Raman Spectroscopy of the Skeleton of the Coral Acropora Cervicornis

Zachary C. Shepard
University of Central Florida

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RAMAN SPECTROSCOPY OF THE SKELETON OF THE CORAL ACROPORA CERVICORNIS

by

ZACHARY SHEPARD

A thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science in the Department of Mechanical Engineering in the College of Engineering and Computer Science at the University of Central Florida Orlando, Florida

Fall Term
2017

Thesis Chair: Dr. Nina Orlovskaya, Ph.D.
ABSTRACT

Coral reefs are an important element of marine ecosystem that are critical to maintain a healthy environment. Unfortunately, in recent years coral reefs are doing poorly and many in parts of the ocean are simply dying. Therefore, study of coral’s structural response to external loads could answer what will happen with their structures, while they exhibit different types of loading. Therefore, the proposition of using in-situ micro-Raman spectroscopy to study skeletons of *Acropora cervicornis* was used. Coral skeleton samples I subjected to mechanical loading studied their vibrational properties by exciting the material with 532nm visible light. A uniaxial compressive load I applied using a MTS universal testing machine and then using the Raman Spectroscopy to study the vibrational response of coral skeletons. Indentations used Vickers Hardness tester and performed 2D mapping of the coral structure around the indentation. If it’s expected that as a result of the proposed research the better understanding of structural stability of the Acropora Cervicornis coral skeletons will be achieved.
ACKNOWLEDGMENTS

I acknowledge and express my appreciation to the people who contributed to this research. I thank my thesis chair and mentor Dr. Nina Orlovskaya for her support and constant guidance throughout this research. Thank you to Nova Southeastern University for providing the corals and Dr. John Fauth for entrusting our research group with the experiments. I also thank Dr. Ghatu Subhash at the University of Florida for providing us access to his lab and indentation machine. Thank you to Dr. Romain Guame, for allowing me to use his diamond saw and for sparking my interest and understanding of materials during his Structures and Properties of Materials class. Special thanks to Alejandro Carrasco-Pena, mentor and now friend, for patience, guidance and expert knowledge of the micro-Raman spectrometer Renishaw Wire helped me tremendously throughout this research and was the key to success.

This research was supported in part by MRI NSF project 133775 Development of a Multi-Scale Thermal-Mechanical-Spectroscopic System for in-Situ Materials Characterization, Research and Training.
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CHAPTER 1.1.1: INTRODUCTION

Considered the rainforests of the sea, coral reefs play are important to the organisms that inhabit the ocean and also to humans [1]. Coral reefs provide a nursery for about 25% of all oceanic life protect the coastlines from waves and tropical storms that can cause the coastlines to erode [2]. Also, have been and continue to be, a contribution to medicine. Corals have been used in treatments for asthma, arthritis, cancer, heart disease, and more [3]. They also are natural filters, purifying water and using some of the carbon dioxide and calcium to produce calcium carbonate skeletons.

In recent years, coral reefs declined worldwide due to the increasing levels of carbon dioxide, which causes oceans to become more acidic. [4]. In addition, when water becomes too warm corals eject their symbiotic algae, which causes the coral to become white like it has been bleached. If corals stay bleached for too long they could also die. This is why the study of corals structural response to the external loads can answer what happens to coral skeletons under different environmental stressors and different types of loading.

I tested the endangered coral *Acropora cervicornis*, which is found mainly in shallow waters when its branches are susceptible to being broken. When a piece of the coral breaks off, it can reattach to the ocean floor and start growing again, which is a form of asexual reproduction. [5]

To better understand *Acropora cervicornis* structure, I used Raman spectroscopy with a 532 nm visible light that will excite the samples. Raman spectroscopy is becoming more widely used because it is inexpensive and non-destructive. The laser sends a constant wavelength to the
sample, which excites the material and emits a wavelength back with certain vibrations characteristic of each molecule. The received wavelength shows peaks at certain Raman shifts. This shows that the coral is made up of materials that are Raman active and these different peaks are the fingerprint of these materials.

**Chapter 1.2.1: Raman Spectroscopy**

Sir Chandrasekhr Venkata Raman was the main contributor of developing Raman spectroscopy, which was invented in 1928. Its purpose was to use scattered light to deduce as much as possible about a material’s chemistry and structure. As light passes through a sample it emits off a unique vibration specific to the material being studied; the light then returns with a slightly different color due to its change in energy. When an electromagnetic wave encounters a molecule or passes through a lattice, scattering occurs if Bohr’s Frequency Condition is satisfied.

\[
\Delta E = h \nu = h c / \lambda = h c \bar{\nu},
\]

When \( \Delta E \) the difference in energy between two quantized states, \( h \) is is Planck’s constant \((6.62 \times 10^{-27} \text{erg s})\) and \( c \) is the velocity of light\((3 \times 10^{10} \text{cm/s})\). Third parameter common to vibrational spectroscopy is the “wavenumber” \( \bar{\nu} \), where

\[
\bar{\nu} = \frac{\nu}{c}.
\]

By combining the frequency equation and wavenumber equation we obtain

\[
\bar{\nu} = \frac{\nu}{c} = \frac{1}{\lambda} \quad (cm^{-1})
\]
Figure 1: Energy units for various portions of electromagnetic spectrum. [6]

Figure 1 shows the different spectra and their transitions expressed in units of were number, wavelength, and frequency. Here we only are concerned with vibrational transitions found in the Infrared and Raman spectra. Even though the other spectroscopies have different origins, we are only going to focus on the Raman and Infrared spectra. Figure 1, we can see the transition between spectra’s is on the $10^4 \sim 10^2 \text{ cm}^{-1}$ region, which is frequency range of the molecules vibrating nuclei. [6]
Figure 2 shows the difference between vibrational and rotational transitions in a diatomic molecule. Even though rotational transitions are seen in gaseous states it is good to see the difference. [6]
In Rayleigh scattering, almost all photons are elastically scattered when light encounters a molecule, about 99.999%. In Raman scattering there is a 0.001%, which is a small portion that will scatter in elastically. This inelastic scattered light undergoes a shift in energy. [6]

The Raman Effect is complementary to infrared spectroscopy. Vibrational transitions can be observed in either infrared or Raman spectra. In Raman, if the bond is covalent vibrations are strong; whereas infrared vibrations are strong if bonds are ionic. [6]

In Raman spectroscopy the vibrational frequency is measured as a shift, either plus or minus, from the initial beam frequency, anti-stokes and stokes. Anti-Stoke lines are equal to the initial beam frequency plus the vibrational frequency, and Stokes is equal to the initial beam frequency minus the vibrational frequency.
frequency minus the vibrational frequency. For a material to be Raman active, the rate of change of polar ability must not equal zero. Also, if the polarizability tensor changes during vibration, the vibration is Raman active. [6]

Unlike diatomic molecules that only have one nuclei, polyatomic molecules have more and each nucleus makes its own harmonic oscillations. These vibrations can be expressed as some number of independent “normal vibrations.” [6]

Figure 4: Atomic motions in normal modes of vibrations in CO$_2$ [6]

Figure 4, represents normal vibrations of CO$_2$, with the circles representing C and O atoms (black circles). The C-O bonds move in three different ways. Figure 4a the C-O bonds stretch which is the symmetric (in-phase) stretching vibration Another normal vibration occurs when one C-O bond stretches and the other shrinks, which is the antisymmetric (out-of-phase)
stretching vibration (Figure 4b). Bending vibrations occurs when atoms move in perpendicular directions and then release simultaneously (Figure 4c). Each vibration’s frequency is different: $\nu_1$ is $1,340 \text{ cm}^{-1}$, $\nu_2$ is $667 \text{ cm}^{-1}$, and $\nu_3$ is $2,350 \text{ cm}^{-1}$. Infrared and Raman instruments observe these normal vibrations. [6]

**Chapter 1.2.2: Raman Spectroscopy of Coral Samples**

In this article [7], Raman Spectroscopy demonstrated the nature of the pigments, bio-mineralized structure and the presence of artificial dyes in corals and pearls from different locations, such as *Hyriopsis cuminigi, Hyriopsis sclegelii, Pinctada margeritifera* and several other pearls. Some of the corals used were *Favia pallida, Pocillopora damicornis, Stylophora pistillata, corallium rubrum*, and more. Raman spectra were recored using Jobin Yvon LabRam microspectrometer (Horiba, Parma, Italy) at the University of Parma (Physics and biology department). It covered a spectral range of $100-6000 \text{ cm}^{-1}$. The micro spectrometer is equipped with notch filter and a thermoelectrically cooled CCD array detector. The excitation was at 473.1 nm from a solid-state laser and at 632.8 nm. Resonance Raman spectra with 473.1nm excitation line are helpful to revealed small quantities of pigments involved in the coral or pearl coloration and were important tests for vibrational properties of the polyenic/carotenoid molecules. [7]
Figure 5: Raman Spectrum of *Corallium rubrum* of different origins and colors. At 473.1 nm and 632.8 nm. [7]

Figure 5 shows the spectra of museum sample corals and other corals with slight chromatic changes from the Mediterranean. Features are mainly viewable at an excitation of 473.1 nm and not at 632.8 nm (as seen as the last line of Figure 1). Red shifts occurred in the v2 frequency and blue shifts in v4 and v1. The strongest shifts occurred in red and pink corals at about 1130-1160 cm⁻¹ and 1515-1530 cm⁻¹.

Red and pink corals were assigned to C-C (v1) and C=C (v1) stretching modes of the polyenic chain. The region of 1000-1020 cm⁻¹ (v3) was assigned to the CH=CH wagging mode in polyene molecules, and the region of 1270-1300 cm⁻¹ (v4) was due to the CH=CH bond in the plane rocking motion of the olefin hydrogen. [7]
Figure 6 compares Raman Spectra of the corals *Stylaster* and *Corallium rubrum*. Features of aragonite (a mineral, calcium carbonate) are present but pigment were different in the two species. Mainly at frequencies distinctive of carotenoids. [7]

Figure 7: Raman Spectrum at 632.8 nm of different “white” aragonite corals. [7]
The spectrum of *Pocillopora damicornis* (Figure 7), shows peaks assigned to celestine, lazurite (L), amorphous carbon (C), and aragonite (A). [7]

**Chapter 1.2.3: Raman vs. XRF Spectroscopy**

Two specific methods that analyze several different coral samples were used. Raman spectroscopy and micro-X-ray fluorescence (micro-XRF) are both non-destructive to the corals. This research explains how Raman testing alone is not enough in distinguishing between different corals. [8]

![Raman Spectra](image)

*Figure 8: a.) Raman Spectra of red corals taking from Mediterranean area (blue line) b.) Raman Spectra of pink corals (green, red, and black lines) and red coral taking from the Pacific area. [8]*

In Figure 8, you can see the very distinctive peaks of the red and pink corals, from both the Mediterranean (Figure 8A) and Pacific areas (Figure 8B). In Figures 8A and 8B, one can tell that the red and pink corals are very similar to each other even though they are from different areas. [8]
Chapter 1.2.4: Biological Control of Aragonite Formation in Stony Corals

With the changing of the oceans and corals becoming scarce in areas around the world; the understanding of what makes up corals and how to preserve or possibly engineer an artificial coral is becoming important. In this article they used a few material science techniques to study the coral, with Raman Spectroscopy being one of those techniques. They hope to understand the processes in which these corals deposit their calcium carbonate skeleton in the form of aragonite. Some experts believe that the increase in pH and the concentrations of calcium and dissolved inorganic carbon species at the calcification site generates metastable conditions suitable for the nucleation of the mineral phase. [10]. Another hypothesis presented was, a template-induced nucleation of the mineral phase mediated by the skeletal organic matrix (SOM) and, in particular, the acid-rich proteins. [10].

The type of coral used in this article was the Indo-Pacific coral Stylophora pistillata. Techniques used were Raman imagining and spectroscopy, Scanning Helium Ion Microscopy (SHIM), and Solid-State Nuclear Magnetic resonance (NMR). These will help show crystallization in the corals so they can understand the relationship between the mineral phase and the skeletal organic matrix.

After they took images of the skeleton of the coral using an electron microscope they found corallites that were on the walls of the pores of the coral. These areas found in the coral are called centers of calcification because these areas seem to be enhanced with organic molecules. And these images support the hypothesis that the skeletal organic matrix is important for the starting of the mineral phase. [9]
Figure 9: Morphological skeletal features common to all stony corals. (A) Combination of SEM images showing the intact surface of a skeletal branch. (B and C) PLM micrographs of a single corallite and a trabecula. (D) EBSD inverse pole figure orientation map of the trabecula [9]
CHAPTER 2.1.1: GOALS

The goal in studying *Acropora cervicornis* under Raman spectroscopy is to understand how the coral reacts to cyclic loading to simulate the ocean currents that they undergo. To evaluate the strength of the corals, figure out ways to grow corals using different propagation methods, so compression tests and Raman spectra tests were performed to understand these coral samples.
CHAPTER 3.1.1: METHODOLOGY

The proposed research will be conducted at the University of Central Florida. Dried *Acropora cervicornis* is the coral being tested in this study. The corals were prepared in two different ways: bleaching with sodium hypochlorite (‘chlorox”) and burying corals in situ to let microorganisms clean them. I labeled the bleached coral samples with “B” and corals clened by micr-organisms in ocean sediment “S”. After the coral samples were bleached or sanded, they then were cut with a diamond saw blade. The length that they were cut at was twice their diameter. A series of tests were applied to the coral samples, one of which is a compression test that was performed axially. A total of 15 samples were prepared, of the 15 coral samples 8 are bleached and 7 were sanded. The 8 bleached were split into groups of four due to the fact that they were cut from different branches of the coral. Three other samples of corals were prepared for hardness testing, two that are bleached and one that will be sanded. The three samples were then hand polished, before the indentations and Raman has been done, as best as possible for Raman Spectral Analysis. A base point of Raman mapping was taking, and then using a nano-indentator at the University of Florida to test the hardness of the coral samples. After the test another Raman mapping was taken then a comparison of the results of the two Raman mappings were taken to see how the coral behaves when stress is applied.

A micro-Raman spectrometer Renishaw Wire was used to study the vibrational spectra of Dried *Acropora cervicornis* coral skeleton. The Raman microscope system contains a laser to excite the sample, which is 514.5 nm line of $Ar^+$ ion laser. This system also contains a spectrograph fitted with holographic notch filters, and an optical microscope. The maximum power generated by this laser is 25 mW. 50% of power was used to test the coral samples,
except for when Raman mapping was taken place at 100% power. The collection time was 10 seconds exposure for each point on the sample. The magnification used when taken the Raman spectrum was with a 100x lens. The focused Raman shift was from 100-2000cm$^{-1}$.

These parameters were used for the initial Raman spectra of the corals and also for after the indentations and compressions were performed.

Figure 10: A photo of Acropora cervicornis 1Bleached
Figure 11: A photo of *Acropora cervicornis* 2Bleached

Figure 12: A photo of *Acropora cervicornis* 3Sanded
Chapter 3.2.1: Initial Raman Spectra’s of Coral Samples

Once the corals were cut in nice uniform cylindrical pieces one coral piece was taken from each group, one from 1Bleached, one from 2Bleached, and one from 3Sanded. They were then polished by hand. Once they were sanded to a mirror finish with ends that were parallel to the surface, the dimensions were recorded and ready to be analyzed by Raman Spectroscopy. See Table 1.

Table 1: Initial Coral Samples

<table>
<thead>
<tr>
<th>Coral</th>
<th>Height (mm)</th>
<th>Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B – 1Bleached</td>
<td>22.28</td>
<td>13.30</td>
</tr>
<tr>
<td>2B – 2Bleached</td>
<td>22.36</td>
<td>12.64</td>
</tr>
<tr>
<td>3A – 3Sanded</td>
<td>13.73</td>
<td>8.56</td>
</tr>
</tbody>
</table>

Three different points of Raman were taken on each coral sample. As you can see from the Figures below the Raman shift does not have significant change between the three coral samples. From some additional research, comparing the Raman shifts from the figures below (Figures 14-22) to Raman Shifts from [10], shows that the found that the chemical composition is close if not almost identical to calcium carbonate ($CaCO_3$).

![Figure 13: Normal Raman Spectra of Calcium Carbonate (calcite, chalk). [10]](image-url)
Figure 14: First point of Raman taken on coral 1Bleached

Figure 15: Second point of Raman taken on coral 1Bleached

Figure 16: Third point of Raman taken on coral 1Bleached
Figure 17: First point of Raman taken on coral 2Bleached

Figure 18: Second point of Raman taken on coral 2Bleached

Figure 19: Third point of Raman taken on coral 2Bleached
Figure 20: First point of Raman taken on coral Sanded

Figure 21: Second point of Raman taken on coral Sanded

Figure 22: Third point of Raman taken on coral Sanded
Chapter 3.2.2: Raman Spectra’s of Coral Samples after Indentations

Next part of this research was to determine the corals hardness at different ten locations on each sample of coral and calculate an average. The indentation machine used was Wilson Instruments Tukon 2100 B, located at the University of Florida in Dr. Ghatu Subhash lab.

Figure 23: Wilson Instruments Tukon 2100 B (Close up, configured to take indentation)
The Tukon 2100B indentation machine as seen above was used to make our 10 indentations on the coral samples (1Bleached, 2Bleached, and 3Sanded). First, the coral samples circumference were wrapped with scotch tape, leaving the tops and bottoms untouched, and then marked on one side of the scotch tape with an arrow. This made it easier to find the general location of the indentations taken. The indentation machine used a force of 50 grams. I used the Vickers hardness equation to determine the hardness.

\[
HV = \frac{2F \sin \frac{\theta}{2}}{d^2} = 1.854 \frac{F}{d^2}
\]
Where, $F =$ load (kgf), $d =$ mean diagonal of the indentation (mm), $\theta =$ angle between opposite faces of a diamond = 136°.

Table 2: Bleached Indentations

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
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<tr>
<td></td>
<td>$d_1$ (µm)</td>
<td>$d_2$ (µm)</td>
<td>kgf</td>
<td>$d_1$ (µm)</td>
<td>$d_2$ (µm)</td>
<td>kgf</td>
<td>$d_1$ (µm)</td>
<td>$d_2$ (µm)</td>
<td>kgf</td>
<td>$d_1$ (µm)</td>
</tr>
<tr>
<td>1</td>
<td>16.21</td>
<td>16.68</td>
<td>50</td>
<td>17.01</td>
<td>16.41</td>
<td>50</td>
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<td>16.7</td>
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<tr>
<td></td>
<td>Hardness (Hv)</td>
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<td></td>
<td>Hardness (Hv)</td>
<td>331.99</td>
<td></td>
<td>Hardness (Hv)</td>
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<td>50</td>
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<td></td>
<td>Hardness (GPa)</td>
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<td></td>
<td>Hardness (GPa)</td>
<td>3.26</td>
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<td>Hardness (GPa)</td>
<td>3.58</td>
<td></td>
<td>Hardness (GPa)</td>
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<td>15.83</td>
<td>16.18</td>
<td>0.5</td>
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<td>50</td>
<td>16.04</td>
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<tr>
<td></td>
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<td>Hardness (Hv)</td>
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<td>Hardness (Hv)</td>
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<td>Hardness (GPa)</td>
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<tr>
<td>d1(µm)</td>
<td>d2(µm)</td>
<td>kgf</td>
<td>d1(µm)</td>
<td>d2(µm)</td>
<td>kgf</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>17.2</td>
<td>17.37</td>
<td>50</td>
<td>17.1</td>
<td>17.58</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hardness (Hv)</td>
<td>310.27</td>
<td>308.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hardness (GPa)</td>
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<td>3.02</td>
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<td>3</td>
<td>4</td>
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<td></td>
<td></td>
</tr>
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Table 5: Mean and Standard Deviation of Coral Hardness

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Figure 25: Mean Hardness of Coral Samples ± 1 Standard Deviation.
Table 6: Curve fit data of 1Bleached.c After Indentation

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<td>71892.4</td>
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<td>Middle of diagonal</td>
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<td>5.87763</td>
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<td>Over Crack</td>
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<td>5.42625</td>
<td>1081.94</td>
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The lowest intensity should be over the crack because there would not be any material for the laser to reflect off of. With the data collected in Table 6 showing the Full Width at Half Maximum (FWHM), to see if strain has occurred compared to the FWHM away from indentation. The points where they experienced the most compression should have the higher FWHM, which we can confirm with Table 6 at Center and middle of indentation. The Raman spectrums with broader peaks have more strain due to compressive stress and less intensity.

Figure 26: Away from Indentation

Figure 27: Center of Indentation

Here is a rough representations of the Raman shift taken from sample 1Bleached of its highest peak (1082 cm\(^{-1}\)). Increase in compression, increase in strain.
Figure 29: Middle of Diagonal

Figure 30: Over Crack

Figure 31: Laser spot over the crack
Figure 32: Raman Mapping over crack

Figure 33: Close up of Raman Mapping over crack

Figure 33 shows the Raman mapping based on the intensity of the peak at 1082cm⁻¹. This is a very coarse plot but still able to detect cracks. Mappings help detect surface and sub-surfaces defects.
Chapter 3.2.2: Raman Spectra of Coral Samples after Compression

The coral samples that undergone compression, were subjected to an increasing load until the samples broke. The dynamic loading machine used was the MTS Criterion Model 43. After a few seconds of compression, we stopped compression at 1000 N focused the lens then took Raman spectrum of the coral. The loading was stopped at this load because this was the point right before the first cracked occurred in the coral, was about 1200 N. Once the Raman was done, the compression test was continued.

Figure 34: Coral under compression by the MTS Criterion Model 43
Table 7: 1Bleached Dimensions

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<td>1B.b UF (Not used)</td>
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<td>1B.d</td>
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Figure 35: Profile and Cross-sectional pictures of 1Bleached samples
Table 8: 2Bleached Dimensions

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Figure 36: Profile and Cross-sectional pictures of 2Bleached samples
### Table 9: 3Sanded Dimensions

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**Figure 37:** Profile and Cross-sectional pictures of 3Sanded samples
Figure 38: Profile and Cross-sectional pictures of 3Sanded samples
Figure 39: Raman taken during Compression of 1Bleached.c

Figure 40: Raman taken during Compression of 2Bleached.c
Figure 41: Raman taken during Compression of 2Bleached.d

Figure 42: Raman taken during Compression of 3Sanded.b
Figure 43: Raman taken during Compression of 3Sanded.c

Figure 44: Raman taken during Compression of 3Sanded.d
Figure 45: Raman taken during Compression of 3Sanded.e
CONCLUSION

The skeleton *Acropora cervicornis* coral is a Raman active material that can be studied by Raman spectroscopy. Four bands at around 151, 203, 702, and 1083 cm$^{-1}$ were generally observed at 50% of laser power, with the exception of the Raman mapping of the indentation of the one of the samples that produced a crack which was at 100% power of the laser. All coral samples had an exposure time of the laser of 10 seconds. After all the Raman spectrums have been taking after the indentation and compression tests and focusing on the dominant peak at 1082 cm$^{-1}$ I found that the indentation and compressions caused deformation in the coral samples which caused strain in the material. This strain was detected from the Raman shift at the dominate peak. To back our assumptions that strain has occurred I performed a curve fit of that peak and found that the intensity of the peak shrunk and the width increased, which is an indicator that strain did occur. The Raman spectra helped us determine the composition of the coral because we were not sure what it was. The collected spectra from skeletons of *Acropora Cervicornis* closely resembled the spectrum of calcium carbonate (CaCO$_3$).
APPENDIX
Dear Dr. Orlovskaya

This letter confirms temporary transfer of 30 skeletons of the stony coral *Acropora cervicornis* from my possession to yours. *Acropora cervicornis* is listed as threatened under the Endangered Species Act. These specimens came from our (Nova Southeastern University) permitted offshore *A. cervicornis* nursery. Per 50 CFR 223.208, transport of legally-obtained specimens is permitted as long as it is not in the pursuit of commerce. The purpose of this temporary transfer is education and research.

A copy of this letter should remain with the specimens at all time. If any questions please contact me at the information provided below.

Sincerely,

David Gilliam, PhD  
Nova Southeastern University  
Halmos College of Natural Sciences and Oceanography  
8000 North Ocean Drive; Dania Beach FL 33004  
Office: 954 262-3634  
gilliam@nova.edu
REFERENCES


