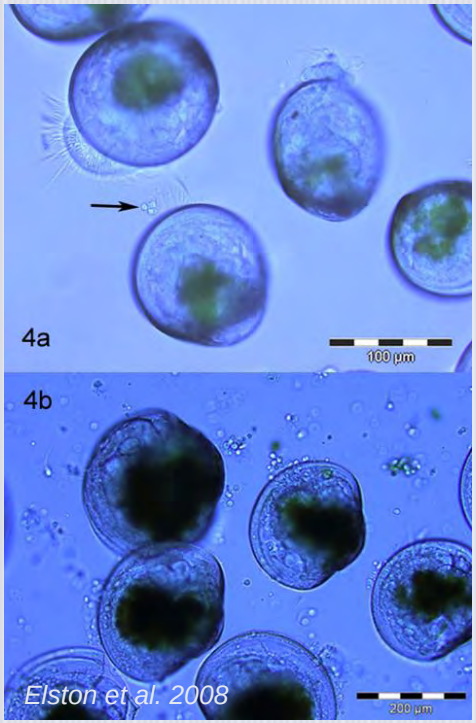
Solutions: drier air and/or air disinfection systems

Temperature Effects: Growth Response of selected bacteria associated with juvenile shellfish morbidity



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 of larvae or juveniles and species**
- **Concentration of pathogenic *vibrios* (dose) in seawater**
- **Temperature of seawater**
- **Growth phase of pathogenic *vibrios***
- **Degree of toxin production by pathogenic *vibrios***
- **Other stress factors**
  - **Water quality, nutrition**

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

- **Larval contamination can carry over to nursery seed....**
- **Particulary if seed are too dense, or if water flow is poor**
  - **Floating upwellers**
- **Invasive infections may also occur and take down large numbers of seed**

Summary

- **Test for and eliminate (reduce), bacterial load starting with highest risk areas**
- **Requires sustained effort and constant management**
- **Water filtration and source:**
  - **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

The purpose of this lab is to expose you to the basics of:

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

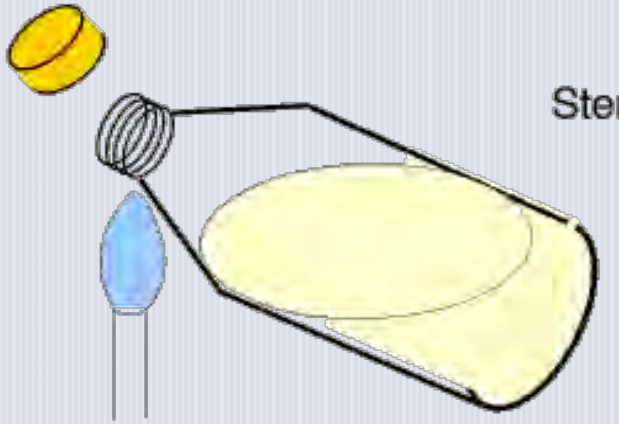
Growth Media

- In order to successfully grow bacteria we must provide an environment suitable for growth.
- Growth media *(singular = medium)* are used to cultivate bacteria.
- Media = mixtures of nutrients that the microbes need to live.
  - Provides a surface & the necessary moisture & pH to support microbial growth.

How is Media Made?

- Measure out a quantity of dry powdered nutrient media, add distilled water, mix well & heat to boiling
- Cap it and autoclave.
  - This is similar to home canning techniques in food preservation.
- 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)*.

"Pouring a Plate"



Neck of agar bottle is passed through flame

Sterilised molten agar is poured in and left to set.

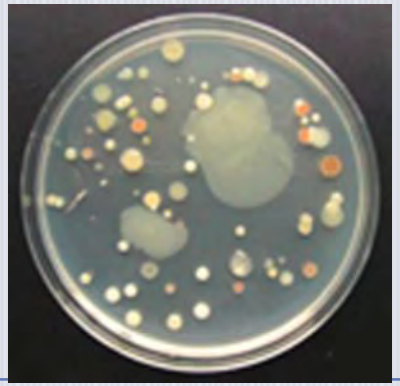
Petri dish lid is opened as little as possible, angled and kept over the base.

- 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
  - Pathogenic

Bacterial Plating Procedure

- Labeling the plate
  - With a sharpie label the bottom half of the plate (media half)
  - Date, initials, collection site, dilutions
- Adding the sample
  - Directly streak sample
    - Quantify
      - Loop (10 μl)
    - Presence/absence
      - Swab
  - Dilute sample in sterile seawater
    - For total counts or…
    - If you suspect high numbers of *Vibrio*

Bacterial Plating Procedure

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

Bacterial Plating Procedure

- 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