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# Focused ultrasound extraction (FUSE) for the rapid extraction of DNA from tissue matrices

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## Abstract

1. Rapid DNA extraction is a critical barrier for routine and fieldable genetics tests for applications in conservation, such as illegal trafficking and fraudulent mislabelling.
2. Here, we develop a non-thermal focused ultrasound extraction (FUSE) technique that creates a dense cloud of high-pressure acoustic cavitation bubbles to disintegrate targeted tissues into an acellular debris, resulting in the rapid release of entrapped DNA.
3. In this work, we demonstrate the proof-of-concept of the FUSE technique by obtaining species identifiable sequences and shotgun sequencing reads from DNA extracted from Atlantic salmon *Salmo salar* tissues.
4. Having mitigated the key risks for this technique, we hypothesize future developments with this technology can be applied to accelerate and simplify DNA extraction from exceedingly difficult samples with complex tissue matrices (i.e. fibrous tissue and timber samples) in both laboratory and field settings.

**KEYWORDS**
DNA barcoding, DNA extraction, focused ultrasound

## 1 | INTRODUCTION

The potential applications of DNA testing methods to combat illegal wildlife contraband (Dormontt et al., 2015; Wasser et al., 2018), determine the integrity of supply chains, (Golden & Warner, 2015) and monitor for invasive species (Dejean et al., 2012) or crop pests and pathogens (Randhawa et al., 2013) are undeniable. However, a critical barrier facing the routine use of DNA testing in supply chain management, conservation and law enforcement is the difficulty in preparing samples for DNA analysis. This process of DNA extraction—collecting samples, lysing tissue and purifying or isolating target DNA—is the most time and labour-intensive step of most DNA tests and typically requires trained laboratory personnel in a fully equipped laboratory to provide target DNA with a sufficient purity for reliable analysis (Buser et al., 2016; Fredricks et al., 2005; Wu et al., 2014).

DNA analysis and test systems have advanced rapidly in recent years, providing systems that can be deployed outside the laboratory to rapidly identify genetic targets of interest through rapid isothermal amplification tests (Niemz et al., 2011; Yetisen et al., 2013) and even provide sequencing reads by identifying which bases are passing through a nanopore (Loman & Watson, 2015; Mikheyev &

Tin, 2014). These systems and methods provide great promise for the integration of DNA testing in the field but require an input of purified, accessible DNA that is currently not possible for all sample types. To overcome this limitation, an array of different benchtop tools and reagents typically have to be utilized in the laboratory to extract accessible DNA from difficult sample types (e.g. coprolites, timber, bone, teeth, desiccated tissue, etc.) which are not easily translated for use in the field. For example, samples with dense tissue matrices often have to be processed under liquid nitrogen to prevent thermal damage to the DNA while the tissue matrix is disrupted (e.g. pulverization; Rachmayanti et al., 2009; Wasser et al., 2008). We envision a system that can be deployed in the field to rapidly disintegrate tissues from a broad spectrum of sample types and provide sufficiently pure DNA for subsequent automated analyses.

Here, we demonstrate the application of focused ultrasound as a novel non-thermal technique for rapid DNA extraction to enable DNA barcoding and shotgun genome sequencing analyses. Focused ultrasound extraction (FUSE) uses focused, high-pressure acoustic pulses that are applied to a target tissue in order to generate a dense cavitation 'bubble cloud' capable of disintegrating the tissue into an acellular debris. This system is based off a technique called histotripsy that is currently being developed as a non-invasive tissue ablation method for medical applications such as benign prostatic hyperplasia (Hempel et al., 2011; Roberts et al., 2014), kidney stones (Duryea et al., 2011), deep vein thrombosis (Maxwell et al., 2011; Zhang et al., 2015), congenital heart disease (Owens et al., 2011; Xu et al., 2010) and cancer (Smolock et al., 2018; Vlaisavljevich, Greve, et al., 2016; Vlaisavljevich et al., 2013). Histotripsy uses high pressure (>10 MPa), short-duration (<20 μs) ultrasound pulses applied at low duty cycles (<1%) to generate a dense cavitation bubble cloud at the focus of the ultrasound transducer (Figure 1; Vlaisavljevich et al., 2015; Vlaisavljevich, Maxwell, et al., 2016; Xu et al., 2004). The cavitation bubbles produced during histotripsy induce very large stress and strain to the tissue, resulting in the complete disintegration of the target tissue into an acellular tissue homogenate with no remaining cellular or extracellular matrix structure (Vlaisavljevich et al., 2013, 2016). Previous studies have shown that single cycle pulses applied by highly focused array transducers can reproducibly generate dense bubble clouds when the peak negative pressure (*p*-) exceeds the intrinsic threshold of water, which is approximately 25–28 MPa (Maxwell et al., 2013; Vlaisavljevich et al., 2017; Vlaisavljevich, Xu, et al., 2016). Figure 1 shows a schematic of histotripsy cavitation formation and tissue ablation from an array transducer used in our laboratory.

In this study, we aim to demonstrate the feasibility of using FUSE to rapidly obtain DNA from tissue samples, to amplify a DNA barcode using a two-step polymerase chain reaction (PCR) to verify DNA integrity, and to sequence the amplification product to validate applications in DNA barcode analysis. While the ultimate goal of this work is to translate this biomedical technology into portable, field-deployable systems that can allow non-technical users to rapidly prepare samples for DNA testing, this initial study aims to demonstrate the first proof-of-principle for this technique to accelerate sample processing and provide useful DNA for species identification tests (e.g. DNA barcoding tests to identify illegal trafficking). We hypothesize that the cavitation 'bubble cloud' can provide for rapid release of encapsulated DNA by disintegrating targeted tissues into acellular debris. This mechanism is similar to how sonic cleaners have been used to break up bacteria for DNA analysis (Taylor et al., 2001). In addition, other focused ultrasonicators have also recently been used for library preparation (Bomsztyk et al., 2019) and sample preparation (Kresse et al., 2018), including testing of clinical sample types and cancer genomics. The histotripsy transducers investigated here offer the potential for further advancing ultrasonic DNA extraction by delivering much higher peak negative pressures and creating dense bubble clouds capable of breaking down even very difficult tissue types. These devices can also be created with many possible geometries and form factors, suggesting

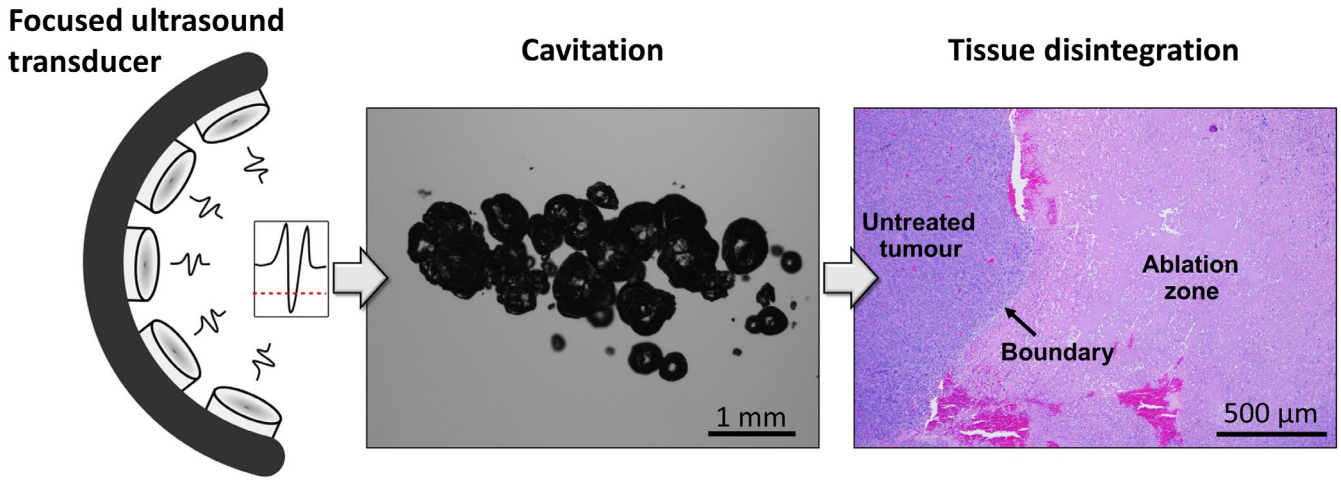



**FIGURE 1** Focused ultrasound pulses create cavitation clouds that effectively ablate complex tissue. Convergent acoustic waves created by individual ultrasonic transducer elements create a point of negative pressure that exceeds the intrinsic threshold of water (illustrated as a red dotted line), creating a dense cavitation bubble cloud. The expansion and collapse of these cavitation bubbles ruptures and erodes tissue into an acellular debris. The image shown is a histological section following a histotripsy treatment of a kidney tumour in a subcutaneous mouse model

the potential for multiple laboratory and fieldwork applications. Functionally, we hypothesize these advantages will allow this technology to address a wide variety of sample types of interest to ecologists and conservationists (such as dried skins, fur, plant tissue and woody tissues), and, more importantly, enable the samples to be processed in the field. The greater pressures and strong shear forces created by this system may also result in substantial fragmentation of the target DNA. Thus, the focus of this work is to determine if continued development of this histotripsy-based FUSE technology is warranted.

## 2 | MATERIALS AND METHODS

In order to demonstrate the proof-of-concept for extraction of amplifiable DNA fragments to provide definitive species identification, samples from muscle tissue of nine separate specimens of Atlantic salmon *Salmo salar*, stored at −20°C before use, were prepared and treated under the following experimental conditions.

### 2.1 | FUSE pulse generation

A custom 32-element 500 kHz array transducer with a geometric focus of 75 mm, an aperture size of 150 mm and an effective *f*-number of 0.58 was used for all experiments in this study (Kim et al., 2014). To generate a short therapy pulse, a custom high-voltage pulser was used to drive the transducers. The pulser was connected to a field-programmable gate array (FPGA) board (Altera DE0-Nano Terasic Technology) specifically programmed for histotripsy therapy pulsing. This set-up allowed the transducers to output short pulses of less than two cycles. A custom-built fibre-optic probe hydrophone (FOPH; Parsons et al., 2006) was used to measure the acoustic output pressure of the transducers. The FOPH was cross-calibrated at low-pressure values using two separate reference hydrophones (Precision Acoustics 1 mm needle hydrophone and Onda HNR-0500) in order to ensure accurate pressures were measured from the FOPH. The lateral and axial full width half maximum (FWHM) dimensions at a geometric focus of the transducer were measured to be 2.3 and 7.1 mm respectively. The acoustic pressures used for all experiments were measured in degassed water at the focal point of the transducer, which was identified using a 3D beam scan. At higher pressure levels ($p$- >23 MPa), the acoustic output could not be directly measured due to cavitation at the fibre tip. These pressures were estimated by a summation of the output focal $p$-values from individual transducer elements. For all samples in this study, a pressure of approximately 0 MPa was applied for histotripsy treatment conditions.

### 2.2 | Visualization of FUSE tissue ablation

For all experiments, a high-speed camera (Blackfly S 3.2 MP Mono USB3 Vision, FLIR Integrated Imaging Solutions) was aligned with the focal zone of the transducer using a focused 50 mm *f*/1.8D lens (Nikon AF, Nikon Corporation) and backlit by a custom-built pulsed white-light LED strobe light capable of high-speed triggering with 1 μs exposures (Figure 2). This set-up resulted in captured images with a resolution of 6.95 μm per pixel.

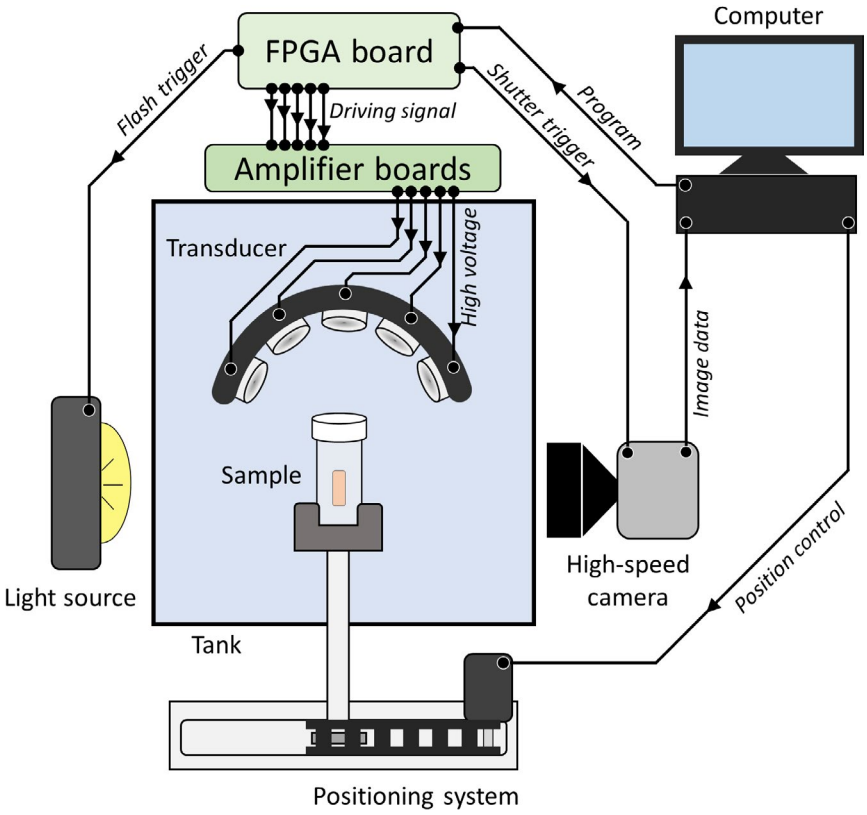



**FIGURE 2** Experimental focused ultrasound extraction set-up. Ultrasonic transducers are driven by an FPGA board and amplifier. High-speed imaging is performed using a strobe and camera controlled by signals from the FPGA board. Custom scripts are delivered to the FPGA board and imaging data are recorded by a computer. A robotic positioning system, controlled by the computer using MATLAB, is used to align the sample in the focus of the transducer array. In this study, tissue samples are housed in an acoustically permeable tube and secured with a custom 3D-printed sample holder

To ensure the destruction of tissue, a sample of *S. salar* muscle tissue (approximately 8 × 8 mm with a thickness of approximately 1 mm) was suspended in agarose gel and treated with 1,000 pulses of focused cavitation at 40 MPa and pulse repetition frequency (PRF) of 25 Hz. The camera was triggered to capture two images for each pulse, one during bubble cloud formation and one between pulses, allowing for the separate visualization of the cavitation bubbles and the resulting lesion. A robotic positioning system controlled by custom MATLAB scripts was implemented to properly locate and align samples.

## 2.3 | Sample preparation and ablation

Nine filets of Atlantic salmon *S. salar* were sampled for processing with the FUSE system. Samples were cut using a sterile scalpel blade into pieces of a consistent shape and thickness (weighing between 25 and 60 mg). In order to minimize the contribution of endogenous, free-floating DNA released post-mortem via seafood processing, all samples were rinsed with 300 μl nuclease-free deionized water (di$H_2O$—Invitrogen) prior to ablation or extraction procedures.

### 2.3.1 | Sample holder and isolation

The sample holder used for this study was a novel design that placed the tissue sample at the focus of the ultrasound transducer. The primary features of the sample holder include the back mount, and the extrusion in which the tube is secured. The sample tube, having a 6.35-mm inner diameter and 1.59-mm wall thickness (Tygon PVC E-1000, McMaster-Carr), is optically transparent and acoustically permeable with <7.1% pressure loss through the tubing. The tube has a locking top through which buffer can be applied to the sample and isolated from the water tank. In this work, samples were ablated in either an enzymatic buffer composed of the Qiagen lysis buffer (Buffer ATL and Proteinase K at a 9:1 ratio, Qiagen Inc.), or a non-enzymatic buffer composed of 1× Tris-EDTA buffer (TE buffer, Integrated DNA Technologies Inc.). Images of the sample holder and transducer are shown in the Supporting Information (Figure S1).

### 2.3.2 | Ablation parameters

For each set of experiments, 10,000 pulses were applied at a PRF of 25 Hz, which results in a process time of 6 min and 40 s. Prior to treatment, the centre of the bubble cloud was first focused on the centre of the sample inside the tube. Transducers then generated a peak negative pressure of approximately 40 MPa. This pressure was confirmed by measurements made with a fibre-optic hydrophone to account for losses through the sample tube.

## 2.4 | Purification conditions

The robustness of the ablation process was investigated with two lysis and purification pathways. The first pathway looked to understand if FUSE treatment excessively fragmented DNA during processing. In this case, DNA was extracted from samples using an enzymatic buffer with and without FUSE treatment ($n = 9$ each group) and purified using silica columns (Qiagen DNeasy blood and tissue kit Qiagen Inc.).

The second pathway looked to understand if the FUSE treatment alone could provide for DNA extraction without surfactants or enzymes. To examine this possibility, DNA was extracted from samples in a non-enzymatic buffer (TE buffer) with and without FUSE treatment ($n = 9$ each group). Preliminary work determined that this buffer was not compatible with silica column purification, so these lysates were purified with alcohol (isopropanol) precipitation.

All lysates were analysed with a Qubit™ 4 Fluorometer (ThermoFisher), and a Nanodrop™ One (ThermoFisher) to determine the quantity of DNA released between histotripsy ablation and controls. For data acquired from Nanodrop™ and Qubit™ measurements, an unpaired student's *t* test with unequal variance was used, with values <0.05 ($p < 0.05$) considered significant.

### 2.4.1 | Qiagen Dneasy™ blood and tissue

A standard Dneasy™ blood and tissue kit (Qiagen Inc.), one of the most common DNA extraction/purification kits, was used for the enzymatic control (QIAGEN, 2006). Samples and controls in this group were treated in a lysis buffer containing 270 μl of Buffer ATL and 30 μl of Proteinase K (0.4 mg). The FUSE-treated samples ($n = 9$) were ablated in 300 μl of lysis buffer. The standard controls ($n = 9$) were soaked in lysis buffer at 56°C and vortexed every 5 min for 15 s until no tissue fragments remained (approximately 45 min). Subsequent purification of both the control and treatment group was performed in silica columns using the standard protocol as recommended by the manufacturer.

### 2.4.2 | Isopropanol precipitation

Control samples ($n = 9$) were soaked in 300 μl of TE buffer for 10 min at room temperature, while FUSE-treated samples ($n = 9$) were ablated in 300 μl of TE buffer. The lysate solution was aspirated to a new tube to avoid any remaining tissue, then mixed with an equal volume of 70% isopropanol. Samples were incubated at 4°C for 30 min to allow DNA to precipitate prior to a 4°C centrifugation at 20,000× *g* for 30 min. All volume was aspirated from the DNA pellet which was then washed twice with 750 μl of 70% ethanol. The samples were left to dry under vacuum for 1 hr, after which the DNA pellet was resuspended with 200 μl TE buffer.

## 2.5 | Quantitative PCR amplification

Polymerase chain reaction primers were designed to target the cyclooxygenase subunit 1 mitochondrial gene (COI) of *S. salar*. The forward primers consisted of the sequence 5′–CGCCCTA AGTCTCTTGATTCG–3′ which was derived from a primer set used to identify *S. salar* from environmental DNA samples (Atkinson et al., 2018). An alternate reverse primer 5′–GTCACTGCCGTC CTTTTGTT–3′ was designed to produce a 550 bp amplicon. The adjustment to a larger amplicon was to test whether the treatment produced DNA templates of sufficient length to provide high-quality amplicon sequencing reads.

Samples were amplified with a total volume of 12.5 μl, composed of 1X PowerUp™SYBR™ Green master mix (Applied Biosystems), 0.5 μM of each primer (Integrated DNA Technologies), 2 μl of nuclease-free water and 2 μl of template. qPCR was performed on an Applied Biosystems 7300 (ThermoFisher) under the following conditions: Initial holds at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. A negative control that contained 2 μl of nuclease-free water instead of a template was performed with each qPCR run to control for contamination. Amplifications were performed in triplicate for each sample and treatment group. Extractions were considered successful if at least two of the three replicates amplified with a threshold (Ct) value within three cycles.

## 2.6 | Sequencing analysis

Lysates obtained from with FUSE treatment and purified with silica columns as well as enzymatic controls were selected for amplicon sequencing, which is still commonly used in DNA barcode tests for mislabelling and illegal trafficking (Bunholi et al., 2018; Chang et al., 2018), and shotgun sequencing.

### 2.6.1 | Amplicon sequencing

PCR products from one successful amplification in both the FUSE and control groups were selected for sequencing. Primer extension sequencing was performed by GENEWIZ, Inc (South Plainfield) using Applied Biosystems BigDye version 3.1. The reactions were then run on Applied Biosystem's 3730xl DNA Analyzer. Consensus sequences were compared to the National Center for Biotechnology Information database using the BLAST tool to confirm the sequence identity was *S. salar*.

### 2.6.2 | Shotgun sequencing

For enzymatic controls and FUSE-treated samples purified with Qiagen DNeasy™ Blood and Tissue kit, 200 ng of DNA from each purified lysate was digested with 1 μl of ApeKI and then ligated to Illumina-compatible adapters with ApeKI overhangs with 1.6 μl of T4 DNA ligase. Each of the P1 adapters had a variable length (4–6 bp) index downstream of the sequencing primer such that it was read immediately preceding the restriction site. The P2 adapter was common across all samples. Following adapter ligation and PCR confirmation, the ligations were pooled and purified with the New England Biolabs Monoch PCR and DNA Clean Up Kit, then 18 cycles of PCR were performed and purified. Fragments in the range of 250–600 bp were selected on a BluePippin™ instrument (Sage Science) and the resulting libraries were visualized on a Bioanalyzer (Agilent 2100 BioAnalyzer). All libraries were pooled at equimolar concentrations based on the fragment distribution from the Bioanalyzer traces and sequenced on an Illumina NovaSeq instrument with an S-Prime flow cell in 2 × 150 bp paired-end mode at the Duke University Center for Genomic and Computational Biology. Raw reads were filtered for quality, filtered for adapter contamination and de-multiplexed using STACKS software (Catchen et al., 2013). Filtered reads were then aligned to the *S. salar* reference genome (Lien et al., 2016) using the Burrows-Wheeler Aligner (BWA) *mem* algorithm, and subsequently converted to BAM format, sorted and indexed with SAMtools (Li & Durbin, 2010; Li et al., 2009). Coverage depth per sample was calculated using the SAMtools *depth* function, and statistical analysis of coverage depth was performed using a Welch's two sample *t* test with values <0.05 ($p < 0.05$) considered significant.

# 3 | RESULTS

The FUSE protocol used in this study provided amplifiable DNA in comparable quantities to standard Qiagen extractions. DNA obtained using FUSE yielded sufficient fragment lengths for high-quality amplicon sequencing reads and definitive species identification. This FUSE protocol also provided lysates from a non-enzymatic buffer that had the same amplification success rates as traditional Qiagen extractions.

## 3.1 | FUSE tissue breakdown

*Salmo salar* samples suspended in agarose gel showed breakdown of the targeted tissue within a few hundred pulses and complete breakdown of the focal region, exposed to the bubble cloud within 1,000 pulses, wherein the tissue was liquefied and became homogeneous with the surrounding gel (Figure 3). Optical images confirmed a dense cavitation bubble cloud was formed after each pulse, and the resulting tissue disruption was observed to increase over the course of the treatment.

Similar results were observed for samples treated in sample tubes for DNA extraction experiments (Figure 4). In these cases, the tissue samples were free to move about in solution, during the treatment, which has the potential to reduce the efficacy of tissue breakdown due to the sample moving in and out of the focal volume. However, for all experiments, complete or near complete breakdown (only a few small tissue fragments remaining) was observed for samples treated with the standard 10,000 pulse regimen used in this

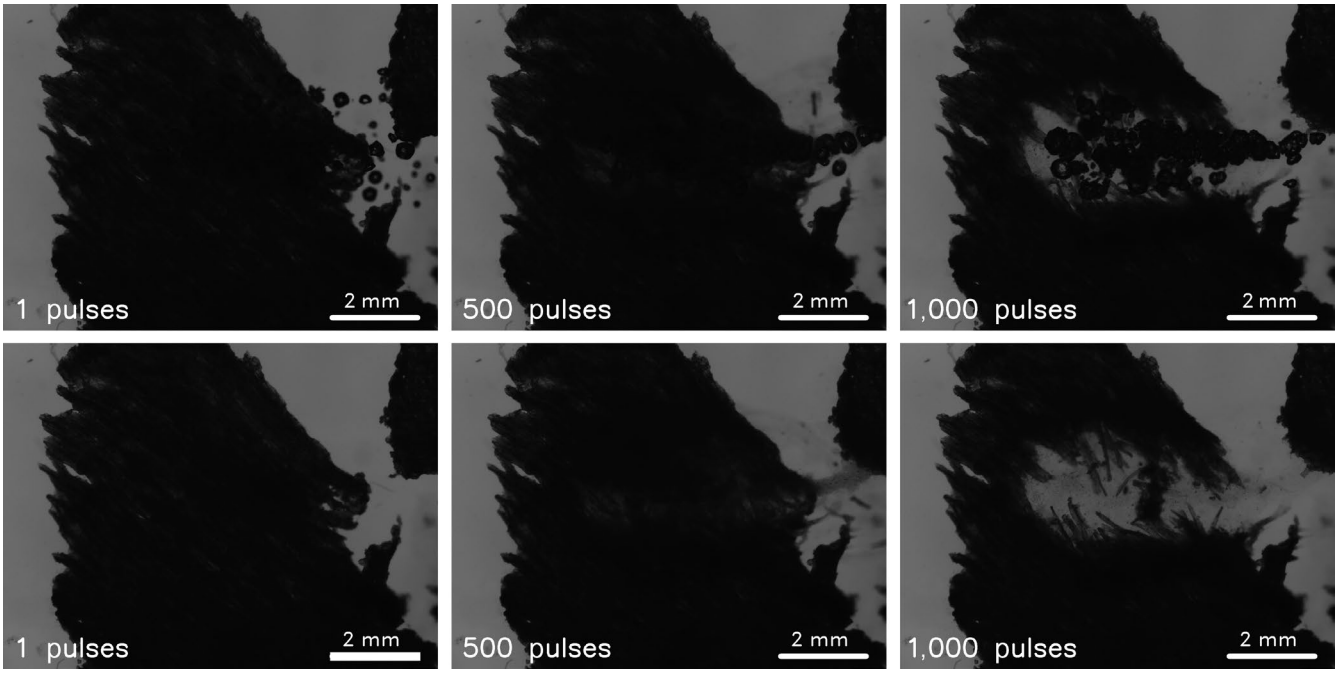



**FIGURE 3** Focused ultrasound extraction pulses effectively ablate *Salmo salar* tissue. Focused cavitation clouds (top row) effectively ablate tissue into an acellular debris (bottom row). Ablation is observed within 500 pulses and complete ablation occurs within 1,000 pulses. With the pulse repetition frequency of 25 Hz used in this study, the depicted process took 40 s. The bottom row shows images of the tissue captured in between pulses

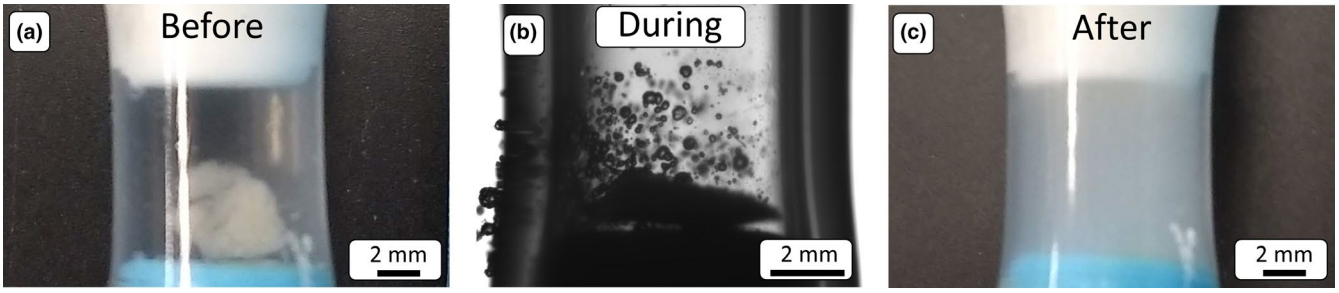


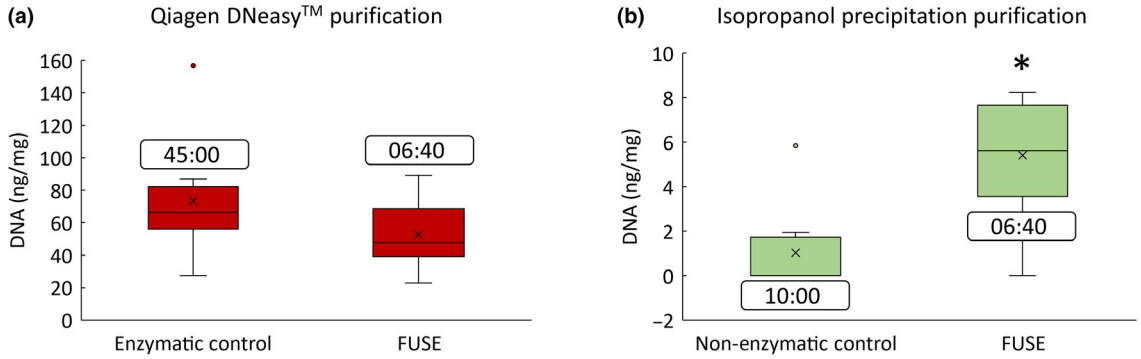

**FIGURE 4** *Salmo salar* tissue is completely disintegrated by focused ultrasound extraction treatment in sample tubes. A piece of *S. salar* tissue (a) treated in the sample holder with TE buffer (b) is ablated into solution with no visible particulates (c)

study (Figure 4). A video from this process is also included in the Supporting Information.

## 3.2 | DNA extraction efficiency

There were no significant differences observed in the quantity of DNA released per milligram of tissue (DNA concentration measurements were normalized by mass to account for differences in starting tissue quantities) between the Qiagen procedure and the FUSE-treated samples with silica column purification. This result indicates that histotripsy is as effective at releasing DNA as traditional thermal processing using Qiagen's lysis buffers (enzymatic control). The histotripsy treatments used in this study took 6 min and 40 s to complete, whereas enzymatic controls took 45 min of incubation at 56°C (with vortexing every 5 min) to achieve the same result. No significant differences were observed in the 260/280 ratios between extraction processes. The 260/230 ratio was significantly lower for FUSE-treated samples than for standard controls (Figure 5). However, the 260/230 ratios for all FUSE-treated samples were still in an acceptable range.

Focused ultrasound extraction treatment was also observed to release significantly higher quantities of DNA when ablated in TE buffer compared to controls that were incubated in TE buffer for 10 min (Figure 5). The mean amount of DNA released by FUSE-treated samples in the non-enzymatic buffer was lower than both FUSE-treated and control samples in the Qiagen lysis buffer. The purity as indicated by the 260/280 and 260/230 ratios was also lower for groups purified with isopropanol precipitation compared to samples purified with the Qiagen protocol.




(c)

| Purification method | Buffer activity | No FUSE | | | | | FUSE | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lysis time (mm:ss) | DNA (ng/mg) | Protein (ng/mg) | 260/280 | 260/230 | Lysis time (mm:ss) | DNA (ng/mg) | Protein (ng/mg) | 260/280 | 260/230 |
| Qiagen DNeasy™ | *Enyzmatic* | 45:00 | 73.4 ± 35.6 | 0 ± 0 | 2.06 ± 0.30 | 2.10 ± 0.04 | 6:40 | 52.5 ± 20.3 | 0 ± 0 | 2.07 ± 0.06 | 1.87 ± 0.30* |
| Isopropanol precipitation | *Non-enzymatic* | 10:00 | 1.0 ± 1.9 | 0 ± 0 | 1.40 ± 0.40 | 0.40 ± 0.09 | 6:40 | 5.4 ± 2.7* | 839.3 ± 553.9* | 1.67 ± 0.19 | 0.37 ± 0.05 |

**FIGURE 5** Focused ultrasound extraction (FUSE) treatment accelerates DNA extraction. Samples treated with histotripsy showed similar DNA release when compared to a standard thermal lysis with enzymatic control buffer (a). Significantly higher DNA release when samples are ablated in a non-enzymatic buffer (b). Table shows how FUSE affects the quality of resulting lysate compared to control buffers (c). The processing time for FUSE and control treatments is shown in text on the graphs. DNA and protein quantification measurements are reported from Qubit™ Fluorometer measurements, and 260/280 and 260/230 ratios are reported from Nanodrop™ measurements. *indicates significant ($p < 0.05$) differences between FUSE-treated groups and respective controls

**TABLE 1** DNA extracted from focused ultrasound extraction (FUSE) treatment can be successfully amplified. All samples treated with FUSE successfully amplified, regardless of lysis buffer and purification method. Sample ratios indicate qPCR replicate groups, the total ratio demonstrates overall sample success. Amplification was considered successful if at least two of the three replicates amplified with a $C_t$ value within three cycles

| | Replicate success | | | |
|---|---|---|---|---|
| | Qiagen DNeasy™ purification | | Isopropanol precipitation | |
| Sample | Control | FUSE | Control | FUSE |
| Ss1 | 3/3 | 3/3 | 3/3 | 3/3 |
| Ss2 | 3/3 | 3/3 | 3/3 | 3/3 |
| Ss3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Ss4 | 3/3 | 2/3 | 3/3 | 2/3 |
| Ss5 | 3/3 | 2/3 | 3/3 | 2/3 |
| Ss6 | 2/3 | 3/3 | 1/3 | 2/3 |
| Ss7 | 3/3 | 3/3 | 1/3 | 3/3 |
| Ss8 | 3/3 | 2/3 | 3/3 | 3/3 |
| Ss9 | 2/3 | 2/3 | 0/3 | 2/3 |
| Total | 9/9 | 9/9 | 6/9 | 9/9 |
| Extraction success | 100% | 100% | 66% | 100% |

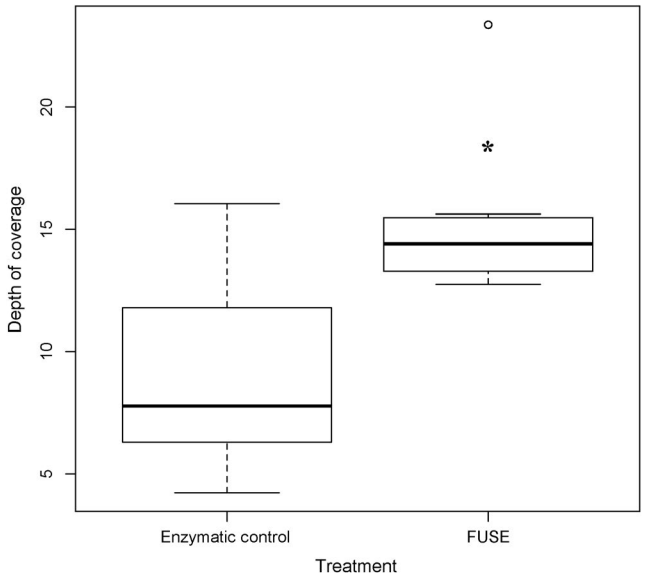



**FIGURE 6** Focused ultrasound extraction (FUSE) treatment provides sufficient DNA for shotgun sequencing. FUSE treatment showed significantly higher read depth compared to the control group, indicating that FUSE treatment can extract DNA for shotgun sequence reads. However, additional studies will be required to determine if this trend provides a meaningful improvement across different sample types and studies. *indicate significant differences ($p < 0.05$) in coverage depth between treatment groups

## 3.3 | DNA amplification

Successful amplifications were obtained for each sample processing condition (Figure 5). Results showed that there was no difference in amplification success rate between control samples and FUSE-treated samples purified under the same conditions (Figure 5). For non-enzymatic lysis buffers purified with isopropanol precipitation, all samples treated with FUSE amplified while multiple samples of the

control group failed to provide consistent amplification across replicates (Table 1).

### 3.4 | Sequencing

All samples sent for sequencing provided high-quality amplicon sequencing reads that matched vouchered *S. salar* COI sequences (Accession numbers: MK295655, MK295654, MK295653) stored in public databases accessed using the BLAST tool. There were no significant differences in the read length or quality score of FUSE-treated samples and the control groups. Forward and reverse sequences were aligned to create a consensus sequence (Metazoan Mitochondrial COX1 / Salmo salar CO1 DNA sequencing with FUSE extractions). There were no damage sites observed between FUSE-treated groups and controls. Furthermore, there were no mutations observed between filet samples, indicating that all filets exhibited the same haplotype (which was expected, as they were all obtained from farm raised stocks in Chile, which likely originated from the same brood stock). Shotgun sequencing showed that FUSE-treated samples had significantly greater read depth than enzymatic controls (Figure 6). While this result does not necessarily indicate that FUSE treatment improves read depth, this result demonstrates that DNA collection from FUSE-treated samples is sufficient for shotgun sequence analyses (Atlantic salmon sequencing to demonstrate the utility of focused ultrasound for tissue ablation and genomic DNA extraction).

## 4 | DISCUSSION AND CONCLUSION

The results from this proof-of-concept study demonstrate that the FUSE protocol can provide accessible DNA for amplification and sequencing with an extraction time of under 7 min compared to the 45-min lysis time that was required to obtain the same result using traditional thermal and enzymatic lysis protocols. DNA obtained using FUSE provides high-quality amplicon sequencing reads for definitive species identification and DNA barcoding and shotgun sequence reads for broader applications.

The purity of lysates produced using FUSE was similar to that of standard control extractions. The 260/230 ratio of the FUSE-treated samples purified with the Qiagen protocol was significantly lower than that of the control samples but was still sufficient for reliable amplification. The reduction in this ratio could be attributed to the release of additional compounds from cells and tissues that were broken down by the FUSE treatment. The quality of lysates purified using the isopropanol precipitation method was substantially lower than the Qiagen purification method. However, FUSE-treated samples purified with isopropanol precipitation exhibited the same amplification success rates as samples purified with the Qiagen protocol. This result suggests that the effects of the FUSE treatment (tissue disintegration from the cavitation cloud activity) may be increasing the accessibility of DNA on its own (e.g. breaking apart proteins or carbohydrates that may be bound to DNA). Although the FUSE treatment was more effective when performed in a buffer containing surfactants and enzymes, the possibility that FUSE causes physical disruption of inhibitory compounds that bind DNA could enable successful DNA extraction for extremely challenging sample types, especially if complementary enzymatic or chemical buffers are used in conjunction with FUSE treatment (which will be further explored in future work).

The FUSE treatment tested in this study is a non-thermal tissue disintegration process, and no samples were heated during FUSE treatments conducted in this work. However, it is possible that the addition of thermal energy could also accelerate the DNA extraction process using FUSE or improve the success rate of DNA extraction from more challenging sample types. Furthermore, the PRF used in this study was only 25 Hz, whereas the PRFs used in clinical histotripsy systems can be much higher (100–1,000 Hz) without inducing any thermal effects. Adjusting the FUSE protocol to use higher PRF would further improve the speed of this extraction technique. For example, if the PRFs were increased to 250 Hz or 1,000 Hz, the extraction process would take 40 s or 10 s respectively. While the PRF in this first study was kept very low to rule out the contributions of any potential thermal effects, future work is ongoing to explore the use of higher PRF FUSE treatments for more rapid DNA extraction. We expect these higher PRF pulsing strategies to enable denser, tougher tissue types (which require a large number of pulses for full disruption) to be treated with FUSE while maintaining a rapid extraction rate.

Overall, the results of this study demonstrate the potential of FUSE as a novel platform for rapid DNA extraction of tissue matrices. This finding, albeit less impactful for simpler tissues like the salmon used in this initial study, could also enable routine work or substantially accelerate process flows with extremely challenging sample types (such as seeds, fibrous tissue, timber, filamentous fungi, etc.), and justifies a comprehensive exploration across a variety of challenging sample types, which is the focus of ongoing and future work. In addition, as part of these ongoing studies, we are currently developing fully integrated FUSE systems that condense this technology into desktop and portable systems that can be used to accelerate and enable DNA extraction process flows in and out of the laboratory. More specifically, we aim to develop portable FUSE systems consisting of miniature histotripsy transducers that can be driven at low power (potentially with off the shelf batteries) while maintaining the characteristic histotripsy bubble clouds needed for efficient tissue ablation. If successful, these portable and low-cost systems would allow for the convenient use of this technology in both laboratory and field settings, as well as the possibility of ablating tissue in preservation buffers to improve sample quality when received by a laboratory. Although our ultimate goal is to develop a single, flexible system that can be used to efficiently breakdown a wide range of sample types, it is possible that the optimal acoustic parameters for DNA extraction from different tissue samples will require customized FUSE systems and treatment parameters that need to be designed into separate devices for specific applications. Our ongoing studies in more challenging sample types are expected to provide significant insight into the optimal acoustic parameters, devices, and bubble cloud dynamics need to efficiently break down specific tissues, which will be essential to determining the

ultimate design of future FUSE systems as well as the readiness of this technology for potential commercialization. Ultimately, our goal is to develop a low-cost, portable, sample preparation system that can be integrated with all types of currently available DNA testing systems in order to address the key bottle necks in the DNA extraction process for a wide range of laboratory and field-based genetic tests while also expanding the use of those systems to more challenging sample types.

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## AUTHORS' CONTRIBUTIONS

H.R.H., D.B. and E.V. conceived the initial ideas for this work; H.R.H., M.H., T.L.H. and E.V. developed the methodology and analysed the data from focused ultrasound experiments; H.R.H., R.H., C.E. and D.B. developed the methodology for qPCR and amplicon sequencing experiments; Q.Z. and J.H. developed and performed the methodology and analysed the data from shotgun sequencing experiments; H.R.H., M.H. and R.H. gathered the data; H.R.H., R.H., C.E., D.B. and J.H. analysed the data from molecular experiments; H.R.H. led the writing of the manuscript. All the authors contributed critically to the drafts and gave final approval for this submission.

## PEER REVIEW

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## DATA AVAILABILITY STATEMENT

All genetic sequences generated in this work are available on GenBank. Amplicon sequences can be accessed with the Accession numbers: MW051095-MW051112 and shotgun sequence data can be access with the Accession number: PRJNA665390.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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