

A Preliminary Investigation of Damage in Marine Mammal Tissue due to Mechanical Loads using Silicone Rubber

By

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4 Abstract

Abstract of "A Preliminary Investigation of Damage in Marine Mammal Tissue due to Mechanical Loads using Silicon Rubber (ECOFLEX) as a Representative Material." by Tarik Matthew Simpson, Sc. M., Brown University, May 2025.

Objectives: Underwater shock waves and other activities that result in mechanical loads can cause harm to a variety of marine life. This problem can be detrimental to marine mammals that rely on echolocation. As an example, underwater impact and acoustic shock waves can cause irreparable damage marine mammal melon (in toothed whales), which assists these animals in hunting and communication. The adverse effects to these creatures caused by underwater impact forces or shock waves could cause long-lasting problems to the ecosystem, such as extinction and the overpopulation of their known prey [2]. It will be of value to understand what happens to the melon or other critical soft tissues in marine mammals under various amounts of force. Surrogate materials such as Ecoflex 300 have been used to investigate the potential mechanical damage to melon. We have conducted preliminary research to use two surrogate materials with different stiffness and applied a variety of forces to study if there is any change (damage) in the material after the force is applied.

Method: Ultrasound tests were run on surrogate Ecoflex samples without any load (control) and after the samples were subjected to mechanical loads and the data was analyzed in GageView Pro. Visual images were taken on an optical microscope to assess any physical damage to the samples.

Results: The average speed of sound through the T1 control is 1030.3 m/s and the T2 control, 971 m/s with the lowest load applied was the 25% strain at a rate of 1×10^{-3} for both samples. There was an minimal increase in the speed of sound for the materials when subjected to mechanical loads.

Conclusion: T1 at 25% and 75% had greater stress values compared to the T2 samples. There is a change in the speed of sound through materials when comparing a sample with no load applied to one that has a load applied. The change in speed of sound through the material is relatively small and further research needs to be done to confirm if this change is significant compared to potential exper-

imental errors. The findings are preliminary groundwork for the research method and measurement types but more research is needed to determine damage to the surrogate material.

5 Introduction

5.1 Background

Underwater explosions occur from many drilling and military operations that cause acoustic shock waves to propagate through the surrounding environment [3] [4][5]. The resulting impact loads from these shock waves can have the unintended consequence of harming marine life and the local ecosystems [6]. Marine mammals are at particular risk to shock load damage due to their reliance on echolocation to communicate and perceive their surroundings via reflected sound waves.[7] This unique sense is controlled using an organ located in the forehead called the marine mammal melon to aid in signal direction and reception.[8] [9]Therefore, it is of importance to understand the role underwater explosions may have in altering marine mammal melon function. In the special case of underwater explosions, a spherical gas bubble is created as depicted in Figure 1 that produce a shock wave to travel through the water medium and apply a load to the marine mammal melon which can cause tissue damage and as a result affect its function [10].

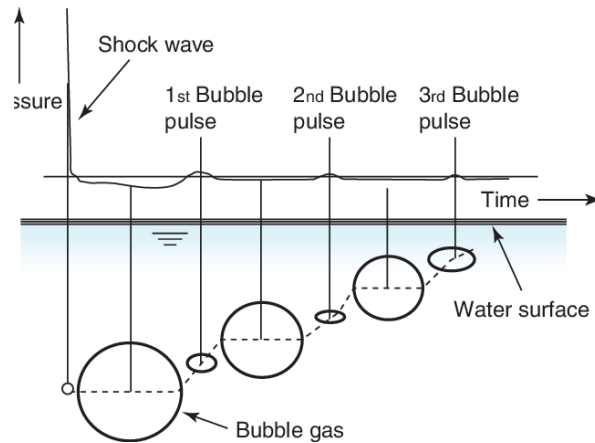


Figure 1: The gas bubble created by an underwater explosion undergoes a series of contraction and expansion in volume, a phenomenon called bubble pulse [1].

5.2 Marine Mammals

Under the taxonomy of Cetacea, there are two main classes of Marine mammals: Odontocetes, commonly referred to as toothed whales, and Mysticetes, commonly referred to as baleen whales [11] [12]. Of these two classifications, the marine mammal melon is unique only to Odontocetes as illustrated in Figure 2 [13] [14]. Toothed whales are pack animals that rely heavily on their echolocation (using a high frequency 150-170 kHz) to communicate with one another and sense spatial surroundings. This organ is critical to their survival and essential for functions such as hunting, mating, and migration. The biological composition of the melon is that of adipose tissue containing varying levels of wax esters and triacylglycerols depending on the subspecies [15]. The melon structure and composition is specialized by species for sound, pitch, and rate of signal propagation [16][17].

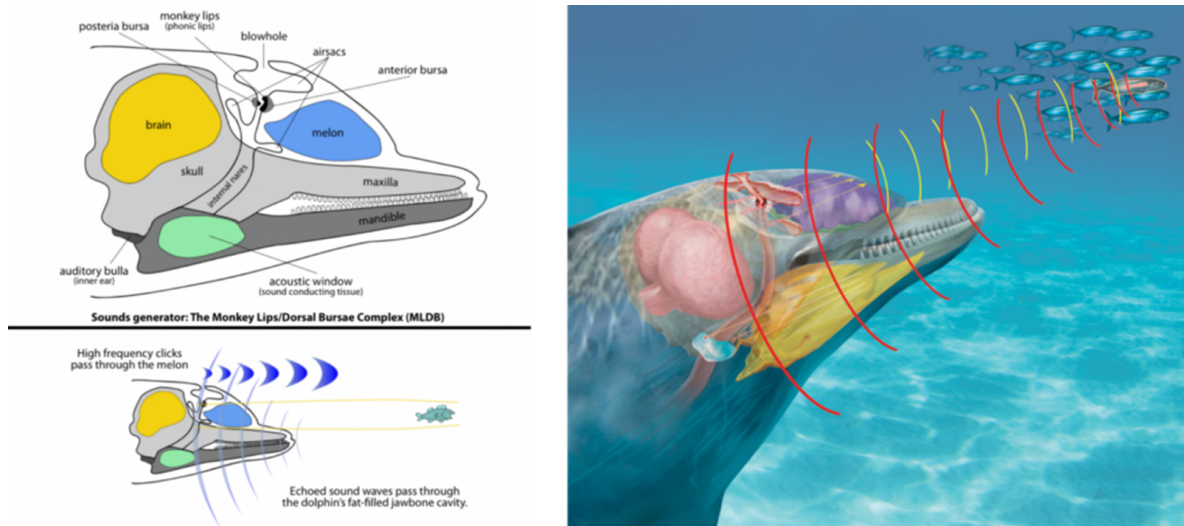


Figure 2: A visual representation on the anatomy and function of the melon.

5.3 Melon Function

The melon is located in the forehead of toothed whales and is composed of adipose tissue of unique composition from traditional fats. This lipid-filled "acoustic fat" tissue contains triacylglycerols, wax esters, and other biological content. There are two regions of the melon: inner and outer [18]. The outer melon typically contains a higher percentage of triacylglycerols and a lower wax ester percentage. The opposite is true for the inner melon, with greater amounts of wax esters than triacylglycerols. Some species of toothed whales entirely lack one of either wax esters or triacylglycerols. It has been

hypothesized that the lipid content and bone structure contribute to the sending and receiving of sound signals by the melon [19] [20] [21]. There exist air sacs within the melon that produce vibrations used to send signals out and receive signals through an "acoustic window" which transmits the sound through the adipose tissue into the inner ear [22] [23]. Damage to the melon can obstruct communication by altering the frequency at which signals are sent and received. These marine mammals play a vital role in maintaining the ecological equilibrium of their ecosystems, so a disruption to an organ vital to survival functions can be detrimental to the environment.

5.3.1 Current Trends in Literature

Previous biological research into the function of the melon, was based on specific subspecies of toothed whales [24]. There are 72 different subspecies of Odontocetes, the melon has been generalized in literature despite compositional differences between toothed whales [25]. The percentage of wax esters, triacylglycerols, and other extracellular matrix components varies depending on the subspecies of toothed whale [26]. Therefore, the material and mechanical properties of the melon and the speed of sound propagation through the tissue may differ between compositions. In addition, different regions of the melon have can also vary in these properties that are often overlooked when studying melon function in relation to tissue damage [27]. In this study, we aimed to qualitatively and quantitatively measure the effect of an applied load to damage the melon and potentially alter the speed of sound through a material of representative mechanical properties. Stress of surrogate material specimens under loads of varying strain and strain rate were measured and observations were made for any physical damage.

6 Methods

6.1 Qualitative and Quantitative Testing

Two mechanical load application methods were performed using a drop tower for moderate rate and universal testing machine (UniVert) for slow rate. A final strain of 25% and 75% was achieved using the UniVert at strain rates of 0.001 s^{-1} to 1 s^{-1} . A single force application using controlled gravitational acceleration of a 0.565 g weight was applied using the drop tower. Four samples of Ecoflex 0030 were

made as surrogate material to the marine mammal melon and tested under each mechanical loading condition, where the stress and strain were also recorded. Ultrasound measurements were collected for the speed of sound and optical microscope images were captured both before the application of a mechanical load on the UniVert or drop tower and after. The speed of sound data was analyzed for each trial of load application and a controlled undamaged sample. Light microscopy images were taken to observe any visual changes such as surface defects. The goal was to measure and observe any changes in the material properties as a result of the various applied mechanical loads. In a previous study, the drop test had an impact crosshead that had a nominal weight of 70 grams (170 g max weight), an impactor diameter of 2.5 inches for 1.5 in. x 0.5 in. samples, and drop tests were conducted for heights of 0.64 cm, 13.3 cm, and 17.8 cm [6]. In contrast, the samples used in our study were 12.5 mm x 10 mm, an impactor weight of 0.565 grams, and a drop height of 120 mm (12 cm) for compatibility with ultrasound equipment.

6.1.1 Ultrasound

A Olympus EPOCH 650 system shown in Figure 3 was used to collect the speed of sound data. This form of ultrasonic flaw detector emits sound waves that travel through a material using a 1 MHz probe that is connected to a 'gate' that detects reflected signals [28] [29] [4]. The speed at which sound is transmitted and the distance the sound travels through the material can be adjusted through calibration of the probe [30] [31] [32] [33]. The speed of sound is measured before and after the application of mechanical loads. Before measuring the speed of sound with the Olympic EPOCH 650, a few parameters had to be taken into account. The gates function is to independently define the digital measurement regions for samples amplitude and time of flight measurements. The gate is defined before a test is run so that a start point for the test is set. Every gate has a specific level that should be set before running a test (for this project, the gate level was set at 3%). The gate was set to detect echoes at their peak to ensure the maximum data was collected with an example plot in Figure 4.

The velocity had to be calibrated to measure the speed of sound through the material by first setting a base velocity to 1000 m/s. This number is based on the average speed of sound through silicone, of which the Ecoflex 0030 is composed. After selecting the autocalibration function, the



Figure 3: Olympus EPOCH 650 (a NDT) flaw detector and 1 MHz probe.

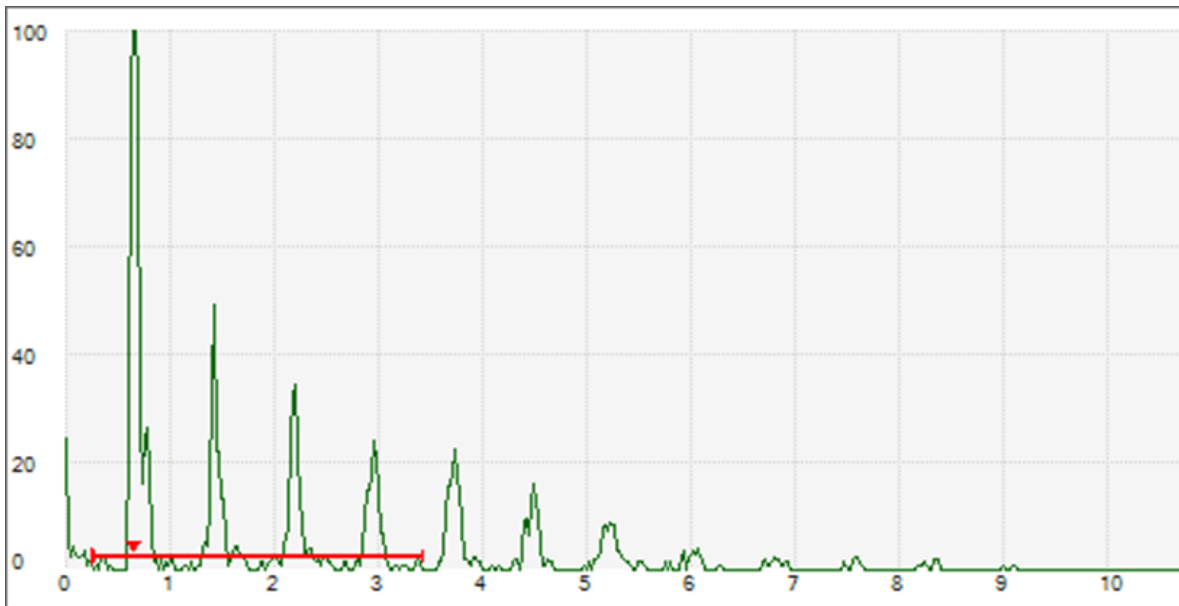


Figure 4: Waveform of a T1 Control sample with the gate level (red bar) set at 3%.

probe would be placed on the surface of the material with ultrasound gel in between. From there, an estimated thickness of the material and image of the waveform is captured. To calibrate a zero position, the estimated thickness of the test specimen is entered. Following calibration, the probe and ultrasound can be used to measure the speed of sound through the trial materials. A summary of the ultrasound settings is in Table 1.

PRF Mode	Auto
Energy	100 V
Mode	P/E (Pulse Echo)
Damp	50 Ohms
Pulser	Square
Freq	1MHz* or 5 MHz (depending on probe used)
Filter	0.5-4 MHz(Varies Depending on Probe and material tested)
Rect	Full
Reject	0%

Table 1: General parameters set for the Olympus EPOCH 650.

6.1.2 Drop Tower

The drop tower (Figure 5) is a mechanical testing device with an adjustable height cross-member with an impact-crosshead attached that is released using a controllable electromagnet to fall along guide rails onto the material specimen [34]. This device can achieve moderate strain rate load application and the impact force can be adjusted using the weight of the impact-crosshead and the height from which it is dropped. The droptower electronics are controlled using an Arduino microcontroller with source-code developed within the Srivastava Research Group to change the impact-crosshead height, release the platform, and record data. The acceleration, velocity, and displacement profiles can be measured and reported for each trial run. To operate the drop tower, one must connect to the WiFi signal of the drop tower controller. From there, one would go connect to the drop tower using the IP of the microcontroller on a web-browser. The user enters the test height, so the cross-member moves to the desired position. The impact-crosshead is manually moved up to the cross-member where the electromagnet engages to hold it in place at the given height. The samples are loaded directly beneath the impactor and the user can begin the test. The electromagnets disengage, releasing the impact-crosshead, and gravity pulls the impactor to make contact with and apply a load to the specimen. After the test runs, the collected data can be exported for analysis.

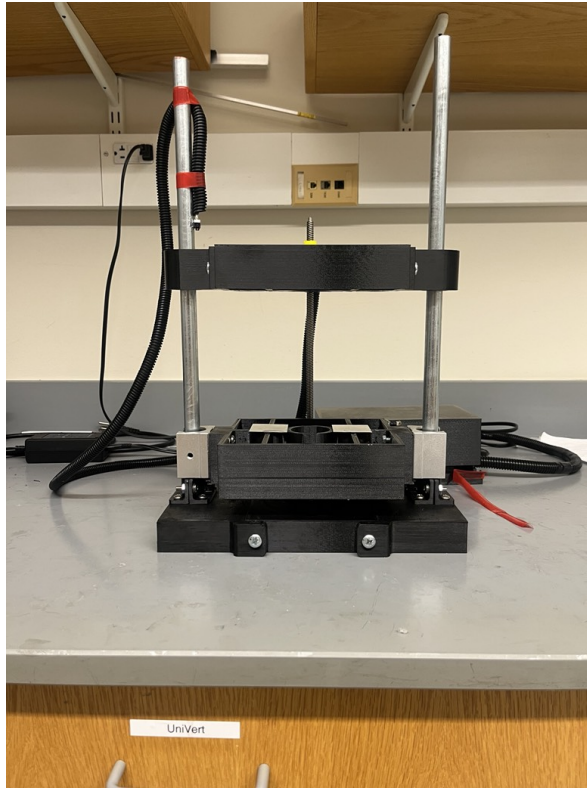


Figure 5: Drop Tower with an adjustable height for the platform to impact materials.

6.1.3 UniVert

The mechanical testing was conducted using a UniVert system (Figure 6), which is designed to perform uniaxial mechanical tests on soft materials. In this study, compression tests were carried out at engineering strains of 25% and 75% at rates of 1 s^{-1} and 0.001 s^{-1} . These specific strain levels were selected to gain a deeper understanding of the material's behavior and the types of defects that may develop as a result of deformation. Prior to testing, the sample's initial dimensions, including height and diameter, were recorded using calipers. To calibrate the displacement, the distance between the top and bottom platforms was manually measured using calipers and then input into the CellScale software. The user then defines the target engineering strain, the time to reach this strain, and the desired data acquisition rate. Before initiating the test, the top platform was adjusted to apply a preload of less than 0.5 N on the sample before the load cell was zeroed. This step is critical to avoid introducing artifacts into the data due to unintended pre-compression or gaps between the platform and the sample.

Once the test trials finished, the data is automatically saved to the computer. The UniVert

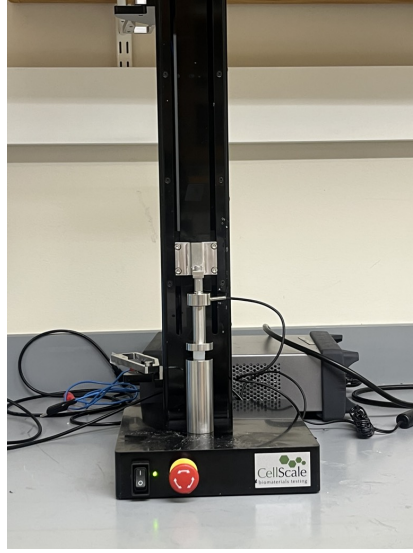


Figure 6: CellScale UniVert used to perform Mechanical Testing

collects the time (in seconds), the size and displacement (in millimeters), and the force (in Newtons). With the data from the software and the manual measurements of sample dimensions, the stress and strain was calculated of each trial. The stress is calculated by the force at all points divided by the area of a cylinder. The equation used to represent engineering stress is:

$$\sigma = F/((D/2)^2\pi)$$

where F is the change in force and D is the diameter of the sample. The engineering strain is obtained by dividing displacement by the initial length l and is represented by the equation:

$$\epsilon = \Delta l/l$$

where Δl is the change in length.

6.1.4 Optical Microscope

The optical microscope used in this study was the Reflected Trinocular model from the EXAMET-5 Microscope Series by UNITRON (Figure 7). This microscope features an adjustable stage and is equipped with four objective lenses of 5x, 10x, 20x, and 50x magnification [35]. Images of the samples were captured both before and after mechanical loads were applied to visualize any structural changes

to the material. To maintain consistency in imaging and orientation, reference marks were placed at the base of each sample surface.

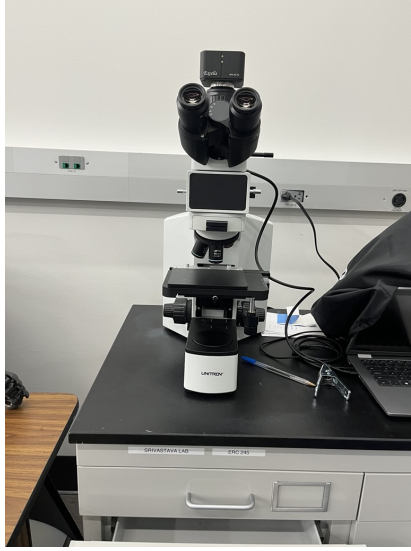


Figure 7: Optical Microscope used to capture images for analysis.

7 Results

7.1 Initial Soft Material Tissue Surrogates

This project aims to perform an experimental study on soft tissue surrogates of marine mammal melon tissue before and after various strains are applied and measure the data qualitatively and quantitatively. This was done by attempting to replicate the melon tissue with another soft material because melon tissue cannot be ethically sourced [36]. The main components found in the melon are triacylglycerols, wax esters, and other biological materials that are unique to Odontocetes [37] [38]. To attempt to mimic the mechanical properties of the melon tissue, different variations of the fatty components found in the melon were combined with gels to provide a matrix such as agarose, gelatin, and collagen.

Limitations were encountered in the approach to incorporate similar components to the native melon tissue. Due to the lack of literature on synthesizing an artificial adipose tissue, the

task proved challenging [39][40]. The efforts to create artificial melons with biological materials did not succeed because of the incompatibility of the hydrophobic lipid components and the hydrophilic matrix components. Combinations of collagen, gelatin, and agarose matrices with triacylglycerols and wax esters resulted in heterogeneous specimens with clear separation of phases. Although the samples solidified into a 3D mold as seen in Figure 8, they were not mechanically representative of a melon sample. [41]

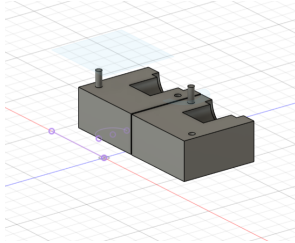


Figure 8: Attempt at synthesizing melon samples using gelatin, wax esters, and triacylglycerols

Since the initial aim of producing a more chemically similar, multi-material melon surrogate was unsuccessful, a different approach was taken using synthetic soft material. Ecoflex 0030 is a silicone-based soft polymer with mechanical properties similar to the marine mammal melon. With its validated formulation and past use in surgical training as a substitute for tissues, it was selected as a surrogate material for the melon in the mechanical tests [38] [40].

7.2 Synthesizing Materials

Different formulations of the Ecoflex 0030 samples were fabricated. The first formulation named as T1 comprises the recommended 1:1 ratio of the two part A and B mixture of Ecoflex 0030. The second formulation named T2, are a 1:3 ratio of one part A to three parts B. Each formulation was mixed until homogeneously dispersed and then was poured into a 12.5 mm x 10 mm 3D printed mold, as seen in Figure 9a. The samples were then placed in a vacuum chamber to degas the specimen and remove any air bubbles which may have been introduced during the mixing process. Samples were then placed into an oven set to 50°C and left to cure over 6 hours. When polymerized, samples were placed into secure storage shown in Figure 9b to prevent any particulates from settling on them and producing imaging anomalies.



(a) CAD model of the molds that were used to make samples



(b) A collection of samples in the container and petri dishes.

Figure 9: The CAD model of the mold used and the samples tested sectioned off for their specific test.

7.3 Speed of Sound

A summary of the measured speed of sound for each trial for the T1 samples can be found in Table 2 and a summary of the measured speed of sound for each trial for the T2 samples can be found in Table Table 3. A table comparing the average speed of sound for the T1 and T2 formulations under each condition can be found in Table 4. A one-way ANOVA with post-hoc Tukey HSD multiple comparisons test was used to compare each strain and strain rate loading condition applied to both T1 and T2 samples.

A graph comparing the speed of sound data for each of the strain and strain rate testing conditions for T1 samples is shown in Figure 10. The 25% strain condition proved to have no significant difference in comparison to the control samples regardless of the rate at which the loading was applied. The samples subjected to 75% strain had a significant change in the speed of sound through the material compared to the control. The samples impacted using the drop tower equipment also saw a significant increase in the speed of sound.

Figure 11 shows the graphical comparison of the speed of sound data for each of the strain and strain rate testing conditions for T2 samples. All conditions had significant different in the speed of sound compared to the control. Most drastically observed with the increase in the speed of sound following impact via drop tower.

Speed of Sound for T1 Samples			
Conditions	Trial 1	Trial 2	Trial 3
25% Strain at a Rate of $1 \times 10^{-3}/s$	1056 m/s	1071 m/s	1062 m/s
75% Strain at a Rate of $1 \times 10^{-3}/s$	1108 m/s	1083 m/s	1096 m/s
25% Strain at a Rate of 1/s	1071 m/s	1056 m/s	1062 m/s
75% Strain at a Rate of 1/s	1095 m/s	1090 m/s	1084 m/s
Drop Tower	1089 m/s	1057 m/s	1083 m/s
Control	1011 m/s	1050 m/s	1030 m/s

Table 2: Measure of the speed of sound of T1 samples after applying specified strain and strain rate conditions.

Speed of Sound for T2 Samples			
Conditions	Trial 1	Trial 2	Trial 3
25% Strain at a Rate of $1 \times 10^{-3}/s$	1046 m/s	1051 m/s	1049 m/s
75% Strain at a Rate of $1 \times 10^{-3}/s$	1090 m/s	1067 m/s	1089 m/s
25% Strain at a Rate of 1/s	1046 m/s	1059 m/s	1079 m/s
75% Strain at a Rate of 1/s	1113 m/s	1098 m/s	1075 m/s
Drop Tower	1080 m/s	1118 m/s	1179 m/s
Control	992 m/s	945 m/s	976 m/s

Table 3: Measure of the speed of sound of T2 samples after applying specified strain and strain rate conditions.

Average Speed of Sound		
Conditions	T1	T2
25% Strain at a Rate of $1 \times 10^{-3}/s$	1063 m/s	1048.67 m/s
75% Strain at a Rate of $1 \times 10^{-3}/s$	1095.67 m/s	1082 m/s
25% Strain at a Rate of 1/s	1063 m/s	1061.33 m/s
75% Strain at a Rate of 1/s	1089.67 m/s	1095.67 m/s
Drop Tower	1076.33 m/s	1125.67 m/s
Control	1030.33 m/s	971 m/s

Table 4: Compiled average speed of sound through both T1 and T2 formulated samples following applied conditions.

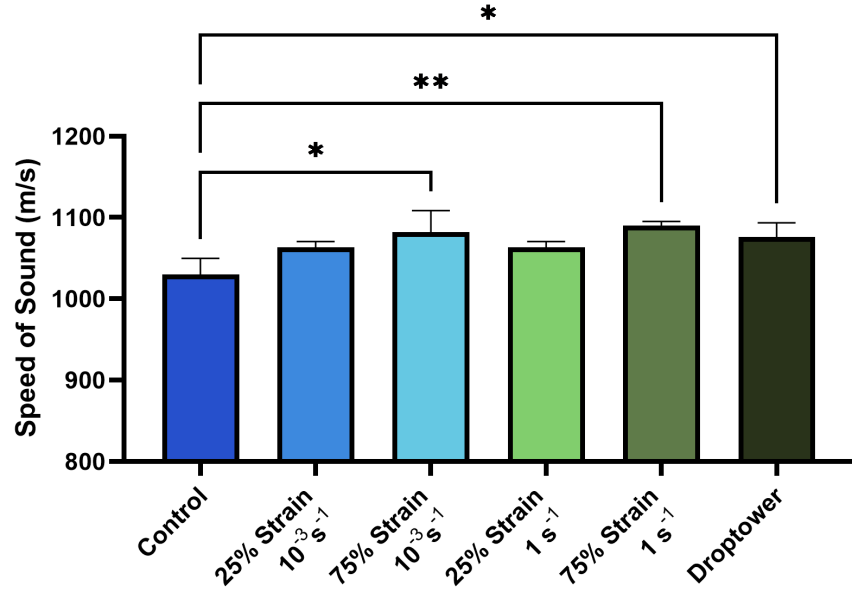


Figure 10: The speed of sound in the T1 samples. * $p<0.5$, ** $p<0.01$.

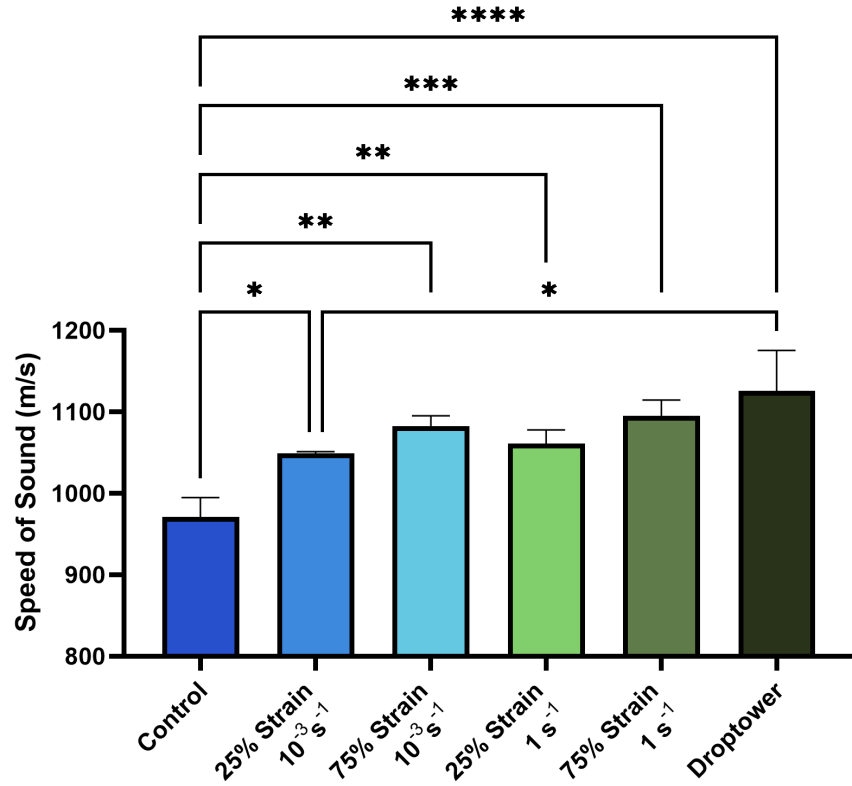
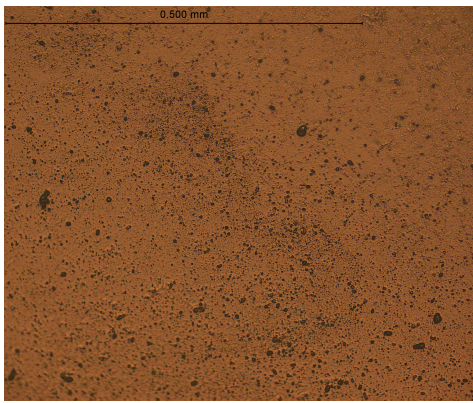


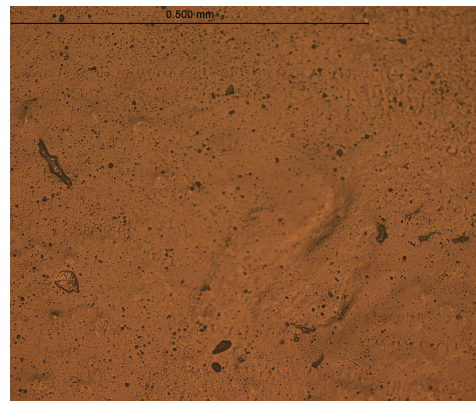
Figure 11: The speed of sound in the T1 samples. * $p<0.5$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

7.4 Microscopic Observations

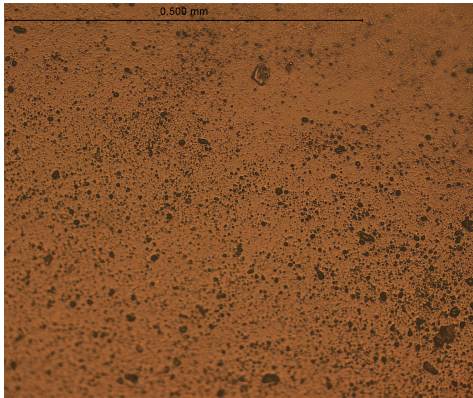
Samples were observed before and after testing as displayed in Figures 12-14, with most samples exhibiting some form of physical changes following the loading application. However, the overall physical damage observed in Ecoflex via the optical microscope was relatively minimal. Due to this and observing a minimal change in the speed of sound through the material after a load is applied, it would not be easy to make scientific conclusions regarding the type of damage each sample underwent, and further experimentation and research are required. This is especially surprising because the speed of sound was observed to increase when greater strain rates were applied to the specimen.



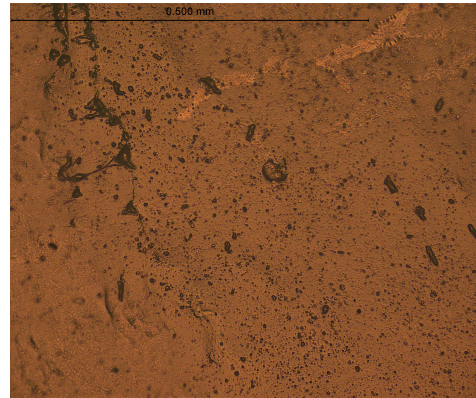
(a) T1 drop tower sample before a load is applied



(b) T1 drop tower sample after a load is applied

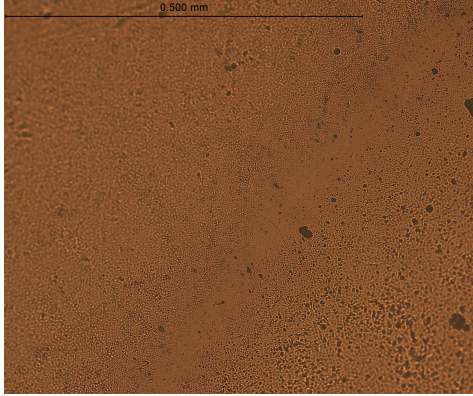


(c) T2 drop tower sample before a load is applied

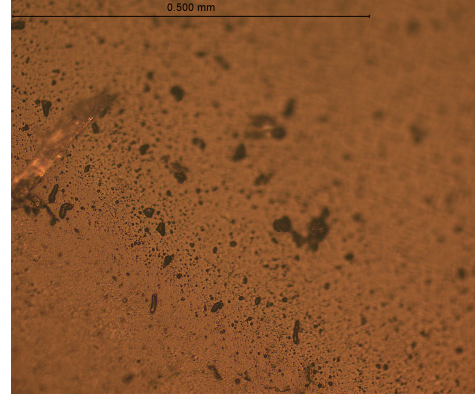


(d) T2 drop tower sample after a load is applied

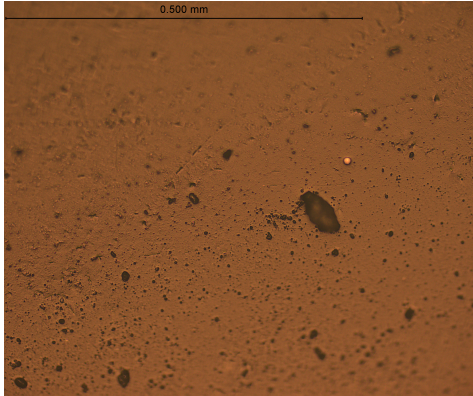
Figure 12: Comparison of T1 and T2 drop tower samples before and after a load is applied taken in the same location.



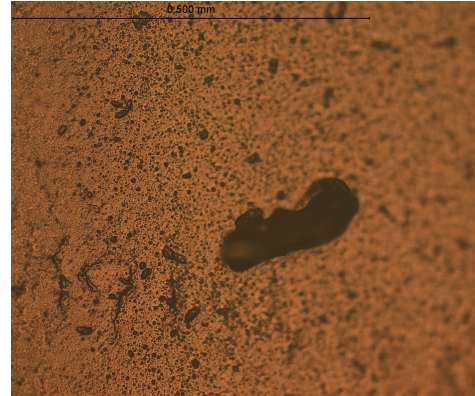
(a) T1 sample at a 25% strain sample and a strain rate of $10^{-3} /s^{-1}$ before a load is applied



(b) T1 sample at a 25% strain sample and a strain rate of $10^{-3} /s^{-1}$ after a load is applied

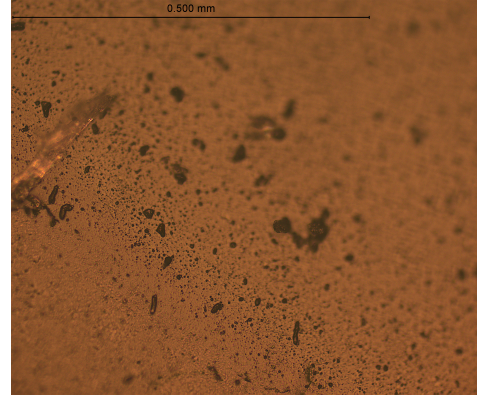
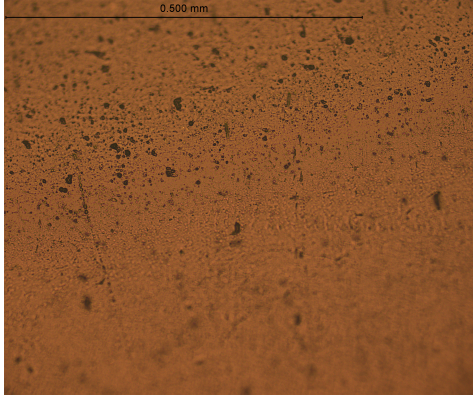


(c) T2 sample at a 25% strain sample and a strain rate of $10^{-3} /s^{-1}$ before a load is applied



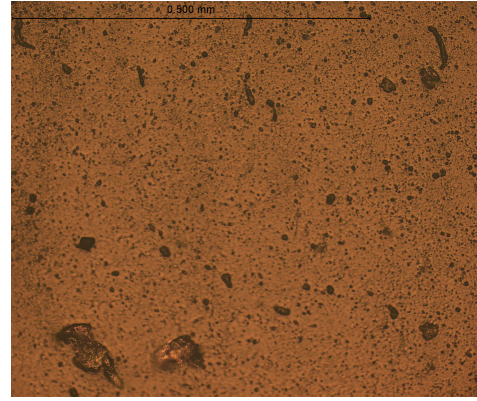
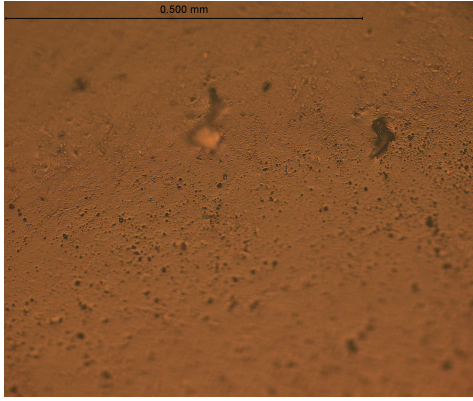
(d) T2 sample at a 25% strain sample and a strain rate of $10^{-3} /s^{-1}$ after a load is applied

Figure 13: Comparison of T1 and T2 at a strain of 25% and a strain rate of $10^{-3} /s^{-1}$ samples before and after a load is applied taken in the same location.



(a) T1 sample at a 75% strain sample and a strain rate of $10^{-3} /s^{-1}$ before a load is applied

(b) T1 sample at a 75% strain sample and a strain rate of $10^{-3} /s^{-1}$ after a load is applied



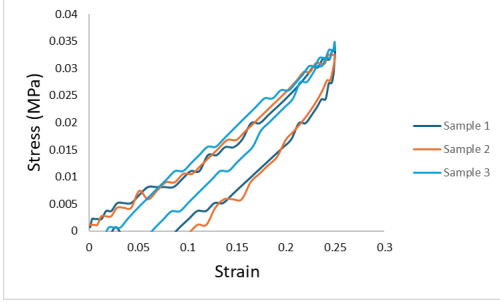
(c) T2 sample at a 75% strain sample and a strain rate of $10^{-3} /s^{-1}$ before a load is applied

(d) T2 sample at a 75% strain sample and a strain rate of $10^{-3} /s^{-1}$ after a load is applied

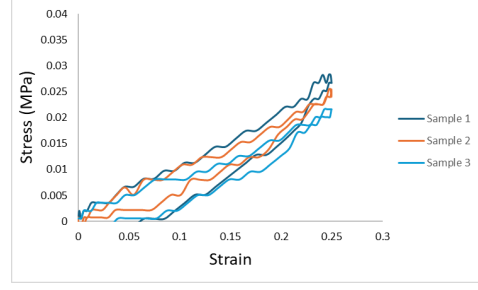
Figure 14: Comparison of T1 and T2 samples at a 75% strain sample and a strain rate of $10^{-3} /s^{-1}$ before and after a load is applied taken in the same location.

7.5 Stress and Strain

The stress-strain curves obtained from the UniVert mechanical testing device for the T1 and T2 samples can be seen in Figure 15 for 25% strain and Figure 16 for 75% strain. T1 had a noticeable higher maximum stress than T2 in both cases of each final strain. The slope of the linear region of the T1 sample is greater than the slope of the region for the T2 sample, signaling T1 formulation being stiffer than the T2 formulation.

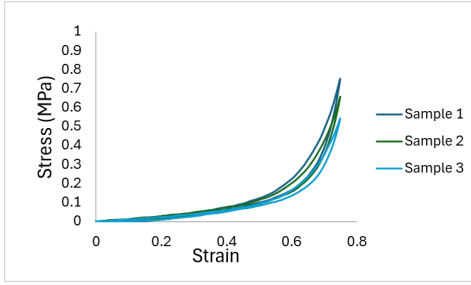


(a) A collective of T1 25% samples on a Strain-Strain plot

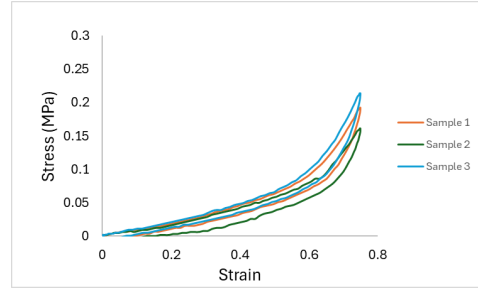


(b) A collective of T2 25% samples on a Strain-Strain plot

Figure 15: Comparison of the Stress-Strain plot of T1 and T2 samples at a strain of 25% at a strain rate of $1/s^{-1}$.



(a) A collective of T1 75% samples on a Strain-Strain plot



(b) A collective of T2 75% samples on a Strain-Strain plot

Figure 16: Comparison of the Stress-Strain plot of T1 and T2 samples at a strain of 75% at a strain rate of $1/s^{-1}$.

8 Discussion and Conclusion

In this project, we have done a preliminary assessment of a surrogate material to marine mammal melon tissue. The initial results show that the speed of sound in the material increased after a mechanical load was applied. The T2 samples of a lower stiffness undergo more qualitative changes than the T1 samples of a higher stiffness.

In a previous study performed using greater loads and higher rates, there was a decrease in the speed of sound for the Ecoflex and melon samples [6]. Considering our conflicting results with previous literature, a few factors that should be considered when comparing the two studies. The

first the rate of load application and maximum strain achieved in our study was lower. Next our specimen size was much smaller at roughly 12.5 mm x 10 mm compared to 1.5 in x 0.5 inches of the previous study. Their drop tower impactor (70 g - 170 g) has a significant amount more weight than the one used in this study (0.565 g), meaning the stress experienced by the samples in our trial was also less. Lastly, they performed a Digital Image Correlation (DIC) analysis in conjunction with the drop tower experiment. This is useful information to inform the conclusions of our study. Continued testing should be done at a wider range of strain rates because it is of interest to identify the cause of having an opposite trend in the speed of sound increasing with an increase of strain rate. The samples we tested with a lower stiffness (T2) undergo a relatively higher increase in the speed of sound upon deformation at low to moderate strain rates than the high stiffness (T1). Therefore, a potential hypothesize moving forward is that there may be voids in the Ecoflex 0030 samples before deformation that may be closing rather than being further damaged to cause the material to become more solid and allow for faster propagation of sound signals. This could be compatible with the findings from previous literature if a threshold strain or strain rate can be identified at which voids in the material transition from closing into fracture and producing larger voids.

The experiments were performed on a surrogate polymeric material, not native marine mammal melon tissue or even another fatty tissue type. Although the mechanical properties of the tissue surrogate are similar to the melon, the change in microstructure that may affect speed of sound transmission may be drastically different. Therefore, little biological conclusions can be made in using Ecoflex 0030 as a substitute for melon tissue. This is especially considering the differences observed in melon composition even between subspecies. However, the mechanical data obtained could still be relevant to how signal transmission can be altered in toothed whales following an applied load, such as an underwater shock wave.

Much future work needs explored to fabricate surrogate materials more representative of melon tissue. Therefore, more representative mechanical measurements and biological changes could be identified as a result of due to mechanical loading damage. The tests in this study were performed

at low to moderate strain rates, so future work could also expand to include test trials at a much greater strains and strain rates. This could be achieved using a Kolsky bar experimental technique to obtain high strain rate loads to better capture the extreme conditions of an underwater explosion. It would be groundbreaking to formulate a composition with melon components such as wax esters and triacylglycerols within a biological matrix. Overcoming the difference in phases of the materials will be the biggest challenge in achieving this, but an emulsifying agent could be a solution to enable mixing of the different ingredients. Ideally, an actual biological specimen to test and study will be provide the best scientific data for this problem, but it is currently not possible to obtain.

9 Appendix

9.1 Additional Drop Tower Information

Other important data are gravity (9.81) and the platform's mass (0.565 g). From here, one can find the acceleration mass (max. acceleration multiplied by mass) and the force. To find the Stress, one would divide the force by the area of the given cylinder.

9.2 Additional UniVert Information

Data is collected through a software called "CellScale," where one inputs the parameters for testing the material. In return, the parameters that will be measured are the force, displacement, and time. [42]

9.3 Additional Ecoflex Information

The advised mixing ratio is a 1:1 ratio of mixtures A and B. The solution cures at room temperature (73°F / 23°C) and no lower than 65°F / 18°C.[43] The cure time is roughly 4-6 hours. Once fully cured, the material will have a tensile strength of 1.38 MPa (200 psi). [44] [45]

9.4 Initial Analysis Protocol 1

- 1 Mix 1% by weight wax ester in 25, 20, 16, 15 and 14%wt of fatty acids. Mix well and heat at 90°C for 10 minutes. Mix them with collagen and let it cool until it becomes gel. Maintain pH by adding
- 2 NaOH solution to help gelation. Keep it undisturbed without shearing to help gelation.

9.5 Initial Analysis Protocol 2

Oil suspension for storage Artificial Melon Tissue Protocols (Gelatin based) Mixing wax esters with gelatin involves a few steps to ensure all components are incorporated well. The main three components will be wax esters, (medium chain) triacylglycerols, and gelatin. There are a few different substances that could be mixed with: Distilled Water (DI), Phosphate Buffered Saline (PBS), and Dimethyl Sulfoxide (DMSO).

DI

- 1 Heat the wax ester to 50-90°C(122-158°F) in a water bath or double boiler until a liquid- like consistency is achieved.
- 2 Add triacylglycerols to the water bath if needed.
- 3 Assure the ratio of gelatin to DI is 1 g: 10 mL
- 4 Heat DI to 37°C for 30 minutes
- 5 Add gelatin, increase heat to 40°C, and stir for an hour
- 6 While stirring, add triacylglycerols and wax ester to the gelatin mixture
- 7 Once the hour is finished, pour into mold
- 8 Repeat process with various concentrations of triacylglycerols and wax ester.

PBS

- 1 Heat the wax ester to 50-90°C(122-158°F) in a water bath or double boiler until a liquid- like consistency is achieved.

- 2 Add triacylglycerols to the water bath if needed.
- 3 Make sure the solution ratio of gelatin to PBS is 1:1.
- 4 Heat and stir at 37°C.
- 5 Add triacylglycerols and wax esters to the PBS: gelatin mixture.
- 6 Once the hour is finished, pour into mold
- 7 Repeat the process with various triacylglycerols and wax ester concentrations.

DMSO

- 1 Heat the wax ester to 50-90°C(122-158°F) in a water bath or double boiler until a liquid-like consistency is achieved.
- 2 Add triacylglycerols to the water bath if needed.
- 3 Heat 8 mL of DMSO at 37 °C
- 4 Add 100 mg of gelatin to the solution and stir for 3 hours
- 5 At the halfway mark, add the wax esters and triacylglycerols
- 6 Continue to heat and stir for the remainder of the time
- 7 Once finished, pour into molds.
- 8 Repeat the process with various concentrations of triacylglycerols and wax ester.

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