INVESTIGATION OF SEAWATER QUALITY ISSUES RELATING TO LARVAL SURVIVAL RATES FOR HAWAII OCEAN SCIENCE AND TECHNOLOGY (HOST) PARK

Submitted to: **NATURAL ENERGY LABORATORY OF HAWAII AUTHORITY**

Submitted by: **Dr. Jim Wyban**

Date Submitted: **June 29, 2024**

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EXECUTIVE SUMMARY

The HOST Park in Kailua Kona is an economic engine for Kona and has been called "the Silicon Valley of Aquaculture". The standout feature of HOST Park is its dual-temperature seawater supply system, a globally unique asset that positions it as a center for ocean science innovation. HOST Park is administered by the Natural Energy Laboratory Hawaii Authority (NELHA) and offers leasable land and office and laboratory facilities including the dual-temperature seawater supply for aquaculture, energy production, and various ocean-related research, education, and commercial projects dedicated to ocean science and renewable energy.

Currently, HOST Park hosts 45 tenants, including aquaculture producers, water bottlers using deep sea water, and renewable energy researchers. HOST Park tenants collectively pay over $2.5 million in annual rent and royalties and their overall economic impact is estimated to be $150M annually including the creation of over 600 jobs Statewide (UHERO 2022). Since 1974, the State of Hawai'i and the Federal government have invested over $150 million in HOST Park.

In 2023, eleven HOST Park tenants formed the Keahole Point Larval Group (KPLG) to tackle challenges they faced in marine animal larval rearing systems, which were linked to potential water quality issues with HOST Park's surface seawater (SSW) system. This group, consisting of some of HOST Park's largest tenants, has encountered unexplained larval mortalities across a variety of marine species. To address these issues, HOST Park engaged Dr. Jim Wyban to conduct an in-depth investigation. The establishment of KPLG and their collective efforts to resolve the larval mortality issue underscore the collaborative, innovative spirit that thrives at HOST Park.

This report examines the challenges impacting larval survival at KPLG farms, identifies potential causes of the water quality issues, and provides recommendations for both HOST Park and KPLG to address these challenges and improve operational efficiency.

HOST Park Seawater Systems

HOST Park operates a dual-temperature seawater supply system, originally designed to support Ocean Thermal Energy Conversion (OTEC) projects. This system is expansive, comprising multiple ocean intake locations. It features pipelines up to 55 inches (1.4 meters) in diameter and seawater pumping capacity up to 42,000 gallons per minute (151 million liters per day). The system has been operating continuously for over 4 decades.

Deep Seawater (DSW) System: This system pumps water from depths of approximately 2-3,000 feet and is capable of flow rates up to 27,000 gallons per minute. The DSW is cold temperature (6°C, 46°F) and contains relatively high concentrations of Nitrogen and Phosphorus nutrients.

Surface Seawater (SSW) System: The SSW system pumps water from depths of approximately 80 feet and supports flow rates up to 15,000 gallons per minute. The SSW is warm temperature (26°C, 76°F) and contains relatively low concentrations of Nitrogen and Phosphorus nutrients. SSW is vital for most aquaculture activities at HOST Park.

Methodology

This investigation involved meetings with members of the Keahole Point Larval Group (KPLG) and HOST Park technical staff, a detailed survey of KPLG members on water quality and larval problems, compilation of KPLG hatchery data documenting larval rearing challenges, site visits to each KPLG hatchery to assess their seawater treatment systems and procedures, discussions with outside experts and a literature review on relevant topics.

Key Observations

A significant water quality issue in the Surface Seawater at HOST Park is impacting marine animal larval rearing, significantly impacting HOST Park companies that are dependent on successful reproduction of their stocks. This ongoing issue led to the formation of KPLG, consisting of 11 tenant companies at HOST Park. Some KPLG companies are some of HOST Park's largest tenants and anchors within the facility. Notably, the persistent water quality problem forced at least one company to cease operations due to failures in raising marine fish larvae in HOST Park.

The most likely causes of the seawater quality issue are:

- **Biofouling** in the SSW pipeline system may release toxic chemical(s) into the SSW.
- ***Vibrio* bacteria** in the system may release toxic chemical(s) into the SSW.

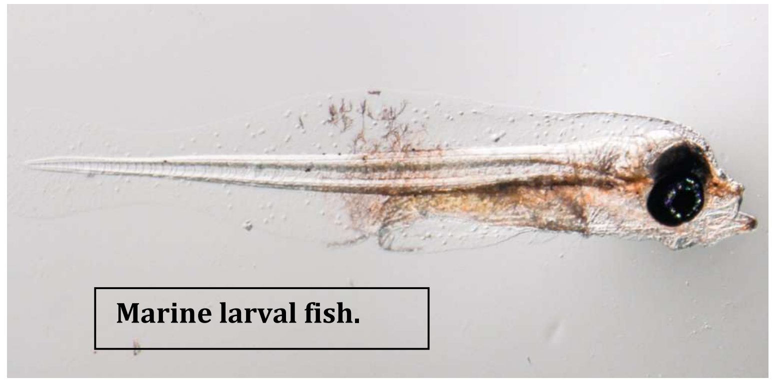
These causes are not mutually exclusive and may be interrelated. A less probable cause might be environmental contamination from nearby sources such as the airport, golf course, or sewage treatment plant. These are highly unlikely given the vast volume of seawater flowing past Keahole Point daily, which would dilute any potential contaminants. Historical data from the last 30 years, including seawater quality measurements in the CEMP monitoring program, have shown no significant changes.

In this investigation, no infectious disease biosecurity risks were identified. None of the KPLG farms showed evidence of reportable, excludable pathogens. Pathology studies of larval animals submitted to professional analysis by several farms have been inconclusive and failed to identify a causative factor. Most of these farms are regularly inspected by licensed professionals who test their animals for reportable pathogens as part of their health certification process required for exporting seedstock or broodstock. All certified farms remain free of any reportable pathogens.

However, *Vibrio harveyi* and *V. corallytiicus*, both recognized pathogens of marine organisms, were found to be widespread in recent SSW samples. *V. harveyi* was also detected in aerial samples at HOST Park. A recent environmental DNA (eDNA) study conducted at HOST Park revealed significant presence of *Vibrio* (0.2-5% of the prokaryote community) in raw SSW samples. In one notable instance, *Vibrio corallytiicus*, a documented disease-causing shellfish pathogen, constituted 40% of the *Vibrio* content in SSW filtered to 5 micrometers. The high levels of *Vibrio* in the seawater may indicate some anaerobic (oxygen-deprived) conditions within the pipeline, which is undesirable for maintaining water quality.

Solutions to date

Operators at KPLG hatcheries have made strides in addressing their larval mortality issues through the application of several chemical treatments to SSW. One such treatment, disinfection with Bio-hydrox, an oxychloride solution, has shown promise in reducing larval mortalities. Bio-hydrox is an approved drinking water and aquaculture treatment for oxidation and disinfection. In some cases, high larval mortalities have persisted in some systems despite the use of Bio-hydrox. Some negative side effects of the Bio-hydrox on larvae have also been observed.



Marine larval fish.

Ozone treatment of SSW has been beneficial for larval fish rearing. Ozone is a powerful oxidizing agent and is widely used in various water treatment processes, including for seawater. It kills bacteria, viruses, and other microorganisms by disrupting their cellular structures, leading to cell death. Ozone also helps break down organic compounds, including natural organic matter and pollutants like oil and pesticides, enhancing the clarity and quality of seawater. It also controls algal growth and reduces toxin concentrations from certain algae, mitigating risks associated with harmful algal blooms.

Ozone treatment is a common disinfection practice in many marine hatcheries and significantly improved fish larval rearing outcomes in 2023 following installation of an ozone system that cost $100,000. Encouraged by these results, several other KPLG companies have recently invested in ozone systems for their hatcheries. Although ozone treatment has improved survival rates, they have yet to return larval survivals to previous levels, prompting ongoing calls for further SSW system decontamination.

Despite these efforts, the fundamental issue of animal mortalities attributed to deteriorating SSW water quality remains unresolved. The mortality problems observed in larval stages suggest possible sub-lethal stressors in the seawater that may also affect the efficiency of HOST Park companies growing post-larval stage animals. One fish company reports excessive broodstock mortalities they attribute to water quality. An abalone company reports excess mortality in adult animals. These problems plus the recurring larval mortalities could be indicative of broader systemic issues, acting as HOST Park's "canary in the coal mine." Discussions in KPLG to consider switching to Recirculating Systems (RAS) rather than the current flow-through systems practices would significantly reduce SSW use and could change the economics of HOST Park. Considering these challenges, the following recommendations are proposed for both HOST Park and KPLG to address and potentially resolve these issues.

Recommendations

The following recommendations are based on the findings in this investigation of the impact of SSW Water Quality on larval rearing at KPLG hatcheries and are divided into near term and long term:

Near Term HOST Park should:

1. Explore the potential of exploiting/converting some existing groundwater wells to become a Hatchery Seawater supply system. This is KPLG's top priority.
2. Continue to communicate with KPLG on issues relating to water quality and efforts to remedy the problems.
3. Implement a monthly high volume flushing protocol to clear the SSW system of potential sediments and contaminants. Consider disposing flushing water to the OTEC sump.
4. Replace existing intake screening on all SSW intake pipes and create a schedule for cleaning/replacing these screens.

Long Term HOST Park should:

5. Conduct a detailed inspection inside the SSW system pipelines, to assess the extent of biofouling in the system.
6. Develop a pigging system for the SSW system to facilitate regular (yearly or as needed) scouring of the pipeline to reduce biofouling in the pipes.
7. Explore potential to clean SSW pipeline using advanced technologies, such as Nanobubbles, to purify SSW supply.
8. Institute bacteria testing to determine amounts and species present in SSW system and the impact on such by flushing, intake screen replacement and nanobubbles.
9. Launch a chemical testing initiative to identify chemical factors in SSW that could be cause of larval mortality including organic toxins, heavy metals, petroleum-based contaminants, pesticides, or others.

Near Term KPLG hatcheries should:

1. Integrate the use of probiotics into hatchery protocols to enhance larval health and resilience.
2. Upgrade hatchery seawater filtration systems to incorporate all of the following "best practices" elements: mechanical filtration (50 um, 10 um, 5 um, 0.5 um), Carbon filtration, and some combination of UV, chlorination or ozone.
3. Implement "best practices" Hatchery filtration system maintenance including regular flushing, acid wash, FW rinse, and dry out.
4. Improve hatchery biosecurity protocols to prevent bacterial contamination.

Long Term KPLG hatcheries should:

5. Replace above ground PVC pipes that may have biofouling buildup to maintain optimal water quality.
6. Install parallel SSW hatchery supply plumbing to facilitate alternating acid wash and dry outs.
7. Install Hatchery bypass plumbing to enable on-farm SSW flushing following interruptions in the SSW system.

INTRODUCTION

The Natural Energy Laboratory of Hawaii Authority (NELHA) is a state agency located at Keāhole Point in Kailua-Kona, Hawaii that operates the Hawaii Ocean Science and Technology Park (HOST Park). HOST Park is globally recognized for its unique dual temperature seawater resources and as a hub for aquaculture innovation. HOST Park provides facilities to support a range of activities from aquaculture to energy production and scientific research.

Economically, HOST Park has a significant impact in the local Kona community and the wider state. According to a report by the University of Hawaii Economic Research Organization (UHERO), the total economic impact of HOST Park's activities was estimated at $145.4 million in 2022. This figure represents the sum of local business sales, employee earnings, tax revenues, and job creation linked to the expenditures of its tenants. These activities support a variety of industries, contributing significantly to local and state economic development.

HOST Park operates the world's largest seawater utility. The system is world renowned for its innovation and scale. Originally designed to allow OTEC R&D and commercial development it includes two massive parallel systems. One system pumps surface seawater (SSW) and one system pumps deep seawater (DSW). OTEC utilizes the temperature differential of these two supplies to drive a turbine to generate electricity. The facility uses a unique deep cold seawater pipeline, one of the few such systems in the world, which draws from a depth of 2-3,000 feet. This cold, nutrient-rich seawater is used in aquaculture and other marine biotechnology applications and also in cooling solutions for renewable energy processes. This system allows for sustainable technology development and research, leveraging the natural deep-sea environment to foster innovation in various scientific fields.

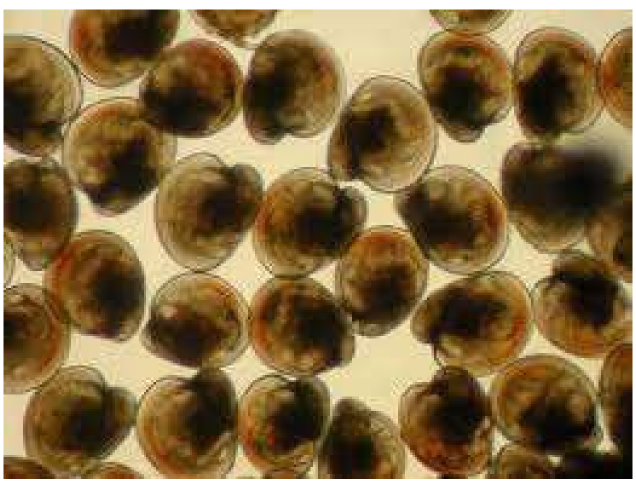
The integration of this advanced seawater supply system with HOST Park's broader economic activities underscores its role as a cornerstone of technological and environmental innovation in Hawaii, with substantial benefits to local communities and the broader economy.

At present, there are approximately 11 private companies at HOST Park who are wholly dependent upon the successful cultivation of delicate and sensitive marine larvae to sustain and grow their aquaculture farming business. As larvae, these species (*i.e.* marine fish, shrimp, abalone, sea urchins, oysters, clams, seahorses, and ornamental fish) represent "canaries in the HOST Park coal mine" as any problematic seawater quality issues can be expected to show up first in the hatcheries. Over recent years, most of the HOST Park-based hatchery managers have experienced poor larval survival when using standardized operating procedures that have previously yielded consistent rates of survival for many years. Hatchery managers report that the larval animals fail to thrive and eventually die, which impacts tenants' profitability and could contribute to company failure. An association composed of HOST Park-based hatchery managers, biologists and company owners formed Keahole Point Larval Group (KPLG) in 2023 to meet monthly and discuss issues of concerns pertaining to hatchery production.

Table 1. KPLG Members

Big Island Abalone	Ocean Era
Blue Ocean Mariculture	Ocean Rider
EcoHarvest	Pacific Hybreed
Indo-Pacific Seafarms	Shrimp Improvement Systems
Jamestown Seafoods	Taylor Shellfish
Moana Technologies	

Discussions with members of the KPLG suggest that these poor larval survival rates may be related to the HOST Park surface seawater system (SSW) due to some unidentified water quality factor(s). The unexplained larval mortalities affect multiple species from diverse animal groups comprised of both vertebrates and invertebrates. While no causative factor has yet been identified, possibilities include some chemical factor(s).



Larval Pacific oysters *Crassostrea gigas*

PROJECT GOALS AND PURPOSE

- Improve the understanding, knowledge and monitoring attributes of the delivered seawater that may be relevant to aquaculture operations using HOST Park-supplied seawater.
- Identify and document water quality factors and the means to monitor and assess them, that may adversely impact larval culture.
- Provide information on such water quality factors that may aid clients in creating and implementing best management practices to mitigate and reduce potential impacts of seawater quality on their operations.

Methodology

This investigation involved meetings with members of the Keahole Point Larval Group (KPLG) and HOST Park technical staff, detailed surveys of KPLG members on water quality and larval problems, compilation of data documenting larval rearing challenges, site visits to each KPLG hatchery to assess their seawater treatment systems and procedures, discussions with outside experts, and a literature review on relevant topics.

Outcomes

A significant water quality issue in the Surface Seawater at HOST Park is impacting marine animal larval rearing thus affecting aquaculture companies that are dependent on successful reproduction of their stocks. This ongoing issue led to the formation of KPLG, consisting of 11 tenant companies at HOST Park. Some of these companies are HOST Park's largest tenants and anchors within the facility. Notably, the persistent water quality problems forced at least one KPLG company to cease operations due to failures in raising marine fish larvae.

The most likely causes of the seawater quality issue are:

- **Biofouling** in the SSW pipeline system may release toxic chemical(s) into the SSW.
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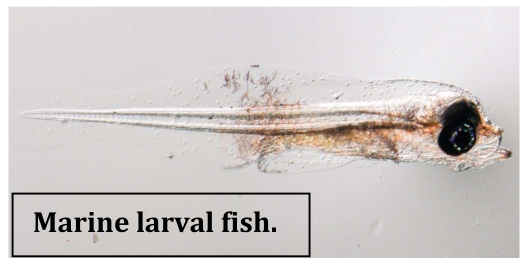
These causes are not mutually exclusive and may be interrelated. A less probable cause might be environmental contamination from nearby sources such as the airport, golf course, or sewage treatment plant. These are highly unlikely given the vast volume of seawater flowing past Keahole Point daily, which would dilute any potential contaminants. Historical data from the last 30 years, including seawater quality measurements in the CEMP monitoring program, have shown no significant changes.

In this investigation, no infectious disease biosecurity risks were identified. None of the KPLG farms showed evidence of reportable, excludable pathogens. Most of these farms are regularly inspected by licensed professionals who test their animals for reportable pathogens as part of their health certification process required for exporting seedstock or broodstock. All certified farms remain free of any reportable pathogens.

However, *Vibrio harveyi* and *V. corallytiicus*, both recognized pathogens of marine organisms, were found to be widespread in recent animal and SSW samples. *V. harveyi* was also detected in aerial samples at HOST Park. A recent environmental DNA (eDNA) study revealed significant presence (0.2-4% of prokaryotes) of *Vibrio* in HOST Park seawater samples. In one instance, *Vibrio corallytiicus,* a well-known shellfish pathogen*,* constituted 40% of the *Vibrio* content in SSW filtered to 5 micrometers. The presence of certain pathogenic *Vibrio* in the seawater may indicate some anaerobic (oxygen-deprived) conditions within the pipeline, which is undesirable for maintaining water quality.

Solutions to date



Marine larval fish.

Operators at KPLG hatcheries have made strides in addressing their larval mortality issues through the application of several chemical treatments to SSW. One such treatment, disinfection with Bio-hydrox, a mineral oxychloride solution, has demonstrated promise in reducing larval mortalities. However, despite the use of Bio-hydrox, high larval mortalities have persisted in some systems and side effects of the Bio-hydrox on animal quality have been observed. Bio-hydrox is an approved drinking water treatment for oxidation and disinfection and is deemed suitable for aquaculture.

Use of ozone in treating SSW has been beneficial for larval fish rearing. Ozone is a powerful oxidizing agent widely used in various water treatment processes, including for seawater. It effectively kills bacteria, viruses, and other microorganisms by disrupting their cellular structures, leading to cell death. Ozone also breaks down organic compounds, including natural organic matter and pollutants like oil and pesticides, enhancing the clarity and quality of seawater. Ozone also controls algal growth and reduces toxin concentrations from certain algae.

Ozone treatment is a common disinfection practice in many marine hatcheries. Ozone significantly improved fish larval rearing outcomes at a KPLG hatchery in 2023. The ozone system installation cost that company $100,000. Encouraged by these results, several other KPLG companies have recently invested in ozone systems for their hatcheries. Although ozone treatment has improved survival rates, the hatcheries have yet to return to previous survival levels, prompting ongoing calls for further SSW system decontamination.

Despite these efforts, the fundamental issue of deteriorating SSW water quality remains unresolved. The problems observed in larval stages suggest possible sub-lethal stresses in the seawater that may affect the efficiency of HOST Park companies in growing post-larval stage animals. The recurring larval mortalities could be indicative of broader systemic issues, acting as HOST Park's "canary in the coal mine." A KPLG fish company has also noted high broodstock mortalities in HOST Park.

RESULTS

HOST Park CEMP

HOST Park operates a Comprehensive Environmental Monitoring Program (CEMP), which plays a crucial role in overseeing and managing the environmental impact of operations at the Keahole Point facility. The CEMP is designed to monitor environmental impact and ensure compliance with environmental regulations and standards set by local, state, and federal agencies.

The program is designed to evaluate the environmental impacts caused by operations at HOST Park. It involves collection of comprehensive data on various environmental parameters, including water quality, biological diversity, and ecological health. It reports these environmental data to the public and other stakeholders.

Water quality monitoring includes a monthly sampling protocol and analysis of temperature, pH, and nutrients. Studies on the biodiversity in the ocean adjoining HOST Park include the status and health of marine flora and fauna. Findings from the CEMP are regularly reported to the governing bodies and the public and listed on HOST Park's website (www.HOSTPark.org).

In the context of current concerns about water quality issues in the SSW system, HOST Park Chief Operations and Science Officer Keith Olson reviewed the CEMP and provided the following comments:

> "During the period from July 2020 to June 2023, no extraordinary groundwater water quality issues were noted. For the same period, HOST Park's ocean transect sampling results were within the historical range of HOST Park's pipeline and ocean transect data set."
>
> "Despite the strong El Nino meteorological conditions, the 2022 coral cover results are in the range of the last ten years of benthic surveys performed at HOST Park. Fish communities have remained relatively stable over the past twenty-eight years of monitoring and chlorophyll-a, a measure of phytoplankton biomass, has never exceeded the State of Hawaii, Department of Health (DOH) limit. Marine water chemistry observations are unchanged since HOST Park's nearshore water quality monitoring inception in 1993. Groundwater water chemistry has been comparatively constant over the past thirty-two years with intermittent anthropogenic nutrient enrichments and associated recoveries.".

These results and findings from surveys of the anchialine pools, nearshore benthic substrate, and nearshore fish assemblages indicate these environments are not exhibiting any signs of detrimental impacts associated with the HOST Park facility.

In summary, the monitoring program conducted over the past 31 years indicates that the fish communities in the area are highly productive and diverse. There are no dramatic declines in abundance or changes in population structure that indicate any detrimental impacts are associated with proximity to the HOST Park facility.

History of Flushing and Pigging at HOST Park

In the early years of HOST Park, there were ongoing projects studying biofouling in the seawater systems at HOST Park. These projects were referenced in the HOST Park EIS filings listed on HOST Park's website. It is common knowledge that open ocean seawater carries a myriad of marine larval forms that cause biofouling problems. In global aquaculture, it is well known that direct ocean intake leads to substantial biofouling so that marine hatcheries usually develop seawater wells to avoid biofouling.

In the 1990s, HOST Park tested a backflush system of the SSW line several times (Jan War, pers. Com). They opened the Kau pump station 24" line to discharge the contents of the backflush into the ocean. The Kau pump intake was inactive at the time. No specific data were gathered at that time and overall impressions were positive for the flushing exercise.

In the 1990s, HOST Park also conducted a single Pigging effort (Jan War, pers. com). This was the only attempt to Pig the SSW lines in HOST Park's history. They purchased a "Polly Pig" from a Houston company. The pig was installed with a forklift into the 28" line at the Light House pump station. It was pushed through the 24" SSW pipeline by seawater pumping pressure down to the ocean discharge at the Kau Station. Jan & Tony Mitchell were waiting at the discharge end of the pipe during the pigging effort. They were seated in their scuba gear at the discharge pipe, and they could hear the pig rumbling through the pipe as it approached. They witnessed 2-3 dump trucks worth of material pushed out of the pipe by the pig and dump into the ocean. Jan noted there were lots of sea urchins and cowry shells in the discharge debris.

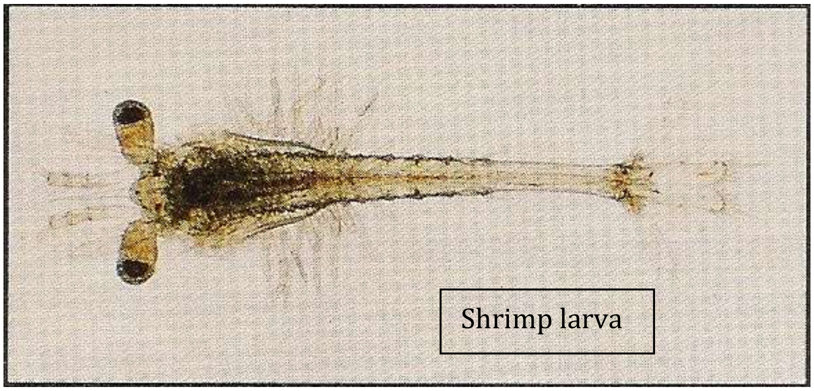
Jan recommends that if another pigging effort is to go forward, HOST Park should seek a permit to discharge the contents directly into the ocean because the contents are seawater plus organic ocean debris from inside the pipeline. Other options are to use a discharge sump to catch the discharge which would be costly and difficult to engineer.

HOST Park Staff Meetings

In January 2024, one on one meetings with HOST Park technical staff were conducted. These included Pam Madden, Water Quality Lab Manager, Alex Leonard, Projects Manager and Keith Olson, Chief Operations and Science Officer. These meetings included detailed discussions of each person's thoughts on the Water Quality issue and possible solutions.

KPLG data

One of the main deliverables of this project is to develop a database of the larval rearing results at KPLG farms to document the impact of the WQ issue on larval rearing. This was quite a challenge because of the nature of individual farm operations. The collected data sets (which are considered proprietary) are listed in Appendix 1.


Shrimp larva

KPLG Site visits

Site visits to each KPLG hatchery were conducted by Dr. Jim in February 2024. These site visits provided additional one on one discussions of the water quality issues impacting each hatchery. In the site visits, the complete seawater system at the hatchery was inspected as well as reviewing their water treatment protocols. In several of the hatcheries it was found that essential elements of a best practice seawater treatment system were missing. In addition, some of the seawater treatment systems were "under engineered" with mismatched filter sizes and plumbing infrastructure. It was also found that some of the seawater treatment systems were not being sufficiently maintained per best practices. Recommendations in the KPLG list address these shortcomings.

Water treatment equipment at a KPLG hatchery.

KPLG Survey

To gain a better understanding of the scope of the problem, a survey was developed to poll the KPLG members to compile specific comments from each company. Nine KPLG companies replied to the survey and are listed in Table 2. It is clear from reviewing Table 2 that the KPLG companies are diverse in the species cultured and have significant experience at HOST Park. The balance of the survey is listed in Appendix 2.

Table 2. KPLG Survey Respondents

Company name	Contact person	Title	Years at HOST	Species Cultivated
Kowa Premium Foods Hawaii Corporation	Ashley von Jetzer	Hatchery manager	26	Abalone *Haliotis discus hanni,* Dulse, sea urchins and sea cucumbers
Shrimp Improvement Systems	Jaime Matsukawa	General Manager	24	*Litopenaeus vannamei* Pacific White Shrimp)

EcoHarvest LLC	Chris Kiser	COO	2	Yellow Tang (*Zebrasoma flavescens*), Blue Tang (*Paracanthurus hepatus*), Multiple Dwarf Angelfish species (*Centropyge sp*)
Pacific Hybreed Inc.	Rachael Taylor	Hatchery Technician	4	Pacific Oysters - *Crassostrea gigas,* Manila Clam - *Venerupis philippinarum, Tetraselmis sp. Chaetoceros muelleri Tisochrysis lutea*
Blue Ocean Mariculture Hatchery	Federico Rotman	VP Hatchery Operations	24	Kanpachi (*Seriola rivoliana*) rotifers, Artemia
Jamestown Seafood	Nathan Tsao	GM	10	Pacific Oyster, Kumamoto Oyster
Ocean Rider Seahorse Farm	Carol Cozzi-schmarr	CEO	26	*Sygnathidae*, copepods, phyto plankton, marine ornamentals (pipe fish and clown fish, cardinal fish, yellow tangs)
Moana Technologies LLC	Geovanni Tolentino	GM	19	*P.monodon*
Indo-Pacific Sea Farms	Gerald Heslinga	President and Founder	29	Macroalgae: *Gracilaria, Ulva, Caulerpa, Halymenia,* Microinvertebrates. no intensive larval production underway.

KPLG Meetings

On February 8, 2024, a KPLG meeting was convened to introduce Dr. Jim to the KPLG, to talk about the SSW Water Quality investigation he was contracted to conduct, and to hear KPLG members thoughts on the WQ issues at HOST Park. 14 KPLG members were present. Following a lively discussion, each attendee was asked to summarize their thoughts on the issue. These were recorded and transcribed and are included in Appendix 3 of this report.

To summarize the issues discussed, KPLG members are experiencing significant mortalities in marine animals larval rearing. In four cases these same tenants had successfully produced marine larvae for many years. A key date in everyone's experience was a mass mortality event in April 2023.

Discussions with outside experts

Outside experts consulted with on the issues of water quality at HOST Park are listed in Table 3.

Table 3. Outside experts consulted in the WQ investigation.

Name and Position
Dr. Micah Brodsky, DVM State of Hawaii Aquatic Veterinarian
Dr. Tom Losordo Aquaculture Engineer
Dr. Ralph Elston Shellfish pathologist
Mr. Jan War Retired Operations Manager HOST Park
Mr. Grant Kunishima GM Kona Bay Shrimp/Hendrix Genetics
Ms. Carol Araki Wyban Retired GM High Health Aquaculture

Bacteria in HOST Park SSW

A group of 4 KPLG companies plus the HOST Park WQ Lab submitted seawater samples for eDNA analysis by AquaBiomics in June 2023. The WQ Lab's samples were collected the day before a SSW shutdown (June 20, 2023) and then after the pumps were turned back on and water was flowing (June 21, 2023).

Environmental DNA (eDNA) sampling from seawater is a powerful method used in marine biology to assess biodiversity and detect the presence of species in aquatic environments. This non-invasive approach involves collecting and analyzing DNA fragments found in water samples, which are shed from organisms through skin, scales, feces, or mucus. DNA is extracted from the material collected by filtration. The DNA fragments are amplified and sequenced to identify the species present. High-throughput sequencing technologies allow for the simultaneous identification of multiple species from a single sample. The primary use of eDNA is to identify which species are present in a particular sample area. This can include detecting rare or elusive species, monitoring biodiversity, and assessing the presence of invasive species.

The data sets received were complex. A sample of the results is listed in Appendix 2 with the KPLG data. One KPLG shellfish company sent their eDNA data to Ralph Elston, a noted shellfish pathologist, and the following are his comments about those data:

"Here are my comments on your seawater analysis:

Raw Seawater analysis. A lot of vibrio. I do not recognize any bad shellfish pathogens from them but need to research a few of them to confirm this statement. BUT, vibrio are facultative anaerobes so the predominance of this group indicates a low oxygen or oxygen deficient condition. This condition will foster Vibrionaceae, which can include known shellfish pathogens.

Filtered surface seawater: This one does contain a smoking gun: Vibrio corralliilyticus (=V. tubiashii) at the high concentration (as computed from DNA analysis minus about 39%, although I am not clear on why there are two listing for this strain). I first isolated this (V. tubiashii) from the HOST Park site at a producer around 2000 (no longer there) having a terrifically bad problem with losses of Pacific oyster larvae and seed due to this pathogen. This information was published in 2008 (reprint attached). While the publication concentrates on a mainland hatchery, it contains quite a bit of information on this isolate from HOST Park, as listed in Table 1 and subsequent figures etc. Some of the other vibrios in your current analysis could be significant but have not been tested for shellfish pathogenicity.

As I am fond of saying, what we know about shellfish pathogenic bacteria is far less than what we do not know. "

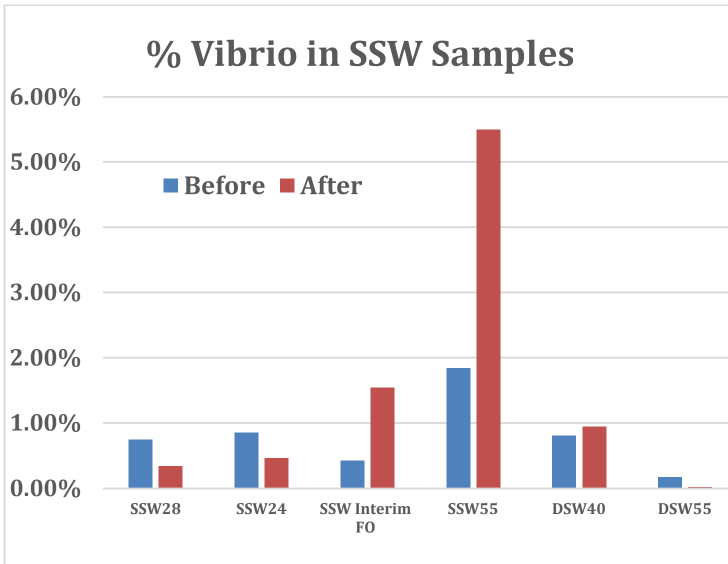
Vibrio is a ubiquitous bacterial group found in seawater and brackish water systems worldwide. Some *Vibrio* species are pathogenic. P. Madden, WQL Manager compiled the eDNA numbers for microbes in the Vibrionaceae family (total of good + bad vibrio) and recorded the %-community composition of Vibrio to create the following table. The last column has the percent *V. coralliilyticus* (bad vibrio) of the entire community. ND = not-detected.

Table 4. Summary of Vibrio Family composition and percent *Vibrio coralliilyticus* based on AquaBiome eDNA data from HOST Park seawater samples (Madden, pers. Com).

Sample ID	Family	Family % Community Composition	V. Coralliilyticus Percent of Community
SSW28 Before	Vibrionaceae	0.75%	0.02%
SSW28 After	Vibrionaceae	0.34%	ND
SSW24 Before	Vibrionaceae	0.85%	0.29%
SSW24 After	Vibrionaceae	0.46%	ND
SSW Interim FO Before	Vibrionaceae	0.42%	0.16%
SSW Interim FO After	Vibrionaceae	1.55%	0.66%
SSW55 Before	Vibrionaceae	1.84%	4.66%
SSW55 After	Vibrionaceae	5.50%	0.52%
DSW-40 Before	Vibrionaceae	0.81%	ND
DSW 40 - After	Vibrionaceae	0.94%	0.13%
DSW-55 Before	Vibrionaceae	0.17%	0.17%
DSW 55 After	Vibrionaceae	0.02%	ND

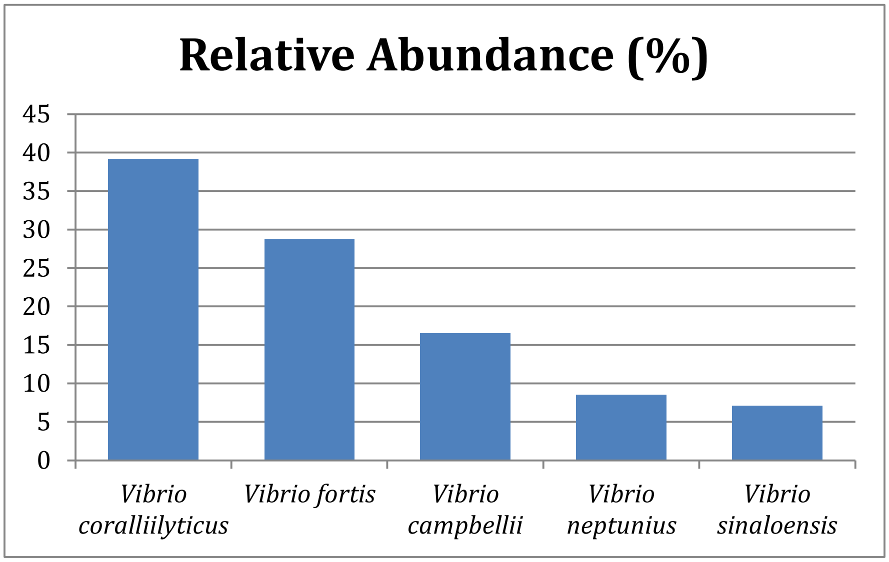
Using these data, the following graph presents the vibrio composition in the various SSW samples comparing Before and After SSW pumping shutdown in June 2023. Two of the samples showed significant increase in Vibrio following SSW interruption. One explanation for this is the restart of the SSW pumping causes a release of Vibrio particles from biofilms inside the pipes. More work is needed to unravel these phenomena.

Figure 1. % vibrio in HOST Park SSW samples collected before and after SSW interruption in June 2023.

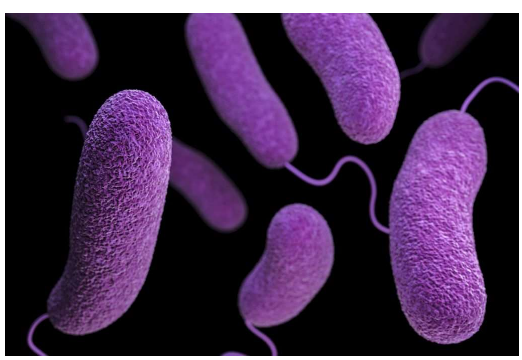


One of the KPLG samples submitted was SSW filtered through 5um filter. This filtration would not remove *Vibrio* but may have in fact concentrated the *Vibrio*. As shown in the following graph, *V. corallytiicus* comprised nearly 40% of the *vibrio* in the sample. There is clearly more work needed in this area and it is strongly recommended that bacteriological testing of SSW be considered.

Figure 2. Relative abundance of *Vibrio* species in SSW in 5 um filtered SSW collected at HOST Park.



One shellfish company that has experienced severe impact of water quality issues has also done a lot of research to find which SSW treatments will alleviate the problem. Comparing many different seawater treatments, larval mortalities remained very high. Their most interesting result was when they used seawater imported from an oyster hatchery in Hilo (Hawaiian Shellfish), they had excellent larval results at HOST Park. This refutes the notion that their consistent problems were caused by technical errors or site-specific problems. They also tested seawater obtained from a HOST Park groundwater well and got good larval results. Two other KPLG shellfish hatcheries have consistently produced shellfish seed at HOST Park for many years. Starting about 2 years ago, they started having significant survival problems and have suffered 100% mortality in the early larval stages (Day 2-10) in their LR systems. While they have been unable to produce post larval seed, they have been able to maintain their businesses by importing post larvae from the mainland and growing them out to seedstock.



Vibrio parahaemolyticus

BACKGROUND AND DISCUSSION

Marine Animal Larval Rearing

The relationship between water quality and larval rearing success in seawater hatchery systems is a critical factor in the aquaculture industry. The quality of water in which larvae are reared directly affects their health, growth, and survival rates. Here are some of the key aspects of this relationship:

1. **Temperature:** Larval organisms often have specific temperature requirements for optimal development. Water temperature influences metabolic rates, digestion, and overall growth. Extreme temperatures can negatively impact larval survival and development.
2. **Salinity:** Larvae of marine species are adapted to specific salinity ranges. Sudden changes in salinity can lead to stress and mortality. Maintaining stable salinity levels is crucial for successful larval rearing.
3. **Dissolved Oxygen:** Adequate dissolved oxygen is vital for larval respiration. Low oxygen levels can lead to hypoxia, negatively affecting larval metabolism and growth. Oxygen levels need to be carefully monitored and maintained in seawater hatchery systems.
4. **pH Levels:** The pH of the water can impact larval development and the availability of essential minerals. Fluctuations in pH can affect enzyme activity and nutrient absorption, influencing overall larval health.
5. **Nutrient Levels**: Proper nutrient levels, including essential minerals and nutrients, are crucial for larval growth and development. Imbalances or deficiencies can lead to deformities, weakened immune systems, and increased susceptibility to diseases.
6. **Toxic Substances**: Presence of pollutants, heavy metals, or harmful chemicals in the water can be detrimental to larval health. Regular water testing and removal of contaminants are essential to prevent negative impacts on larvae.
7. **Microbial Quality:** Microbial quality of seawater is critical to prevent the proliferation of harmful pathogens. Regular monitoring and management of waterborne diseases are essential for successful larval rearing.
8. **Biosecurity Measures:** Implementing biosecurity measures helps prevent the introduction of pathogens and diseases to the hatchery. Quarantine procedures, water treatment, and controlled access help maintain a healthy environment for larvae.
9. **Water Flow and Circulation:** Adequate water flow and circulation ensure the distribution of nutrients and oxygen throughout the rearing system. Stagnant water can lead to localized poor conditions, impacting larval health.
10. **Monitoring and Control Systems:** Advanced seawater hatchery systems utilize automated monitoring and control systems to maintain optimal water quality parameters. This helps in real-time adjustments to ensure a stable and conducive environment for larval rearing.

In summary, maintaining optimal water quality parameters in seawater hatchery systems is essential for the success of larval rearing. Regular monitoring, control measures, and a comprehensive understanding of the specific requirements of the targeted species contribute to creating a healthy and productive environment for larvae.

HOST Park Seawater System

HOST Park's seawater supply system is globally unique in its innovation and scale. The facility uses a deep cold seawater pipeline which draws from a depth of up to 3,000 feet. This cold, nutrient-rich water is not only used in aquaculture and other marine biotechnology applications but also in cooling solutions for renewable energy processes. HOST Park also utilizes surface seawater, which is warmer, around 26 degrees Celsius (79 degrees Fahrenheit). This system allows for a dual-temperature water supply system that can be used for various research and commercial purposes, including temperature-controlled experiments and aquaculture.

Surface Seawater System (SSW)

Surface seawater, being easily accessible, is used primarily for operations that do not require the unique properties of deep seawater. It serves a variety of purposes, including the culture medium for most of HOST Park's aquaculture ventures.

Deep Seawater System (DSW)

HOST Park's DSW system utilizes water drawn from deep below the surface of the ocean, typically from depths of 2-3,000 feet (approximately 650-750 meters). The water at this depth is cold (4 degrees Celsius or 39 degrees Fahrenheit) and nutrient rich. DSW pumping system employs high-capacity submersible pumps located offshore at the specified depth. These pumps are designed to handle large volumes of water, which are transported through pipelines extending from the deep sea to the surface. The seawater is carried through specially designed, corrosion-resistant HDPE pipelines. These pipelines are crucial for maintaining the integrity and quality of the seawater as it is transported over long distances. The cold deep seawater has an additional utility in renewable energy projects, particularly for ocean thermal energy conversion (OTEC) systems, which generate renewable energy by exploiting the temperature difference between deep and surface waters.

The unique properties of the deep seawater are used across multiple fields, including aquaculture and energy production. For instance, cold seawater is ideal for cultivating certain species of marine life, which require specific temperature conditions.

The HOST Park seawater supply system exemplifies state-of-the-art engineering and environmental management, making it a cornerstone of technological and ecological research in Hawaii. This system not only supports local industries but also contributes to global advancements in marine science and renewable energy.

The HOST Park has played a pivotal role in the development and demonstration of Ocean Thermal Energy Conversion (OTEC) technology. OTEC technology, which leverages the temperature difference between warmer surface water and colder deep water to generate energy, saw its first practical tests in the 1970s. HOST Park was established as an ideal site for this technology due to its access to both deep cold and warm surface tropical waters.

In the late 1970s and early 1980s, HOST Park developed its first seawater intake and pumping systems. The initial system was designed to draw cold deep seawater (DSW) from depths of about 2,000 feet and warm surface seawater (SSW) from shallower depths. These intakes were crucial for conducting OTEC research, which requires a significant and continuous flow of both types of water.

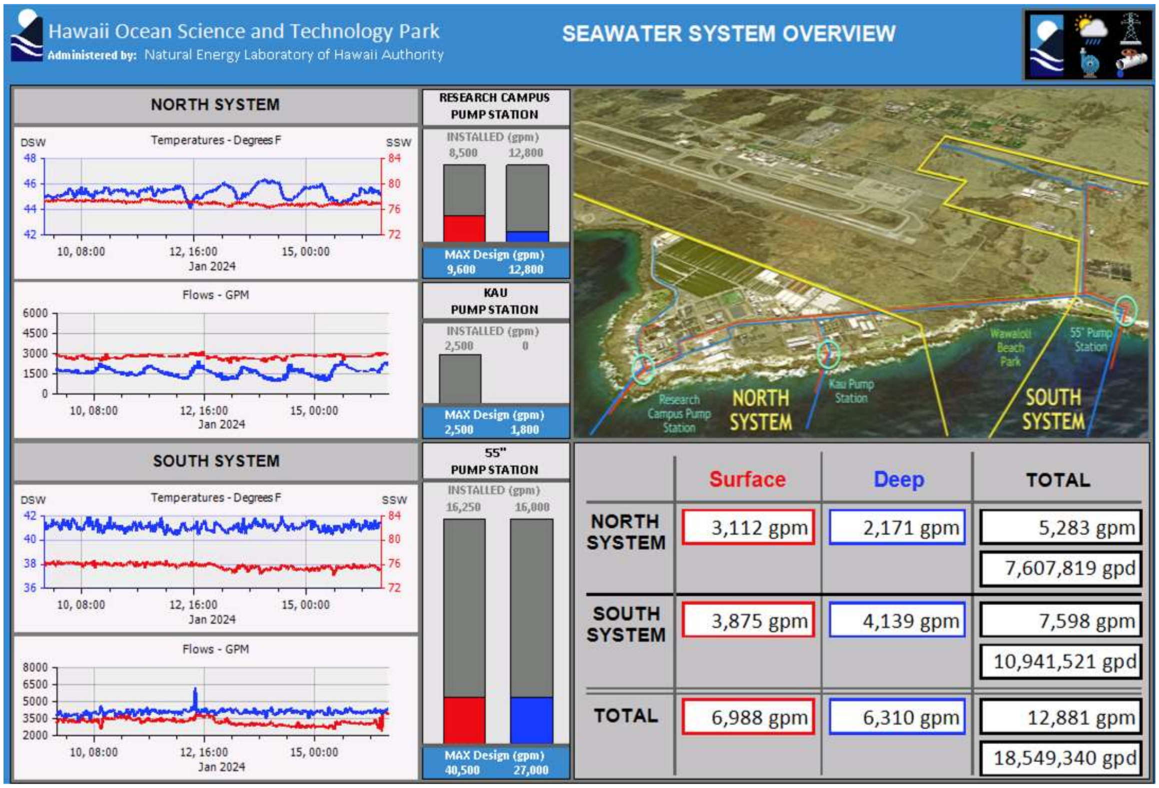
Throughout the 1980s and 1990s, HOST Park expanded its infrastructure to support larger and more sophisticated OTEC experiments. This included enhancements to the pumping systems and the addition of secondary pipes and pumps to increase redundancy and operational flexibility.

Discharge systems were carefully designed to minimize environmental impacts. After being used in the OTEC process, the mixed seawater is discharged at specific depths to ensure it blends smoothly with surrounding water, avoiding thermal pollution and protecting marine life.

Since the 2000s, HOST Park has continued to serve as a hub for OTEC research and development, supporting various international projects and partnerships. The facilities have been upgraded with modern technology to improve efficiency and environmental compliance.

HOST Park's commitment to OTEC is a significant part of its mission to advance green energy technologies. By providing critical infrastructure and expertise, HOST Park helps pave the way for future commercialization of OTEC technology.

Figure 3. HOST Park Seawater system overview (Jan 2024).



Biofouling:

In the early years of HOST Park there was significant concern about biofouling in the seawater pumping systems. There were research projects focused on that issue because of biofouling's potential to reduce pumping efficiencies in the OTEC system. Those concerns about biofouling were primarily focused on pumping efficiency. As interest in OTEC declined and aquaculture became the dominant user of HOST Park seawater, pumping efficiency concerns diminished because HOST Park's installed capacity far exceeded aquaculture needs. For example, while the SSW system has a pumping capacity of 15,000 gpm, it is routinely pumping at 7,000 gpm. Likewise, DSW has a capacity of 27,000 gpm, but currently pumps at 6,000 pgm. Thus, pumping efficiency in the current context is not so important so concerns about biofouling have diminished. But biofouling and its products can have a significant impact on the delicate organisms and larval animals that HOST Park tenants are rearing. In the global aquaculture industry and especially the hatchery (larval rearing) industry, biofouling is of great concern because of the toxicity that it can generate. Biofouling occurs when marine organisms, such as barnacles, mussels, and algae, attach themselves to the inner surfaces of pipes, filters, and other components. This can reduce water flow, increase pressure, and introduce unwanted organisms and toxic chemicals into the aquaculture system. Factors caused by biofouling in seawater systems include:

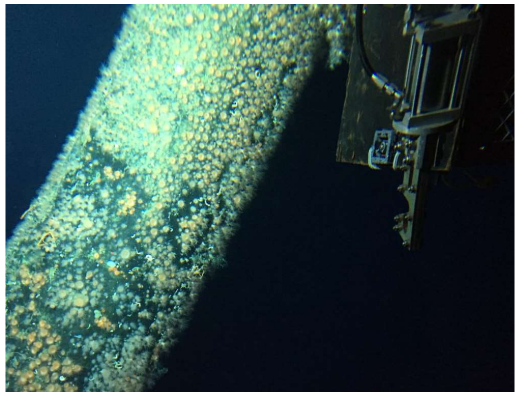
1. **Sediment Accumulation**: Sediments, including sand, silt, and organic matter, can accumulate in seawater delivery systems. Sedimentation can lead to clogging of pipes, reduce water quality, and provide a substrate for the growth of harmful bacteria and pathogens.

Sediment gathered from 25" pipe near Kau pump station. (photo credit Jan War)

2. **Microbial Growth**: Bacteria, viruses, and other microorganisms can proliferate in seawater delivery systems. If not responsibly managed, these microbial populations can lead to waterborne diseases that affect aquatic organisms.
3. **Toxic Chemical Contaminants**: Marine biofouling organisms can produce toxic chemical pollutants. These contaminants may have toxic effects on marine life and accumulate in the aquaculture system over time.

Regular maintenance, monitoring, and preventive measures are essential to mitigate the buildup of biofouling in seawater delivery systems. Filtration, chemical treatment, and biosecurity protocols can help maintain water quality and minimize the risk of contamination, ensuring the health and productivity of aquatic organisms in aquaculture operations.

Biofouling on HDPE DSW pipeline
offshore at HOST Park.



Costs of Biofouling

1. **Increased Energy Consumption**: Biofouling increases surface roughness and mass, leading to higher hydraulic resistance and, consequently, a significant increase in energy costs required to maintain flow rates.
2. **Maintenance and Cleaning Costs**: Frequent shutdowns are necessary for cleaning and maintenance to remove biofouling, leading to high operational costs including labor, equipment, and downtime.
3. **Toxicity Problems**: Biofouling can release toxic substances into the seawater.
4. **Equipment Wear and Corrosion**: Biofouling organisms can accelerate the corrosion of metal parts and degrade materials, leading to faster wear and tear and shorter lifespans of components.
5. **Reduced System Efficiency and Capacity**: Accumulation of biofouling can block intake screens, reduce the diameter of pipes, and impair the function of critical components, thereby decreasing overall system efficiency and capacity.

Chemicals produced by tropical biofouling organisms

Biofouling organisms that accumulate in tropical seawater pumping systems can include a variety of marine species such as bacteria, algae, barnacles, mussels, sponges, and other sessile organisms. These organisms often produce various chemicals as part of their biological processes, particularly those involved in their defense mechanisms against predators and competitors, as well as in communication and adhesion. Some of the key chemical groups produced by marine biofouling organisms that are toxic to marine larvae include:

1. **Bioactive Compounds:** Many marine organisms produce bioactive compounds that can have antibacterial, antifungal, antialgal, or antifouling properties. These are particularly common in sponges and some types of algae.

2. **Extracellular Polymeric Substances (EPS):** These are complex mixtures of proteins, polysaccharides, lipids, and DNA that are secreted by bacteria and other microorganisms. EPS help in adhesion to surfaces and in the formation of biofilms, providing structural integrity and protection to the microbial community.
3. **Toxins and Allelopathic Chemicals**: Certain algae and bacteria produce toxins that can deter predators or inhibit the growth of competing species. Some algae, such as dinoflagellates, can produce toxins as a defense mechanism. These toxins can be harmful to marine life and even to humans in cases where they accumulate in seafood.
4. **Enzymes**: Biofouling organisms often produce enzymes to facilitate nutrient acquisition from the environment or to degrade competitive biofilms. These enzymes can include proteases, lipases, and carbohydrases. Biofouling organisms produce enzymes to assist in adhering to surfaces or to protect against hostile environmental conditions. These enzymes can also contribute to the degradation of materials.
5. **Quorum Sensing Molecules**: Bacteria use these chemical signals to communicate with each other, especially in the formation and maintenance of biofilms. These molecules can regulate a wide range of functions, including biofilm formation, virulence, and resistance to antimicrobial agents. Bacteria in biofilms communicate through quorum sensing, where chemical signals regulate gene expression in response to population density. These molecules can influence various aspects of biofilm development and stability.
6. **Metal Chelators (Siderophores):** Some bacteria produce these compounds to sequester iron from their environment, which is crucial for their growth and metabolism.
7. **Antifoulants:** Some marine organisms produce natural antifoulants to prevent other organisms from settling on them. These can include various halogenated compounds, peptides, and fatty acids.
8. **Corrosion-Inducing Compounds:** Some bacteria, such as sulfate-reducing bacteria (SRB), produce hydrogen sulfide, which can accelerate corrosion of metal surfaces.
9. **Polyketides**: Polyketides are a diverse group of secondary metabolites produced by bacteria, fungi, and other marine organisms. They often exhibit potent biological activities, including antibacterial, antifungal, and antifouling properties. Specific polyketides can deter the settlement and growth of marine larvae, effectively acting as natural antifouling agents.
10. **Terpenoids (Isoprenoids):** Terpenoids are another large and varied class of naturally occurring organic chemicals derived from five-carbon isoprene units arranged in multiple ways. Many marine organisms, including corals and sponges, produce terpenoids, some of which are known to be toxic to marine larvae. These compounds can interfere with the physiological processes of larvae, preventing their attachment and growth on surfaces.
11. **Alkaloids**: Alkaloids found in marine environments are often produced by sponges, tunicates, and certain types of algae. These nitrogen-containing compounds are known for their bioactivity, including toxicity to fish, invertebrates, and even other marine microorganisms. Alkaloids can act as potent toxins that disrupt cellular processes in marine larvae, leading to mortality or repellence.
12. **Phenolic Compounds**: Phenolic compounds, including simple phenols and complex polyphenols, are common in marine algae and higher plants. They have antioxidant properties

but can also act as toxins that inhibit the growth of competing organisms, including marine larvae. These compounds can affect the settlement and development stages of marine larvae, making surfaces unattractive or inhospitable for colonization.

13. **Peptides and Proteins:** Some peptides and proteins produced by marine organisms have antimicrobial and toxic properties. Bioactive peptides, such as those derived from marine cyanobacteria and other microorganisms, can be particularly effective in controlling the settlement of marine larvae due to their targeted biological activity.
14. **Fatty Acids and Their Derivatives:** Certain unsaturated and modified fatty acids have been identified as having antifouling properties. These compounds can alter the lipid composition of larvae membranes, disrupting their normal development and survival.
15. **Sulfated Polysaccharides:** Produced mainly by marine algae, sulfated polysaccharides can inhibit various stages of larval development and settlement. Their mode of action might involve the disruption of signal pathways necessary for larval settlement and attachment.

Sponge fouled pipeline at KPLG tenant's farm at HOST Park. (photo credit BOM)

Understanding the chemical production of these organisms can be crucial for designing effective antifouling strategies and for the management of marine and coastal infrastructures, such as seawater pumping systems.

Impact on Larvae

The impact of these compounds on marine larvae can include disrupted development, altered behavior, reduced survival rates, impaired energy production and mortality.

Flushing and Pigging for Biofouling Removal

Effective management of biofouling in tropical seawater pumping systems is crucial for maintaining operational efficiency, reducing costs, and prolonging equipment life. It requires a multifaceted approach combining physical, chemical, and potentially biological strategies tailored to the specific conditions and needs of the system. Regular monitoring and proactive maintenance are key to controlling biofouling and mitigating its impacts effectively.

Flushing involves the high-velocity flow of water through the system to dislodge and remove biofouling organisms and debris. The process typically uses clean seawater, and the flow rate and pressure must be carefully controlled to ensure effective removal of fouling without damaging the system infrastructure. Flushing is simple and quick to implement and is effective for dislodging loose biofouling materials. Flushing can be used frequently with minimal disruption.

But flushing is less effective against hardened or heavily calcified deposits. Repeated flushing may be necessary. Flushing requires high volumes of water which can be costly.

In Hawaii's tropical climate, the rate of biofouling is accelerated by warm water temperatures. Regular pigging and flushing schedules are necessary to keep up with the rapid growth of biofouling organisms. Both methods should be evaluated for their potential environmental impact, especially in sensitive tropical marine ecosystems. The disposal of biofouling debris and the use of any chemicals in flushing need to comply with local environmental regulations.

Pigging involves the use of a device known as a 'pig', which is sized to fill the pipeline diameter. It is inserted into the pipeline or conduit and travels through the system, driven by the pressure of the seawater inside the pipeline. Pigs come in various forms including foam, spherical, brush, and scraper pigs. The choice of pig type depends on the nature of the fouling, the sensitivity of the pipeline material, and the required cleanliness level.

The operation involves inserting the pig at an access point and retrieving it at another, often with the assistance of flow reversal or additional pushing mechanisms. As the pig travels through the pipeline, it scrapes off or pushes out biofouling debris such as mussels, barnacles, algae, and microbial slimes. It is particularly effective in removing soft deposits and can be used in conjunction with chemical treatments for harder deposits.

Pigs are highly effective for regular maintenance to prevent severe biofouling accumulation; they can be adapted to different sizes and types of pipelines and require minimal chemical use.

Not all pipelines are suitable for pigging. Pigs can miss areas or get stuck, requiring complex retrieval operations. Initial setup costs for pigging infrastructure can be high.

Environmental impact of pigging discharge

The environmental impact of discharging the contents after a pigging event aimed at cleaning up biofouling can be significant. These impacts largely depend on the nature of the biofouling, the composition of the discharge, the location of the outfall, and local marine biodiversity. Here are some of the key environmental considerations:

1. **Release of Organic Material:** Discharging the contents removed by pigging, which can include a mix of marine organisms like mussels, barnacles, algae, and microbial slimes, introduces a large quantity of organic material into the water. This sudden influx can lead to:

- Decreased Oxygen Levels: The decomposition of organic material consumes oxygen, potentially leading to hypoxic conditions that can stress or kill aquatic life.
- Nutrient Enrichment: The breakdown of organic matter releases nutrients (such as nitrogen and phosphorus), which can promote algal blooms. Some blooms, particularly those involving harmful algae, can produce toxins detrimental to marine life and human health.

2. **Physical Impact on Marine Life**

The physical process of discharging large volumes of water and biological debris can have direct physical impacts on marine life:

- Sediment Disturbance: The force of the discharge can resuspend bottom sediments, leading to increased turbidity that can smother benthic habitats and reduce light penetration, affecting photosynthetic organisms.
- Direct Harm: Smaller marine organisms, such as plankton and larval stages of various species, can be physically harmed or killed by the discharge process.

3. **Regulatory and Social Concerns**

- Compliance with Environmental Laws: Discharges must comply with local, national, and international regulations that protect water quality and marine ecosystems.
- Public Perception and Stakeholder Impact: The environmental impacts of such discharges can affect the social license to operate, especially for industries reliant on public trust and resource stewardship.

4. **Mitigation Strategies**

- Treatment Before Discharge: Treating the discharge to remove or neutralize harmful components before it is released into the environment.
- Controlled Release: Modifying the rate or location of the discharge to minimize environmental impacts, such as releasing during high tide or in deeper waters.
- Monitoring and Assessment: Implementing thorough monitoring before, during, and after discharge to assess impacts and effectiveness of mitigation measures.
- Ecological Risk Assessment: Conducting detailed assessments to understand the ecological risks associated with discharge, particularly focusing on sensitive or protected areas.

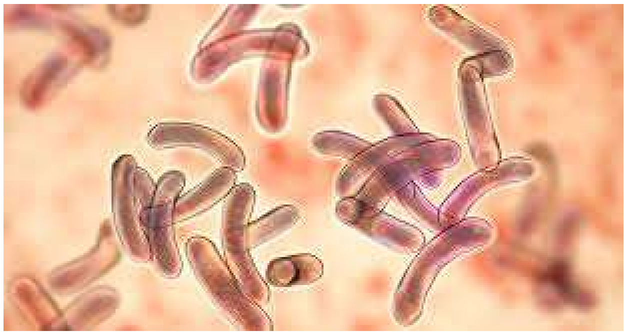
By carefully considering and managing these impacts, operators can significantly mitigate the environmental risks associated with discharging the contents of a tropical seawater pipeline after pigging events.

Presence and Risk of Vibrio Species at HOST Park

This section details the identification and disease potential of various *Vibrio* species found in the HOST Park seawater. Some of these bacteria are recognized for causing disease in marine aquaculture species. Understanding the presence and characteristics of these pathogens is crucial for effective management and preventive strategies.

Vibrio species are ubiquitous gram-negative bacteria inhabiting marine environments worldwide. Given Hawaii's tropical climate and extensive marine activities, the presence of these bacteria poses significant aquaculture health risks. This section identifies common *Vibrio* species in HOST Park, assesses their potential to cause disease, and suggests measures to reduce these risks.

Disease causing Vibrio at HOST Park



Vibrio coralliilyticus

Vibrio coralliilyticus is a temperature-regulated aquatic pathogen that causes coral bleaching and acute death and striping off disease in post larvae small abalone *Haliotis diversicolor*. It has also been reported to cause infection in other fish and shellfish, such as rainbow trout (*Oncorhynchus mykiss*), larval brine shrimp (Artemia spp.), Pacific oyster larvae *C. gigas*, and the European flat oyster (*Ostreaedulis*). *V. coralliilyticus* forms a biofilm on the surface of aquatic animal tissues and organs that has high resistance to stress, antibiotics and the host immune system, resulting in mass mortality of infected animals.

Although primarily known for its pathogenic effects on corals, *Vibrio coralliilyticus* is considered a major pathogen in marine bivalve seed production. It caused a crash of the oyster hatchery industry in Korea in 2015.

V. coralliilyticus has also been identified as a fish pathogen. It has been associated with infections in several species of fish, leading to diseases characterized by symptoms such as skin ulcers, tail rot, and lethargy. The bacterium's ability to thrive at higher temperatures makes it a particular concern in aquaculture as water temperatures rise, either seasonally or due to climate change.

V. coralliilyticus does release toxins, which are involved in its pathogenic effects on marine organisms, especially corals and shellfish. It secretes a range of anti-eukaryotic toxins, enhancing the bacterium's ability to infect and cause disease in its hosts.

Vibrio tubiashii

Vibrio tubiashii is a serious pathogen in shellfish, particularly affecting bivalve larvae such as oysters, clams, and scallops, leading to high mortality rates in hatcheries. It causes diseases such as larval vibriosis and bivalve gastroenteritis. *V. tubiashii* was identified as the cause of significant shellfish disease at HOST Park in 2000. This bacterium has also been linked to disease outbreaks in shrimp farms, causing similar symptoms and significant economic losses.

V. tubiashii is capable of producing various virulence factors, including toxins and enzymes that contribute to its pathogenicity. Management of *Vibrio* infections in aquaculture settings typically involves improving water quality, using probiotics, and in some cases, administering vaccines developed to protect against specific strains. Additionally, good husbandry practices and biosecurity measures are critical to preventing the introduction and spread of *Vibrio* pathogens in aquaculture operations.

Vibrio harveyi

Vibrio harveyi is a well-known pathogen in marine aquaculture, causing significant diseases in both shrimp and fish. In shrimp, *Vibrio harveyi* is one of the primary bacterial pathogens affecting shrimp farming, particularly species like *Penaeus monodon* (black tiger shrimp) and *Litopenaeus vannamei* (whiteleg shrimp). It causes a disease known as luminous vibriosis due to its bioluminescent properties. The infection leads to symptoms such as lethargy, reduced feeding, and high mortality rates. In severe cases, it can cause a systemic infection that leads to rapid death.

In fish, *Vibrio harveyi* causes various infections known collectively as vibriosis. This can manifest as skin ulcers, fin rot, and systemic infections that often result in high mortality. It affects a wide range of fish species used in aquaculture, including seabass, grouper, and bream.

Vibrio harveyi produces several virulence factors, including hemolysins, proteases, and toxins that contribute to its pathogenicity. These factors damage host tissues and impair the immune responses of the infected organisms. Managing infections caused by *Vibrio harveyi* in aquaculture settings involves a combination of strategies, including: Good husbandry practices including maintaining optimal water quality and nutrition; biosecurity measures to preventing the introduction of the pathogen into aquaculture environments; use of probiotics and competitive exclusion by introducing beneficial bacteria that can outcompete *Vibrio harveyi*; and vaccination in vertebrates (fish) can provide immunity against specific strains of the pathogen.

Other common *Vibrios* found in HOST Park seawater include: *V. parahaemolyticus, V. vulnificus, V. alginolyticus.*

Preventing Vibrio disease

Vibrio infections pose a significant health risk to tropical marine aquaculture. Typical hatchery filtration systems using 5 μm (micrometer) filtration will not remove *Vibrio* from water. Vibrio are small rod-shaped bacterium with dimensions generally within the range of about 0.5 to 0.8 μm in diameter and 1.4 to 2.6 μm in length. These dimensions are smaller than the pore size of a 5 μm filter, allowing the bacteria to pass through the filter with ease.

To effectively remove *Vibrio coralliilyticus* and other similar bacteria, a filter with a pore size of 0.2 μm or 0.45 μm is recommended. These smaller pore sizes are capable of physically blocking the bacteria from passing through, thus providing a more effective method for bacterial removal from water samples in both laboratory and environmental settings.

Mitigating the impact of Vibrio-related diseases is crucial for maintaining healthy sustainable aquaculture practices. Here are several interventions and strategies:

1. **Biosecurity Measures:** Implementing stringent biosecurity measures can help prevent the introduction and spread of *Vibrio* pathogens. This includes quarantine procedures for new stock, regular monitoring of water quality, and disinfection of equipment and water supply.
2. **Probiotics and Competitive Exclusion:** Introducing beneficial bacteria into the aquaculture environment can help outcompete pathogenic *Vibrio* species. Probiotics can enhance the host's immune response, improve water quality by breaking down organic waste, and inhibit pathogen growth through competitive exclusion.
3. **Vaccination:** Developing and administering vaccines against specific Vibrio strains can provide fish with immunity against these pathogens. Vaccination has been effective in reducing the prevalence and severity of diseases caused by Vibrio in aquaculture.
4. **Genetic Selection:** Breeding and selection for resistance to Vibrio infections can reduce the incidence of disease. Genetic improvements can enhance the innate immune capabilities of aquaculture species, making them less susceptible to infections.
5. **Environmental Management:** Maintaining optimal water quality and temperature can limit the growth of Vibrio populations. Proper management of organic matter and nutrient levels can reduce the environmental conditions favorable to Vibrio proliferation.
6. **Phage Therapy:** Utilizing Bacteriophages and Endolysins that specifically target Vibrio species is a promising area of research. Phages and Endolysins can be applied to control bacterial populations without affecting other microorganisms and without the risks associated with antibiotic use.

Implementing these interventions requires a multidisciplinary approach, combining microbiology, veterinary sciences, environmental science, and aquaculture management. Such integrated efforts can help minimize the impact of Vibrio-related diseases on marine aquaculture and natural ecosystems.

Chemical treatments to reduce *Vibrio* in SSW

In nursery and growout systems, adding lime, or calcium hydroxide, to seawater with high *Vibrio* counts is an effective method to disinfect the water. Lime is highly alkaline and significantly raises the pH level when added to seawater which kills most *Vibrio* species that prefer a neutral to slightly alkaline environment (pH around 7 to 9). The high pH denatures proteins and disrupts the cell membrane integrity of the bacteria, leading to cell death. This process effectively reduces the population of *Vibrio* bacteria in the water.

Nanobubbles to Manage Biofilms and Biofouling

Nanobubbles is an emerging new technology that uses extremely small bubbles, typically measuring in the nanometer range, that have unique properties that make them effective for various applications, including cleaning seawater delivery pipes. The process involving nanobubbles in cleaning pipes is often referred to as "nanobubble cleaning" or "nanobubble technology for pipe cleaning."

Nanobubbles are generated using specialized equipment that can produce bubbles with diameters on the nanoscale. Nanobubbles have a much higher surface area compared to larger bubbles. This increased surface area enhances their cleaning efficiency as they come into contact with contaminants on the surfaces of the pipes. Nanobubbles have the ability to collapse and create microscale shockwaves through a process known as cavitation. This cavitation effect generates localized energy that helps dislodge and remove contaminants from the surfaces of the pipes. Nanobubbles can penetrate biofilms, which are thin layers of microorganisms that can accumulate on the interior surfaces of pipes. The small size of nanobubbles allows them to reach and disrupt biofilms more effectively than larger bubbles.

1. Nanobubbles can selectively target and clean specific types of contaminants, including algae, bacteria, and organic deposits. This targeted cleaning ability minimizes the need for harsh chemicals and reduces environmental impact. The use of nanobubbles for cleaning pipes can reduce the dependence on chemical cleaning agents. This is particularly beneficial in applications where minimizing chemical residues in the seawater is a priority.
2. **Energy Efficiency**: Nanobubble systems are designed to be energy efficient. The small size of the bubbles and the efficient generation process contribute to reduced energy consumption compared to traditional cleaning methods.
3. **Non-Destructive Cleaning**: Nanobubble cleaning is generally non-destructive to the infrastructure of the pipes. The gentle yet effective cleaning action helps preserve the integrity of the pipes over time.
4. **Environmental Sustainability**: The use of nanobubbles in pipe cleaning aligns with environmental sustainability goals. By minimizing the use of chemicals and energy, nanobubble technology offers a cleaner and more eco-friendly approach to maintaining seawater delivery systems.
5. It's important to note that the effectiveness of nanobubble cleaning may depend on factors such as water chemistry, pipe material, and the specific types of contaminants present. Application-specific considerations and system design play a crucial role in optimizing the performance of nanobubble technology for cleaning seawater delivery pipes.

Ultrafiltration to remove *Vibrio*

Ultrafiltration can be used to remove *Vibrio* from water. An Ultrafiltration device, called AQQA-system is currently being demonstrated at HOST Park by Pacific Filtration Systems. In preliminary tests of this system, the ultrafilter removed 97-99% of the bacteria present in SSW passed through the ultrafilter.

The AQQA®-system is a submerged ultrafiltration system, which combines proven and new, unique features. The filter is submerged into the untreated water. With a gentle pressure the water is sucked into the filter, leaving all particles and bacteria on the outer surface of the membrane. An optimized flow of air bubbles and water achieves a constant cleaning effect with high shearing forces on the membrane surface. This cleaning effect alone can keep the filter clean for up to one year. The AQQA®-filter is the first filter with solid plates that can be back-flushed by reversing the flow and pressing a cleaning liquid into the filter. By doing this, all pores are kept open and the filter can operate sustainably with a much higher output. In normal operation, the filter remains in the water and does not have to be removed from the tank, as was the case with filters of the first generation.

A bacteria removal sampling was conducted by Microbiology Consultants where bacterial content of incoming NELHA SSW and seawater after AQQA filter were determined. The following table shows the results.

Table 5. Pacific Filtration Systems Ultrafilter Testing at Keaohole Point. NELHA Surface Seawater was tested before and after filtration on 6/14/2024.

	Marine Agar	**Marine Agar**	**TCBS**
	Large CFU	**Pinpoint CFU**	**Vibrio count**
	CFU/100mL	**CFU/100mL**	**CFU/100mL**
Pre-filter	9,700	1,840,000	3,000
Post-filter	28	34,000	86
% Bacteria Removed	**99.71%**	**98.15%**	**97.13%**

24hr incubation CFU = Colony Forming Unit

These data show that the AQQA filter removed 97-99% of the bacteria in the SSW. These data confirm AQQA's literature on the device.

A comparison of the bacteria content of the unfiltered SSW in this study can be compared to historical SSW data collected between 2005 and 2007 in the following table. These data show the quantity of bacteria in SSW has not changed significantly.

Dates	**Vibrio CFU/100mL**	**Total Count Marine Agar CFU/100mL**
2005-2007	2,015	12,237
2024	3,000	9,700

Recommendations

The following recommendations are based on the findings in this investigation of the impact of SSW Water Quality on larval rearing at KPLG hatcheries and are divided into near term and long term:

Near Term HOST Park should:

1. Explore the potential of exploiting/converting some existing groundwater wells to become a Hatchery Seawater supply system. This is KPLG's top priority.
2. Continue to communicate with KPLG on issues relating to water quality and efforts to remedy the problems.
3. Implement a monthly high volume flushing protocol to clear the SSW system of potential sediments and contaminants. Consider disposing flushing water to the OTEC sump.
4. Replace existing intake screening on all SSW intake pipes and create a schedule for cleaning/replacing these screens.

Long Term HOST Park should:

5. Conduct a detailed inspection inside the SSW system pipelines, to assess the extent of biofouling in the system.
6. Develop a pigging system for the SSW system to facilitate regular (yearly or as needed) scouring of the pipeline to reduce biofouling in the pipes.
7. Explore potential to clean SSW pipeline using advanced technologies, such as Nanobubbles, to purify SSW supply.
8. Institute bacteria testing to determine amounts and species present in SSW system and the impact on such by flushing, intake screen replacement and nanobubbles.
9. Launch a chemical testing initiative to identify chemical factors in SSW that could be cause of larval mortality including organic toxins, heavy metals, petroleum-based contaminants, pesticides, or others.

Near Term KPLG hatcheries should:

1. Integrate the use of probiotics into hatchery protocols to enhance larval health and resilience.
2. Upgrade hatchery seawater filtration systems to incorporate all of the following "best practices" elements: mechanical filtration (50 um, 10 um, 5 um, 0.5 um), Carbon filtration, and some combination of UV, chlorination or ozone.
3. Implement "best practices" Hatchery filtration system maintenance including regular flushing, acid wash, FW rinse, and dry out.
4. Improve hatchery biosecurity protocols to prevent bacterial contamination.

Long Term KPLG hatcheries should:

5. Replace above ground PVC pipes that may have biofouling buildup to maintain optimal water quality.
6. Install parallel SSW hatchery supply plumbing to facilitate alternating acid wash and dry outs.
7. Install Hatchery bypass plumbing to enable on-farm SSW flushing following interruptions in the SSW system.

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