Supplemental Report: Biosecurity Benefits of EC

Vibrio Suppression Technology

Trane was contracted to review NaturalShrimp (OTCQB: SHMP) current and anticipated offering utilizing electrochemistry. Their patented product offering "Vibrio Suppression Technology" addresses multiple concerns for marketable shrimp. This technology utilizes a modified electrocoagulation (EC) system which not only provides the aforementioned reduction in ammonia in the shrimp grow out tanks, but additionally, a well-documented antimicrobial effect. This paper will address the benefits of EC's antimicrobial effect, how it compares to alternative Superintensive Production Facility model designs, and the current microbial challenges all facilities face.

The mission of the company is to provide a proven, game-changing solution to provide healthy and accessible product that is cost-effective and sustainable. Based 150 miles from the closest body of saltwater outside of San Antonio TX, the NaturalShrimp product can be effectively produced anywhere in the country

Livestock Operations- Aquaculture Marketplace

Livestock operations are land- and water-based animals generally raised in superintensive production or concentrated animal feed operations for food consumption. Each livestock facility is responsible for establishing biosecurity protocols to ensure pathological bacteria, viruses and fungal outbreaks are kept to an absolute minimum. This paper will address water-base, or aquaculture facilities.

In 2017, the aquatic-based protein global marketplace was in excess of $175 Billion[1]. The United States is a minor aquaculture producer, ranked 16th in 2016 —but it is the leading global importer of aquatic protein. By value, nearly 90 percent of the seafood we eat comes from abroad, over half of it from aquaculture. These oversea companies are government subsidized, have low labor costs, and a reduced regulatory oversight. Because of these low cost imports, the U.S. seafood trade deficit (East Asia only) has grown to $14 billion in 2016[2], of which $5.1 billion consists of fresh and frozen shrimp. This number dwarfs the established domestically grown shrimp aquaculture of slightly more than 2%[3]. This trade imbalance is an opportunity to seek a domestic solution.
That ideal solution for the US market is an offering that is environmentally sustainable, food safe, can be located anywhere, and is not hindered by existing superintensive production technologies and razor thin margins.
This is the mission of NaturalShrimp.

Superintensive Production Technologies

Developing a business model that cost-effectively permits high density shrimp production in a relatively small space is challenging. Shrimp health and product yield are highly dependent on site factors such as water and feed quality, physical environment, and population density. These factors are no different

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[1] https://www.businesswire.com/news/home/20170925006405/en/Global-Aquaculture-Market-2017-2022-Market-Expected-Reach Access July 2019
[2] NOAA Fisheries, US Aquaculture Production Report 2017. https://www.fisheries.noaa.gov/national/fisheries-united-states-2017
[3] https://www.fisheries.noaa.gov/national/aquaculture/us-aquaculture

from any livestock in that they have a strong impact on their immune systems. As a result, product survival is relatively low in shrimp farming. In fact, a product survival of 50% is acceptable for shrimp production facilities.
Having a solution that reduces product mortality to less than 10% is a game changer in the shrimp aquaculture marketplace.

Another relevant factor in any livestock operations (especially shrimp) is delivery of fresh product through logistics. Logistics carries with it not only a transportation cost, but also a Carbon Intensity cost. Carbon Intensity is a global initiative, utilizing a scoring methodology to quantify human-created emissions (trucks, planes, and production facility CO2 equivalent emissions) that directly impact Climate Change. Reducing the Carbon Intensity due to logistics is directly related to the location of a production facility. Reducing the distance from "farm to plate" reduces Carbon Intensity. Domestically, a rise in consumer preference is seen in purchasing locally sourced food as opposed to transported. Locally sourced food also benefits the local economy. Companies are also starting to report greenhouse gas emissions created at their facilities as part of corporate stewardship programs to further encourage product acceptance.

Production Facility Design

Commercial shrimp superintensive production facilities all share common features and challenges. They house and feed shrimp in various tanks, raceways, or ponds based on their siting preferences and growth phase. The major differences lie in the way they manage waste water. If the facility is close to seawater, the vessels are replenished through recirculation of fresh seawater. Waste containing high amounts of nitrogen, is also rejected through the same route. Care must be maintained in the environmental management of the site of supply water and rejected waste to ensure no environmental imbalances occur such as excessive nitrogen buildup creating algae blooms. It is estimated that 65% of the waterways which include coastal shores are already compromised due to excessive discharge of nitrogen nutrients.[4] The supply waters can also introduce common bacteria that thrive in warm coastal waters.[5] Some of these common bacteria are potentially pathogenic to humans. These bacteria are discussed in more detail in the Superbug section.
The other two models are inland-based, and do not have access to fresh water. The most common model manages their nitrogen waste through bacteria-based communities that co-exist with the shrimp. These bio-communities are called bio-flocs and contain bacteria that provide benefits such as nitrogen and sulfur digestion and improved water quality stability[6]. This stability is noteworthy since the bio-floc serves as a defense mechanism against a bacteria called Vibrio. This species is included in the aforementioned pathogenic bacteria[7] seen in the first facility design.

There are several issues with bio-floc: a) it can act as a potentially pathogenic bacteria; b) the biological and nutrient controls are slow and not fully effective; c) Due to bio-floc's inability to remove all targeted nutrients and Vibrio, it must be constantly monitored through lab and culture testing to ensure that the

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[4] https://oceanservice.noaa.gov/facts/eutrophication.html
[5] https://www.cdc.gov/vibrio/faq.html
[6] Gavin Burnell, G., Allan, G., 2009, New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management" by Elsevier Science. Elsevier Science, p. 1995
[7] Ibid p. 1995. See also reference 5 for more detail on Vibrio.

bacteria load is maintained at proper levels. Improper management of the bacteria will create an uncontrollable and catastrophic reductions in production levels.
The second model is NaturalShrimp offering. It does not utilize bio-floc nor outside coastal water sources, which improves market yield and reduced production risks. This offering is discussed in detail further in the paper.

Superbugs

In order to better understand the necessity of biosecurity policies, we need to discuss the microbial challenges that are present in the aquaculture marketplace. There is a large amount of public data that attest to issues regarding the quality of supply associated with superintensive production facilities. This is most frequently seen in the 90% imported shrimp, such as the rejected shipments arriving from Eastern Asia into the US and Canada[8]. Some of these foreign businesses operate unregulated, which encourage poor business practices. Documented examples include low quality protein feeds such as improperly processed chicken manure[9], poor or no biosecurity, which then gives rise to the utilization of pesticides and human antibiotics to improve product survival[10].
A recent large study discussed concerns about food-acquired Antibiotic Resistance (ABR) in humans. The important point to this systematic review was to study if ABR contaminated food would impact vital human drug therapies[11]. The study was only limited to food sold in the UK, and was inconclusive of human transmission of ABR. However, documented pathogens, antibiotics, dead insects and other harmful products were found in the food[12]. Their presence continues the discussion that ABR pathogens and antibiotics could be involved in human-acquired ABR. In technical terms, these pathogens are called Extended Spectrum Beta-Lactamases (ESBLs). ESBLs are also known as “Superbugs”, and are resistant to treatments with commonly used antibiotics such as penicillin and cephalosporins. ESBL-producing bacteria were first isolated and discovered in Europe in 1983 but are now a world-wide problem[13]. Superbugs have presented themselves in humans as necrotizing fasciitis (skin eating), intestinal, and blood infections. These conditions are close or direct relatives to pathogens present in shrimp farming.

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[8] Reported rejected shipments are only caught through spot inspections by the FDA. It is estimated that a large percentage of tainted product is missed simply due to the inability to test all incoming shipments.
[9] Properly processed chicken manure is used for animal feed, including aquatic species. https://www.feedipedia.org/node/66 Access July 2019
[10] There is some marketing double-talk regarding a drug class called Ionophores. These drugs are included in the antibiotic classification. Some companies claim “Antibiotic Free” or ABF, when in fact they Ionophores is a drug that operates similarly to antibiotics. There are numerous impartial articles that discuss its applications: https://drsarahskinner.com/2017/06/carboxylic-ionophore-toxicity-in-small-animals/ Accessed July 2019
[11] https://www.food.gov.uk/sites/default/files/media/document/amr-systematic-review-final-report-2016_0.pdf
Food testing with particular reference to pork and poultry meat, dairy products, seafood and fresh produce on retail sale in the UK, Royal Veterinary College and Safe Food Solutions Doc # FS102127, 2016
[12] Like the UK, not all imported shipments in the US are tested by the FDA, leaving obvious opportunities for tainted shrimp to enter the US food market. Rejected products include human antibiotics, pesticides, and dead pests.
[13] Numerous sources: Sibhghatulla Shaikh, 2015, Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment, Saudi J Biol Sci. 2015 Jan; 22(1): 90–101. And Rewat, D., Nair, D. Extended-spectrum Beta-lactamases in Gram Negative Bacteria, Journal of Infectious Diseases, Sept 2010, And, Extended Spectrum Beta-Lactamases (ESBLs)
Certain strains of bacteria are

Two specific ABR organisms, E. coli[14] and Vibrio, are the most frequently seen causative agents in East Asia reported human illness outbreaks, and are associated with antibiotic abuse (see reference 10).

Vibrio

One of the most common pathogen present in shrimp and mollusks is Vibrio. This large bacteria family has several disease-causing species that produce cholera, necrotizing fasciitis (flesh eating disease), and the more common gastroenteritis. Vibrio species account for a significant proportion of human infections in the US from the consumption of raw or undercooked shellfish[15]. These pathogens exist in warm coastal waters, and are monitored by Centers of Disease Control (see reference 5).
The NaturalShrimp design does not utilize bio-floc nor outside coastal water sources, which improves market yield and reduced production and health risks. In addition, NaturalShrimp's patented design provides a further layer of biosecurity, ensuring that pathologic bacteria growth is immediately treated in the production process.

Solution

For the past four years, NaturalShrimp has been developing a solution that would allow them to discontinue using the common bio-floc process that is ubiquitous in the shrimp aquaculture industry. Several tests later, they submitted and was granted a patent for their solution. For the past year, they have operated the system in production mode, growing Pacific White shrimp to full maturity. This solution, called Vibrio Suppression Technology, brings several critical benefits to the marketplace.

1) Size- Vibrio Suppression Technology equipment is compact and permits entire installations to be installed practically anywhere, including in urban areas.
2) Environmental- instead of relying on bacteria to partially reduce nutrient levels in their waste water, the nutrients are destroyed through an electrochemical process. These nutrients are largely generated through shrimp feces and uneaten feed.
3) Product Safety- Utilizing the same electrochemical process, Vibrio and other pathogens have been visibly removed from the grow tanks, as demonstrated in the attached culture plate images.
4) Employee Safety- Removes the potential for bio-floc acquired bacterial infections through cuts or abrasions in the workplace.
5) Improved Immune Systems- Physical environment has a direct impact on survivability of the shrimp. This benefit is largely due to the two benefits detailed in items 2 and 3 above. Pathogens can decimate a shrimp population through failure to thrive, or succumbing to disease. Nutrients, in the form of ammonia, also generate stress in the shrimp, and is the leading issue in poor product growth.
6) Self-sustaining- The electrochemical process maintains the water quality throughout the growth cycle of the shrimp. It additionally buffers the water, which further reduces OPEX costs, removing the necessity to add the commonly seen additive, sodium bicarbonate.
7) Improved product quality- this solution provides locally sourced fresh shrimp.

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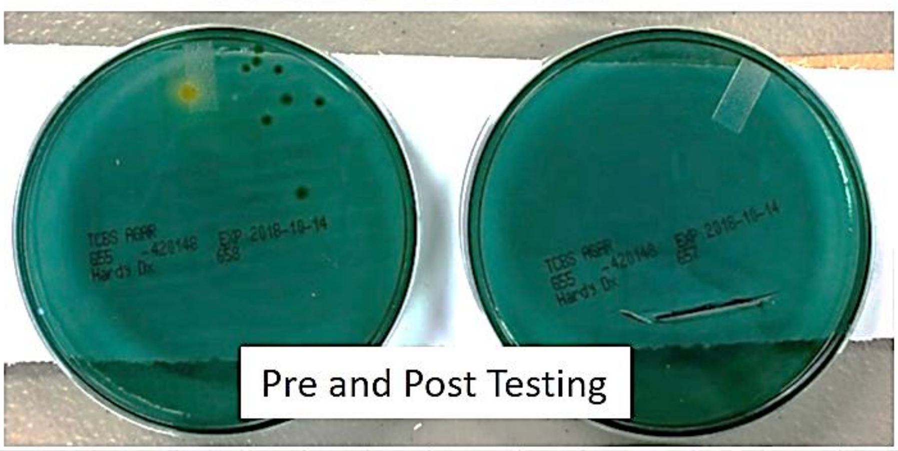
[14] Ibid p. 10
[15] Charles A. Kaysner, C.A. and Angelo DePaola, A. 2004, Bacteriological Analytical Manual (BAM), Chapter 9 Vibrio, p.1 https://www.fda.gov/food/laboratory-methods-food/bam-vibrio

Part of the acceptance testing of the solution required two metrics to be totally or drastically reduced- mortality and pathogen testing.

1) Product Survival- The survivability of the shrimp has increased due to the operation of the Vibrio Suppression Technology system.
2) Pathogen Testing- Agar plate testing is routinely performed to monitor the growth of Vibrio. For systems operating Bio-floc, there is an acceptable level of Vibrio allows due to the high eradication costs. It does come at a cost however, with reduced survivability. The 3 images below represent this scenario, before and after the Vibrio Suppression Technology system introduction. The top image represents the level of Vibrio in a Bio-floc system, shown in yellow and dark green dots. The 2 images below the top image represent the acceptance testing of the EC system. The image on the bottom left, Pre, shows a small outcrop of environmentally background Vibrio taken from the growout tank housing the shrimp. The second image on the bottom right, Post, shows a clean agar plate, indicating that the Vibrio was destroyed. Note the dominant green population in the Pre, indicating early onset over the slower growing yellow species. Both are equally removed by the Vibrio Suppression Technology system. The chart below the 3 images helps identify the 11 relevant strains of Vibrio by color.



	V. alginolyticus	V. cholerae	V. fluvialis	V. furnissii	V. hollisae	V. metschnikovii	V. mimicus	V. parahaemolyticus	V. vulnificus	A. hydrophilia **	P. shigelloides **
TCBS agar	Y	Y	Y	Y	NG	Y	G	G	G	Y	G