SAMPLE PROCESSING & ANALYSES – Role of the Support Team

Module 6

Support Team Land or On-Water

- **Three Person Team:** 2 processors & 1 snorkeler
- **Collect information for a ‘case history’**
  i.e., site history, inputs to the system, weather conditions, unusual conditions or observations
- **Photo documents above water line operations**
- **Stabilizes, processes, and ships samples**

Snorkeler

- Monitors Pelican Floats for Survey Team
- Ferries samples to the boat, sent from divers

Sample Processing Topside

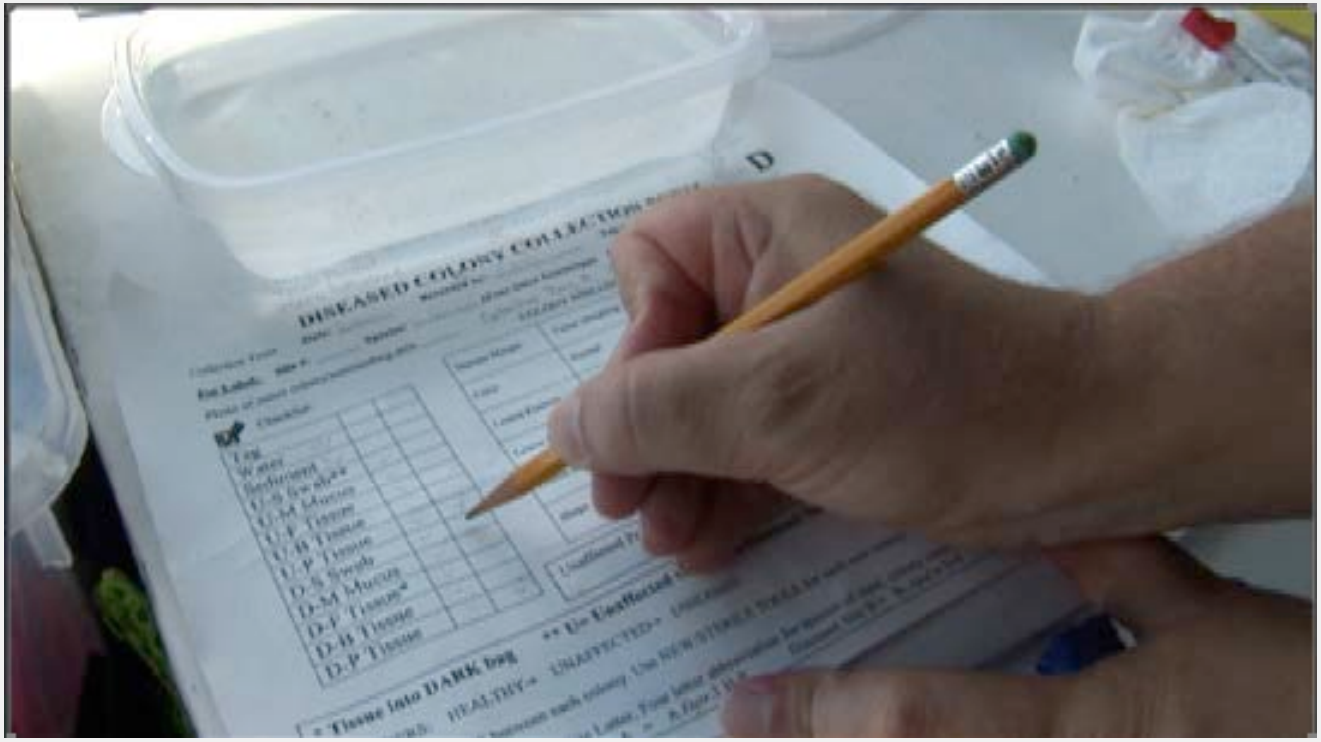
Support Team- Sample Technician

Each sample has a predetermined experimental or analytical role, which defines how each will be processed on the boat.

Specific Sample Processing Due to Time Sensitivity

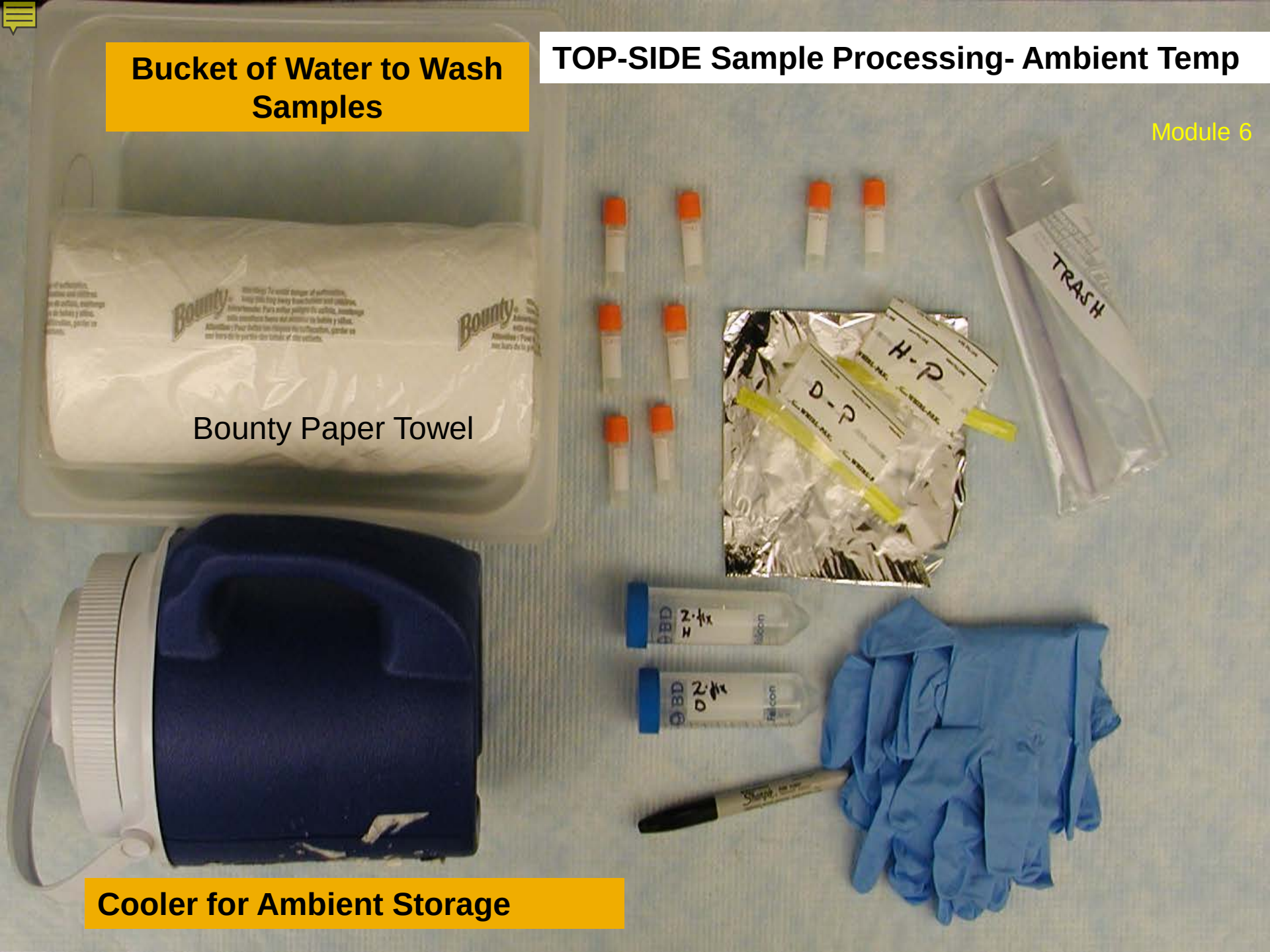
***Note- these are opposite the order of Sampling**

Tissue-P – Most Time Sensitive
Tissue-F
Tissue-B
Swab Mucus
Sediment
Water



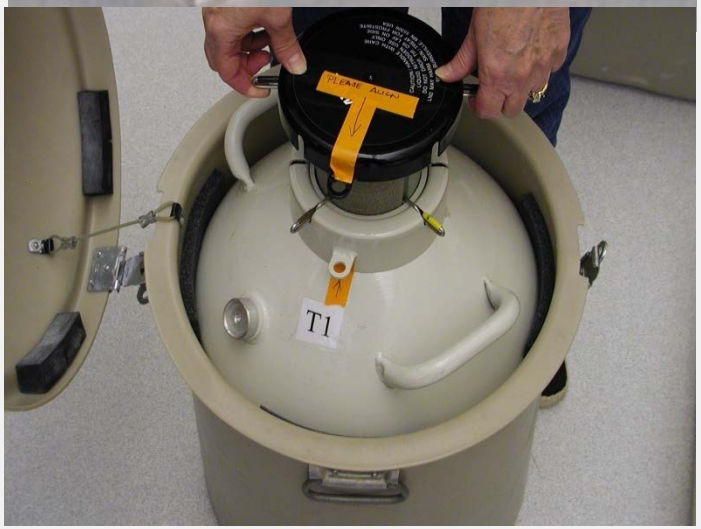
TOP-SIDE Sample Processing- Ambient Temp

Module 6



Tissue-Protein

- **Most Time Sensitive Samples**
- Need to be processed in shaded area away from direct sunlight
- Rinse mucus from tissue by swishing in clean seawater
- Dab on Bounty® paper towel
- Wrap in aluminum foil with a waterproof paper label
- Place in Cryoshipper



Tissue-Histology

Histological samples

- Transfer tissue biopsy to a 50cc polypropylene tube with 25 mL of fixative, i.e., Z-fix diluted 4:1 with sterile ASW (35ppt) for 5cm divot or branch; 3-5 ml fixative for 1.5cm divot

- Ratio of tissue to fixative:

at least 1:10

- Hold at ~25°C (Store in cooler)

- DO NOT FREEZE

Tissue-Microbiology

Microbiology samples (H-B, U-B, D-B, R-B)

- Keep in Whirlpak ®, add sterile artificial sea water if needed, keep at ambient temperature in cooler until processed
- At field lab, homogenize with sterile mortar and pestle
- Flash freeze half of homogenate in dry shipper
- Plate other half of homogenate on marine agar or similar media

Tissue-Microbiology

Microbiology samples (H-B, U-B, D-B, R-B)

- At field lab, homogenize with sterile mortar and pestle

Tissue-Microbiology

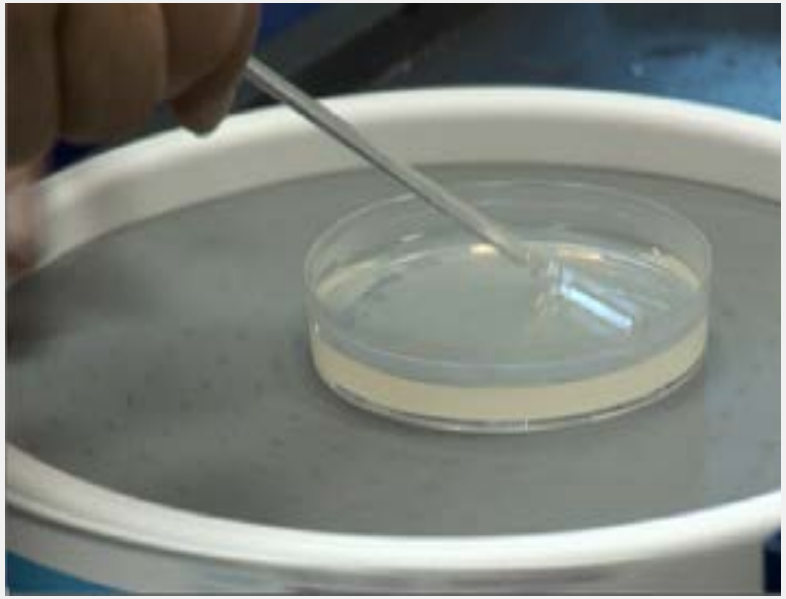
Microbiology samples (H-B, U-B, D-B, R-B)

- Flash freeze half of homogenate in dry shipper for molecular analyses

Tissue-Microbiology

Microbiology samples (H-B, U-B, D-B, R-B)

- Plate other half of homogenate on marine agar or similar media



SWAB Processing

- Epicenter swabs
  - break off tip
  - place tip in cryovial and into cryoshipper
  - DO NOT ATTEMPT WITH COMMON COTTON SWABS

MUCUS Processing

- Mucus will be collected in 3ml syringes; H-M and D-M
- These samples will be split into cryovials for either molecular analysis or conventional microbiological culturing

MUCUS Processing

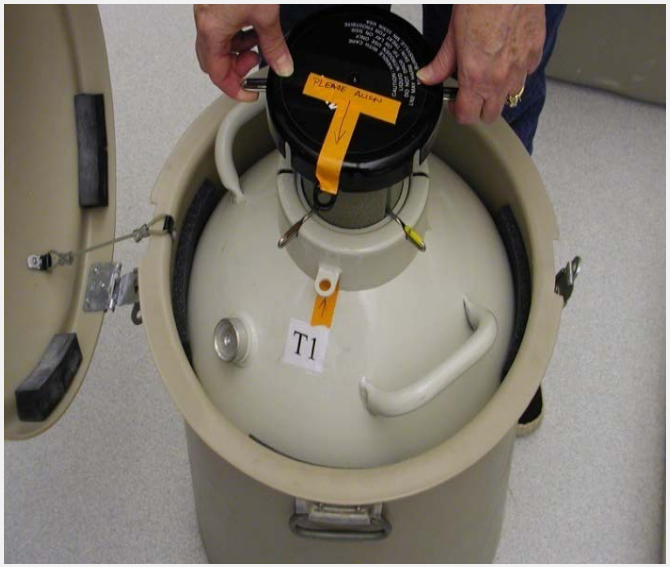
- **Molecular samples (half)**
  - Placed in a cryogenic vial
  - Immediately flash freeze in a dry shipper
- **Microbiology samples (half)**
  - Keep at ambient seawater temperature in screw top vials
  - Culture on media as soon as possible

SEDIMENT for Molecular & Microbiology

Module 6

Sediment

- Invert tube and shake
- Loosen cap and decant water into 2mL cryovial
  - Store at ambient temperature
  - Plate at Field Lab
- Leave air space,
- ~2cm between sample and cap
- Cap sediment tightly
- Freeze in dry shipper



WATER

Molecular sample (half)

- Transfer from syringe to 2.0 mL cryovial
- Place in dry shipper

Microbiology sample (half)

- Transfer from syringe to 2.0 mL cryovial
- Keep at ambient temperature

Other Collections

- Contaminants/ Toxicology
- Parasitology

Specialized collection techniques are required for these analyses.
A specialist in each field should be called on as conditions warrant.

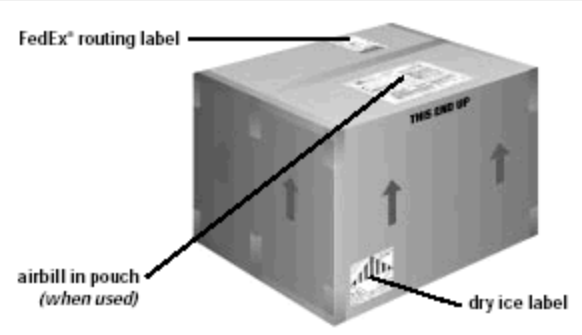
Each day of response….

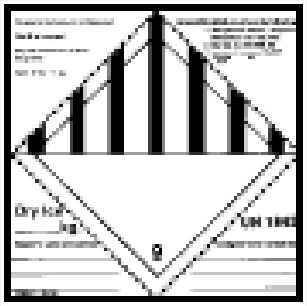
Logistics Chief and Support team

- Catalog samples
- Track and label all samples
- Label and link digital photos to samples
- Download GPS coordinates and upload to GIS
- Prepare to ship time-sensitive samples

SHIPPING

- **Contact Lab**
- **Ensure proper packaging**
  - **$25K DOT fines for leaky samples**
- **Label dry shippers as non-regulated to avoid concerns.**
- **Follow up with lab to ensure arrival of samples <u>to appropriate person</u>**





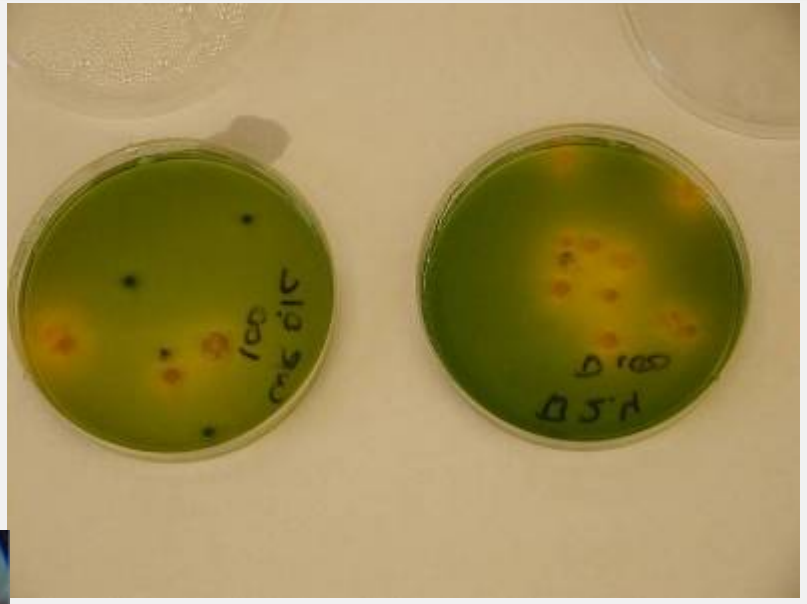
FedEx Dry Ice Label

Typical Analyses from Tissue Samples

- Biochemical
- Cellular Diagnostic
- Microbiology
- Molecular Microbiology
- Histology

Sample Analyses:
Biochemical & Microbiology Samples

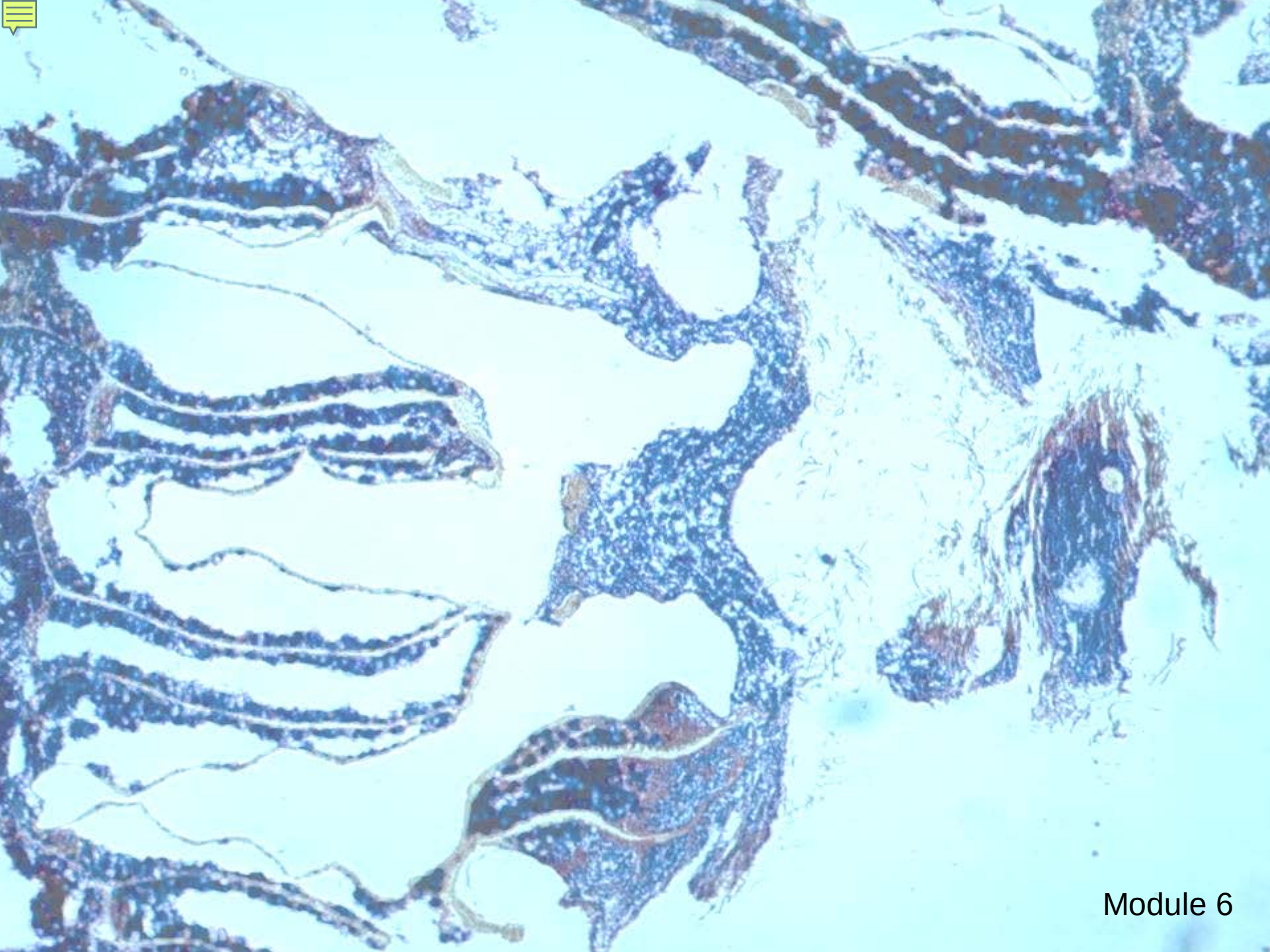
- Swabs
- Coral Mucus
- Coral Fragment
- Water
- Sediment



Histology Samples

Tissue

- Clippings
- Cores

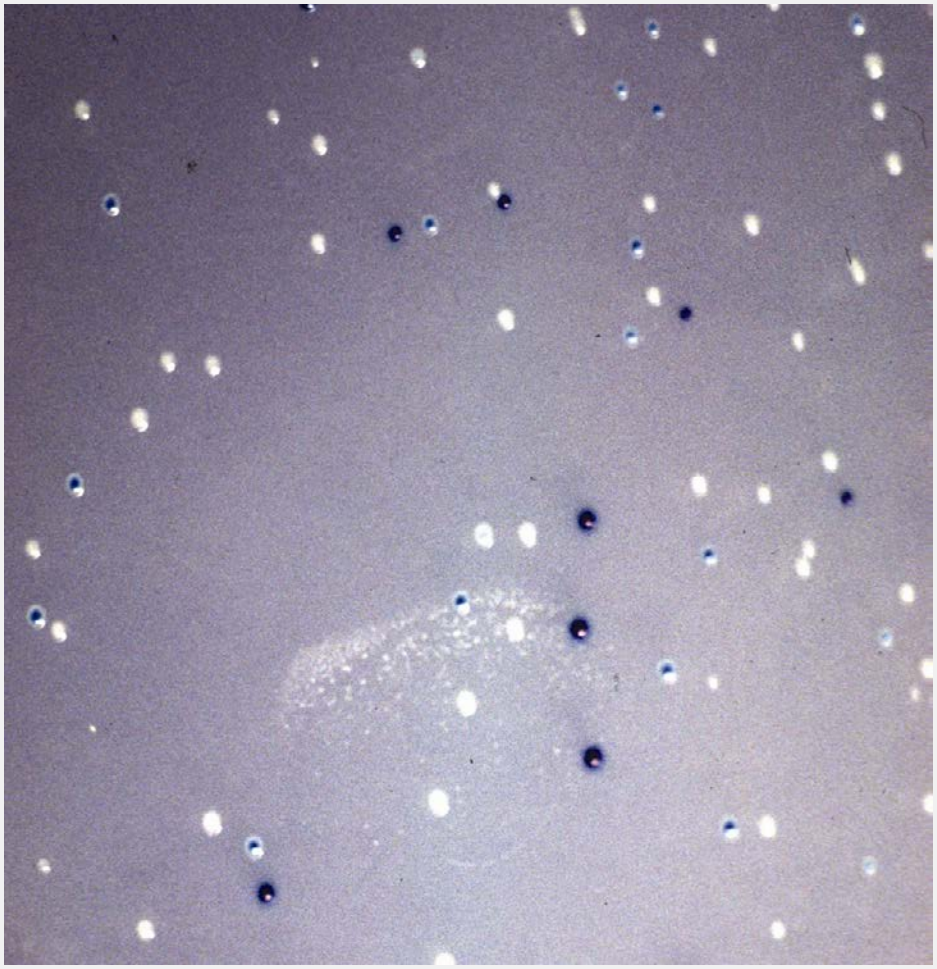


Module 6

Sample Analyses

Microbiology

Culture-dependent:
- used to isolate and identify coral-associated microbes from lesions, healthy tissue or mucus



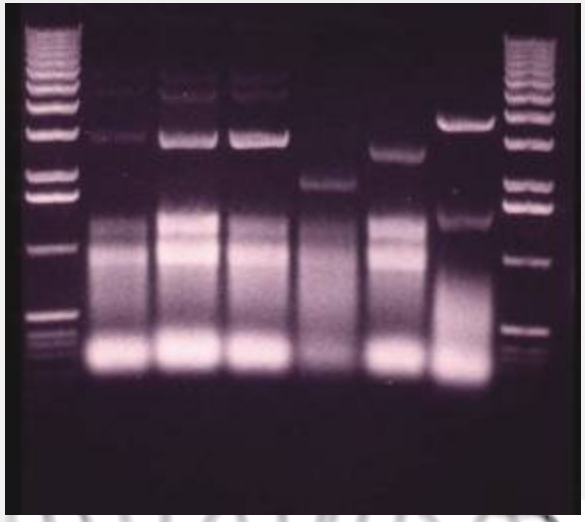
Sample Analyses

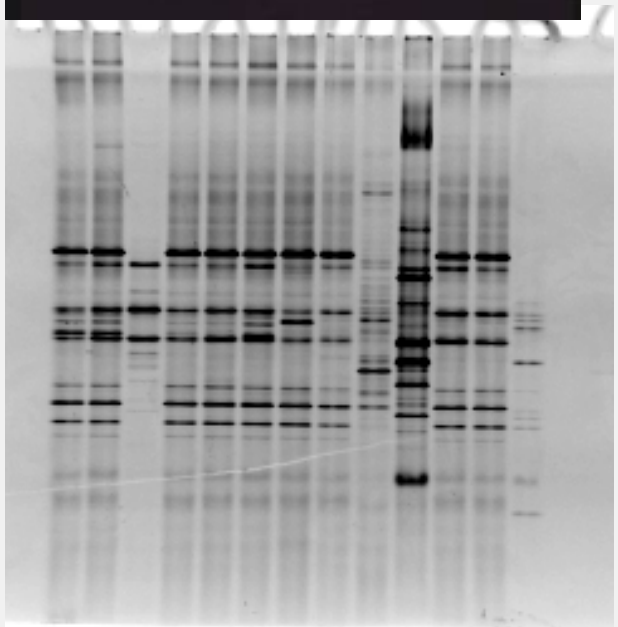
Molecular Microbiology

Culture-independent:

- isolate DNA and RNA from coral lesions, healthy tissue or mucus

- use ribosomal or other sequence information to identify bacterial community members and evaluate changes in those communities





Sample Analyses

Biochemical - Cellular

- Analyses measure levels of indicators of physiological status or pathological state of the organism. These include indicators of such as protein metabolism, genetic integrity, anti-oxidants, detoxification.

- Identification of bacterial proteins may aide in the identification of the disease-causing agent in a coral disease outbreak.