FINAL STUDY REPORT

The efficacy of a novel water sterilization system for potential fish culture applications

Study Number: HMSC-165

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Regulatory status:	GRP
Authors:	Study Director: Dr. Ehab Misk Facility Management: Chris Bridger
Sponsor:	Pontic Technology LLC

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Marine Science Centre

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(Page 1 of 3)

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This Final Study Report has been reviewed and approved by:

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Michael Papadopoulos
Sponsor Representative
Pontic Technology LLC

15-5-2023
Date: DD-MON-YY

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Study Number: HMSC-165

This Final Study Report has been reviewed and approved by:

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Chris Bridger
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16-MAY-23
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FINAL STUDY REPORT

The efficacy of a novel water sterilization system for potential fish culture applications

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I have prepared this Final Study Report and agree that it contains all necessary details that were performed during the study as described.

This study was conducted according to the Study Plan following the principles of Good Research Practice (GRP) and applicable regulatory requirements.

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Ehab Misk — 16-MAY-23

Dr. Ehab Misk
Study Director
Huntsman Marine Science Centre

Date: DD-MON-YY

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List of Abbreviations

CFU – Colony Forming Unit

CMA – Corn Meal Agar

ITS - Internal Transcribed Spacer

JPL - Jet Propulsion Laboratory

LLC - Limited Liability Company

MA – Marine Agar

MB – Marine Broth

MWP – Microwell Plate

PBS – Phosphate Buffered Saline

rRNA – Ribosomal RNA

SDB – Sabouraud Dextrose Broth

SFW – Sterile Fresh Water

SMF – Study Master File

TDSS – Thermal Disinfection Steralization Systems

TSA – Tryptic Soy Agar

TSB – Tryptic Soy Broth

The efficacy of a novel water sterilization system for potential fish culture applications

1 Summary

- This study aimed to test a novel water sterilization instrument (Pontic EP-EH-200 Prototype) to neutralize the following:
    - Oomycete spores from *Saprolegnia parasitica* in spiked freshwater;
    - *Aeromonas salmonicida* – a freshwater bacteria; and,
    - *Tenacibaculum tordalis* – a seawater bacteria.
- Oomycete evaluation
    - *Saprolegnia parasitica,* identified by 18S rRNA gene ITS sequencing, used to generate spores to achieve a target concentration of 10,000 spores per liter.
    - In seven independent trials (1 to 7), the efficacy of the device to neutralize *Saprolegnia* growth was tested post passage.
    - Triplicate samples (i.e., technical replicates) were collected at ambient temperature (10-20 °C), 50 °C, 80 °C, 100 °C and 120 °C in tests #1 to 4 while ambient, 80 °C, 100 °C, 120 °C and 140 °C temperatures were tested in experiments #5 to 7.
    - Viable spores counts were evaluated in each sample using a 96-well microwell plate (MWP) method.
    - The average efficacy was more than or equal to 98.73% when the instrument temperature was 100°C – 140°C.
- Bacteria evaluation
    - *Aeromonas salmonicida* (freshwater bacterium) and *Tenacibaculum tordalis* (seawater bacteria) were tested. In addition, the results from incidental inclusion of a sediment bacteria (*Alteromonas naphthalenivorans)* were also reported.

- Bacteria stock solution was passed through the TDSS system and samples were collected and quantified by Colony Forming Units (CFU).
- *Aeromonas salmonicida* had more than 99.99% reduction following treatment by the instrument at 80°C and 100% elimination at 100°C and above.
- *Tenacibaculum todarodis* reduction was more than 99.9% at 50°C and 100% at temperatures of 80°C and above.
- *Alteromonas naphthalenivorans* reduction was less than 90% at 50°C but 100% at temperatures of 80°C and above.

- In conclusion, the Pontic EP-EH-200 Prototype was effective to neutralize oomycete spores from *Saprolegnia parasitica* in spiked freshwater. Efficacy was also demonstrated for the freshwater bacteria *Aeromonas salmonicida* and the seawater bacteria *Tenacibaculum tordalis* and *Alteromonas naphthalenivorans* at varying temperature settings.

The efficacy of a novel water sterilization system for potential fish culture applications

2 Objectives

The study sponsor (Pontic Technology LLC) is a multidisciplinary group of scientists, engineers, and technologists from NASA's Jet Propulsion Laboratory (JPL) in Pasadena, California. This study tested the Pontic Technology Thermal Disinfection Sterilisation System (TDSS), which was developed to provide high-volume, continuous flow sterile water at a competitively low operating cost, for use in target aquaculture applications.

This study tested the novel water sterilization instrument (Pontic EP-EH-200 Prototype) to validate the capability and efficacy of the water sterilization system for possible aquaculture and hatchery applications by neutralizing the following:

1- Oomycete spores from *Saprolegnia parasitica* in spiked freshwater;

2- *Aeromonas salmonicida* – a freshwater bacteria; and,

3- *Tenacibaculum tordalis* – a seawater bacteria.

3 Methods

3.1 Instrument

The TDSS technology aims to achieve sterilization by superheating water, while maintaining in a liquid state, to eliminate any contaminants in an economical and environmentally responsible way, no matter the level of contamination of the source. Sterilization is achieved through high heat, pressure and dwell time in which temperature and pressure can be changed while the process is operating thereby allowing sterilisation from a variety of microbes with varying thermal inactivation temperatures using a single device.

The sterilization instrument was maintained and operated according to the Sponsor's instructions (Appendix 1).

3.2 Testing *Saprolegnia*

Saprolegnia parasitica isolate was previously isolated from Atlantic salmon by the Huntsman Fish Health Laboratory and characterized using 18S rRNA gene ITS sequencing.

The isolate was maintained at Huntsman on Corn Meal Agar (CMA) or hemp seeds incubated at 21°C or 4°C. The spores were produced according to Hatai and Hoshiai (1993). Briefly, *Saprolegnia* was grown in Sabouraud Dextrose Broth (SDB) to collect mycelia. Mycelia were then washed with Sterile Fresh Water (SFW) and then split to stimulate extensive spore formation.

3.2.1 Experimental Design

Saprolegnia parasitica spores were collected, the solution concentration quantified using a hemocytometer, and then diluted with filtered (0.2 µm) SFW to achieve a target concentration of 10,000 spores per liter in a 20-liter clean bucket.

In seven independent trials (1 to 7), the efficacy of the instrument was tested to neutralize *Saprolegnia* growth in samples collected from the effluent post-passage. Triplicate samples (i.e., technical replicates) were collected at ambient temperature (10-20°C), 50°C, 80°C, 100°C and 120°C in tests #1 to 4 and ambient, 80°C, 100°C, 120°C and 140°C in experiments #5 to 7.

The capacity of the instrument to affect *Saprolegnia* growth was quantified for each stock solution and all collected samples as described by Thoen et al. (2010). Briefly, 100 µl of each sample were inoculated in each well of a 96-well microwell plate (MWP) filled with 100 µl of SDB media then incubated at 21 °C and monitored for growth. The number of wells showing *Saprolegnia* growth was counted using an inverted microscope (10x).

3.2.2 Instrument Use

The instrument was used without initial decontamination of the interior water treatment pathway in experiments #1 to 4. The interior water treatment pathway of the instrument was subsequently decontaminated by passing 20 L of 5% sodium hypochlorite solution followed by 60 L of sterile freshwater through it for the remaining experiments #5 to 7.

3.2.3 Inclusion

All experiments that resulted in more than 50% recovery of *Saprolegnia* growth from the collected Ambient temperature effluent sample were considered to be valid and included in the statistical analysis.

3.2.4 Measurement of Efficacy

The percent efficacy was calculated using Abbott's formula as the percentage of the difference between mean positive wells at Ambient temperature and each of the temperature groups divided by the mean count of the Ambient temperature group.

Efficacy at each temperature was calculated as follows:

Percent efficacy = 100 * (MA – MT) / MA

Where: MA = arithmetic mean counts in the Ambient temperature group

MT = arithmetic mean counts in the temperature group

3.2.5 Statistical Analysis

All statistical analysis was completed with STATA (STATA CORP. Ver 14). All completed Data Capture Forms (DCFs) and entered data were subjected to Huntsman quality control processes in accordance with SOPs.

A negative binomial regression model was applied to mean positive well counts of the technical replicates for all experiments for each temperature. The temperature groups and cleaning step were

included as categorical variables in the model. Robust standard errors for the parameter estimates were used. Significant variable effects at a level of 0.05 were kept in the model. The model assumptions and goodness of fit validation were also assessed.

3.3 Testing Freshwater and Seawater Bacteria

3.3.1 Bacteria

Two bacterial species were tested, *Aeromonas salmonicida* and *Tenacibaculum tordali*. Both bacteria were cultured and identified using 16S rRNA sequencing. *Aeromonas salmonicida* was isolated from Atlantic salmon and cultured on Trypticase Soy Agar/Broth to represent a fresh/brackish water bacteria. *Tenacibaculum tordali* was isolated from lumpfish and cultured on Marine Agar/Broth to represent seawater bacteria.

3.3.2 Instrument Use

The bacteria trials followed those completed earlier for *Saprolegnia* and therefore the interior water treatment pathway of the instrument was subsequently decontaminated by passing 20 L of 5% sodium hypochlorite solution followed by sterile freshwater or seawater until there was no more detectable chlorine in the effluent water (HACH DR 900 – Method 8021).

3.3.3 Experimental Design

Both cultures were propagated on Broth medium and growth curves were established to determine the best time to test cultures such that bacterial cultures were in the log-phase of growth. The cultures were then centrifuged and washed with PBS before being resuspended into 20L of cold-filtered (0.045 μm) fresh or seawater to reach expected target concentrations of 1E7 and 1E5 CFU/mL.

The suspension was allowed to pass through the Pontic EP-EH-200 device (~0.26 l/min). Samples were collected from both the suspension and the effluent water at several temperatures including

Ambient, 50℃, 80℃, 100℃ and 120℃ in three 25 ml sterile test tube/sample. Residence time of ~5-10 minutes were allowed before sampling after the instrument reached the target temperature.

3.3.4 Bacterial Quantifications

The bacterial content of the samples were quantified using Colony Forming Units (CFU). Briefly, the collected samples were diluted with 10-fold serial dilutions in PBS. All tubes were gently vortexed and 100 µl was taken from each sample then evenly spread on TSA or MA and monitored for growth. All plates with more than 50 colonies or that had only one colony were discarded to allow reliable estimation of the concentration.

3.3.5 Bacterial Reduction

The reduction of the bacterial concentration in the samples, expressed as CFU, from the suspension (Influent) to the Effluent samples following passage through the instrument was estimated as log10 reduction using the following formula:

$$Log_{10}\ Reduction = -Log_{10}\left(\frac{N_{Suspention}}{N_{Effluent}}\right)$$

The conversion from log10 reduction to percent reduction is provided in Table 1.

Table 1 Log10 removal conversion to percent reduction

Log_{10} Removal	Percent Reduction
1	90
2	99
3	99.9
4	99.99
5	99.999
6	99.9999
7	99.99999
8	99.999999

4 Study Record Maintenance

Data were collected in compliance with the specific procedures referred to or described in the Study Plan and in compliance with any Amendments that were in effect at the time the study was conducted. Upon study completion, all original or authenticated copies of the study records collected for the study were placed into the Study Master File (SMF) to be archived by the Sponsor as per local, regulatory, and company requirements.

5 Results and Discussion

5.1 Testing *Saprolegnia*

5.1.1 Inclusion

The spore positive wells in the all 96-well plates were visible and counted using an inverted microscope to ensure all positive wells were included (Figure 1).

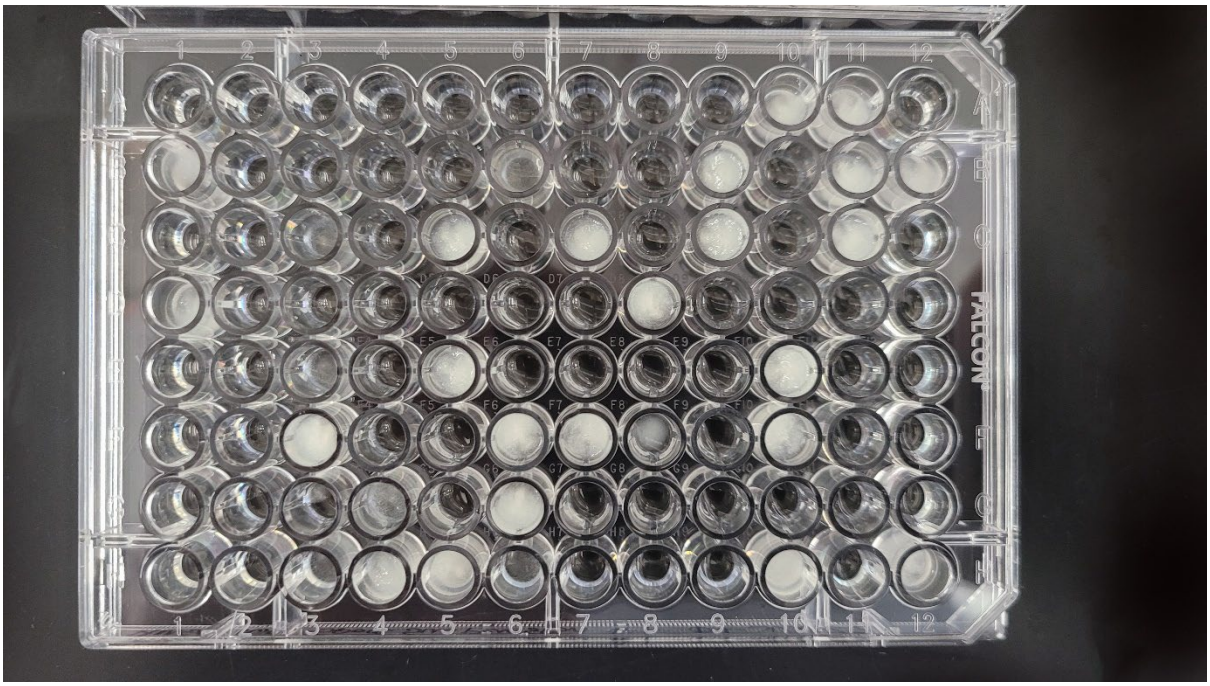

Figure 1 Evaluation of spore concentration on a 96-well plate showing visible *Saprolegnia* growth after seeding with *Saprolegnia* spore suspension.

The counts in the stock were evaluated and percent recovery from the Ambient temperature was calculated (Table 2). A recovery rate of more than 50% was considered to be the cutoff value for inclusion. Trial #5 was excluded from the study due to a very low recovery rate of 3.9%.

Table 2. Recovery rate for *Saprolegnia* spores after passing the stock solution through the instrument at Ambient temperature. Trial # 5 was excluded from further statistical analysis due to the low recovery rate below 50%.

Trial Replicate	Mean Positive Well Count		Recovery %
	Stock	Recovered	
1	27.7	16.0	57.8
2	22.3	15.0	67.2
3	16.7	14.7	88.0
4	18.3	15.0	81.8
5	17.0	00.7	03.9
6	15.3	09.3	60.9
7	17.0	09.0	52.9

5.1.2 Measurement of Efficacy

A significant reduction in *Saprolegnia* counts was observed by increasing the temperature of the instrument (Figure 2).

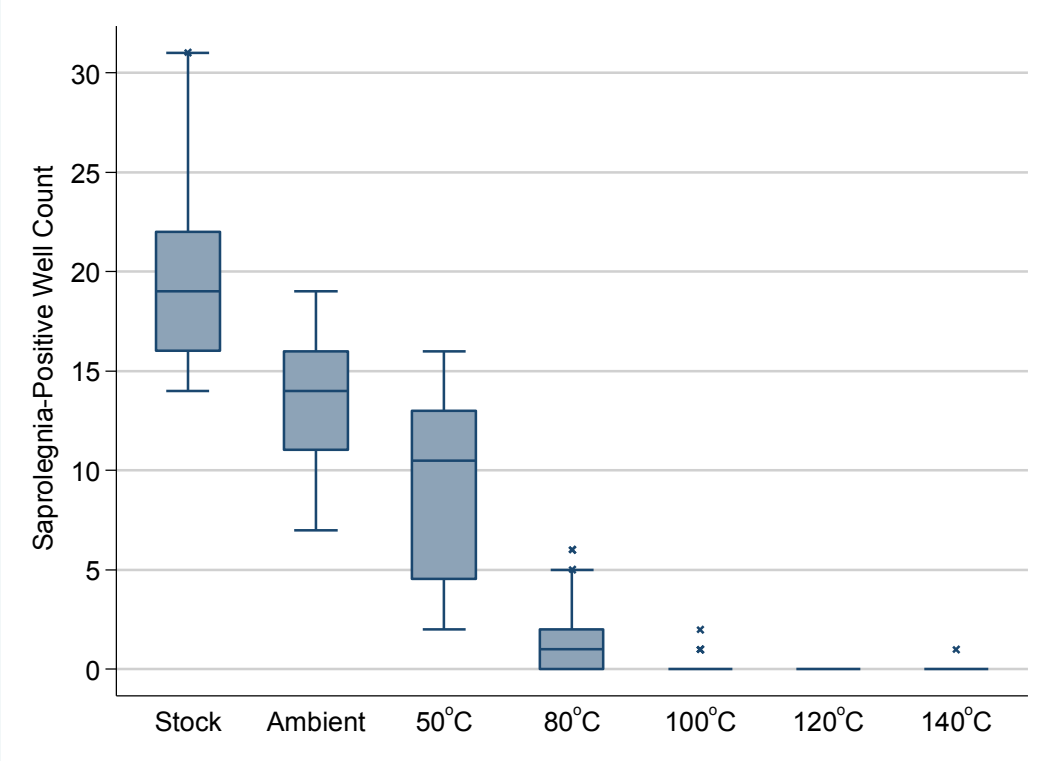

Figure 2. *Saprolegnia* well counts box-plot from all trials and technical replicates at each temperature group.

The efficacy of neutralizing *Saprolegnia* spores was calculated at each temperature from all the independent trials as described in the methods (Section 3.2.4). The results (Table 3) indicated that the average efficacy was more than or equal to 98% when the instrument temperature was above 100°C. At 140°C, the average efficacy was 98.2 percent, however, it is important to consider that this temperature was only tested twice and the 120°C had a 100% efficacy in six independent tests.

Table 3. The mean count and standard deviation of positive wells and the mean efficacy to neutralize *Saprolegnia* spores at each temperature group compared to the Ambient temperature group.

Exposure Temperature	Mean count at Ambient	Std. at Ambeint	Mean count at Temperature	Std. at Temperature	Mean Efficacy of Inhibition %	Std. of Efficacy of Inhibition	Number of Independent Experiments	Number of Replicatss Per Experiement
50°C	15.2	0.6	9.4	5.3	36.9	36.0	4	3
80°C	13.2	3.1	1.7	1.5	83.6	17.4	6	3
100°C	13.2	3.1	0.3	0.3	98.0	1.7	6	3
120°C	13.2	3.1	0.0	0.0	100.0	0.0	6	3
140°C	9.2	0.2	0.2	0.2	98.2	2.5	2	3

5.1.3 Regression Analysis

The statistical analysis of mean well counts for the three technical replicates in all trials was tested using a negative binomial regression analysis. The outcome variable was the average positive counts in the technical replicate and the independent variables were temperature groups (categories) and cleaning (Categories: before and after). The results of the model indicated that cleaning had no significant effect (P= 0.457) on the model so it was removed.

The model estimated an overdispersion parameter with CI [2.95e-06 – 107.978] indicating that the negative binomial model was a better choice for the data than the Poisson model. The Anscombe residuals were normally distributed using the Shapiro-Wilk test for normal distribution. The Deviance goodness-of-fit (6.859449, P=0.999) fitted better than in the Poisson model (24.68202, P=0.423).

The results indicated that the 80 to 140°C temperature treatment groups were statistically different from Ambient temperature control groups. The 50°C temperature treatment group was not statistically different from the Ambient temperature control (Table 4).

Table 4. Negative binomial model analysis for average positive wells count data in all temperatures.

Temperature	Coef	Robust SE	Z	P>|z|	Confidence Interval	
50°C	-.3352273	.2622813	-1.28	0.201	-.8492891	.1788346
80°C	-2.034074	.3415759	-5.95	0.000	-2.703551	-1.364598
100°C	-3.858494	.3540443	-10.90	0.000	-4.552408	-3.16458
120°C	-20.02581	.4249059	-47.13	0.000	-20.85861	-19.19301
140°C	-4.369396	.7247886	-6.03	0.000	-5.789956	-2.948837
Intercept	1.479086	.0901734	16.40	0.000	1.302349	1.655822

Furthermore, the 50°C temperature treatment group was significantly different from all the higher temperature treatment groups ($p<0.000$ in all) indicating that the 50°C temperature treatment group was not much different from the Ambient temperature control group. Also, the 80°C temperature treatment group was significantly different from the 100°C (χ^2 =14.74, P=0.0001), 120°C (χ^2=1152.14, P<0.000), and 140°C (χ^2=8.72, P=0.0032) temperature treatment groups. Finally, the 120°C temperature treatment group was significantly different from both the 100°C and the 140°C temperature treatment groups (P<0.000 in all). Although the 120°C and 140°C temperature treatment groups were found to be statistically different there was no biological relevance to this finding. Therefore, increasing sample size and further testing are expected to find no difference between these two highest temperature treatment groups .

5.1.4 *Saprolegnia* Conclusions

The results from this analysis show that the instrument was effective in neutralizing *Saprolegnia* spores at various temperatures, including 80°C, 100°C, 120°C, and 140°C. The average efficacy above 100°C was 98.73%.

5.2 Testing Freshwater and Seawater Bacteria

5.2.1 *Aeromonas salmonicida* freshwater bacteria

The bacterial growth on TSB in a pre-test serial dilution indicated that 48 hrs of growth was suitable to produce enough bacterial growth for the testing of this species (Figure 3).

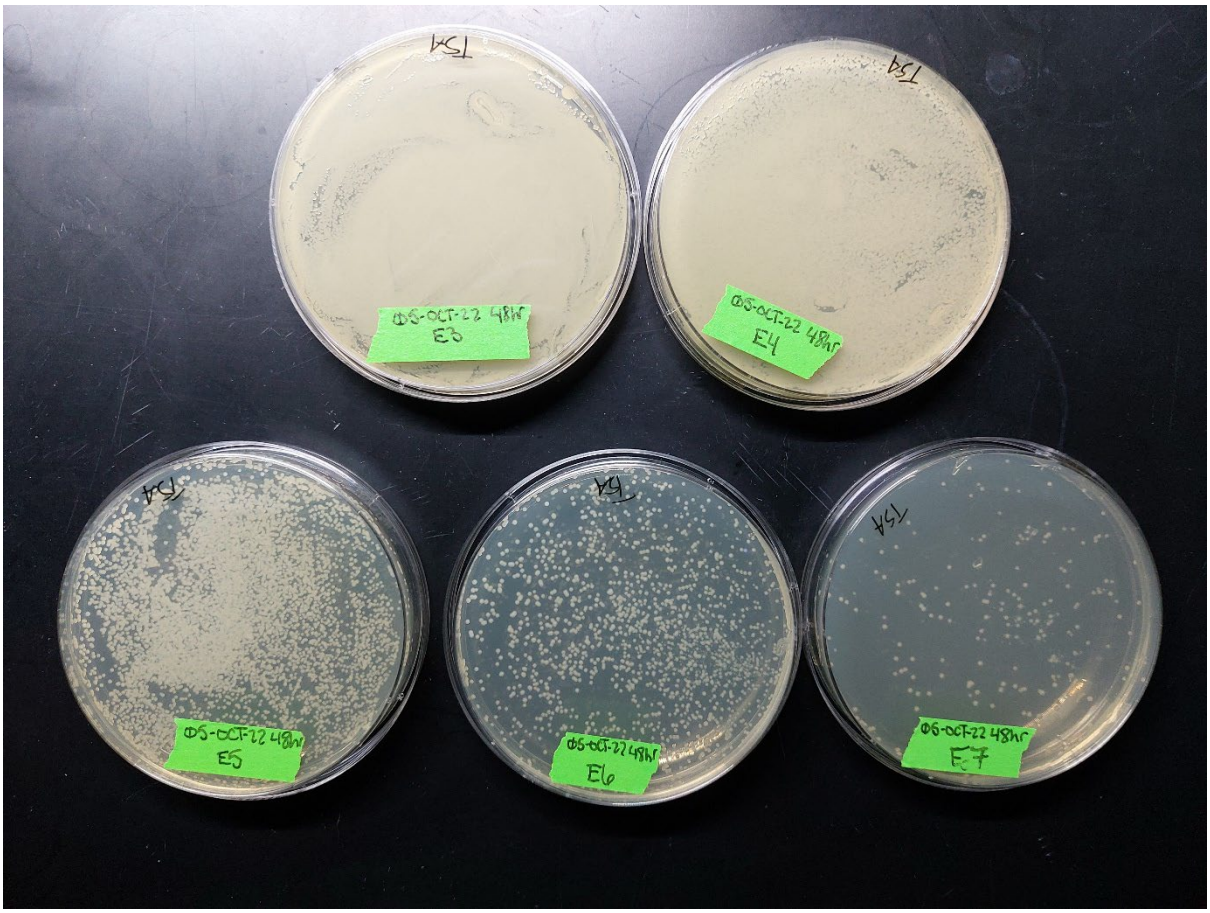

Figure 3 CFU of serial dilutions of *Aeromonas salmonicida* cultures from TSA broth after 48 hrs.

The average concentration of the bacteria in the suspension was 4.1E6 CFU. The log10 reduction following the passage of the suspension through the instrument then collected in the effluent samples was calculated and presented in Table 5.

Table 5 Log10 reduction of *Aeromonas salmonicida*

Sample	Rep.	Concentration (CFU)	Log_{10} Reduction	Log_{10} Reduction Average	Log_{10} Reduction Standard Deviation
Ambient	1	3.00E+06	0.136	0.060	0.131
Ambient	2	5.05E+06	-0.091		
Ambient	3	3.00E+06	0.136		
50C	1	2.00E+03	3.312	3.009	0.329
50C	2	9.00E+03	2.659		
50C	3	3.60E+03	3.056		
80C	1	4.00E+01	5.011	4.703	0.435
80C	3	1.65E+02	4.395		
100C	1	0.00E+00		NA	NA
100C	2	0.00E+00			
100C	3	0.00E+00			
120C	1	0.00E+00		NA	NA
120C	2	0.00E+00			
120C	3	0.00E+00			

The results indicated that *Aeromonas salmonicida* had more than 99.99% reduction following treatment by the instrument at 80℃.

5.2.2 *Tenacibaculum todarodis* seawater bacteria

The bacterial growth on MB in a pre-test serial dilution indicated that 72 hrs of growth was suitable to produce enough bacterial growth for the testing (Figure 4).

The results indicated that the *T. todarodis* culture from the suspension was contaminated before entering into the Pontic EP-EH-200 instrument for sterilization. The contaminating bacterium was identified by 16S rRNA sequencing as *Alteromonas naphthalenivorans* (Mi Jin et al., 2015).

The two bacteria were easily discernible on the same culture plate by distinct colony morphology and color (Figure 5). This allowed testing to proceed with both colonies counted separately and log10 reduction values were estimated for each bacteria type with the assumption that there were no overlapping colonies.

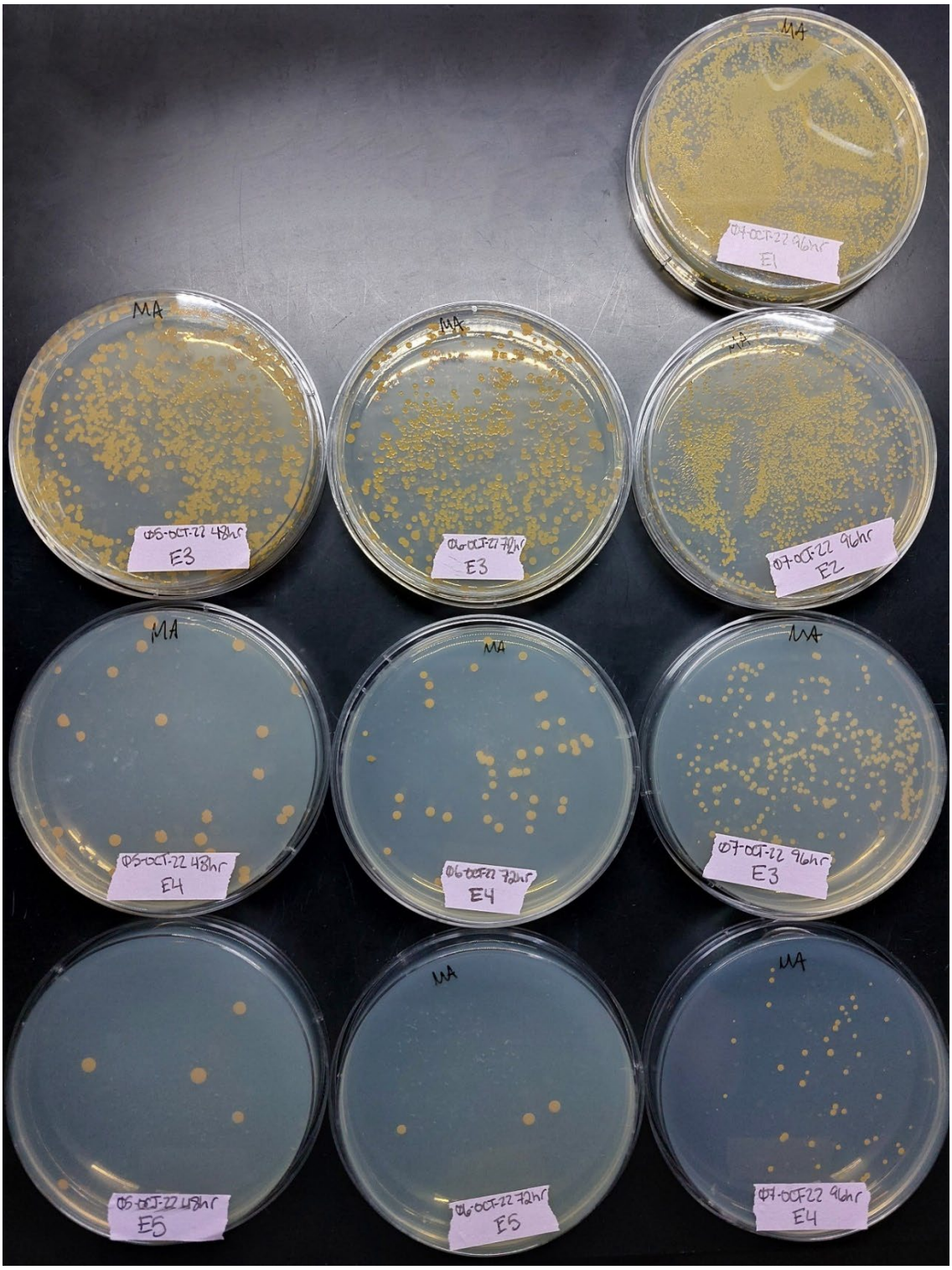

Figure 4 Growth of *Tenacibaculum todarodis* at 48hr, 72hr and 96hr.

Figure 5 Plate showing growth of (1) small slower growing yellow colonies of *Tenacibaculum todarodis* and (2) large creamy colonies of the contaminating sediment bacteria *Alteromonas naphthalenivorans.*

The results indicated that for *Tenacibaculum todarodis* the reduction was more than 99.9% at 50 ℃ and 100% at temperatures of 80℃ and above (Table 6) while *Alteromonas naphthalenivorans* reduction was less than 90% at 50℃ but 100% at temperatures of 80℃ and above (Table 7).

Table 6 Log10 reduction for *Tenacibaculum todarodis*

Sample	Rep.	Concentration	Log_{10} Reduction	Log_{10} Reduction Average	Log_{10} Reduction Standard Deviation
Ambient	1	1.50E+05	0.186	0.235	0.111
Ambient	2	1.00E+05	0.362		
Ambient	3	1.60E+05	0.158		
50C	1	6.50E+01	3.549	3.611	0.058
50C	2	5.50E+01	3.621		
50C	3	5.00E+01	3.663		
80C	1	0.00E+00	NA	NA	NA
80C	2	0.00E+00			
80C	3	0.00E+00			
100C	1	0.00E+00	NA	NA	NA
100C	2	0.00E+00			
100C	3	0.00E+00			
120C	1	0.00E+00	NA	NA	NA
120C	2	0.00E+00			
120C	3	0.00E+00			

Table 7 Log10 reduction of *Alteromonas naphthalenivorans*

Sample	Rep.	Concentration	Log_{10} Reduction	Log_{10} Reduction Average	Log_{10} Reduction Standard Deviation
Ambient	1	1.00E+05	-0.022	-0.110	0.125
Ambient	2	1.50E+05	-0.198		
50C	1	2.20E+04	0.635	0.702	0.249
50C	2	3.05E+04	0.493		
50C	3	1.00E+04	0.978		
80C	1	0.00E+00	NA	NA	NA
80C	2	0.00E+00			
80C	3	0.00E+00			
100C	1	0.00E+00	NA	NA	NA
100C	2	0.00E+00			
100C	3	0.00E+00			
120C	1	0.00E+00	NA	NA	NA
120C	2	0.00E+00			
120C	3	0.00E+00			

5.2.3 Bacteria Conclusions

The results of the analysis indicated that the EP-EH-200 showed a very high efficacy to decontaminate water from representative bacteria in both freshwater and seawater. The use of 80°C eliminated ~99.99 of the freshwater bacterium and 100% of seawater bacteria. Although the *Tenacibaculum todarodis* culture stock was contaminated with *Alteromonas naphthalenivorans*, the two bacteria were easily distinguishable by colony morphology allowing inhibition estimation for both types of bacteria.

6 Conclusion

The Pontic EP-EH-200 Prototype was effective to neutralize oomycete spores from *Saprolegnia parasitica* in spiked freshwater. Bacterial efficacy was also demonstrated for the freshwater bacterium *Aeromonas salmonicida* and the seawater bacteria *Tenacibaculum tordalis* and *Alteromonas naphthalenivorans* at varying temperature settings.

Installation of a Pontic EP-EH-200 water sterilizer in freshwater culture operations would be expected to eliminate an average of 98% of *Saprolegnia parasitica* spores when operating at a temperature range of 100°C -140°C. This same operational temperature would also eliminate 100% of the infectious fish bacterial species tested in this Study. Lowering the operational temperature to 80°C was still efficacious to clear 83.6±17.4% of *Saprolegnia* spores.

It is noteworthy that present freshwater hatchery systems have no means to effectively neutralize *Saprolegnia* spores within incoming freshwater sources but must resort to prophylactic treatments of the fungus after infections are visibly present. Reducing or eliminating the viable spores of the untreated freshwater source supply may remove the need for expensive and time consuming prophylactic treatments within culture operations.

Future studies will need to scale the Pontic EP-EH-200 instrument to treat the larger volume of incoming water required for aquatic animal culture conditions. A future study may also explore whether the sterilization process causes any negative effects for the cultured species. This type of Study will demonstrate target animal safety following water sterilization using the Pontic EP-EH-200 instrument.

7 References

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Hatai, K., Hoshiai, G.-I., 1993. Characteristics of Two Saprolegnia Species Isolated from Coho Salmon with Saprolegniosis. Journal of Aquatic Animal Health 5, 115-118.

Mi Jin, H., Hyun Kim, K., Ok Jeon, C., 2015. Alteromonas naphthalenivorans sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from tidal-flat sediment. International Journal of Systematic and Evolutionary Microbiology 65, 4208-4214.

Appendix 1 - Operating Procedures for the Pontic EP-EH-200 Prototype Unit

Pontic Technology
Germ-Free, Clean Water from any Source

Operating Procedures for the Pontic EP-EH-200 Prototype Unit

Startup Procedure

1. Place the unit on a flat surface with access to power, feed fluid and drainage.
2. Open the lid and remove the storage caps from the IN, OUT and VENT ports. Save the for re-installation after testing.
3. Connect the feed hose to the IN port.
4. Connect the sample port to the OUT port.
5. Connect the "U" shaped tube to the VENT port.
6. Route the feed hose to the fluid source and the sample port to drainage.
7. Ensure the electrical operation switch is in the OFF position and the temperature setpoint knob is OFF (fully CCW).
8. Connect the power cord to the unit and plug it in to a power source (208/220VAC, 30 Amps).
9. Fully open the flow valve.
10. Turn the ON/OFF switch to the ON position. The internal centrifugal pump should come ON and fluid should flow from the reservoir through the unit and out the sample port. NOTE: You may need to prime the pump by holding the feed hose above the unit and charging with fluid.
11. Once fluid starts flowing, let it flow at full flow for 10 minutes with the flow valve fully open to flush out the system.
12. Close the flow valve to establish a 0.3 liter per minute (100 GPD) flow rate from the sample tube. NOTE: Do NOT fully stop the flow.
13. Slowly turn the operating temperature control knob CW to the desired setpoint as shown on the front screen. NOTE: Look for the "SP=xxx.x" – it is toward the right of the screen in the middle. DO NOT set the temperature setpoint above 160 deg C.
14. The actual fluid temperature is shown on the same line to the left of the screen. When the Actual temperature is within +/- 5 deg C of the setpoint, the unit is ready for testing.
15. To change the temperature during testing, rotate the temperature control knob to the desird temperature and monitor the actual temperature until it is +/- 5 deg C from the setpoint.
16. To change the flow rate during testing, open/close the flow valve to the desired flow rate. NOTE: Do NOT fully close the flow valve when the unit is running.

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Germ-Free, Clean Water from any Source

Shutdown Procedure

1. Rotate the temperature control setpoint fully CCW.
2. Fully open the flow valve.
3. Monitor the actual fluid temperature until it is BELOW 80 deg C. CAUTION: The fluid coming out of the sample port can be > 80 deg C.
4. Once the actual fluid temperature is BELOW 80 deg C, move the electrical ON/OFF switch to the OFF position. The internal pump should stop.

Storage Procedure

1. Fully close the flow valve.
2. Remove the power cord from the unit and the power source.
3. Remove the Feed hose, sample port and Vent tube
4. Install storage caps on flow ports.
5. Ensure cover screws are secured (tighten them down for transport).
6. Store the power cord, feed hose, sample port and U-shaped vent tube in the storage case.
7. Close and secure the lid.