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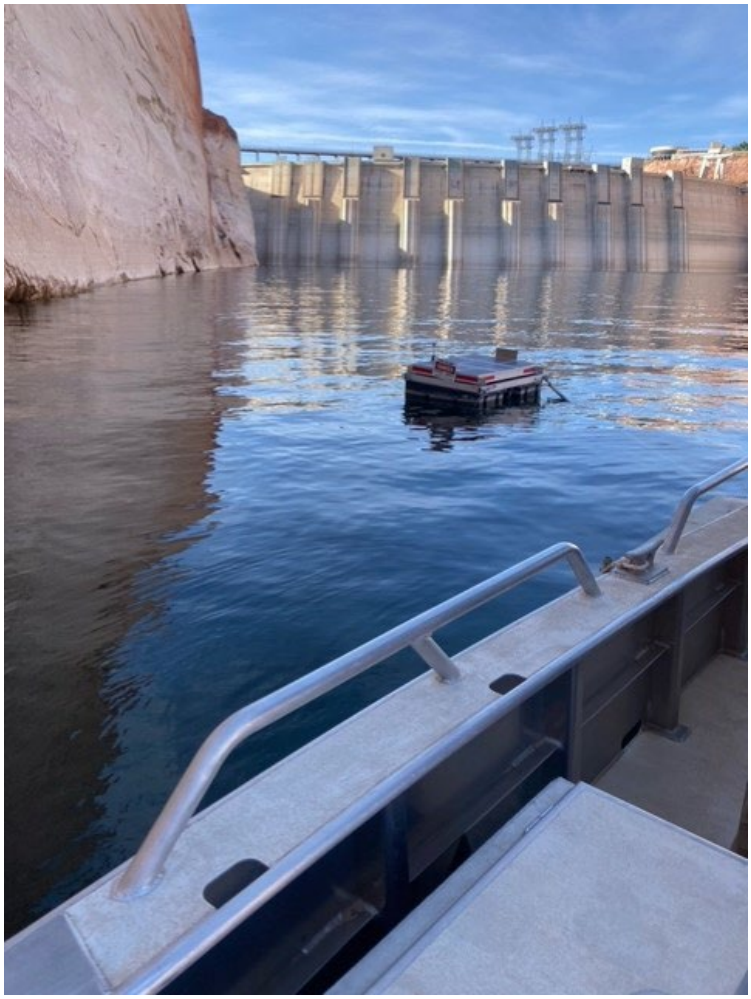
Ultrasonic Transducer Field Test for Quagga Mussel Settlement Control

Science and Technology Program
Research and Development Office
Final Report No. ST-2024-20061-01



U.S. Department of the Interior

March 2024

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
31-03-2024	Research	2020-2024

4. TITLE AND SUBTITLE
Ultrasonic Transducer Field Test for Quagga Mussel Settlement Control

5a. CONTRACT NUMBER
RR4888FARD2003001/ F228A

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
1541 (S&T)

6. AUTHOR(S)
Sherri Pucherelli[1], Biologist
Yale Passamaneck[1], Biologist
Clifton (Shane) Mower[2], Environmental Protection Specialist
Colleen Allen[3], Aquatic Invasive Species Coordinator
Quetzal Elmore[3], Aquatic Invasive Species Supervisor, Wahweap District
Daniel Sedgwick, Bio-Science Technician (formerly with Glen Canyon)

5d. PROJECT NUMBER
Final Report ST-2024-20061-01

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
[1] Bureau of Reclamation, Technical Service Center
1 Denver Federal Center, 6th Avenue and Kipling, Building 67, Denver, CO 80225
[2] Bureau of Reclamation, Upper Colorado Region Power Office
125 South State Street, Salt Lake City, UT 84138
[3]National Park Service, Glen Canyon National Recreation Area
691 Scenic View Drive, Page, AZ 86040

8. PERFORMING ORGANIZATION REPORT NUMBER
EcoLab-F228A-2024-01

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Science and Technology Program
Research and Development Office
Bureau of Reclamation
U.S. Department of the Interior
Denver Federal Center
PO Box 25007, Denver, CO 80225-0007

10. SPONSOR/MONITOR'S ACRONYM(S)
Reclamation

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
Final Report ST-2024-20061-01

12. DISTRIBUTION/AVAILABILITY STATEMENT
Final Report may be downloaded from https://www.usbr.gov/research/projects/index.html

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The goal of this study was to investigate if commercially available ultrasound transducers can be used for invasive dreissenid mussel settlement control on submerged infrastructure such as intakes, gates, screens and trashracks. Ultrasound transducers designed for algae control were deployed at two locations at Lake Powell and the accumulation of invasive mussel settlement, algae species, and overall biofouling was observed within and outside of the range of the transducers over a one-year period. The ultrasound transducer tested in this study did not have an obvious impact on mussel settlement, algae species composition, or overall biofouling.

15. SUBJECT TERMS
quagga mussels, zebra mussels, settlement control, ultrasound

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	THIS PAGE			Sherri Pucherelli
U	U	U		32	19b. TELEPHONE NUMBER *(Include area code)* 303-445-2015

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Acknowledgements

The Science and Technology Program, Bureau of Reclamation, sponsored this research. Thank you to our partners at the National Park Service at Glen Canyon National Recreation Area, for providing a boat and in-kind assistance for this study, including Alexandria Popores and Destynee Robidoux. Thank you to Ray Ripka and staff at Glen Canyon Dam for maintaining and installing the ultrasound units. Thank you to HydroBioscience for answering technical questions about the ultrasound units and providing guidance.

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prepared by

Bureau of Reclamation, Technical Service Center
Sherri Pucherelli
Yale Passamaneck

Bureau Of Reclamation, Upper Colorado Region Power Office
Clifton (Shane) Mower

National Park Service, Glen Canyon Recreation Area
Colleen Allen,
Quetzal Elmore
Daniel Sedgwick

Peer Review

Bureau of Reclamation
Research and Development Office
Science and Technology Program

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SHERRI PUCHERELLI

Digitally signed by SHERRI PUCHERELLI
Date: 2024.03.27 11:00:56 -06'00'

Prepared by: Sherri Pucherelli
Biologist, Technical Service Center, Hydraulic Investigations and Laboratory Services

JACQUE KEELE

Digitally signed by JACQUE KEELE
Date: 2024.03.27 11:03:29 -06'00'

Peer Review by: Jacque Keele
Biologist, Technical Service Center, Hydraulic Investigations and Laboratory Services

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Executive Summary

Invasive zebra (*Dreissena polymorpha*) and quagga (*Dreissena bugensis*) mussels pose a significant biofouling concern to submerged infrastructure. The larval mussels can colonize hard surfaces and can form dense colonies that impede the flow of water. Maintaining and operating hydropower facilities in invasive quagga and zebra mussel infested water bodies can be expensive and complex. Reclamation is investigating and deploying proactive measures to reduce the impacts of mussels at Reclamation managed infrastructure to reduce the risk of equipment failure and increased maintenance. The goal of this study was to investigate if commercially available ultrasound transducers can be used for invasive dreissenid mussel settlement control on submerged infrastructure such as intakes, gates, screens and trashracks.

Ultrasound transducers designed for algae control were deployed at two locations at Lake Powell and the accumulation of invasive mussel settlement, algae species presence, and overall biofouling accumulation was observed within and outside of the range of the transducers over a one-year period. The accumulation of invasive mussels and other biofouling species was collected from settlement plates at 0.5, 2, 4, 6, 8, and 10 meters. Samples were collected after two, six-month exposure periods. The number and average size of settled mussels was determined, the total dry weight of the accumulated biofilm was measured, and metabarcoding analysis of the algae species present was completed. The results indicate that the ultrasonic transducers tested in this study did not have an obvious impact on mussel settlement or size, algae species composition, or overall biofouling.

1. Introduction

Invasive zebra (*Dreissena polymorpha*) and quagga (*Dreissena bugensis*) mussels pose a significant biofouling concern to submerged infrastructure as the larval mussels can colonize hard surfaces and can form dense colonies that prevent the flow of water. Maintaining and operating hydropower facilities in invasive quagga and zebra mussel infested water bodies can be expensive and complex. Reclamation is investigating and deploying proactive measures to reduce the impacts of mussels at Reclamation managed infrastructure to reduce the risk of equipment failure and increased maintenance. The goal of this research project was to test the effectiveness of ultrasonic waves for the prevention of invasive mussel settlement for potential use on critical water facility infrastructure such as trash racks, intakes, gates, and screens.

Ultrasonic transducers are currently sold for the control of algal blooms. Ultrasonic sound vibrations produce critical resonance frequencies that can impact algae gas vesicles, vacuoles and plasmalemma cell lining. Exposure to these vibrations cause the cell membranes to break or tear, which can result in death of the algae cell.

There have been cases studies that suggest ultrasound treatment has significantly reduced or eliminated biofilm accumulation. It is hypothesized that anaerobic bacteria sense ultrasound as a form of water turbulence which may prevent colonization within the treated zone, aerobic bacteria typically accumulate on top of the anaerobic base layer, and without it there may be less biofilm accumulation. While studying the impact of ultrasonic waves on algae, researchers also noticed a reduction in overall biofouling, including native mussel settlement (information provided by HydroBioScience). Elkem Solar (Kristiansand, Norway) installed ultrasound in their sea water intake, and visual inspections have indicated that there has been a reduction in biofilm, algae, and salt-water mussel fouling (case study provided by HydroBioScience).

The impact of ultrasound on mussel settlement has not been studied, but it is theorized that ultrasonic waves might prevent the growth of biofilm and potentially limit food availability for settled mussels, preventing their growth and survival after settlement. This study was designed to investigate the effectiveness of ultrasound waves in preventing invasive quagga mussel fouling and accumulation, as well as the colonization of other biofouling species that may influence mussel survival. Ultrasound would be an appealing treatment because it is easy to deploy and operate and does not cause harm to species other than algae. A study by Getchell et al. (2022) found that ultrasound did not cause avoidance behavior or physical harm to seven recreationally and ecologically important fish species and tadpoles.

This study was conducted at Lake Powell, AZ where the quagga mussel population is well established and reproduction typically occurs throughout most of the year. Mussel fouling has been observed on the Glen Canyon Dam trash racks and gates, and in recent years has significantly progressed (Kubitschek and Vermeyen, 2019). In some areas the trash racks have become completely occluded by mussel fouling, but there is still enough open space where head loss has not

occurred (Kubitschek and Vermeyen 2019). Mussel fouling on the fixed wheel gates has been significant and when gates are pulled for regular maintenance, the removal of mussels requires several days of effort (personal communication with Shane Mower). Colonial hydroid (*Cordylophora caspia*) is another invasive species that is present at Lake Powell, which also has significant biofouling potential (Pucherelli et al. 2016).

Ultrasonic transducers were installed at the Glen Canyon buoy line and at the Stateline breakwater. Settlement plates were hung within and outside of the range of the ultrasound at both locations. Mussel settlement, biofouling weight, and biofilm composition were assessed and compared between plates to determine if there was an impact of ultrasound exposure.

2. Methods

Study Sites

The study was conducted at Lake Powell, AZ in the Glen Canyon National Recreation Area. The two sites selected for the study were the Glen Canyon Dam buoy (GCB) and the Stateline breakwater (SLB). The locations were selected because they each provided an existing floating structure to which settlement plates and the ultrasonic transducers could be attached. The structures were also long-enough so that control plates could be installed outside of the effective range of the transducer. The GCB is in the forebay of Glen Canyon Dam and it is constructed of approximately 6 meter long, concreate buoys attached to each-other with 1 meter long chains (Figure 1). The buoys are attached to the canyon walls with 1.8 meter long cables. The buoy line is regularly adjusted to accommodate fluctuating water levels. The depth of water at the GCB is approximately 122-152 meters. The SLB is a floating structure constructed from large tires and is located upstream of the GCB, near the Stateline marina. The length of the SLB is approximately 183 meters and the water depth is around 30 meters.

Figure 1. Buoy line located upstream of Glen Canyon Dam (GCB study site).

Collection of Pre-Study Mussel Settlement Data

An analysis of mussel settlement was conducted at each site prior to the installation of the ultrasound units. The data provided information about baseline mussel settlement at each location where plates were to be installed. To collect baseline data a single string of settlement plates were deployed on each end of the buoy line (near each canyon wall) and on each end of the Stateline breakwater. The strings of plates consisted of black PVC plates (14.6 cm by 14.6 cm) which were strung on plastic coated metal cable so that they would hang at 2, 4, 6, 8, and 10 meters below the surface. Each plate had a hole punched in the center at the top and bottom which allowed for the wire rope to be threaded through. The string of plates were attached to the floating structures by looping the cable around the structure and securing with a U-bolt. A 3-lb dive belt weight was secured to the end of the plate string to keep the plates hanging vertical in the water column.

National Park Service (NPS) staff installed the first set of plates on 9/3/2021 and collected the settled mussels on 10/13/2021 (plates were in the water for 41 days). Unfortunately, at the time of collection it was discovered that a stirring of plates at the GCB site was missing. The plates were re-deployed to start a second test on 10/13/2021. A replacement string of plates was also installed at this time. The plates remained in the water until 12/7/2021 (56 days) at which time mussel settlement was collected and the plates were removed from the water.

Settled mussels were removed from both sides of the plates using a razor blade. The mussels were collected in a tray, the plate was rinsed with deionized water, the sample was poured into a sample bottle and preserved with ethanol. The samples were sent to the Reclamation Ecological Research

Laboratory (Eco Lab) where the number of mussels collected from each plate were counted using cross-polarized light microscopy.

Installation of Ultrasonic Transducers and Settlement Plates

Two solar powered Quattro-DB® ultrasonic transducers (Hydro BioScience®), which had been purchased by Reclamation for the purpose of algae control at a different waterbody, were used for this study. The transducers were powered by solar panels mounted on a floating raft. The transducer hung from the center of the raft at 2 meters below the surface. The units tested in this study did not produce cavitation and operated at 148 db. The transducers emit two zones of frequency, a low range of 41 kHz centered with 34 kHz bandwidth and a high range 200 kHz centered with 10 kHz bandwidth. The total frequencies per cycle is 2024 with a 34 minute cycle. The transducers are expected to have a 60-meter effective radial distance range for biofilm control and a 150-meter effective radial distance range for algae and diatom control.

Reclamation employees from Glen Canyon Dam constructed a brace so that the ultrasound rafts could be securely attached to the floating structures and would remain at a fixed, 3-meter distance away from the attachment point. The ultrasound unit was installed near the east canyon wall at the GCB site (Figure 2) and on the southern end of the SLB site (Figure 3). All components of the ultrasound units were confirmed to be operational prior to installation. After installation, the units were turned on and a decibel meter was used to confirm operation.

Figure 2. Installation of the ultrasound solar raft on the east side of the GCB Site.

Figure 3. Ultrasound solar raft on the south end of the SLB site, prior to placement.

Twelve strings of settlement plates were constructed for the study. Black PVC plates (14.6 cm by 14.6 cm) with holes drilled at the top and bottom were strung onto plastic coated metal cable. The plates were positioned so they would hang at 0.5, 2, 4, 6, 8, and 10 meters below the surface. A 3-lb dive belt weight was secured to the end of each plate string to keep the plates hanging vertical in the water column.

Settlement plates were deployed on 1/27/2022. Six strings of plates were installed at each study site (Figure 4). The strings of plates were attached to the floating structures by looping the cable around the structure and securing with a U-bolt. Three strings of plates were installed within the range of the transducer and three strings were installed outside of the range of the transducer at the other end of the floating structures. At the GCB site a string of plates was installed in-line with the transducer, approximately 3 meters upstream. Another string of plates was installed 6.7 meters to the west, near the west canyon wall and the third string was installed 6.7 meters to the east of the transducer. The three other strings of plates were installed near the eastern most end of the GCB in the same configuration, and at the same distances away from the canyon wall. These plates were out of the effective range of the transducer and were considered the control plates. Six strings of plates were installed in the same configuration (Figure 4) at the SLB site (Figure 5). Three strings of plates on the south-end of the SLB were installed 6.7 meters apart with the center string of plates installed in-line with the transducer. The three strings of plates installed on the north-end of the SLB were considered the control plates as they were out of the effective range of the transducer.

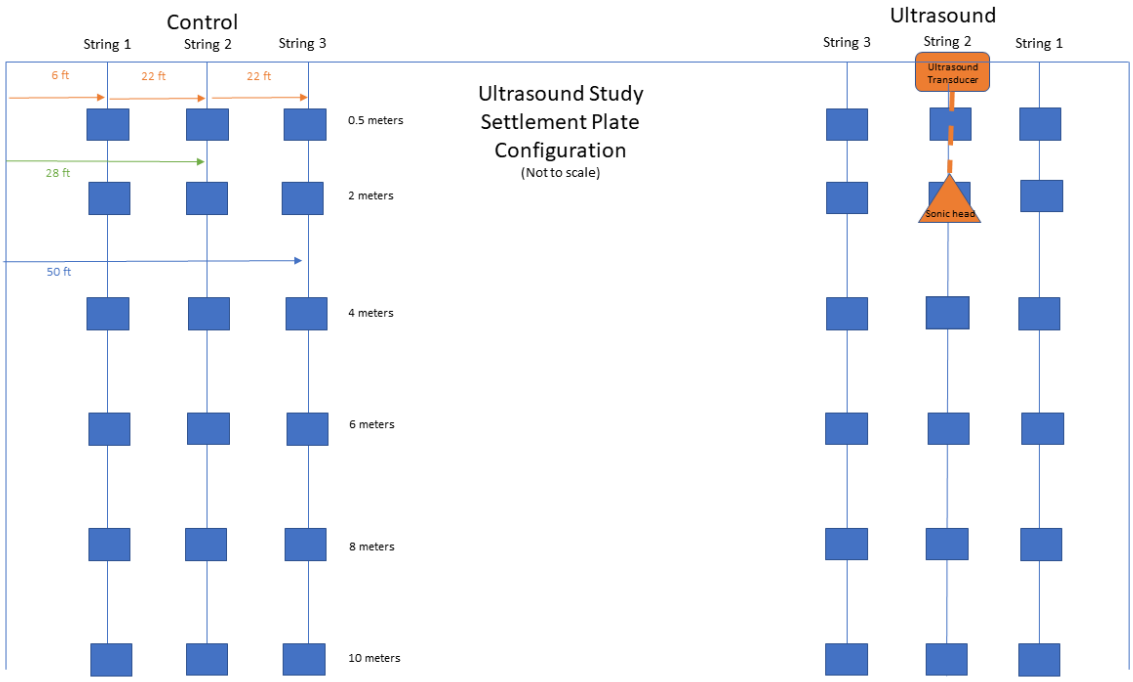

Figure 4. General settlement plate configuration.

Figure 5. Installation of settlement plates by NPS staff at the SLB site.

The plates remained in the water for one year and the biofouling that accumulated on the pates was collected twice. The first collection occurred on 7/19/2022 after a 6-month exposure period (173 days) between January and July (Test 1). The second collection occurred on 2/7/2023 after a 6-month exposure period (203 days) between July and February (Test 2). During the study, monthly site visits were conducted by the NPS to confirm the plates and transducer were in place. If a string of plates went missing within the first three months of a test, a new string was deployed upon discovery. If a string went missing later in the test period it was not replaced.

During monthly visits, a decibel meter was used to confirm that the transducer was operating. The transducer at the SLB site was not working during the May site visit and it was able to be fixed and put into operation two days after detection, but it is not known how long the unit was inoperable. The transducer was also found to be inoperable during the June site visit. It was removed for a month for repairs. The ultrasound plates at the SLB were not exposed for two weeks before the conclusion of Test 1 and for two weeks at the beginning of Test 2.

Mussel and Biofilm Collection

At the conclusion of both 6-month tests, each settlement plate was pulled out of the water and the mussels and biofilm attached to the entire front surface, and half of the back surface of each plate was removed with a razor blade and collected in a tray (Figures 6 and 7). The plate was rinsed with de-ionized water, which was also collected in the tray (Figure 8), and then the sample was poured into a pre-labeled sample bottle and preserved with ethanol.

Samples were also collected for genetic metabarcoding analysis of epiphytes (see "Algae Metabarcoding Sample Collection" section for methods). Those samples were always collected before the mussels and biofilm samples were collected, and they were only collected from a sub-set of plates. The sample was collected from half of the back side of the plate. After the metabarcoding sample was collected the remaining biofilm was scraped from the plate and discarded.

After the first samples were collected the plates were re-deployed for another 6-month exposure period. After the second set of samples were collected the plates and the ultrasound transducers were removed. The transducers were decontaminated according to the NPS decontamination protocol.

Figure 6. Collection of settled mussels and other biofouling species at the SLB site after Test 2.

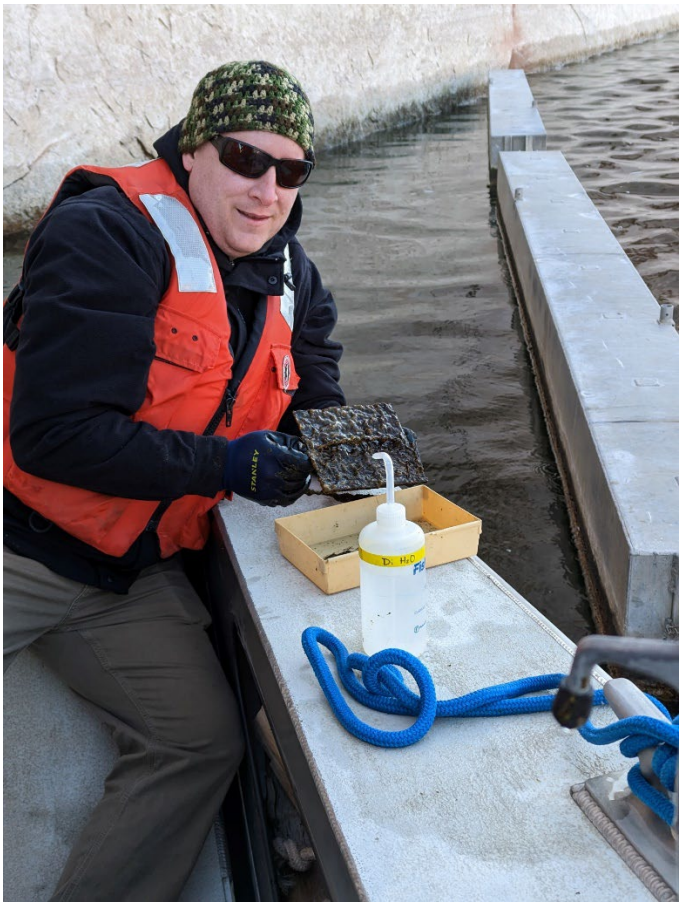

Figure 7. Collection of settled mussels and other biofouling species at the GCB site after Test 2.

Figure 8. Settlement sample collected in a tray prior to preservation.

Mussel and Biofilm Sample Analysis

The preserved settlement samples were sent to Reclamation's Ecological Research Lab. The first set of samples from the GCB and SLB sites were analyzed under cross-polarized light microscopy and the total number of mussels in each sample was determined. Veligers that may have been incidentally collected in the sample were not included in the total count. The shell length of five of the largest mussels in each sample was measured and recorded. There was more mussel settlement during the second test, especially at the SLB site, so it was only possible to do a count of mussels from Test 2 samples collected at the GCB site.

After mussel counts were complete, the samples were poured through a 64-µm sieve to remove the preservative and rinse water. The sample was poured into a labeled and pre-weighed aluminum tray, which was then placed in a drying oven at 105ºC for 24 hours. The sample was weighted and the dry weight of the sample was calculated by subtracting the weight of the container.

Mussel settlement and the dry weight of all biofouling organisms was examined to determine if there were differences between the accumulation on plates exposed to the transducer and those that were not exposed to the transducer. The average size of the largest mussels collected was also compared to determine if mussel growth was impacted by ultrasound.

Algae Metabarcoding Sample Collection

Samples for metabarcoding of epiphytes were collected from each settling plate on one control string and one ultrasound exposed string at both the GCB and SLB sites. Collections were

conducted on July 19, 2022 and on February 7, 2023. Samples were collected by scraping biofilm off the settling plates using a sterile plastic spatula (Figure 9). For each plate, approximately 1 inch square of the plate was scraped, and the biofilm was placed in a sterile 2-ml screwtop tube containing 1-ml of absolute ethanol. At each site a field blank was collected by opening a 2-ml screwtop tube containing ethanol for exposure to the air for two minutes prior to collection of field samples from the plates. After collection, sample tubes were kept on ice for transport to the Eco Lab, and then stored at -80ºC until processed for DNA extraction.

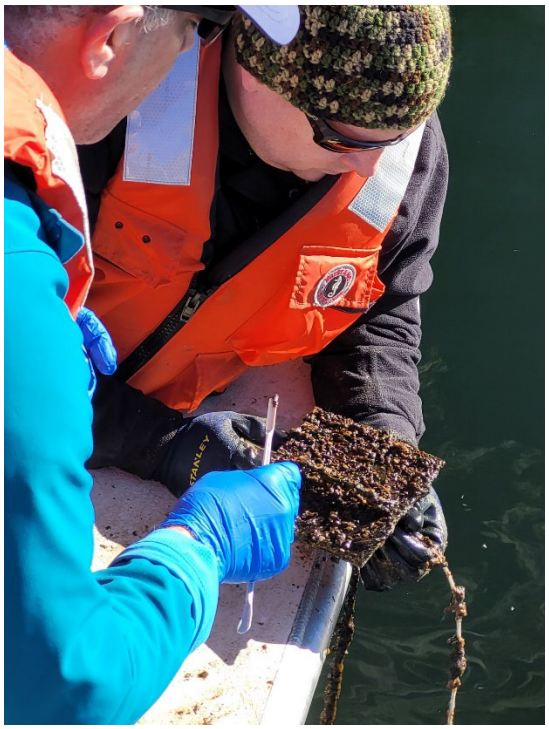

Figure 9. Collection of biofilm with a sterile spatula for metabarcoding analysis of algae.

DNA Extraction, Metabarcode Sequencing, and Data Analysis

DNA extraction and sequencing were performed by Jonah Ventures laboratory (Boulder, CO). PCR amplification targeted the V5 region of the 23S rRNA gene (forward primer: 5'-GGACAGAAAGACCCTATGAA-3'; reverse primer: 5'-TGAGTGACGGCCTTTCCACT-3'). These primers designed to amplify from both cyanobacteria and the chloroplast genome of photosynthetic eukaryotes. Amplified sequence variant (ASV) identification and taxonomic assignment were performed by Jonah Ventures using a custom data pipeline. Statistical analyses of the data were performed using QIIME2 software (Bolyen et al., 2019).

3. Results

Pre-Study Settlement Analysis

Prior to installation of the ultrasound transducers mussel settlement was detected at each site and location, but less mussel settlement was observed at the GCB site than the SLB site. Less settlement was observed at both sites during the first test that occurred over 41 days between September and October 2021. Unfortunately, the string of plates installed on the east end of the GCB was found to be missing at collection. Only 7 mussels were found on the remaining 6 plates on the west end (Figure 10). At the SLB site, 5,118 mussels were found on the 6 plates on the south end, and 6,366 were found on the 6 plates on the north end (Figure 11). More settlement was observed at both locations during the second test that occurred over 56 days between October and December 2021. A total of 481 settled mussels were collected from 12 plates at GCB (Figure 10), and 34,945 mussels were collected from 12 plates at SLB (Figure 11).

At the GCB site, there was a 62% difference between settlement observed at the two extents of the buoy line, with more mussels settled on the west end (315 mussels) and less settled on the east end (166 mussels). The east end is where the ultrasound unit would be installed. On the west end, more mussels were observed at all depths, except at 4 meters. During the first test at the SLB site, there was a 22% difference between settlement observed at the two extents of the breakwater structure, with less mussels settled on the south end (5,118 mussels) and more settled on the north end (6,366 mussels). The south end is where the ultrasound unit would be installed. During the second test at the SLB site, there was a 26% difference between settlement observed at the two extents of the breakwater structure. This time there were more mussels settled on the south end (19,754 mussels) and less settled on the north end (15,191 mussels).

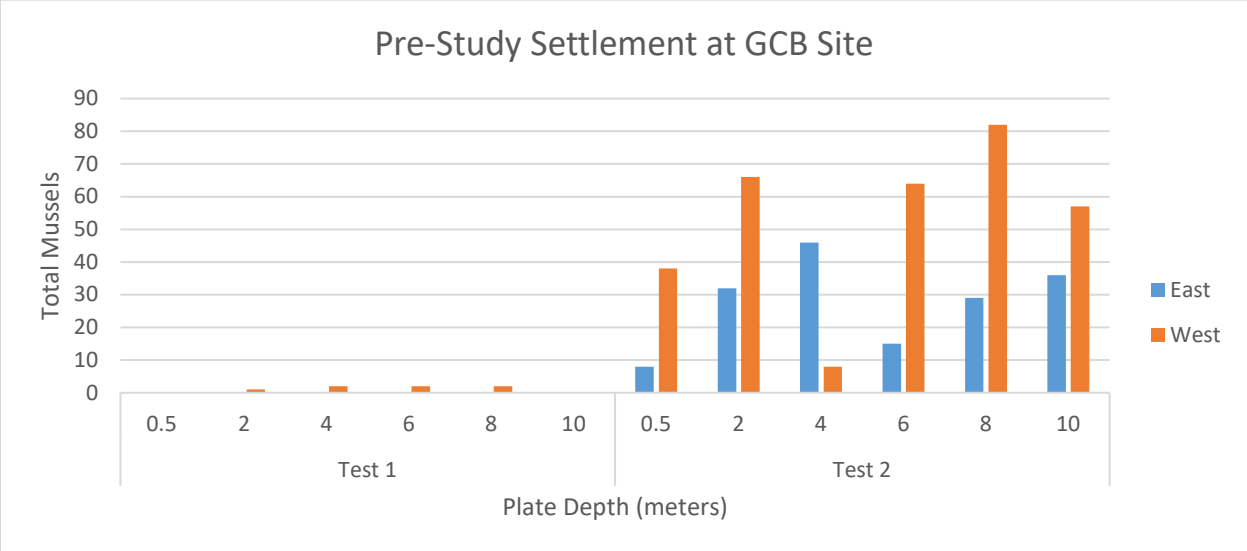

Figure 10. Quagga mussel settlement observed on plates the GCB site between September and October 2021 (Test 1) and October and December 2021 (Test 2) prior to installation of the ultrasound transducer. The ultrasound transducer would be installed on the east end of the buoy line. The plates on the east end were missing at the conclusion of Test 1.

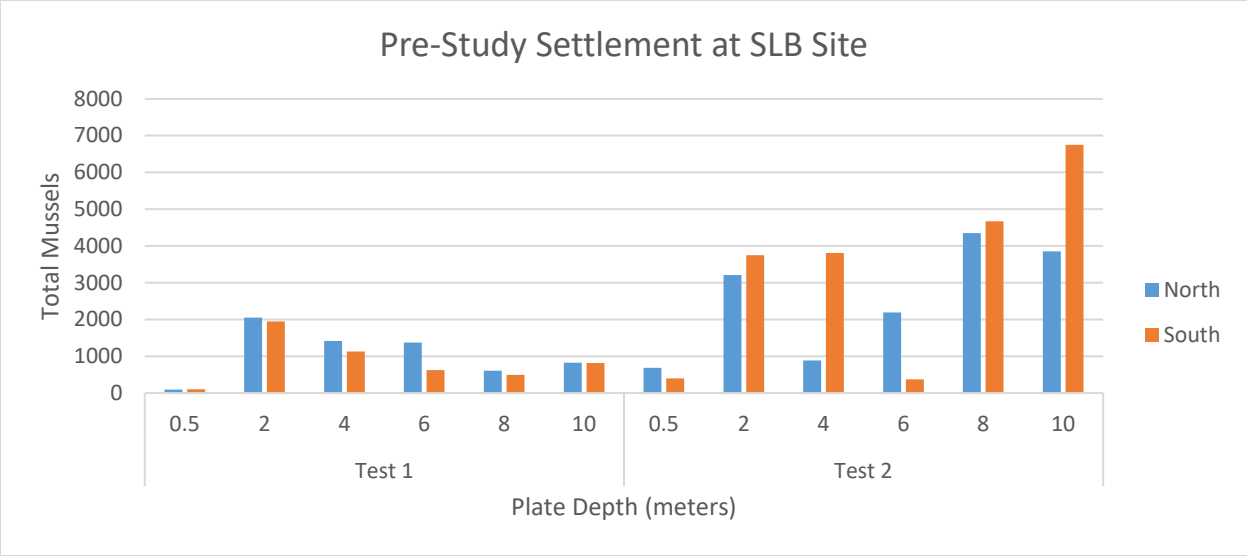

Figure 11. Quagga mussel settlement observed on plates the SLB site between September and October 2021 (Test 1) and October and December 2021 (Test 2) prior to installation of the ultrasound transducer. The ultrasound transducer would be installed on the south end of the buoy line.

Ultrasound Impact on Mussel Settlement

Mussel settlement was observed at each site, but more mussels were collected from the SLB plates than the GCB plates. A total of 5,966 mussels were collected from plates at the GCB site while 64,126 were collected from plates at the SLB site. Additionally, less mussel settlement was observed during the Test 1 time frame (January 2022-July 2022) compared with the Test 2 time frame (July 2022-February 2023) (Figure 12). So many mussels were collected from the SLB plates during Test 2 that it was not feasible to do a mussel count. Unfortunately, several of the strings of plates were found to be missing at the time of collection. At the GCB, the strings of plates closest to the canyon walls (String 1) were missing at the conclusion of each test, and the string of plates closest to the ultrasound transducer (String 2) at SLB was missing at the conclusion of the first test.

Mussel settlement was not eliminated or dramatically reduced on plates exposed to the ultrasound transducer, but on average, mussel settlement was less on plates exposed to the ultrasound treatment (Figure 12). During the first test at the GCB site there as an average of 5,413 mussels/ m^2 on plates exposed to the ultrasound treatment and 4,945 mussels/ m^2 on plates that were not exposed. During the second test there was an average of 25,036 mussels/ m^2 on ultrasound exposed plates and 39,246 mussels/ m^2 on control plates. At SLB, there was an average of 40,071 mussels/ m^2 on ultrasound plates and 71,273 mussels/ m^2 on control plates (Figure 12). When analyzing settlement by depth, certain plates exposed to ultrasound had greater mussel settlement than the corresponding control plate. After the first test 7 of 12 plates hanging in range of the transducer at GCB had greater mussel settlement than the corresponding control plates (Figure 13), and 3 of 12 plates in range of the transducer at SLB had greater settlement (Figure 14). During the second test at GCB, 3 of the 12 plates had greater settlement (Figure 15).

Ultrasound effectiveness is expected to decline with distance from the transducer, therefore it was hypothesized that the greatest impact on mussel settlement would occur on the plates closest to the transducer. Plates at 0.5 m and 2 m on string 2 were the closest as they were installed directly in-line with the transducer. During the first test at GCB there was more settlement on these two plates compared to the control plates (Figure 13), and during the second test the plate at 0.5 m had greater settlement than the control and the plate a 2 m had less settlement than the control (Figure 15). Plates on strings 1 and 3 were further away from the transducer than plates on string 2, so it was expected that greater settlement reduction might occur on string 2 plates. This pattern was not observed. Mussel settlement on control plates changed with depth, generally increasing with increasing depth. This pattern was also observed on the plates exposed to ultrasound. Because no clear pattern of settlement reduction was observed it is likely that the differences in mussel settlement are due to natural settlement variability.

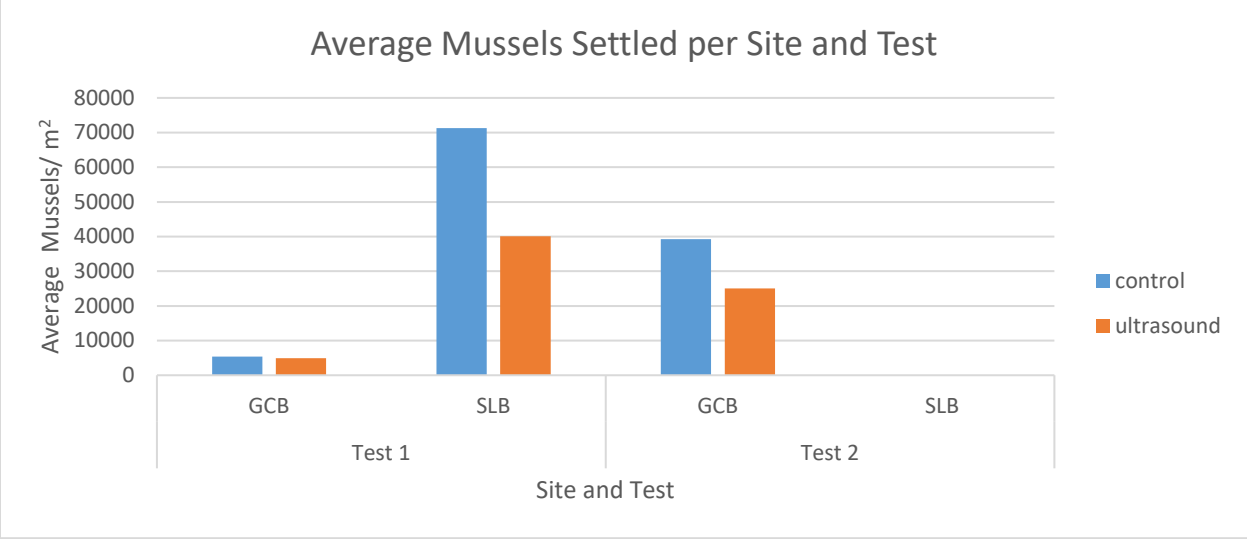

Figure 12. Average mussels settled per square meter within and outside (control) of the range of the ultrasound transducer at the GCB and SLB sites.

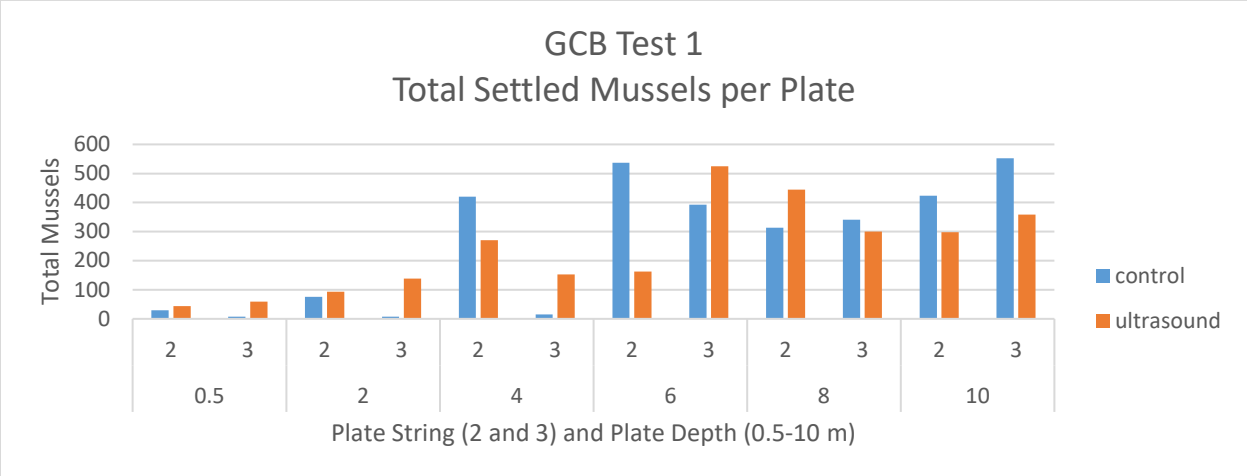

Figure 13. Total mussels settled on each plate at the GCB site at the conclusion of Test 1 (173 days from 1/27/2022- 7/19/2022).

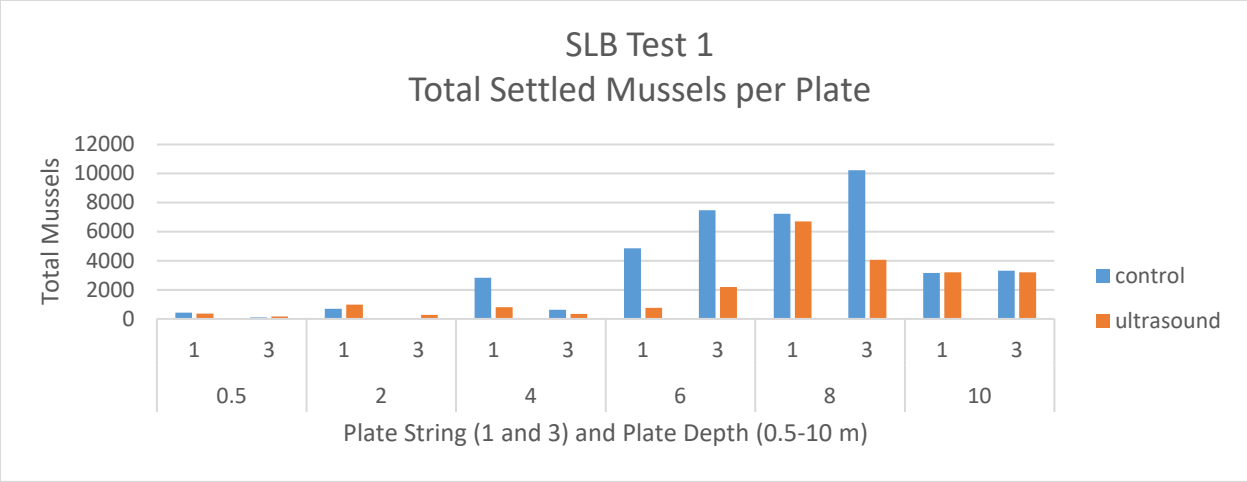

Figure 14. Total mussels settled on each plate at the SLB site at the conclusion of Test 1 (173 days from 1/27/2022- 7/19/2022).

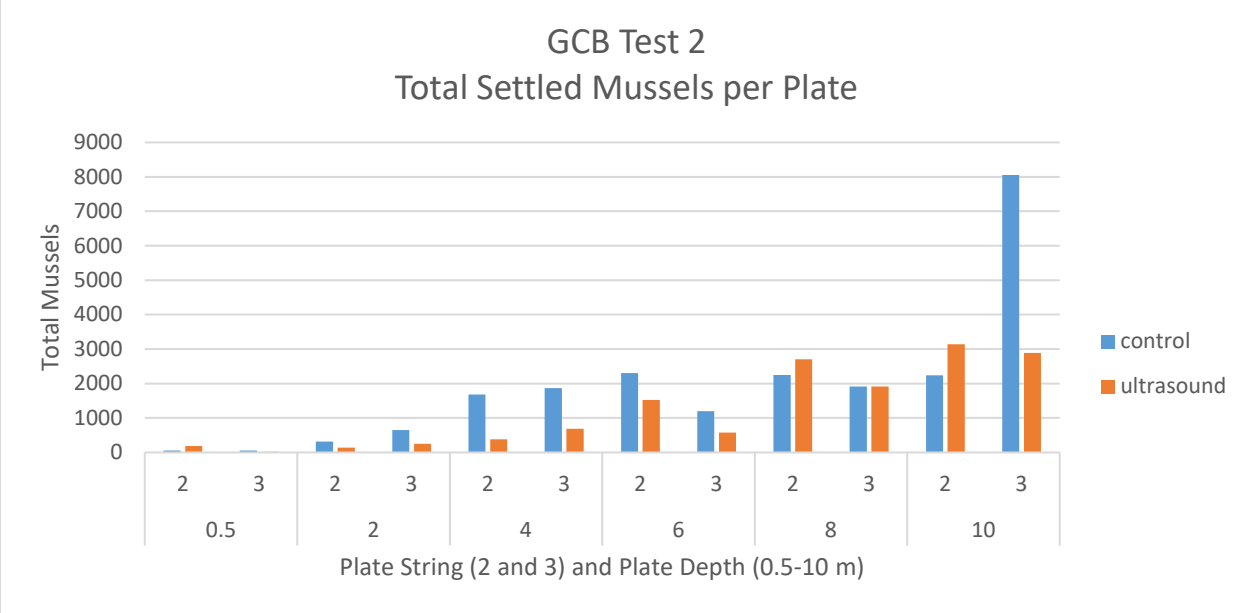

Figure 15. Total mussels settled on each plate at the GCB site at the conclusion of Test 2 (203 days from 7/19/2022-2/7/2023).

Ultrasound Impact on Mussel Size

There was no obvious difference in the size and shell condition of mussels that were exposed to ultrasound and those that were not. The average size of the largest mussels observed on each plate in the treated and untreated locations were comparable at both sites (Figures 16 and 17).

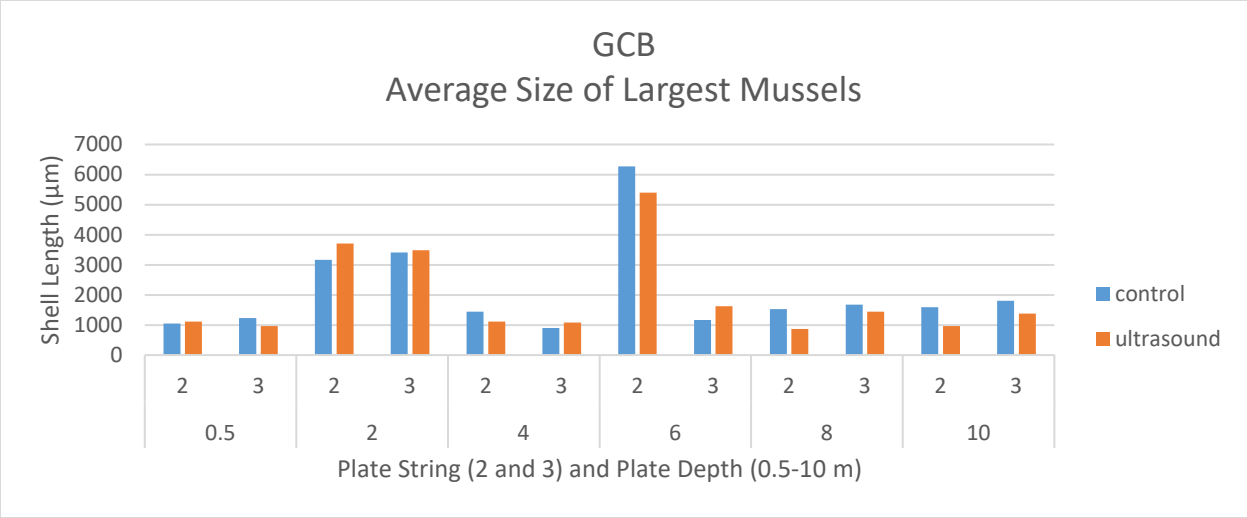

Figure 16. Average shell length (µm) of the largest mussels collected on plates at the GCB site.

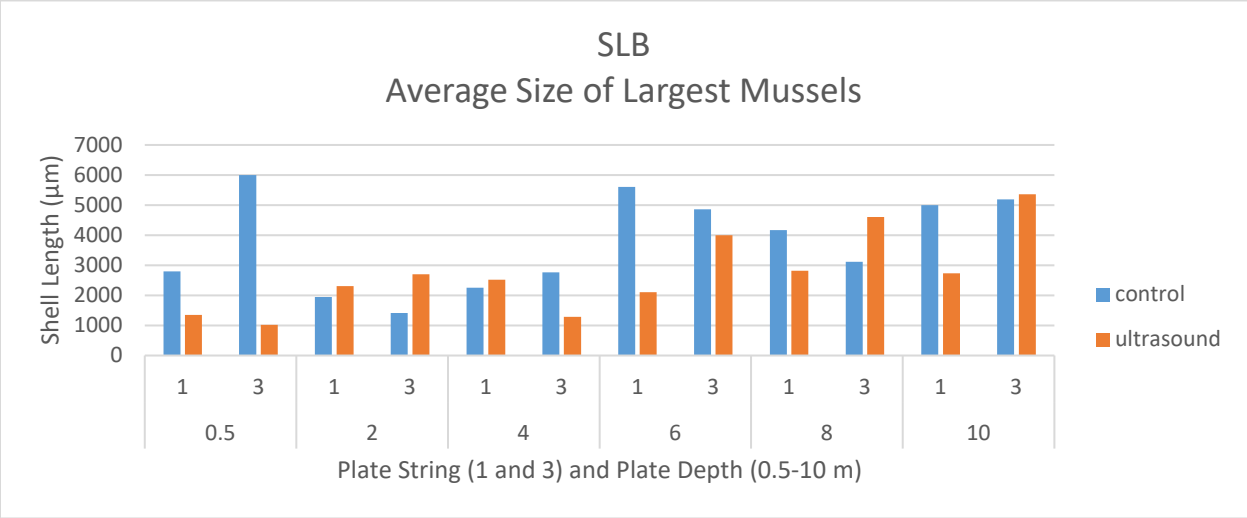

Figure 17. Average shell length (µm) of the largest mussels collected on plates at the SLB site.

Ultrasound Impact on Overall Biofouling

Quagga mussels, colonial hydroid, and algae were the primary biofouling organisms that were visually observed on the settlement plates. The average dry weigh of all accumulated biofouling organisms was found to be greater on plates exposed to ultrasound at GCB during both tests and at SLB during the second test (Figure 18). The dry weight was consistently greater within the range of the ultrasound at all depths and sites (Figure 19). Ultrasound exposure did not reduce overall biofouling, and the dry weight of settled organisms within the ultrasound range followed the trend observed outside of the treatment area. Like the mussel counts, the plate closest to the transducer (the 2 m plate on string 2) did not experience reduced biofouling, in fact these plates had greater biofouling accumulation than the control (Figure 20). The dry weight appears to be somewhat correlated with the number of settled mussels, especially during the second test, because of the greater number of mussels observed (Figures 20 and 21). Dry weight was also greater at shallower

depths where fewer mussels were observed, this is due to the thick mats of algae that had accumulated (Figures 20 and 21).

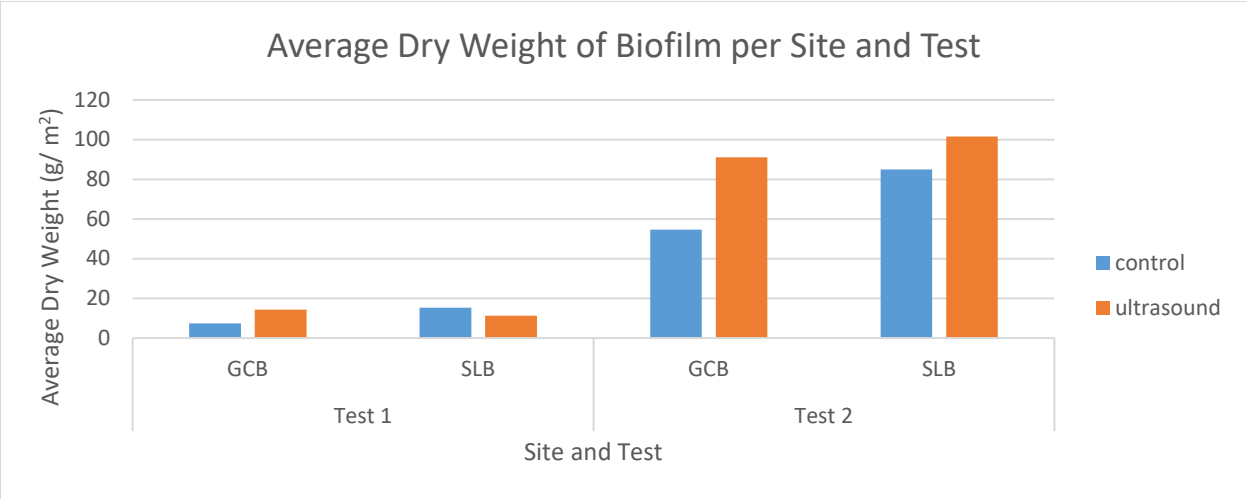

Figure 18. Average dry weight (g/m^2) of organisms settled on plates within and outside (control) the range of the ultrasound transducer at the GCB and SLB sites.

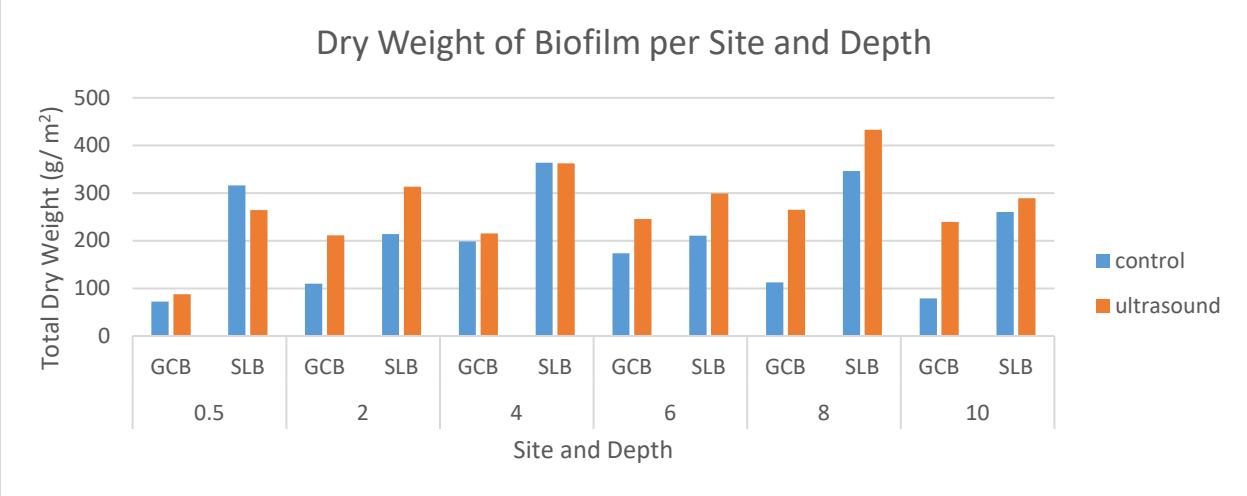

Figure 19. Total dry weight (g/m^2) of organisms settled on plates at each depth within and outside (control) the range of the ultrasound transducer at the GCB and SLB sites.

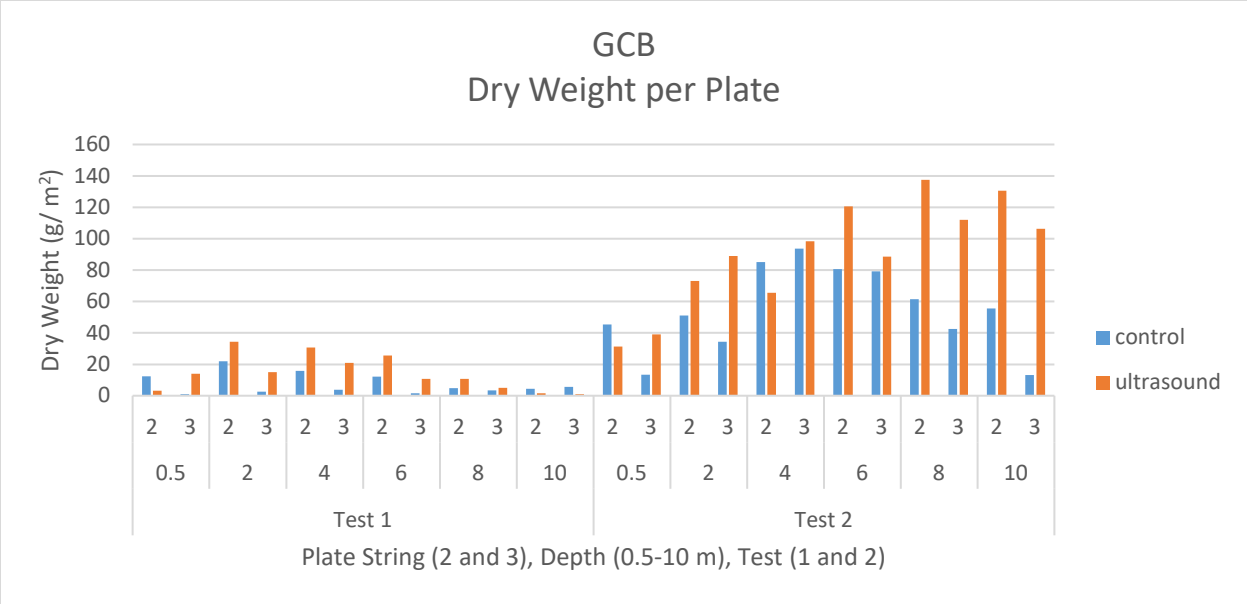

Figure 20. Dry weight of organisms collected from each plate at the GCB site at the conclusion of Test 1 (173 days from 1/27/2022- 7/19/2022) and Test 2 (203 days from 7/19/2022-2/7/2023).

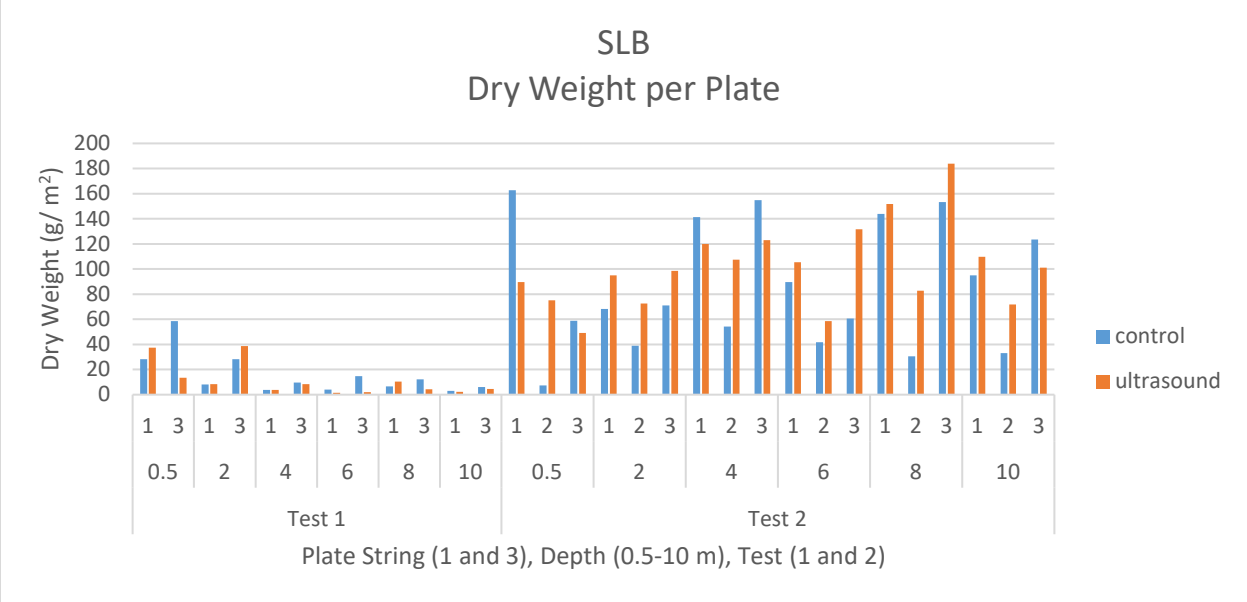

Figure 21. Dry weight of organisms collected from each plate at the SLB site at the conclusion of Test 1 (173 days from 1/27/2022- 7/19/2022) and Test 2 (203 days from 7/19/2022-2/7/2023).

Algae Metabarcoding Results

A total of 56 samples were sent to Jonah Ventures for DNA extraction, PCR sequencing, and DNA sequencing, including 24 control samples, 24 ultrasound treated samples, and 8 field blanks. Raw Illumina DNA sequencing reads across the dataset averaged 19,400 reads per sample. After bioinformatic steps including denoising and taxonomic assignment, an average of 6,968 assigned reads were recovered per sample (excluding field blanks, discussed below). One field sample, from

the 2 m plate of the ultrasound exposed string at SLB sit sampled on February 7, 2023, had no reads that received taxonomic assignment.

Across the samples, a total of 1,067 unique amplified sequence variants (ASVs) were identified and given taxonomic assignments. Of these ASVs, 838 were unique to a single sample, suggesting that many of the recovered ASVs may be attributable to PCR or sequencing errors. The impact of these ASVs on statistical analyses is negligible given that they generally had low read counts and given that diversity measurements used factored in sequence abundance.

Taxonomic assignment was performed at seven levels, from kingdom to species, based on matching to sequences in a reference database. ASVs with a single match were assigned to the level of species. ASVs matching to multiple reference sequences were assigned to the most inclusive taxonomic rank. Only 115 ASVs were assigned to the level of species, while 759 ASVs were assigned to the level of class (Figure 22).

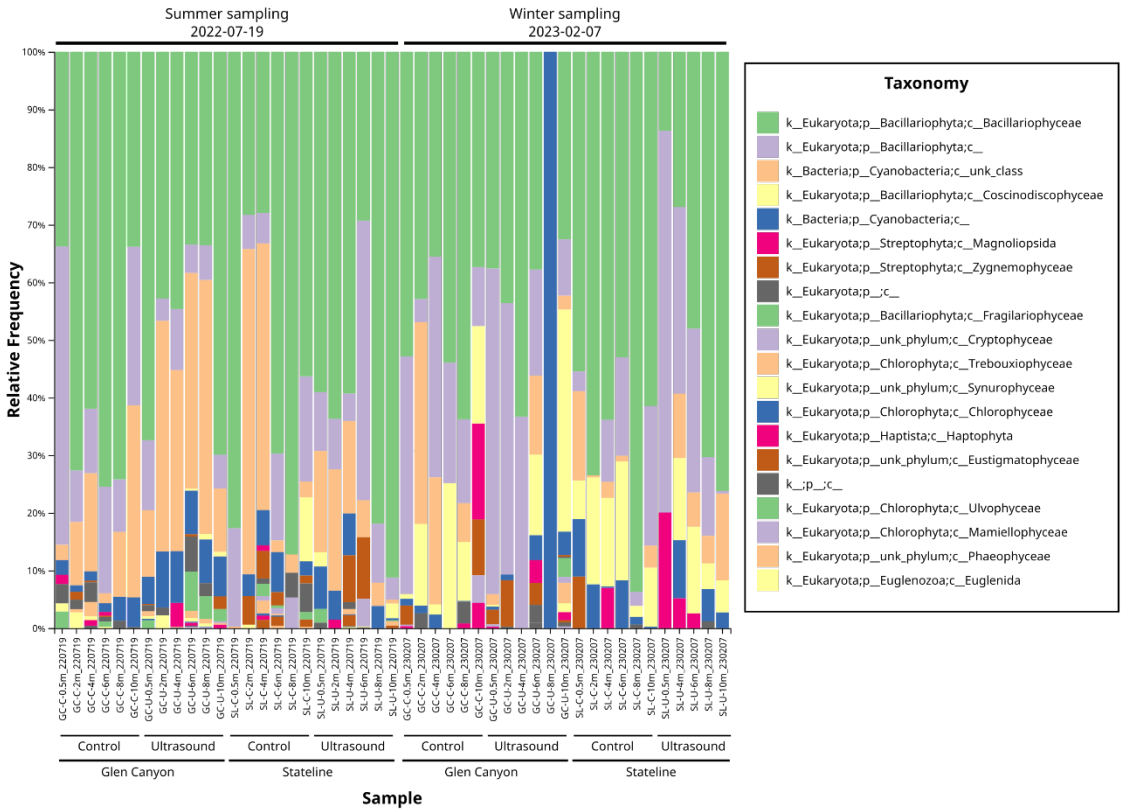

Figure 22. Bar plot of taxon frequency across samples. Bars correspond to relative frequency of taxa grouped at the level of class.

Between Sample Comparison: Alpha-diversity and Beta-diversity

The primary goal of DNA metabarcoding was to test whether differences in algal populations would be observed between ultrasound treatments and controls. Influence of the additional variables of plate depth, sample site (GCB versus SLB), and sampling date (winter (Test 1) versus summer (Test 2)) was also evaluated.

Samples were initially evaluated for alpha-diversity, which refers to the ASV richness within samples. Comparisons of Shannon's diversity index using the Kruskal-Wallis test found no significant differences in ASV richness between samples ($p > 0.05$).

Beta-diversity, which is the diversity of ASV composition between samples, was evaluated using weighted UniFrac distances. Weighted Unifrac distance was chosen as the metric of beta diversity because it incorporates phylogenetic distance, meaning that it accounts for the fact that ASVs with similar sequences are closely related at represent less diversity than ASVs with greater sequence divergence. Weighted Unifrac distance is also a qualitative measure of sample dissimilarity, accounting for the differences is ASVs frequency between samples. This is key because it was expected that the same species/ASVs would likely be identified across samples, but that their relative frequencies might vary with one or more of the tested parameters (ultrasound treatment versus control, sampling site, plate depth, and sampling date).

Weighted Unifrac distances were evaluated using a PERMANOVA test. Beta diversity was significantly different between samples collected in the summer and in the winter ($p = 0.002$). Beta diversity was not found to be significantly different based on treatment type (ultrasound versus control), plate depth, or sample site ($p > 0.05$). Beta diversity was further evaluated for the influence of these variables by evaluating data from the two sampling dates separately, to remove the demonstrated influence of seasonality. For both the summer and winter collected sample sets, again no significant difference in beta diversity was observed based on treatment type, plate depth, or sampling site ($p > 0.05$).

The significant difference in beta diversity between samples collected in the summer and winter is consistent with the overall trends observed in taxon frequency. While most samples were dominated by ASVs matching to reference sequences for diatoms (Bacillariophyta), many samples from the summer sample collection had a high proportion of ASVs matching to cyanobacteria (Figure 22). In samples collected in the winter the proportion of cyanobacteria ASVs was generally quite low, while the proportion of diatoms, and in particular those in the class Coscinodiscophyceae, was higher (Figure 22).

The difference between samples from the summer and winter was also born out in principal coordinates analysis (PCoA) of the weighted Unifrac distances. Samples from the summer and winter generally sort into two sperate clusters in the PCoA of weighted Unifrac distances (Figure 23). In contrast, there is no clear separation of control and ultrasound samples in the PCoA of weighted Unifrac distances (Figure 24).

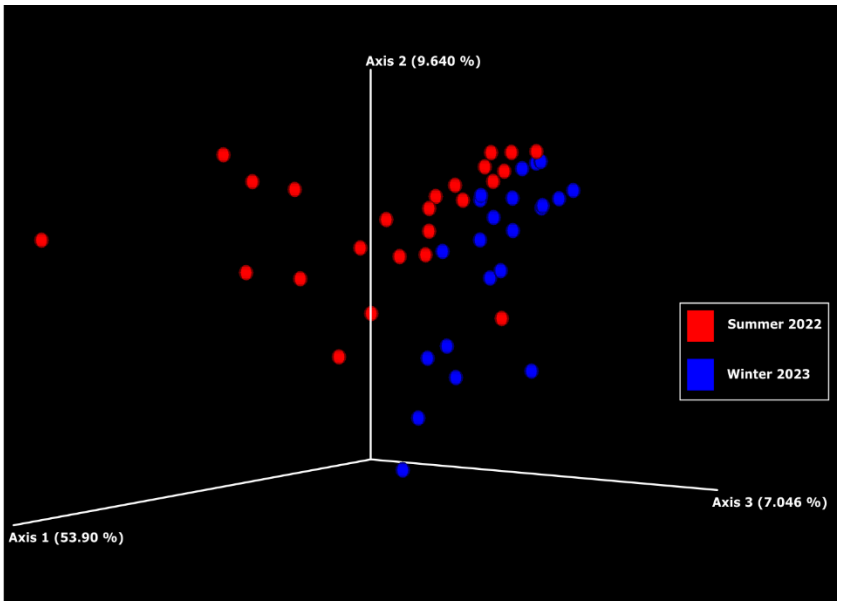

Figure 23. Emperor plot of principal coordinates analysis for sampling date from weighted Unifrac analysis. Sample collected in the summer are shown in red. Samples collected in the winter are shown in blue.

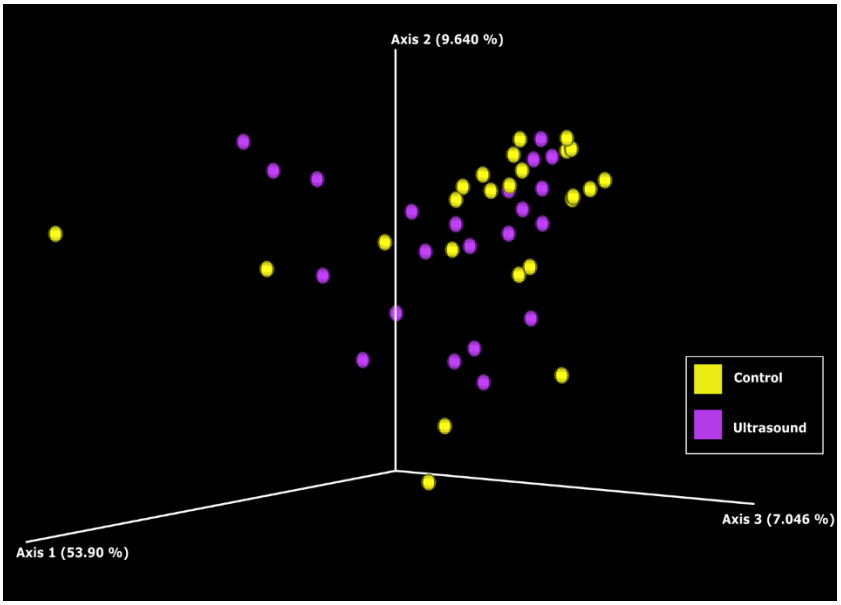

Figure 24. Emperor plot of principal coordinates analysis for control and ultrasound samples from weighted Unifrac analysis. Control samples are shown in yellow. Ultrasound samples are shown in magenta.

4. Conclusions

The goal of this study was to determine if a commercially available ultrasound transducer designed for algae control would impact invasive quagga mussel settlement. The ultrasound transducer tested

in this study did not have an obvious impact on mussel settlement, algae species composition, or overall biofouling. Although the total number of settled mussels was consistently less within the range of the transducer it is not possible to determine if this was due to natural settlement variability or an effect of the transducer. Settlement and biofilm accumulation within the range of the transducer followed a similar trend to control plate accumulation, suggesting that most of the difference could be attributed to naturally occurring variability. The design of this study did not allow for the detection of small impacts of the treatment due to the implicit variability associated with a field study. The pre-study settlement analysis confirmed that mussel settlement was variable at the locations where treated and untreated plates would be installed. Therefore, only complete or obvious settlement reduction would indicate that the treatment was having an effect. Additionally, small reductions in settlement would not be overly beneficial for large structures that are continuously exposed to mussel fouling. A small reduction might reduce the frequency of cleaning but would not meaningfully address the fouling issue.

The original hypothesis was that mussel settlement may be impacted by ultrasound exposure because it creates an inhospitable environment with a reduction in food availability. It was expected that mussels would be able to settle in the range of the transducer but they might not grow as quickly or may be compromised so that they would not survive long-term. Mussel size was not found to be different on the treated plates when compared to the control. Additionally, the accumulation of biofilm was greater on the ultrasound exposed plates, with no obvious reductions in algae species, suggesting that food availability was not likely reduced.

It is possible that the one-year duration of this study was not long-enough to observe the impacts of ultrasound on mussel survival. Additionally, it is possible that the settlement substrate (PVC plates) utilized in the study may have reflected the sound waves, possibly reducing the effectiveness. Therefore, additional research may be warranted to investigate the conditions in which ultrasound may effectively reduce biofouling.

Study Data Location:

- Share Drive folder name and path where data are stored:TSC (\\BOR\DO) (T:), Jobs, DO, _NonFeature, Science and Technology, 2020-PRG-Ultrasound for Mussel Control
- Point of Contact name, email, and phone: Sherri Pucherelli, spucherelli@usbr.gov, 303-445-2015

5. References

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