Battle of the filter feeders: bacterial transmission in the presence of ascidians

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Abstract: Marine diseases are a major cost to fisheries and aquaculture industries, and as we continuously rely on marine organisms for protein, we increase our interaction with illnesses like gastroenteritis from undercooked oysters. An additional danger to aquaculture industries are the infestation of invasive ascidian species that can compete for space and food thus creating smaller harvest yields. For this proposal, the goal is to take two existing problems in aquaculture and discover if invasive can filter feedings ascidians can alter the transmission of *Vibrios* bacteria in the water column. If the benefits of using ascidians as biofilters outweighs the risks, then the management of marine diseases can be viewed from a new perspective.

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INTRODUCTION

Economically, marine diseases are a major cost to fisheries and aquaculture, as at least 67 diseases are known or have a potential negative affect. Of these diseases, 28 percent affect mollusks (Lafferty et al. 2015). There are two ways that marine diseases can negatively affect oysters. First, diseases can decrease growth, and increase mortality leading to decreased harvest. Second, diseases can pose risk to human health when oysters are consumed.

As we increasingly rely on aquaculture for protein, the chance of infection of humans from eating marine organisms increases (Broglia and Kapel 2011). *Vibrio* bacteria are abundant in marine and estuarine environments, and at least 12 are harmful to humans (Chen et al. 2011). *Vibrio* are responsible for a majority of the bacterial health risks associated with the human consumption of oysters, because these bacteria have evolved to resist degradation in oysters (Pruzzo et al. 2005). If infected oysters are consumed raw or undercooked they can infect humans with gastroenteritis and systemic infections that can lead to bacteria in the blood or blood poisoning followed by the development of skin lesions (West 1989).

Another concern in fisheries and aquaculture are invasive species, as they can reduce species diversity and the composition of sessile communities (Blum et al. 2007). As climate change brings rising ocean temperatures, it simultaneously increases the threat of invasive species, as alien species display higher growth rates at increased temperatures relative to native species (Stachowicz et al. 2002). Ascidians, or sea squirts, can live solitarily or colonially, and many are successful invasive species. Ascidians are filter feeders, move seawater through their oral siphon and can collect bacteria in the cilated pharyngeal basket surrounded by mucous (Pechenik 2015). The filtration rate of ascidians

varies with body size, seawater temperature, and concentration of particles (Petersen and Riisgard 1992). This finding may affect the way bacteria is transmitted in the water column with different filter feeders present.

Little is known about how ascidians digest, filter, retain, or emit pathogens in their environment (Stabili et al. 2016). The interaction between ascidians and bacteria depends on the type of bacteria (Burge et al. 2016). In a lab study, Stabili et al. (2016) found that the solitary ascidian *Styela plicata* was more efficient at retaining *Vibrio* bacteria than the colonial *Polyandrocarpa zorritensis*, and hypothesized S. plicata would be more suitable for purifying water in marine ecosystems. *P. zorritensis* was also found to digest coliform bacteria in a separate study (Stabili et al. 2015). Most studies explore the invasive nature of ascidians and their settlement and recruitment (Stachowicz et al. 2002). Implementing the use of invasive filter feeders as a potential biofilter for bacteria should be considered carefully, as these species are usually a threat to natural resources and biodiversity (Stabili et al. 2015).

There is a need to develop new management strategies for marine diseases, as climate change may impair immune responses and increase diseases in marine organisms, such as oysters (Travers et al. 2009). In addition to altering the host response to disease, the transmission of pathogens may be altered by the presence of filter feeders (Burge et al. 2016). Changes to the host, disease, or environment may alternatively benefit the host by limiting or removing the disease in the population (Burge et al. 2014). Bivalves can retain pathogens, which makes them a suitable estimator for pathogens present in ecosystems (Burge et al. 2016), and little is known about the transmission of pathogens to ascidians. The

need for research on the transmission of pathogens will create a foundation for aquaculturists to make more informed management decisions regarding diseases.

The goal of this study is to explore the transmission of bacteria to oysters in the presence of another filter feeder, the ascidian. The use of an invasive species to potentially alter the transmission of bacteria to farmed oysters may give a new reputation to the ascidian.

OBSERVATIONAL OR THEORETICAL MOTIVATION FOR RESEARCH

Since 1970, aquaculture has grown by three-fold (Diana 2009), and Diana et al. (2013) makes the argument that it is important that the growth of aquaculture does not adhere to the mistakes made during the growth of agriculture. The intensified growth of agriculture produced pollution in coastal and inland watersheds, global habitat destruction, and most importantly to this study the increased use of antibiotics and pesticides to increase yields (Tilman et al. 2001).

The threat of pathogenic bacteria becoming resistant to antibiotics from the excessive usage was demonstrated (Kang et al. 2017) who found that up to 90.9% of presumed *Vibrios parahaemolyticus* isolates showed resistance to antibiotics.

Rohlheiser et al. (2012) explore chemical, mechanical, and biological treatments to reduce fouling species that compete for space with commercially harvested oysters. Although this study does not focus on antibiotics as a mechanism to remove fouling species, the study involves the treatment of water which may impair water quality.

Motivation for this research comes from the need to establish the most effective way to manage two of aquacultures main complications with the least amount of damage

to the environment that has been widely known from the production of agriculture. Avoiding the use of antibiotics or biological treatment to water will prevent pathogenic bacteria from becoming resistant to antibiotics, and keep unnatural materials out of the water column.

RESEARCH QUESTIONS

1. How does the concentration of bacteria in Pacific Oysters (*Crassostrea gigas*) change the presence of ascidians? - are ascidians altering the transmission of pathogens to oysters?
2. How are ascidians reacting to bacteria? – are they absorbing, filtering or digesting them?

HYPOTHESES

1. Concentration of bacteria in Pacific Oysters in the presence of ascidians

   H_0: No change in the concentration of bacteria in oysters

   H_a: Bacteria will increase

   H_a: Bacteria will decrease

2. Ascidian reaction to bacteria

   H_0: No bacteria will be found in ascidians

   H_a: Ascidians will absorb bacteria (become infected – amplifying bacteria)

   H_a: Ascidians will retain bacteria (store bacteria)

   H_a: Ascidians will digest the bacteria

3. How will the water quality change with increased density of ascidians?

   H_0: No change in water quality

   H_a: Bacteria will increase

H_a: Bacteria will decrease

EXPERIMENTAL DESIGN AND METHODS

Species selection

The Pacific Oyster (*Crassostrea gigas*) will be selected for the purpose of this research as this species is accessible to for cultivating in a lab as well as highly commercialized and will be relevant to the results of this study.

The club tunicate (*Styela clava)* will be selected for this research because this species falls into the same genus as Stabili et al. (2016) study where *S. plicata* was more efficient at purifying water. It is also important to note that the clubbed tunicate is labeled with highest invasive concern (WDFW 2017), which makes this species relevant to the goals of this study.

Vibrios bacteria will be selected for this study because at least 12 species are harmful to human health when consumed in undercooked or raw oysters (Chen et al. 2011).

Collection

Oyster species *Crassostrea gigas* will be raised in laboratory conditions to eliminate any existing infections that occur in the wild and to match conditions of commercially raised oysters.

Oysters will be selected for experimentation based on similar size and overall health before maturity to simulate oysters used for commercial harvest.

Previously identified ascidians will be collected and specimens will be cleaned of all debris and macrofouling organisms such as algae to keep conditions consistent between each ascidian group.

Specimens will be placed into a 100 L fish tank with filtered sea water for 48 hours to acclimate them to laboratory conditions.

Ascidians will be selected for experimentation based on similar size and consistent coloration and overall health after the acclimation period.

Filtration

Three densities of ascidians will be used to see if there is a comparison between the density of ascidians and the bacteria found one constant density of oysters. Controls for each density of ascidian and oysters will be created for comparison (Figure 1). Each group will be repeated 3 times to improve the validity of results.

After the duration of the experiment the oysters and ascidians will be tested for the concentration of bacteria they retained using Thiosulfate-Citrate-Bile Salts-Sucrose Agar (TCBS) to isolate *Vibrios* bacteria and quantify them by colony forming units (CFU) (Kobayashi).

Ascidian bacteria reaction

The control tanks will be used to investigate if the ascidians are digesting the bacteria or if they are retaining the bacteria in their stomach. Control groups will be use to assess if there is less bacteria in the water column, and more bacteria found in the ascidian, then the ascidian is retaining the bacteria and may be storing the bacteria that

can infect organisms later. After the duration of the experiment the control group of ascidians will be analyzed to determine if the bacteria was digested, absorbed, or retained.

Water quality

The initial and final concentration of bacteria in the tanks will be recorded to investigate water quality. Water quality will be measured by comparing initial concentration of *Vibrio* bacteria to final concentration of bacteria using TCBS.

Analysis

Statistical analysis will be performed to identify any significant difference between the concentration of bacteria between the groups of oysters and ascidians, and water quality between each of each of the groups.

ANTICIPATED RESULTS

Based on the literature reviewed the anticipated results are as followed:

1. Concentration of bacteria in Pacific Oysters in the presence of ascidians

H_0: No change in the concentration of bacteria in oysters

- Control oysters and oysters paired with all concentrations of ascidians did not show any significant difference raising the question if ascidians have any affect on the transmission of bacteria in the water column.

H_a: Bacteria will increase

- Depending on the filtration speed of each species, the results may vary. If oysters are more efficient at filtering water, then they will have increased levels of bacteria.
- Pruzzo (2005) discusses the ecological role that *Vibrios* bacteria have with bivalves. The bacteria have been known to withstand purification techniques and are still found in bivalve tissues. The *Vibrios* bacteria may favor the Pacific Oyster over the Club tunicate.

H_a: Bacteria will decrease

- If the ascidians are faster and more efficient at filtering water, then the ascidians may take in more of the bacteria in the water column.

2. Ascidian reaction to bacteria

H_0: No bacteria will be found in ascidians

- The Pacific oysters are more efficient at filtering water, and the club tunicates will not be infected with bacteria.

H_a: Ascidians will absorb bacteria (become infected – amplifying bacteria)

- Stabili et al. (2015) discusses ascidians being able to store bacteria and act as a reservoir for bacteria. If this is the case then this species of ascidian is not ideal for the use as a biofilter in aquaculture.

H_a: Ascidians will retain bacteria (store bacteria)

- The ascidians may just store the bacteria without it being harmful to the surrounding environment.

H_a: Ascidians will digest the bacteria

- Ascidians may digest the bacteria for nutrient content.

3. How will the water quality change with increased density of ascidians?

H_0: No change in water quality

- Both filter feeders did not retain any of the bacteria present in the water;

H_a: Bacteria will increase

- Bacteria multiplied inside ascidian and was released into the water column;

H_a: Bacteria will decrease

- Oysters will absorb a significant amount of bacteria in water decreasing the bacteria in the water column.
- Ascidians absorbed a significant amount of bacteria in the water decreasing the bacteria in the water column
- As the density of ascidians increases, the water quality will increase because there are more filter feeders present. This will allow for the oysters to have less bacteria (Figure 3).
- No significant difference between the absorption of bacteria was found between the oysters and ascidians, but the concentration of bacteria in the water column decreased. Ascidians may have a maximum capacity in which they can filter bacteria Figure 3 shows the interpretation determined by the blue line that as the density of ascidians increase, then the water quality (measured by the concentration of bacteria) remains the same at intermediate and high levels of bacteria.
- A high volume of ascidians may be needed to see any significant result in the quality of water as shown in figure 3 where low and intermediate densities of ascidians yield low water quality.

- There is the possibility that the ascidians cause mortality in the oysters, which means the possibility of ascidians being biofilters to reduce bacteria transmission to oysters is not a possibility. Ascidians can completely cover bivalve crops and compete for nutrients and space (Forrest and Atalah 2017)

DISCUSSION

The presence of ascidians may change the transmission of bacteria to other organisms, and will be studied in this experiment. This investigation will be able to provide much needed information on how ascidians filter bacteria. Ascidians are usually considered pests on aquaculture farms, and their invasive characteristics have been widely studied. This alternative perspective of viewing a more positive effect of dense ascidian populations may provide alternative ways to manage disease in aquaculture. If the benefits outweigh the risks, ascidians could be used as biofilters to reduce bacteria in the water column.

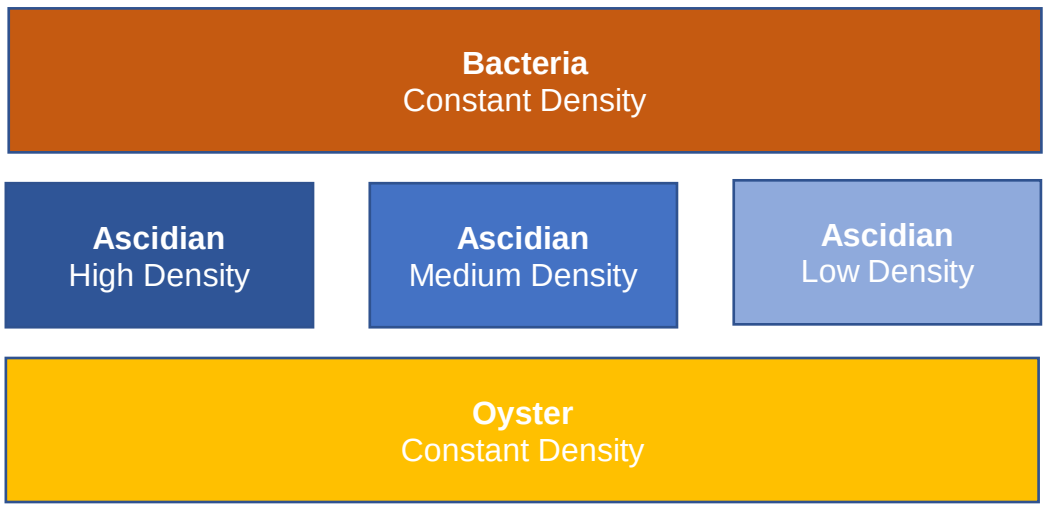

Figure 1 – Visual of groups designated for study. Bacteria concentration and oyster density will remain constant while the density of ascidians will change.

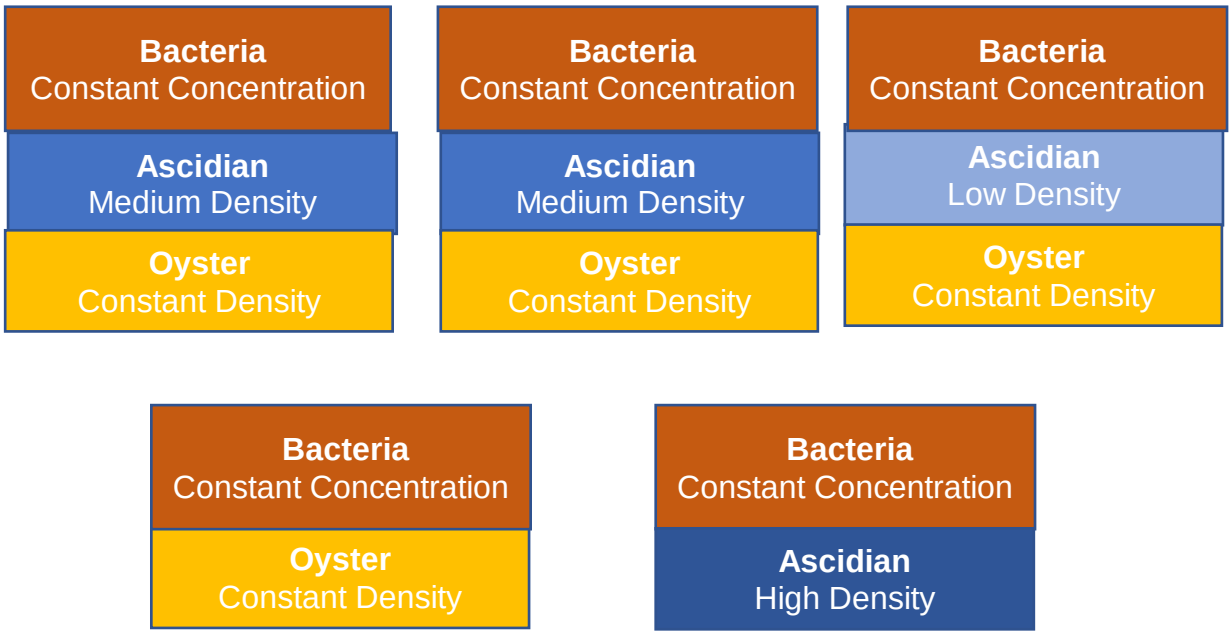

Figure 2 = Visual guide of groups designed to test all hypotheses. Each group will be repeated 3 times to improve validly of results.

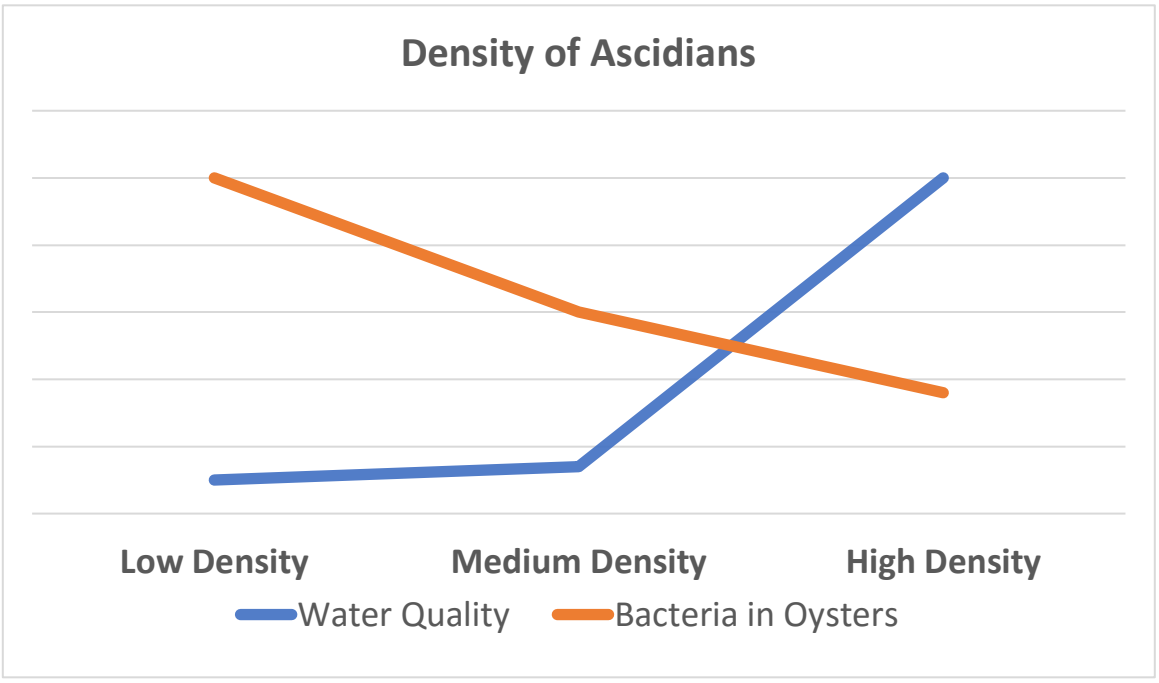

Figure 3 – Interaction plot showing potential outcome to this study. Water quality does not increase until there is a high density of ascidians, suggesting that ascidians are only able to purify the water in high numbers. As the density of ascidians increase, *Vibrios* in oysters decrease because there are more filter feeders in the water take in pathogens.

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