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A Microcantilever Sensor Array for the Detection and Inventory of Desert Tortoises

T. L. Porter, T. R. Dillingham
Northern Arizona University, Dept. of Physics and Astronomy, Flagstaff, AZ 86011

R. J. Venedam
National Security Technologies, LLC, Las Vegas, NV 89183

Abstract

We have designed and tested of a portable instrument consisting of a small infrared camera coupled with an array of piezoresistive microcantilever sensors that is used to provide real-time, non-invasive data on desert tortoise den occupancy. The piezoresistive microcantilever (PMC) sensors are used to obtain a chemical “signature” of tortoise presence from the air deep within the dens, and provide data in cases where the camera cannot extend deep enough into the den to provide visual evidence of tortoise presence. The infrared camera was used to verify the PMC data during testing, and in many cases, such as shallower dens, may be used to provide exact numbers on den populations.

Introduction

The desert tortoise (*Gopherus agassizii*) occurs in North America primarily in the southwest Mohave and Sonoran desert regions, from western Arizona to southeast California, southern Nevada, and southwestern Utah. These tortoises may be found living in a variety of different terrains; the primary factor for a given area is its suitability for the tortoises to find food and construct dens in which to live. Desert tortoises feed on grasses, wildflowers, cactus fruit, or other edible native plants, and also derive most of their water intake from these plants.

As early as 60 years ago, desert tortoise populations in many areas reached densities as high as several hundred per square mile. However, systematic declines in the populations eventually led to the desert tortoise being listed as a threatened species in 1990.[1] Today, areas that once housed large tortoise densities may now contain less than 50 tortoises per square mile. Contributing factors for this decline include drought, destruction of habitat, predation, and recently identified upper respiratory tract disease (URTD)[2,3]. The desert tortoise URTD is a chronic disease, caused by a mycoplasma organism attached to respiratory surfaces, that eventually leads to severe occlusion of the tortoise nares and subsequent destruction of the respiratory epithelium[4,5].

Despite the near consensus opinion regarding the systematic decline in the desert tortoise population, obtaining accurate inventories of desert tortoises in the wild remains difficult. Depending on the nature of the terrain in which they live, desert tortoise dens may extend into the ground a distance of several feet to over 15 feet. Because these creatures spend approximately 95 percent of the time within their dens, and because of the difficulty (or impossibility) of peering into the dens unaided, obtaining occupancy data from an area survey of numbers of tortoise dens is highly inaccurate. One current method of obtaining tortoise population data is to simply look for “tortoise sign” in and around the entrance to a tortoise den, hoping to observe recent tortoise tracks, fresh soil movement, or other indications that a particular den is actually occupied by one or more tortoises.

In this paper, we describe the design and testing of a portable instrument consisting of a small infrared (IR) camera coupled with a two or three element array of piezoresistive microcantilever (PMC) sensors[6,7] used to provide real-time, non-invasive data on desert tortoise den occupancy. The PMC sensors are used to obtain a chemical “signature” of tortoise presence from the air deep within the dens. The IR camera was used to verify the PMC data during testing, and in many cases, such as shallower dens, may be used to provide exact numbers on den populations.

Experimental

PMC sensors provide a simple, low-cost, and robust platform for the detection of a variety of analytes. In the basic PMC sensor, a PMC is embedded or partially embedded into a sensing material. The sensing material is usually a polymer, composite polymer, or bio-composite material that when functionalized acts as a probe for the desired analyte[8]. Upon analyte exposure, reactions with the sensor probe material result in a volumetric change in the sensing material, which is recorded as a resistance change within the PMC. The volume change in the sensing material may be due to diffusion of the analyte species into the sensing material, probe-target binding on the material surface or bulk, or surface or bulk chemical reactions between the analyte and sensing material. We have previously used PMC sensors in a variety of sensing applications. These include the sensing of volatile organic compounds (VOCs) [6, 7, 9], personal hydration monitoring[10], carbon monoxide gas[11], single-strand DNA detection[12], protein detection[13], and viral detection[14].

PMCs used in these experiments were designed by Cantimer, Inc., Menlo Park, CA. The PMCs are approximately 200 microns in length and 40 microns wide. The cantilevers extend into a small circular area on each die to contain the sensing material and also to protect the cantilever during handling. Each cantilever die also contains an integrated thermistor to be used for temperature correction in applications where it is needed. Figure 1 (top) shows a photograph of a single cantilever die. For sensor assembly, a measured amount of sensing material is laid onto the edge of a Si substrate. The liquid material size and volume are controlled by a precision dispensing unit. While the polymer is still wet, the microcantilever tip is positioned to contact the polymer with a micromanipulator. The substrate is then bonded to the chip using epoxy. A graphical representation of the sensor assembly process (circular die area is omitted for clarity) is shown in Figure 1 (bottom).

Electronics for PMC sensors are simple, as only the cantilever resistance is measured during a sensing event. For this project, we use a single-chip AD7793 24-bit A-D converter which functions as a 6½ digit multimeter to directly measure the cantilever resistance. For the tortoise probe, the PMC sensors were assembled into a small aluminum case, with the AD7793 chip and a USBMicro SPI to USB converter chip (USBMicro USB421). The output of the SPI to USB converter chip was fed into one port of a small, two-port USB hub. A small, USB IR camera (TopGear model WC725) was modified to fit in our Al enclosure and plugged into the second port of the USB hub. We also equipped the sensor assembly with four IR emitting light-emitting diodes for IR illumination inside the tortoise dens if needed. Figure 2 shows the components of the tortoise probe assembly.

In the tortoise sensor probe, both the IR camera and the PMC sensors share a single USB hub. Thus, the interface to a laptop computer for both the PMC sensors and the IR camera may be through a single USB cable. We used a 15-foot long USB cable, threaded through the center of a 12-foot 3/8-inch drain auger to provide the laptop computer interface and a means of threading the sensor probe down through the entrance of a tortoise den. The entire assembly is shown in Figure 3 (top), while a close-up photo of the probe itself is in Figure 3 (bottom). For these experiments, we plugged the probe into a laptop computer. The laptop was equipped with LabView software running our own sensor interface program[15].

Results and Discussion

We chose to use three different organic polymers in the PMC sensor array. The polymers were poly (vinyl alcohol) (PVA), poly (epichlorohydrin) (PECH), and cross-linked poly (vinyl alcohol (PVAc). These polymers exhibit different solubility parameters (PVA=21, PECH=19.7 $MPa^{1/2}$) or different physical parameters (PVAc) and thus should help in producing an appropriate “signature” for determining the presence or absence of tortoises in their dens. The PVAc is a proprietary polymer from Bay Materials (Menlo Park, CA) that has previously been tested as a sensing material for hydration monitoring in humans[10]. Figure 4 shows data taken from a tortoise den occupied by a single, medium sized (8-inch shell) male tortoise versus data taken by the same sensor inside of an empty den. The upper line in the chart shows the signal from the PVAc sensor when held just outside of the den entrance (0–20 s) and then inserted into the den to within 6–8 inches of the tortoise (as indicated using the IR camera). The depth of this particular den was approximately 6 feet. The lower line shows data taken from a similar den but unoccupied by a tortoise.

In Figure 5, data from all three sensors taken from occupied tortoise dens is summarized in a single chart. The data has been normalized (or offset) to place the starting sensor voltage from all three sensors at the same value for comparison. Since the individual sensors are constructed by hand, the starting sensor resistance, and thus the voltage measured across each sensor, is slightly different for each individual sensor. The summary responses indicated in Figure 5 are averages from six different occupied tortoise dens, including one den that contained two tortoises. In this chart, the left bars show the average sensor voltages from just outside of the den entrance, while the right bars show the average sensor voltages from within the dens for an approximate 40 s exposure. Sensor data (normalized) from unoccupied tortoise dens is shown in the chart in Figure 6. Here, there is little variation in sensor voltage from positioning the sensors just outside of the dens (left bars) and inserting them into the dens (right bars). It is not known how long these tortoise dens had been unoccupied.

From the sensor data taken from occupied dens versus unoccupied dens, it is clear that some type of signature indicating the presence of tortoises in their dens is indicated. What is not known, however, is the exact nature or cause of these sensor signals. Owing to its higher solubility parameter, PVA will respond to a greater extent to the presence of water vapor than will PECH (the solubility parameter for water vapor; is 47.9 $MPa^{1/2}$). The crosslinked polymer, PVAc, will also respond to water vapor; however, we do not have accurate solubility parameter data on this material. These organic polymers will also respond volumetrically to the presence of other gases or vapors. Both methane and carbon dioxide gases may be present in greater abundances in occupied tortoise dens than in unoccupied dens. Volatile organics present in the occupied dens might also contribute to the overall sensor signal patterns. Common organic polymers are subject to the partitioning of organic molecules from the gas phase into the polymer material. This effect has been used to produce PMC sensors that respond to the presence of VOCs in air[6,7]. It is known that the chin gland of a desert tortoise emits chemicals and organic vapors of various types. Chromatographic analysis of these chin gland secretions has shown the presence of caprylic, capric, lauric, myristic, palmitic, palmitoleic, stearic, oleic, and linoleic fatty acids[16]. These compounds, when volatilized, will also contribute to the overall sensor array signature from the tortoises. In the context of the present study, it would be difficult if not impossible to decouple all of the various possible contributions to the overall sensor array signal from all of the possible individual gaseous or organic components that might be present in the tortoise dens. Here, we seek to only demonstrate that the array does indeed provide a reliable signal that may be used to differentiate between occupied and unoccupied tortoise dens.

The IR camera attached to the sensor probe package was used in this study to confirm the presence or absence of tortoises in their dens while acquiring PMC sensor data. Four small Al “skids” were attached to the probe package to facilitate pushing the probe into the dens when the soil in the dens was too loose, causing the probe to bind up in the loose soil. In Figure 7, we show a still photo taken from this camera, interfaced to the same laptop computer as the PMC sensors, showing an alert male tortoise in his den. In cases where the camera could spot the tortoises in the dens, the PMC array simply provided redundant data. In other cases, however, the PMC array can be used to confirm the presence of a tortoise even when the IR camera could not. This would be the case in tortoise dens in which the length of the den extends beyond the direct reach of the camera. In our case, the camera cable was approximately 12 feet long, with a focal range of 4–12 inches. In these cases, the PMC array could still respond to the presence of various signature gases or vapors in the den, even if the array was not positioned at the bottommost extent of the den.

Conclusion

We have designed and tested a small, portable probe to be used in the inventory of desert tortoises. The probe consists of a small gas detector array of PMC sensors and an infrared camera. The probe assembly is powered and interfaced to a portable computer through a single USB port. Data taken from the sensor array shows that an identifiable signature is obtained when the probe is inserted into an occupied tortoise den. We hope to continue development of this probe, possibly extending its use to other species that live in burrows or underground dens.

Acknowledgements

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Figure 3. The entire tortoise probe assembly (top) and a close-up photo of the probe itself (bottom).

Figure 4. Data taken from a tortoise den occupied by a single, medium sized (8-inch shell) male tortoise versus data taken by the same sensor inside of an empty den. The upper line in the chart shows the signal from the PVAc sensor when held just outside of the den entrance (0–20 s) and then inserted into the den to within 6–8 inches of the tortoise (as indicated using the IR camera). The depth of this particular den was approximately 6 feet. The lower line shows data taken from a similar den, but unoccupied by a tortoise.

Figure 5. Data from all three sensors taken from occupied tortoise dens is summarized in a single chart. The data has been normalized (or offset) to place the starting sensor voltage from all three sensors at the same value for comparison. The summary responses indicated are averages from six different occupied tortoise dens, including one den that contained two tortoises. In this chart, the left bars show the average sensor voltages from just outside of the den entrance, while the right bars show the average sensor voltages from within the dens for an approximate 40 s exposure.

Figure 6. Sensor data from unoccupied tortoise dens is shown in this chart. Here, there is little variation in sensor voltage from positioning the sensors just outside of the dens (left bars) and inserting them into the dens (right bars).

Figure 7. Still photo taken from the probe infrared camera, interfaced to the same laptop computer as the PMC sensors, showing an alert male tortoise in his den.

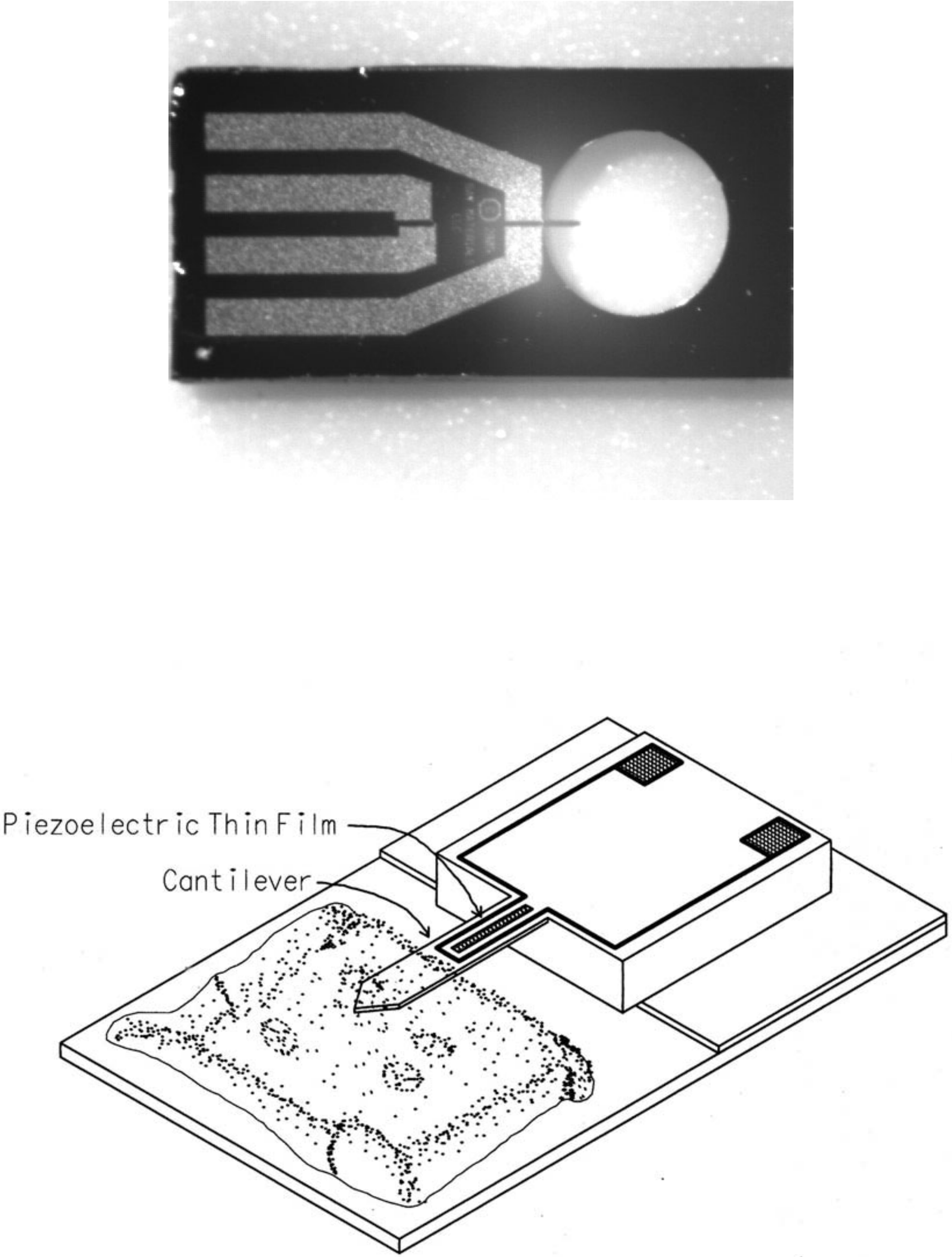

Figure 1

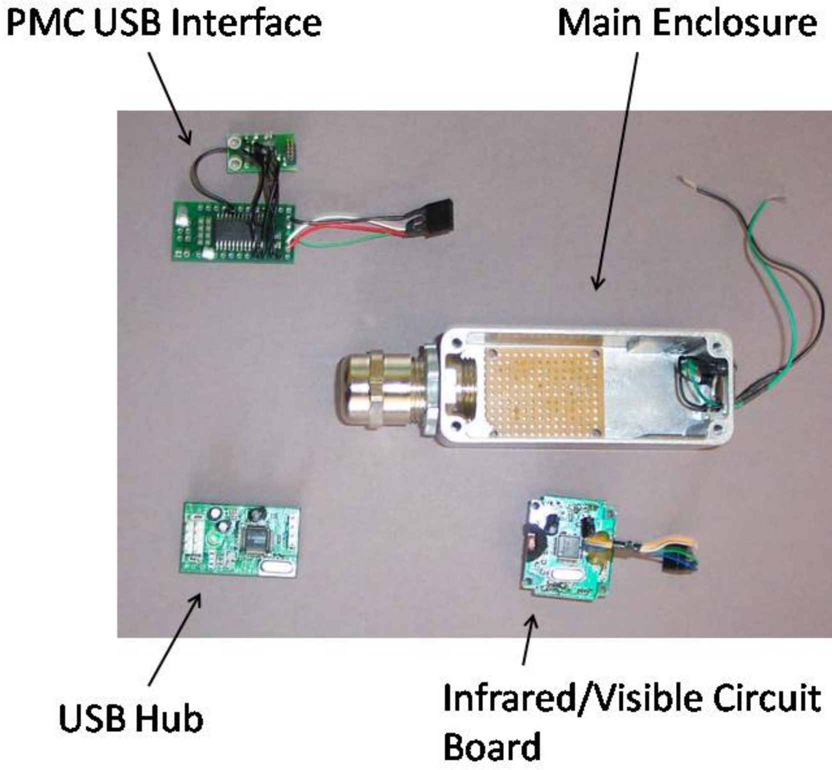
PMC USB Interface
Main Enclosure
USB Hub
Infrared/Visible Circuit Board

Figure 2

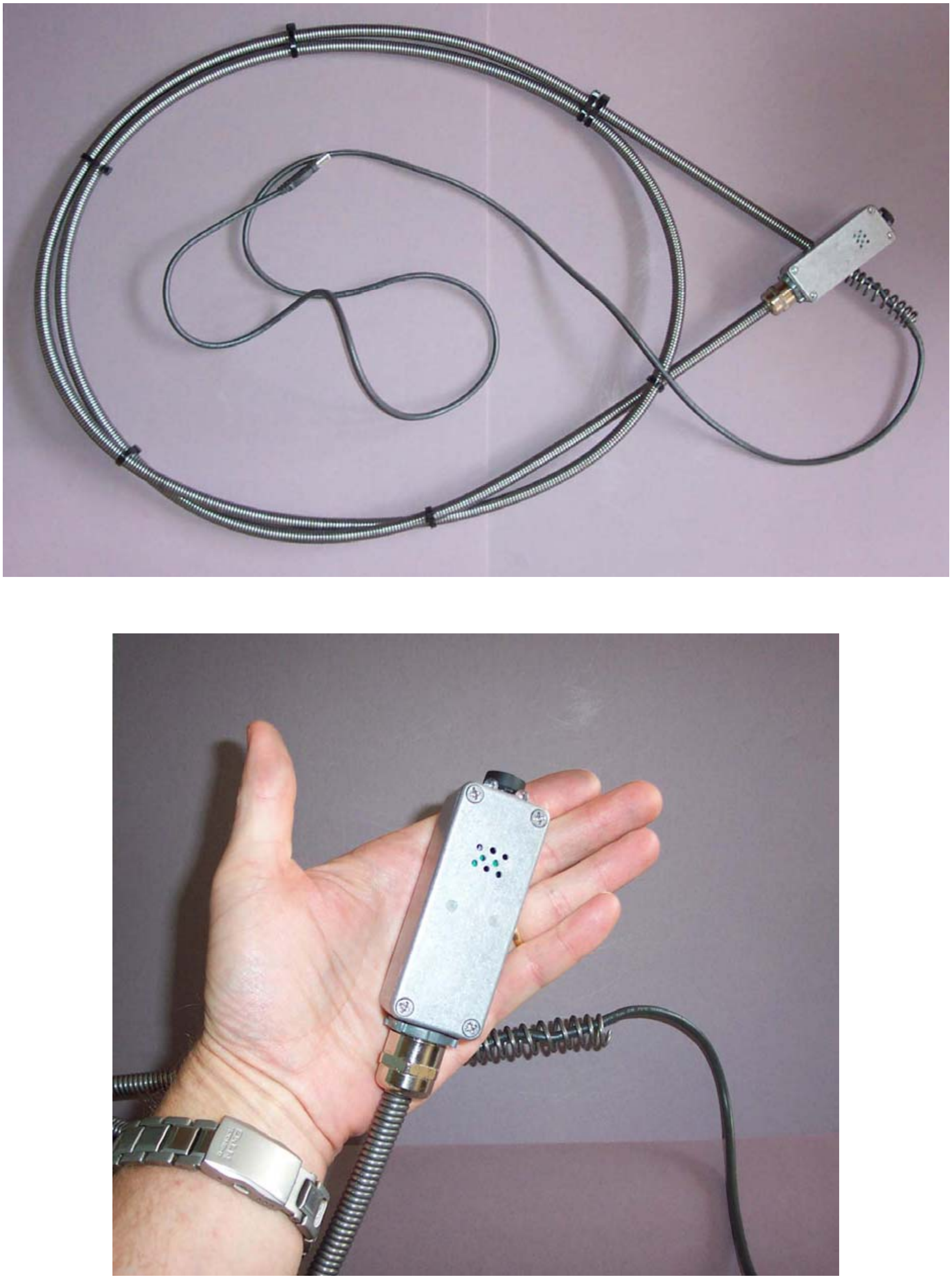

Figure 3

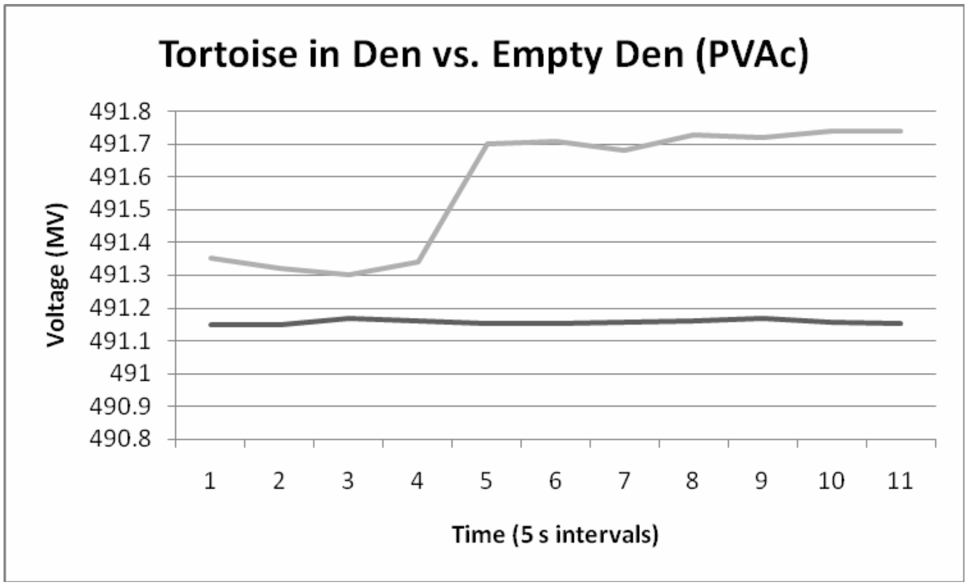
Tortoise in Den vs. Empty Den (PVAc)
Voltage (MV)
491.8
491.7
491.6
491.5
491.4
491.3
491.2
491.1
491
490.9
490.8
1
2
3
4
5
6
7
8
9
10
11
Time (5 s intervals)

Figure 4

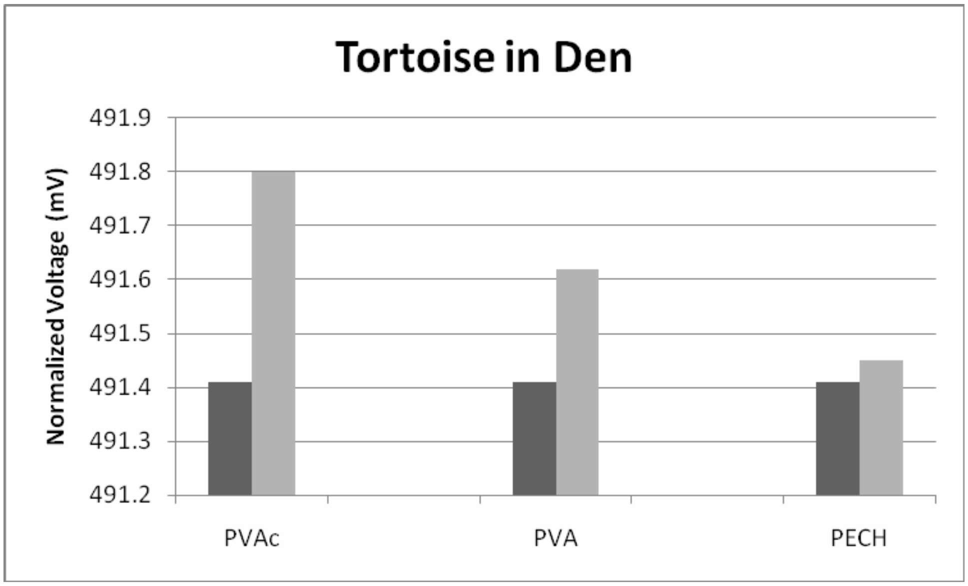
Tortoise in Den
Normalized Voltage (mV)
491.9
491.8
491.7
491.6
491.5
491.4
491.3
491.2
PVAc
PVA
PECH

Figure 5

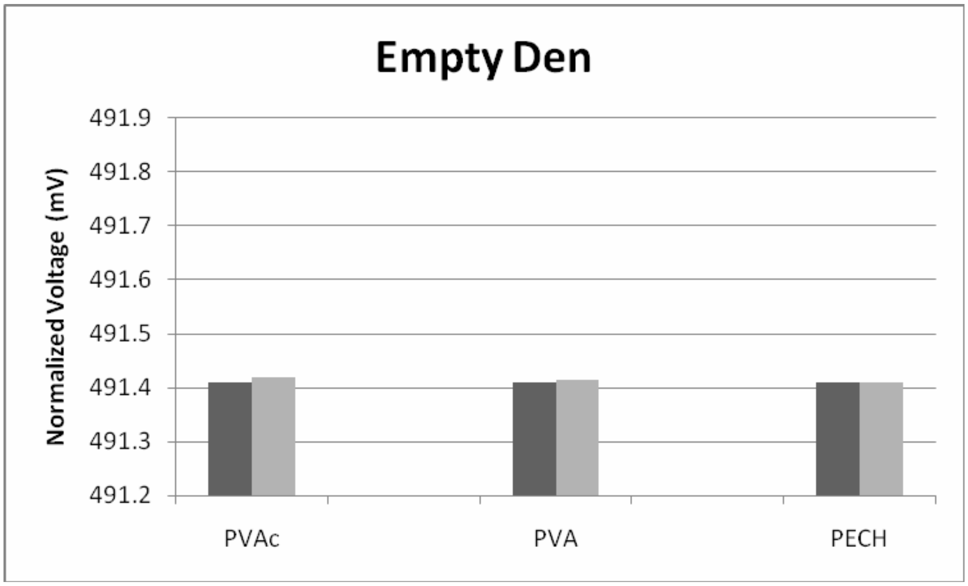
Empty Den
Normalized Voltage (mV)
491.9
491.8
491.7
491.6
491.5
491.4
491.3
491.2
PVAc
PVA
PECH

Figure 6

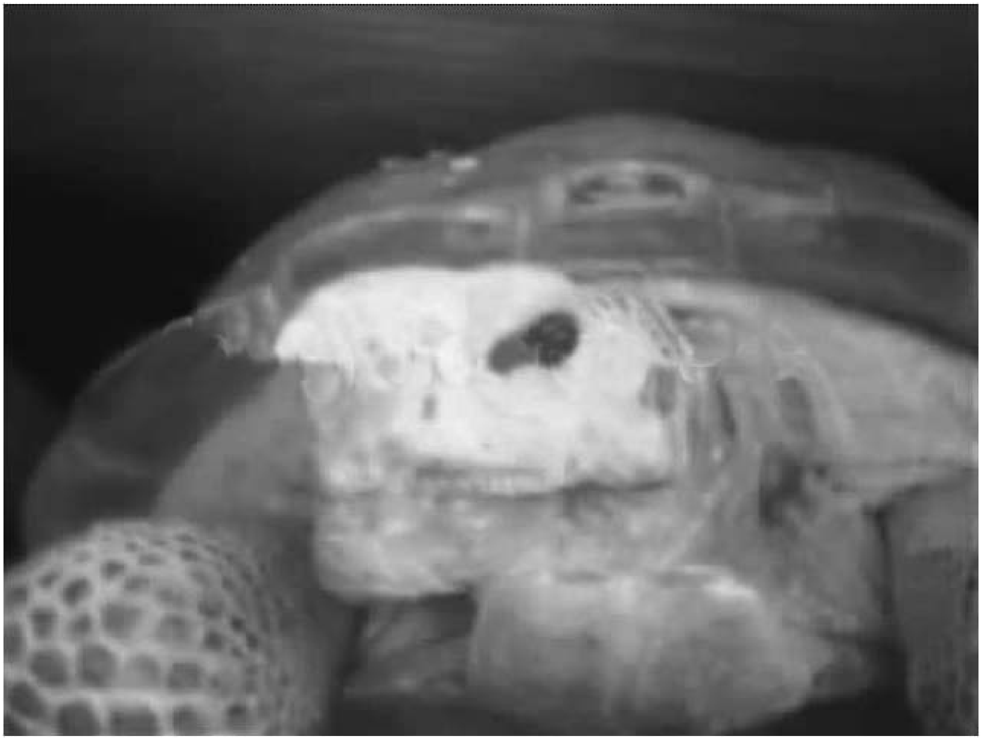

Figure 7