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## Determining the Cause of Abalone Mortality

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#### About Big Island Abalone Corporation (BIAC)

- BIAC operates a 10-acre aquafarm, growing and selling live abalone. It is located on the Kona Coast at the Natural Energy Laboraty Hawaii Authority (NELHA) complex.
- They currently produce about 100 tons of live abalone per year and sell them to vendors in Hawaii, Japan and the Continental U.S.

#### **Project Background**

- Periodic high mortality (80-90%) in 4-5 month-old abalone, generally correlated with the full/new moon.
- Mortality does not affect all tanks simultaneously nor does it affect all tanks.
- The abalone exhibit decreased movement and lose strength in their foot, but otherwise do not exhibit unusual characteristics.



### **About My Project**

- Two main parts:
  - Test two tanks (chosen at random) containing a cohort of abalone that are four to five months old
  - Test the tanks and abalone that experience mortality.



A tank with light abalone mortality



A tank with heavy abalone mortality

### Testing Parameters

#### **Effect on Abalone**

рН	Water that is too acidic or too alkaline can kill the abalone.
Dissolved Oxygen	Low quantities of oxygen in the water can suffocate the abalone.
Bacteria Swabs	Presence of pernicious bacteria can kill the abalone or compromise their immune systems.
Hemocytometer	Protists or other organisms present in the incoming seawater can kill the abalone or compromise their immune systems.
Parasite	Parasites are known to target only specific subsets of abalone and can spread rapidly.

# Results

#### Parameters

#### Results

рН	No significant changes.
Dissolved Oxygen	No significant changes.
Bacteria Swabs	Significant changes seen in quantity, both in the observable amount in the tank and the number of colonies cultured on plates.
Hemocytometer	Not enough cells to discern reliable results from.
Parasite	Organisms that could be parasites have been observed.

### **Outcomes: Bacteria**

- The amount of bacterial colonies seen on plates increased when comparing samples from infected and unifected tanks.
- The amount of bacteria visible to the naked eye also increased in infected tanks.
- It is unknown if this response contributes to the abalone mortality or if it is a byproduct.







### **Outcomes: Parasite**

- Currently the parasite looks like the most promising cause of mortality.
- <u>However</u> this has not been definitely proven.
- Research shows that BIAC's abalone exhibit characteristics consistent with infection by the parasite *Labyrinthuloides haliotidis*.
- These characteristics include the age of mortality, the high rates of mortality, the decreased foot strength and the lethargy.
- Suggested treatment methods include treating every liter of incoming seawater with 25mg of chlorine.



L: Uninfected abalone R: Infected abalone



Possible stained *L. haliotidis* from a live abalone sample



Infected abalone "sitting up" away from the side of the tank



Possible *L. haliotidis* in abalone tissue

### **Next Steps**

- Discern the latent period of abalone mortality:
  - 50 abalone from an uninfected tank have been placed in an infected tank and separated.
  - 50 abalone from an uninfected tank have been separated from the rest of their tank as a control.
- Test the treatment method for the parasite, if confirmed:
  - 25mg of chlorine per 1 liter of sea water, treated for 20 minutes.



### Acknowledgements

- Cecilia
- Big Island Abalone (and everyone who works there!)
- The Akamai Workforce Initiative, especially Lisa, Austin and Dr. J

### Methods/Approach

- pH: Use a pH probe to measure the pH of three separate locations near the outtake valve of each tank and average the values together to compute a representative pH.
- Dissolved Oxygen (DO): Same approach as used for pH.
- Bacteria Swabs: Swab the sides of the tank, near the outtake valve, and culture on both Thiosulfate-citrate-bile salts-sucrose agar (TCBS) and Marine agar plates. Subculture on ChromAgar plates to further discern bacterial types.
- Hemocytometer: Screen incoming and effluent water with 5µm and 20µm and 5µm screens respectively. Dilute with 2ml of deep sea water (to preserve live organisms trapped on the screen), stain cells and count and study under a microscope using a grid.
- Parasite: If abalone mortality occurs study the abalone under a microscope to look for signs of parasite presence.

### **Major Milestones**

- Sampling occurs every Wednesday and there will be 5 sample days.
- Two of those samples have been taken.
- Abalone mortality in a 4-5 month-old cohort will be used to determine parasite presence.



