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A CTD Biotag for Mid-sized Marine Predators

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A CTD Biotag for Mid-sized Marine Predators

By

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy
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Dedication

To Russell.
Acknowledgments

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Note to Reader

Note to Reader: The original of this document contains color that is necessary for understanding the data. The original dissertation is on file with the USF library in Tampa, Florida.
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Abstract

Biologging tools for investigating the study of fine-scale linkages between animal behavior and the physical microstructure of the marine habitat are technically limited by substantial size, high cost or low sensor resolution. However, recent advances in electronic technologies and process techniques present attractive alternatives to current tag designs. Motivated by the need for a low-cost, compact CTD biotag for medium-sized marine animals, the University of South Florida Center for Ocean Technology developed a multi-sensor biotag for quantitative measurements of ocean salinity. This dissertation describes the development and performance of a novel CTD biotag used for animal-borne measurements of the physical microstructure of marine ecosystems.

Printed circuit board processes were used to fabricate a liquid crystal polymer-based conductivity, temperature and depth sensor board. Tests performed in the laboratory exhibited good sensor repeatability between the measured and the predicted variables indicating that the initial design and fabrication process is suitable for the construction of a CTD sensor board. The conductivity cells showed good sensor integrity for the entire conductivity range (0-70 mS/cm), thus demonstrating the potential for a highly resolved salinity system.
The CTD sensor board was integrated into two initial multi-sensor biologging systems that consisted of reconfigurable modular circuit boards. The design and initial performance of a 4-electrode conductivity cell circuit was discussed and preliminary tests showed a sensor accuracy of 0.0161 mS/cm. A potential packaging material was analyzed for use on the temperature and pressure sensors and initial tests showed good sensor sensitivities (-2.294 °C/K Ω and 1.9192 mV/dbar, respectively).

Underwater packaging of the biotag was presented in this work along with three different field observations. Vertical profiles of conductivity, temperature and depth in the Gulf of Mexico were obtained and compared to a commercial instrument. On the West Florida shelf, conductivity, temperature, depth and salinity data were obtained from loggerhead turtle deployments. Data collected showed that the tagged turtle encountered a highly variable salinity range (30.6-35.3) while at depth (20 m). This data trend captured was in agreement with shelf characteristics (tidal fluxes and water mass features) and moored instruments. Finally, observations that were undertaken in Bayboro Harbor showed no biofouling to the conductivity electrodes during a 14 day deployment. This biotag is the first to use a PCB-based low-cost CTD to collect animal-borne salinity measurements.
Chapter 1: Introduction

Advanced biologging tools have an extraordinarily wide range of analytical capabilities including ecological research, physiological studies, in-situ environmental surveys, climate variability observations and conservation applications (Kooyman and Ponganis, 1998, Block, 2005, Burger and Schaffer, 2008, Costa et al., 2008, Charrassin et al., 2008, Boersma et al., 2009). These animal-borne tags record data from free-ranging organisms as they travel through their natural environment.

Development of biologging tools at the University of South Florida (Center for Ocean Technology) has focused on a miniaturized multi-sensor biotag for mid-sized marine predators (see appendix 1 for instrument specifications). Miniaturization, multi-sensor capabilities and low-cost are significant issues for biologging technology because these advancements tremendously improve researchers’ abilities to characterize critical environments. The central goal of this dissertation was to develop and deploy a low-cost, miniaturized biologging instrument that measures conductivity, temperature and depth, and thereby to evaluate the tag’s capabilities to determine physical structure use by mid-sized marine predators such as seabirds, fish and reptiles.

Multi-sensor biologging devices require technological advancements to miniaturize the electronics and develop inexpensive sensor fabrication processes. Chapter 2 addresses development of a miniature low-cost multi-
sensor board with flexible interconnects that can be used to determine ocean salinity (Broadbent et al., 2010a). In order to reduce the cost and allow for small scale-production, PCB processing techniques were used to construct a miniature rigid/flex salinity measurement device. Initial conductivity, temperature and pressure sensor characterizations were discussed. The polymer sensor subassembly developed in this work was then integrated with additional sensors and electronics to assemble a CTD biotag.

Animal-borne salinity data has the potential to define the importance of physical water mass features to the ecology of small marine animals. Chapter 3 describes the development of a smaller, less expensive CTD biotag with emphasis on circuit board design and conductivity cell circuitry (Broadbent et al., 2010b). In order to protect the temperature and pressure sensors from the harsh seawater environment, a soft-gel encapsulant was evaluated and preliminary sensor characterizations were discussed.

Large diving marine predators have been used to collect ocean conductivity, temperature and depth, but smaller animals have been overlooked due to technological limitations (Lydersen et al., 2002, Hooker and Boyd, 2003, Biuw et al., Bailleul et al., 2007, Boehme et al., Charrassin et al., Costa et al., 2008). Chapter 4 explores the evaluation of physical ocean structure wherein CTD biotags were used to investigate (a) in laboratory comparisons of conductivity and temperature, (b) in-situ comparisons of CTD vertical profiles, (c) field deployments on loggerhead turtles (Broadbent et al., 2011).
Biofouling has long been considered a primary limiting factor in terms of sensor measurement accuracy and deployment longevity for autonomous oceanographic instruments. This is also true for animal-attached sensors. Therefore, an additional experiment to determine the effect of biofouling on the performance of the exposed conductivity cell was performed and discussed in Appendix 2.

The ability to actively measure ocean salinity and other parameters (e.g. acceleration, compass direction, light and GPS location) while attached to a broader range of animals establishes this miniaturized, inexpensive CTD biotag as a uniquely promising new biologging tool for mid-sized marine predators.

Note to Reader

Portions of this chapter have been previously published (Broadbent et al., 2010a) and are utilized with permission of the publisher.

Abstract

The design, fabrication and initial performance of a single substrate, miniature, low-cost conductivity, temperature, depth (CTD) sensor board with interconnects are presented. In combination these sensors measure ocean salinity. The miniature CTD device board was designed and fabricated as the main component of a 50 mm x 25 mm x 25 mm animal-attached biotag. The board was fabricated using printed circuit processes and consists of two distinct regions on a continuous single liquid crystal polymer substrate: an 18 mm x 28 mm rigid multi-metal sensor section and a 72 mm long flexible interconnect section. The 95% confidence intervals for the conductivity, temperature and pressure sensors were demonstrated to be ±0.083 mS/cm, 0.01 °C, and ±0.135 dbar, respectively.
Introduction

**Oceanographic Biologgers.** Oceanographic biologgers are miniature animal-attached instruments for logging and/or relaying data about a marine animal’s movement, behavior, physiology and/or environment. These biologgers are capable of allowing insights into the lives of many free-ranging marine species including mammals, birds, fish and reptiles. Specialized biologgers can contain many different sensors that measure temperature, depth, light levels, conductivity, swim speed, acceleration, geomagnetic fields, EGC and heart rate, although the most widely used is the temperature depth recorder (TDR) (Block, 2005, Hooker and Boyd, 2003, Ponganis, 2007). TDRs contain internal temperature and pressure sensors, electronics, memory and batteries. Technologically advanced biologgers can measure conductivity, temperature and depth (CTD) which allows researchers to calculate salinity. Salinity information can define the importance of physical water mass features, such as frontal systems, currents, eddies or ice edges, to the ecology of marine animals.

Commercially available biologgers, such as SMRU 9000 CTD-SRDL and Star-Oddi DST CTD, are capable of measuring salinity and have dimensions of 105 mm x70 mm x 40 mm and 15 mm x 46 mm, respectively. These CTD biologgers mounted on marine animals have been able to provide information about marine animal behavior while simultaneously monitoring the environment experienced by the swimming individual (Fedak, 2004). However, the use of current CTD biologgers is limited by substantial size, high cost and/or low accuracy.
Recent research has developed a micro-fabricated salinity sensor system for use on fish (Hyldgard et al., 2008). This chip-based sensor system was fabricated using conventional microelectromechanical system (MEMS) techniques and materials. Although the multi-sensor size is quite small (4 mm x 4 mm), the traditional MEMS silicon fabrication process can require expensive equipment coupled with a cleanroom environment. To reduce the cost and allow for small-scale production, we have developed a miniature, low-cost multi-sensor board with flexible interconnects that can be used to determine ocean salinity.

This work builds on earlier efforts of one of the authors (Broadbent et al., 2007 and Broadbent et al., 2007), which concentrated on the fabrication of conductivity and temperature sensors on liquid crystal polymer (LCP) substrates. The work reported here describes a different; lower cost, more easily fabricated approach to building a conductivity cell, as well as integrating that device with readily available commercial sensors to create a complete CTD measuring system.

Motivated by the need for a low-cost, compact CTD biologger for medium-sized marine animals, we used printed circuit board (PCB) processing techniques to fabricate a polymer sensor subassembly which could be integrated with the electronics of a biotag. This paper presents the development of a miniature part-rigid, part-flexible LCP conductivity, temperature, depth (CTD) and wet/dry sensor board with standard flex-PCB edge-connectors. This novel single-substrate design incorporates a rigid single-sided conductivity cell, thermistor and piezoresistive pressure sensor and a thin, flexible interconnect cable and edge
connector. CTD design, fabrication and experimental characterization are discussed.

**Liquid Crystal Polymer.** LCP is an organic thermoplastic dielectric material developed for use in single and multilayer flexible printed circuit boards, and its properties make it an ideal substrate for mounting rigid oceanographic sensors and building flexible electrical interconnects for them. LCP is formed by rigid and flexible monomers that link together to form fibrous crystalline chains that maintain a unique crystalline order in liquid or melt phase (Culbertson, 1995). This crystalline structure exhibits excellent electrical, thermal and chemical properties. LCP has a low and stable dielectric constant (2.9 at 10 GHz) and dielectric loss (0.0025 at 10 GHz); good dimensional stability, tensile strength and tensile modulus; extremely low moisture absorption (<0.04%) and gas permeability; a low and controllable coefficient of thermal expansion (0–30 ppm °C−1); and very high chemical resistance. LCP manufactured for high performance printed circuit boards is laminated to copper foil, although the surface can be conditioned with strong concentrated bases at elevated temperatures or oxygen plasma etch to allow additional metallization (Wang et al., 2001). LCP is available with several different melting temperatures, allowing multiple-layer bonding or lamination. These properties, and its low cost, make LCP well suited to the small-run fabrication of miniature sensors for many environments. Recent LCP sensors include humidity, pressure, flow and touch (Dean et al, 2007, Palasagaram and Ramadoss, 2006, Wang et al, 2001). By contrast, traditional rigid PCB (FR4) and flexible polymer (Kapton) materials will
saturate with water over time (having moisture absorption of up to 4.0% by mass), causing radical changes in their electrical properties, which would be incompatible with accurate sensor measurements.

Design

The design of the CTD board was driven by several goals: small size (25 mm width), accurate salinity measurements (0.2%), ease of interfacing to circuit electronics, rapid development and prototyping, robustness to moisture, temperature and pressure, repeatability between conductivity cell devices, and low manufacturing cost for small production runs (10–100 units). To satisfy these requirements, our solution was to fabricate a single-sided rigid/flex LCP-based CTD sensor board with an interconnect region.

Size and Shape Constraints. The CTD biologging instrument was designed to be small and compact in size so that it could measure oceanographic parameters while deployed on medium-sized marine animals such as penguins, turtles, sharks and tuna. The biologging device was designed to have modular circuit boards which could be rearranged to fit alternative packaging architectures, depending on the species the device was to be deployed on and/or the battery life required. The initial size constraints were 50 mm x 25 mm x 25 mm with a weight of 65 g. The device was composed of six circuit boards including the board holding the sensors for conductivity, temperature and pressure. This CTD board is connected by a 16-way connector to a signal conditioning and A/D conversion board. To meet the size and weight requirements, the CTD board needed to be as thin as possible and to have a
flexible portion so that it could be connected to the analog signal conditioning board while positioned on top of the board stack (Figure 1).

Figure 1. Organization of the circuit board stack for the CTD biotag which includes the CTD sensor board, wireless communications board (WCB), digital sensor board (DSB), processor/memory board (PMB), analog signal conditioning board (ASCB), battery charger board (BCB), battery and battery/charger board induction coil.

**Sensor Performance Requirements.** Conductivity, temperature and pressure data are all used in the calculation of water salinity, so the required accuracy for each sensor type can be derived from the salinity accuracy requirement. For a commercial CTD probe, salinity accuracies of ±0.01 are typical and similar accuracies are achieved by the macro CTD units fitted to some types of large marine biologgers (Fedak, 2004). However, the required accuracy in many biologger applications is somewhat lower, with absolute accuracy being less important than repeatability or precision. In this paper we will consider the precision of the sensors. High precision or high repeatability means that the sensor outputs are consistent over a short period while subject to the same input on a number of occasions. Repeatability can be used in
conjunction with a calibration process to produce data whose accuracy is limited by the precision achieved by the sensors.

Other factors are also important in assessing sensors: a linear response to inputs makes calibration and interpolation between calibration points straightforward; appropriate sensitivity reduces the need for electronic amplification and the range of values that can be measured must match the range required for the application. Therefore, we address linearity, sensitivity and range, as well as short-term repeatability (or precision) in this paper. Table 1 summarizes the sensor requirements.

The pressure sensor accuracy requirement is the accuracy needed to effectively monitor the behavior of marine species, rather than its effect on the accuracy of salinity measurements (the pressure accuracy required for salinity calculations is much less than the figures above).

Table 1. Sensor Performance Requirements

<table>
<thead>
<tr>
<th>Sensor</th>
<th>Range</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity</td>
<td>0 to 70 mS/cm</td>
<td>0.2%</td>
</tr>
<tr>
<td>Temperature</td>
<td>-5 to 30 ºC</td>
<td>0.1 ºC</td>
</tr>
<tr>
<td>Salinity</td>
<td>2 to 42</td>
<td>0.2%</td>
</tr>
<tr>
<td>Pressure*</td>
<td>0 to 2000 dbar (gauge)</td>
<td>±0.2 dbar (0 to 50 dbar)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±20 dbar (50 to 2000 dbar)</td>
</tr>
</tbody>
</table>

*The pressure sensor accuracy requirement is the accuracy needed to effectively monitor the behavior of marine species, rather than its effect on the accuracy of salinity measurements (the pressure accuracy required for salinity calculations is much less than the figures above).
**Conductivity Cell Design.** Salinity is the concentration of dissolved salts in water and is derived by using the Practical Salinity Scale of 1978 (PSS 1978) (Lewis, 1980) (Appendix 3). The PSS 1978 is a dimensionless ratio of seawater conductivity to that of a potassium chloride (KCl) solution of known concentration. This conductivity ratio is dependent on temperature and pressure, so seawater salinity at depth is most accurately calculated by obtaining simultaneous *in situ* measurements of electrical conductivity, temperature and pressure (depth). The three measurements are entered into the UNESCO Equation of State algorithm and salinity is calculated (Fofonoff and Millard, 1983). Since all three measurements are needed to calculate salinity, the proximity of the sensors to one another is critical to obtaining an accurate salinity measurement for a given volume of water. Therefore, the conductivity cell, thermistor and pressure sensor were situated within millimeters of each other on the CTD board.

The conductivity measuring technique used in the CTD board was by direct measurement of the resistance of the seawater between two sense electrodes separated by a fixed distance. The cell constant, $C$, is generally used to describe conductivity cells (Equation 2.1). Equation 2.2 is used to convert measured conductivity to specific conductivity with the consideration of the cell constant:

$$ C = \frac{L}{A} $$

(Equation 2.1)
\[
\frac{1}{\rho} = \kappa = \frac{1}{R} \times \frac{L}{A},
\]

Where \( \rho \) is the volume specific resistivity, \( \kappa \) is the specific conductivity, \( R \) is the measured resistance, \( L \) is the length of the electrodes and \( A \) is the cross-sectional area.

Adjusting the cell constant by modifying the cell geometry will influence the behavior of the cell through the interrelated factors: measurement range, sensitivity and electrical current density (Hyldgard et al., 2008). Based on these data, we chose a four-electrode rectangular bar geometry for the conductivity cell design that had 7 x 1mm drive electrodes separated by a distance of 7 mm.

The conductivity cell was designed to be measured using a four-wire impedance measurement technique, consisting of two drive electrodes and two sense electrodes (Figure 2). This approach has the advantage of virtually eliminating the effects of lead resistance and inductance errors between the conductivity meter and the conductivity cell. A four-wire connection was particularly important in this application, as the conductivity cell board was connected to the signal conditioning board by a zero-insertion force connector, rather than a permanently soldered connection. This meant that the resistance at the connector could change each time the device was assembled and could change over time (for example, as the potting material used to encapsulate, the biotag became saturated with water). A four-wire resistance measurement is unaffected by changes of this type.
Figure 2. Diagram showing the conductivity cell conductance measurement technique. The multimeter provides a fixed ac current to the drive electrodes while reading the ensuing voltage drop across the sense electrodes. The seawater conductance is taken as the ratio of the drive current to the measured voltage.

It is usual when making direct measurements of water conductivity to use an ac drive voltage. Driving the circuit with a dc voltage would result in impedance errors that occur due to the presence of an electrolytic double layer capacitance (DLC) at the electrode-to-water interface, as well as parasitic electrode-to-electrode capacitances (Hyldgard et al., 2005). We measured the variation of cell capacitance with frequency and found that the optimum ac frequency was in the 10–100 kHz range; any frequency in that range would be equally satisfactory for making the measurements. Below 10 kHz the double layer capacitance at the drive electrodes began to influence the impedance, while above 100 kHz the parasitic capacitance of the conductivity cell substrate
contaminated the conductivity measurements. In the 10–100 kHz range, the measured impedance is dominated by the actual seawater conductivity. An operating frequency of 16 kHz was selected for the device, as this could be conveniently generated on the signal conditioning board, and was low enough to allow a relatively low sample rate A/D converter to be used. Also, a sufficiently low drive current was chosen to minimize the effects of undesired electrolysis at the conductivity cell/seawater interface. Electrolysis takes place predominantly when the drive electrodes are subjected to the dc drive signal, or any ac signal with a dc offset, and can cause metal degradation which can lead to output drift during long-term measurements. If the drive voltage is sufficiently high, damage to the drive electrodes can occur. To ensure that the voltage at the drive electrodes stayed below 50 mV for all measured conductivities, a fixed drive current of 0.5 mA was used.

The conductivity cell electrodes and wet/dry sensor must be in direct contact with the seawater. They needed to be fabricated using non-corrosive metals on a non-absorptive substrate (LCP) and be situated on the surface of the biotag. They consisted of a thicker base layer metal, which increases the electrode cross-sectional area, and a very thin (to minimize cost) surface layer metal. Nickel was selected as the base metal because it has excellent corrosion resistance to seawater and it can be uniformly electroplated directly onto LCP. Because nickel is not malleable and can crack if the substrate is flexed, which can lead to erratic performance of the conductivity cell, an additional layer of LCP was laminated to the nickel-plated portion of the device.
Generally platinum or platinum black is used for the surface of conductivity cells. Platinum black drastically increases the electrode surface area, thereby reducing polarization impedances (Hunt, 1995, Jacobs et al., 1990). However, it is not ideal for animal-attached marine applications due to its powder-like properties which cause it to be easily removed by abrasion. Preliminary tests showed that platinum electrodes performed as well as platinum black electrodes (Figure 3). Therefore, we chose to construct the conductivity cell and wet/dry sensor with nickel, gold and platinum metals, where the gold is used as an adhesion layer between the nickel and platinum.

The conductivity range the CTD biotag is expected to encounter in field testing is between approximately 2 and 70 mS/cm which corresponds to a measured resistance range of 16–554 Ω for the conductivity cell. The CTD’s drive circuitry was designed to provide a 0.5 mA square wave signal to the drive electrodes. When this drive level is applied to the expected seawater resistance range seen by the conductivity cell, the voltage that occurs at the sense electrodes will provide a high resolution, but still having a low current density due to the larger electrode cross-sectional area. The overall dimensions of the conductivity cell were 8 mm x 9 mm with a 7 mm distance between the drive electrodes.
Figure 3. The conductance measured (mS) as a function of water conductivity (mS/cm) for the Pt and Pt-Pt-Black conductivity cells. The plots are shown with a fitted linear regression.

**Temperature Sensor Design.** Conductivity is strongly dependent on temperature and compensation for this effect is required to achieve an accurate measurement. The temperature effect is linear and can be described in Equation 2.3:

\[ C_t = C_{25} (1 + \alpha(t - 25)) \],  \hspace{1cm} 2.3

where \( C_t \) is the conductivity at \( t \) °C, \( C_{25} \) is the conductivity at 25 °C, \( t \) is the temperature °C, and \( \alpha \) is the temperature coefficient.

The PSS 1978 compensates for temperature effects, but requires very accurate temperature data. For high salinities, a measurement error of 0.1 °C
gives the same salinity error as a conductivity measurement error of 0.1 mS/cm. The required accuracy is higher than that offered by off-the-shelf temperature sensors with digital interfaces, so designing our own highly accurate temperature sensing system was necessary. Temperature sensing in the CTD board was performed using a miniature (2.00 x 1.25 mm) commercially available surface mount chip thermistor (Murata NCP21XW223J03RA) with a specified resistance of 22 kΩ at 25 ºC. This device was chosen for its long-term stability, ensuring that re-calibration due to drift would not be regularly required. This thermistor has a negative thermal coefficient, i.e. its resistance varies inversely with temperature, and its response is inherently highly nonlinear. To achieve a more linear response in the desired temperature range, a 40 kΩ surface mount chip resistor was placed in parallel with the thermistor in the CTD circuit. The resistor in combination with the 22 kΩ thermistor optimizes the linear temperature response over a 0–20 ºC range.

**Pressure Sensor Selection and Design.** The pressure sensing component in the CTD circuit consists of a miniature piezoresistive sensor cell (Keller Series 1 TAB) which provides an output voltage as a function of absolute pressure. The output range is dependent on the chosen pressure range and the excitation current used. The Keller sensor uses piezoresistive sensor elements in a Wheatstone bridge configuration and usually requires the addition of a resistive compensation network to provide a 0 V output at atmospheric pressure. The Keller pressure sensor chosen for this design has a 200 bar maximum pressure range to correspond to the maximum expected operating depth of the
biotag instrument (2000 m). In addition to their small size, another advantage of using a chip thermistor and the Keller pressure sensor is that both can readily be soldered to the CTD’s plated metal pads.

**Fabrication**

**A Novel PCB Fabrication Process for the Rigid Sensor Board with Flexible Interconnects.** The CTD board was effectively divided into two areas. The first one was 100 µm thick and used copper traces; this was the flexible portion of the board, which could be wrapped around the biotag to reach the connector on the analog signal conditioning board. The second rigid region was 225 µm thick and contained the conductivity cell, thermistor, pressure and wet/dry sensors.

To construct the conductivity, temperature, pressure and wet/dry sensor board, a novel subtractive and additive PCB fabrication process was developed. The process involved additional lamination and metallization of the LCP substrate coupled with traditional photolithography techniques to define the two regions (rigid and flexible) of the multi-sensor board. The LCP material that we chose to use was ULTRALAM® 3850 and 3908 bonding film (Rogers Corp, USA), which is delivered with copper plating 18 µm thick on both sides. A detailed schematic of the fabrication process is shown in Figure 4.
Figure 4. CTD sensor board fabrication sequence. (a) Double Cu-clad LCP. (b) Copper etch and lamination of bond layer and second LCP layer. (c) Electroless nickel deposition. (d) Gold electrochemical deposition. (e) Platinum deposition on conductivity cell and wet/dry sensor.

(a) Copper metal was chemically etched from the designated rigid sensor area of a 100 µm thick LCP substrate.

(b) A rigid 200–225 µm thick region of LCP was constructed. This was done by laminating an additional 100 µm layer of LCP to the initial LCP substrate using a 50 µm thick LCP bonding film. The bonding film has a lower melting temperature than the substrate layer and was melted using heat (247 ºC) and pressure (13.6 bar) to adhere the substrate layers together without changing their physical characteristics.

(c) The rigid section of the LCP substrate surface was conditioned for metallization. This was done by chemically etching the LCP surface with a strong concentrated base (KOH). This procedure was necessary to micro-
etch the LCP surface for better adhesion between the LCP and the nickel metal.

A liquid palladium catalyst was deposited in the micro-etched LCP surface. This catalyst was also deposited on a 1 mm thick section of the remaining copper metal, ensuring proper adhesion of the nickel to the copper. A thin seed layer of electroless nickel metal (0.30 µm) was chemically plated to the palladium catalyst on the LCP. The seed layer was necessary to ensure an adequate etch of the nickel metal once patterned with the CTD board artwork.

(d) Using printed circuit board (PCB) photolithography techniques, the multi-sensor and interconnect artwork was patterned. A negative thin-film photoresist (Riston FX950, DuPont, USA) was laminated to the LCP substrate. Then, a film acetate photomask of the CTD board was aligned on the LCP substrate. The mask was aligned so that the wider interconnects were positioned over the copper-nickel metal overlap section. Using a light box the artwork pattern was exposed onto the LCP substrate and then developed. The pattern was completed by chemically etching the unwanted electroless nickel and copper metals away. Since the copper etchant (ammonium persulfate) did not attack the nickel metal, the copper was etched first. The nickel seed layer and the palladium catalyst were then etched using aqua regia (HCl: HNO₃, 3:1).
The electroless nickel seed layer was re-activated by removing surface oxidation and contaminants with a conditioner (C-12 Activator, Puma Chemical, USA). Then, the chemical deposition of nickel was continued until an approximate thickness of 17 $\mu$m was obtained.

A thin layer of protective and electrically conductive gold was then electroplated to the electroless nickel and copper metals.

(e) A 250 nm layer of platinum metal was electroplated to the conductivity cell and wet/dry electrodes.

The Completed Rigid/Flex CTD Board. The overall dimensions of the rigid/flex CTD board are 90 mm x 28 mm (Figure 5). The rigid 225 $\mu$m thick CTD sensor region is 18 mm x 28 mm and the flexible copper-gold interconnects are 72 mm in length. Figure 6 shows the rigid/flex CTD board wrapped around and connected to a test circuit board via a 1 mm high, 16-pin, 0.5 mm pitch, top contact connector. If future packaging constraints require it, longer or shorter interconnects can be used. The four wire measurements used for all the sensors (temperature and pressure, as well as conductivity) show that the length of the interconnect board will not affect the accuracy of the system.

An independent wet/dry sensor was fabricated to sense whether the animal was in the water or not. Wet/dry measurements can be used to determine when particular biotag sensors should be on or off to maximize sensor longevity and battery life. The wet/dry sensor was designed to operate independently of the conductivity cell to provide continuous measurements without the power drain of continuously operating the conductivity cell (or the possible increase in
contamination and drift due to such continuous operation). It consists of two small conductive pads fabricated with nickel, gold and platinum metals, exactly as for the conductivity cell electrodes. To minimize the area of the system in contact with water, we positioned the wet/dry sensor next to the conductivity cell. A section of the traces leading to the sensors and the solder pads were constructed with a larger trace width (1 mm). This was to prevent etch-out and under-cut of the traces at the copper-nickel junction caused by the different etching rates of each metal.

The solder pads for the thermistor, pressure sensor and surface-mount resistors had to be constructed to accommodate the melting temperature and the solder float thermal properties of the LCP material. LCP has a relatively low melting temperature (315 °C) and solder float temperature (288 °C), and the high solder temperatures required for lead-free solder will melt LCP unless oversize pads are used. The large pads rapidly conduct heat away from the solder, making it much easier to solder to the board without melting the LCP. The result of using these oversize solder pads was that the boards were quite easy to solder and a 100% success rate was obtained in assembling CTD boards.
Figure 5. A photograph of the completed rigid/flex CTD board (90 mm x 28 mm). Shown is the (a) Pt rectangular bar conductivity cell, (b) thermistor, (c) pressure module, (d) Pt wet/dry sensor, (e) oversized leads and pads, (f) flexible Cu-Au leads and (g) flexible Cu-Au connector fingers (0.5 mm pitch). The arrow shows the Cu-Ni-Au overlap area where the rigid section begins.

Figure 6. Photograph of the fabricated rigid/flex board wrapped around and connected to the test circuit board, with the (a) conductivity cell (7 x 9 mm), (b) thermistor and (c) pressure module. The wet/dry sensor is placed in the upper left corner (d). The red boxed area shows the platinum coated conductivity cell and wet/dry electrodes that will be exposed to seawater.
Results and Discussion

The CTD sensors were tested individually to establish the feasibility of the CTD board design and fabrication process for the oceanographic multi-sensor biotag. To demonstrate that the interconnect region of the board would flex sufficiently as the design required, sensor measurements were taken while the CTD board was wrapped around and connected to the test circuit board via the 16-pin connector.

Conductivity Cell Performance. Preliminary assessment of the CTD board fabrication process was done by building six CTD boards. Nickel thickness of the conductivity cells was measured using x-ray fluorescence to assess the reproducibility of the nickel plating process. The nickel thickness of the drive electrodes of all conductivity cells was measured. The average nickel thickness ranged from 16.3 to 17.4 µm with a standard deviation of 0.395 µm. This variation of nickel deposition was most likely due to having to plate each CTD board independently (due to the small size of the plating bath), so slight variations in nickel plating rates occurred. This variation could be minimized by increasing the size of the plating container which would minimize the influence of small variations of temperature and chemical compositions. The final stage of biotag construction is a calibration of the assembled CTD board with the analog interface electronics board, which will take account of the differences in the cell constants between CTD boards.

A Tegam 3550 four-wire LCR meter was used to measure the conductance of the conductivity cells when placed in KCl standard solutions.
The Tegam was programmed to measure conductance using the same constant current and frequency that is generated by the analog interface circuitry of the biotag (i.e. 16 kHz drive frequency and 0.5 mA drive current). Before being tested, the conductivity cells were cleaned with weak hydrochloric acid (10%) to remove surface contaminants. Initial tests were performed at ambient room temperature and no temperature compensation was performed. Six KCl standard conductivity solutions (2, 10, 30, 40, 50, 70 mS/cm) were used to test the cells. A series of 500 conductance measurements were averaged and recorded per conductivity solution for each device and shown in the graph shown in figure 7.

![Graph showing the conductance measured as a function of water conductivity for the six fabricated conductivity cells. The plots are fitted with a linear regression.](image-url)

Figure 7. The conductance measured as a function of water conductivity for the six fabricated conductivity cells. The plots are fitted with a linear regression.
The measured conductance (mS) can be seen to be a linear function of the conductivity and linear regression statistics confirmed this. The coefficient of determinations ($R^2$) for the cells ranged from 0.9981 to 0.9993 and indicated excellent linear correlation between the measured (mS) and predicted variable (mS/cm) for all six of the LCP-based conductivity cells. The gradients ranged from 0.8677 to 1.0305 mS (mS/cm)$^{-1}$, which implies that the physical scale or sensitivity of the conductivity cell was suitable to measure the entire conductivity range (0–70 mS/cm). The results show a variation in response of the individual conductivity cells as the water conductivity increased (70 mS/cm 25 ºC standard deviation = 3.853). The solution temperature greatly affects the actual conductivity measurement, especially at high conductivities, and temperature compensation was not performed on these initial tests. This discrepancy is most likely explained by the standard solution temperature variation between CTD boards when sampled. More extensive conductivity cell calibration data with temperature compensation will be provided in an additional publication. As previously discussed, we were not trying to build interchangeable conductivity cells, so this variation was not considered important.

More significant than the difference in response between devices is the repeatability, the extent to which each device will yield the same conductance measurement when exposed to the same conductivity solution at the same temperature. Five hundred conductance measurements were taken and averaged in each conductivity standard per conductivity cell to allow this characteristic to be assessed. The worst-case standard error for any of the
devices ranged from 0.014 mS/cm at 2 mS/cm to 0.043 mS/cm at 70 mS/cm (though typical standard errors were somewhat better than this). Assuming that the distribution being measured is Gaussian, this would imply that the 95% confidence interval of measurements was ±0.028 mS/cm at 2 mS/cm and ±0.083 mS/cm at 70 mS/cm.

Figure 8 shows that this confidence interval is actually smaller than the calculated Tegam LCR meter measurement error (0.01% of range; ranges used were 100 and 1000 Ω) in nearly all cases. The only significant exception is the measurements at low conductivities for CTDB (b); it is thought that experimental error is responsible for this result (probably contamination of the conductivity solution). The strong implication is that the Tegam measurement error dominates the overall error, and that the device would deliver higher precision if more accurate conductance measurements were possible. Our conclusion was that the conductivity cell precision was better than 0.2% at all conductivities except 2mS/cm (where it was 0.5% or better) and was sufficient for use in the biotag device.

In practice, a variety of other issues make this level of accuracy difficult to reproduce in the field. The factors that can affect real-world performance include temperature variability of the interface electronics with temperature; the presence of environmental electrical fields; biofouling on the electrodes or LCP; electrolysis damage to the electrodes; and the presence of non-biological surface contaminants.
Figure 8. The variation of the conductance for each device. The standard error of the measurements was calculated, then converted to a 95% confidence interval, and expressed as a percentage of the measured conductance. The black line is the LCR meter measurement error.

**Temperature Sensor Performance.** The CTD thermistor was tested using a 612 digit multimeter (Agilent 34410A) which was programmed to take fourwire resistance readings over a range of temperatures. The four-wire measurement technique was used to reduce lead resistance and inductance measurement errors in the measured resistance readings; the same technique was also implemented for the temperature measurements on the biotag device itself. The CTD board with linearized thermistor was placed in a deionized water container in a water bath chamber and resistance measurements were acquired from 5 to 35 °C. This temperature range was chosen because it closely resembled the water temperatures that the final device would encounter in the
field. The water temperature was measured using a calibrated thermistor with an accuracy of ±0.01 (US Sensor USP3201), measured using an Agilent 34405A multimeter. Ten roughly simultaneous readings (each reading being the average of 300 samples) were taken from the calibrated thermistor and the CTD thermistor at each temperature increment. As expected, the response is not completely linear (the linearization resistor discussed earlier only provides approximate linearization), so a linear regression for the whole temperature range could not be used to determine how repeatable the thermistor readings were. Instead, a regression was performed on the ten CTD thermistor and ten calibrated thermistor average readings obtained at each temperature setting. If a Gaussian error distribution is assumed, this gives a 95% confidence interval for the repeatability of the thermistor better than ±0.02 °C throughout the temperature range (figure 9). The achievable precision is expected to be somewhat better than the confidence interval achieved; the graph shows that the standard error rose sharply for temperatures of 25 °C and 30 °C, which seem likely to be artifacts, as there is no obvious physical explanation for the device behaving radically differently at these temperatures. The bulk of the readings (those at 5, 10, 15, 20 and 35 °C) indicate that a standard error of 0.005 °C or better is achievable, giving a 95% confidence interval of about 0.01 °C.
Pressure Sensor Performance. The pressure sensor used was capable of measurement to 2000 m, and the manufacturer’s data sheet specifies 1% error throughout the operating pressure range, an error of ±20 m. For this application, the accuracy of shallow depth readings (0–50 m) was important, while the accuracy at higher pressures was not critical. The pressure sensor was therefore tested to determine the achievable precision at depths in the 0–50 m range (corresponding roughly to 0–50 dbar). The tests were conducted in air using a small pressure chamber, the internal pressure of which was monitored with a highly accurate pressure gauge (±0.25%, 0–75 psi). The Keller pressure sensor we used requires four compensation resistors to linearize its response.
and to obtain a null (0 V) output at atmospheric pressure. To calculate the compensation resistor values, the pressure sensor was initially characterized using a 4 mA excitation current. The corresponding output voltage was then used to find the required resistor values for zero output at atmospheric pressure. Once the proper compensation resistors were included, the output voltage was measured over a range of pressures, again using a 4 mA input current. One pressure sensor measured three separate cycles from 0 to 50 dbar and the results are shown in figure10. The preliminary linear regression data showed excellent correlation between the measured (mV) and the predicted (dbar) variable with $R^2$-square values of $>0.9999$ for each independent run. To check the precision of the sensor in the 0–50 dbar range, a multiple linear regression was performed against both temperature and depth. The standard error of the residuals from this regression was then calculated (0.069 dbar). Assuming that the distribution of error is Gaussian, a 95% confidence interval (1.96 standard deviations) was found to be $±0.135$ dbar (i.e. $±0.135$m in depth), showing that the sensor met the biologists’ requirements for precision in the 0–50 m depth range. Calibration to achieve this level of accuracy would have to involve mapping both the temperature and pressure responses of the device.
Conclusions

The microfabrication of a rigid/flex CTD device board for oceanographic measurements has been described. An effective miniature low-cost four-electrode conductivity cell coupled with cheap commercially available transducers (thermistors and pressure sensors) was manufactured using PCB processes and LCP material.

Preliminary individual sensor results have verified that the sensors performed as intended. All sensors exhibited good repeatability between the measured and the predicted variables indicating that the initial design and fabrication process is suitable for the construction of a rigid/flex multi-sensor board. The conductivity cells showed good individual sensitivities (0.8677–
1.0305 mS/cm) and high repeatability for the entire conductivity range, thus
demonstrating sensor integrity. The CTD sensor package largely met the
performance requirements described in table1, and would offer useful, precise
CTD and salinity information for marine biologger applications.

This multi-sensor board demonstrates the feasibility of PCB fabrication
processes coupled with LCP material for the construction of miniature low-cost
environmental sensors. Future work intended includes sensor characterizations,
determination of salinity measurements and instrument packaging.
Chapter 3: A Low-Cost, Miniature CTD for Animal-Borne Ocean Measurements

Note to Reader

Portions of this chapter have been previously published (Broadbent et al., 2010b) and are utilized with permission of the publisher.

Abstract

The study of fine-scale linkages between animal behavior and the physical microstructure of the marine habitat is essential for understanding the ecology of many marine animals. Animal-borne salinity data has the potential to define the importance of physical water mass features to the ecology of marine animals. Recently CTD (conductivity, temperature and depth) data loggers mounted on large marine mammals (pinnipeds and cetaceans) have been able to capture direct qualitative information on the physical microstructure of the foraging environment and microhabitat. In order to understand the physical environment of smaller marine animals (penguins, fish and reptiles) a miniature, inexpensive CTD biotag is being developed. The biotag circuit boards are of a modular design so that several prototypes for different marine species can be developed. Currently two designs (A and B) have been fabricated and they measure 50 x 25 x 25 mm and 85 x 25 x 15 mm (unpotted), respectively.

The biotag has additional internal sensors that are managed by a low-power microcontroller. The complete multi-sensor system measures
conductivity, temperature, pressure, light, three-axis acceleration, three-axis magnetic fields, wet/dry and GPS. CTD measurements are used to calculate salinity and must be in close proximity to one another and the seawater. Therefore a novel CTD board was fabricated. The conductivity sensor was fabricated using printed circuit board (PCB) techniques and integrated with MEMS (micromechano-electrical system) sensors, a thermistor and piezoresistive pressure module, on a liquid crystal polymer substrate (LCP). A four-electrode conductivity circuit that measures electrical resistance was designed. In this paper the biotag initial design is presented along with the conductivity cell circuit and preliminary CTD characterization data.
Introduction

This paper describes the continued development of a low-cost, miniature multi-sensor biotag used to acquire data on marine animals. Previous development efforts of one of the authors are described in Broadbent et al. (2007, a and b), with a more in-depth presentation of the CTD (conductivity, temperature, depth) sensor board fabrication presented in Broadbent et al. (2010). The work reported here presents the description of the initial multi-sensor biologging systems and describes in detail the conductivity cell circuit. Preliminary conductivity, temperature and pressure data are presented from one of the potted CTD sensor boards.

The study of fine-scale linkages between foraging behavior and the physical microstructure of the marine habitat is essential for understanding the effects of environmental change on marine animals. Such studies will help to define the importance of physical water mass features, such as frontal systems, currents, eddies, or ice edges, to the distribution and abundance of many marine animals. In the past, acquiring fine-scale physical oceanographic measurements within foraging areas were constrained to two approaches: ship-based surveys or moored sensors. Both are expensive and only provide coarse-scale data that have a limited applicability to the foraging of marine animals.

Recently developed animal-borne biologgers have provided information about marine predator behavior while simultaneously monitoring the environment experienced by the swimming individual. CTD data loggers mounted on marine animals have been able to capture direct qualitative information on the physical
microstructure of the foraging environment and microhabitat (Lydersen et al., 2002, Hooker and Boyd, 2003, Biuw et al., 2007). However, the use of current CTD biologgers is limited by several factors including substantial size, high cost and/or low accuracy (Hyldgard et al., 2005). The development of a smaller, less expensive CTD biologger could provide the opportunity to study the physical microstructure encountered by small to medium-sized, free-ranging marine animals such as penguins, fish and reptiles.

Magellanic penguins (Spheniscus magellanicus) are central-place foragers that depart from and return to a single nest location. These penguins are opportunistic feeders that prey on small schooling fish that form dense concentrations which are highly associated with oceanographic structures such as fronts and tidal mixing zones (Williams, 1995). Recent satellite transmitter tracking (Platform Transmitter Terminals, PTT) coupled with remote sensing data has revealed important foraging behaviors dependent on oceanographic patterns (Figure. 11). It was shown that: 1) foraging locations coincide with tidal mixing fronts and 2) foraging trips showed a distinct pattern. These penguins meander until they reach the foraging area, spend time catching prey and then swim rapidly and directly back to the colony (Boersma et al., 2009).

This insight into the penguins foraging behavior is quite fascinating, but biologists could gain an even greater understanding if the tags used had multiple sensors that measured physical ocean parameters, behavioral and geolocation data simultaneously. Motivated by this need we designed a small, low-cost
biotag that measures conductivity, temperature, pressure, light, acceleration, magnetic fields, wet/dry and GPS.

Figure 11. Photo of a Magellanic penguin with a satellite PTT tag attached to its lower back.

Instrument Description

The biotag was designed to allow for accurate data acquisition at a required frequency and for long periods (up to 30 days) through optimally controlled power consumption while still conforming to minimum size and weight constraints. Since the device will be completely potted, to protect the device circuitry from the harsh ocean environment, with no connectors available to connect to the outside world, wireless data communication will be used for data retrieval and software upgrade. For the same reason, it was also designed to include an inductive battery charging mechanism with the intent to reuse the device for multiple missions. It was also desirable to make the device modular
and therefore reconfigurable by placing the various sensors and circuits, according to their functions (analog sensor, digital sensor, processor, etc.), on individual circuit boards. This allows for flexible configurations which are mainly dependent on the size and profile requirement of the animal species the device is to be deployed on. Another benefit of a modular design is the ability to readily add other sensors or capabilities (e.g. acoustic monitoring) at a later time by simply placing them on a new board that can then be easily attached to the biotag stack.

**System Boards Description.** The biotag prototype consists of modular circuit boards that can be rearranged into alternative configurations; i.e. the boards can be stacked in a single column or placed in a single or multiple rows of various arrangements. The initial prototype used in testing was single stacked but since penguins require a device with a lower and more hydrodynamic profile, a multi-column design was also created. The two board arrangements are shown in figure 12.
As can be seen in the previous figure and figure 13, the device is composed of six printed circuit boards, an interconnect board, and a rechargeable battery (a GPS antenna is also shown). Miniature push-on board-to-board connectors are used to interconnect the system boards. The only redesign required to change the biotag’s board configuration is in connector placement and the geometry of the interconnect board. Each board was designed according to its circuitry function and is specified as follows:

1. Processor/memory board (PMB) containing the PIC microcontroller, power management chip, flash memory and real-time-clock generator.
2. Digital sensor board (DSB) containing GPS, light sensor, accelerometer and magnetic compass.
3. Analog signal conditioning board which contains the signal filtering and amplification circuitry as well as the analog to digital (A/D) converter for the CTD sensor.

4. Wireless communications board (WCB) which holds the communications module for wireless data retrieval.

5. Battery/Charger board (BCB) holding the inductive charging coil and circuitry that connects to the rechargeable Li-ion battery. This holds also a magnetic reed switch which is used as a power switch to toggle the device on and off.

6. Conductivity/Temperature/Depth sensor board (CTDB) which is a flexible liquid crystal polymer (LCP) board that is comprised of the conductivity cell electrodes, a thermistor, pressure sensor and electrodes for wet-dry sensing.

At the heart of the biotag are the low power microcontroller and the power management chips. To minimize power consumption, most sub-systems can be kept in low power or turned off completely. All sensor boards have been designed with individual power lines so they can be powered on or off separately if their function is not required; e.g. the ASCB when the test animal is not under water. The GPS receiver also has a dedicated power control so that it can powered off while other digital sensors on the DSB are still able to operate during foraging dives. The power management hardware is also able to place the GPS and the WiFi modules in sleep mode. This is especially important for fast GPS data retrieval since we don’t want to power down the device between operations;
i.e. cold starts take significantly longer than warm starts. The combination of optimum hardware design and software control ensures that the time required to adequately power each sensor for accurate readings is balanced with the need to reduce the overall power draw to a minimum.

Currently, the biotag’s communication link will be made using a WiFi module. As stated before, wireless data retrieval as well as the ability to upload software changes is essential after the biotag has been completely potted (except for unpotted areas for the conductivity and wet-dry sensor electrodes). By placing the WiFi module in a separate board (the WSB), other communication options can be investigated and can readily be implemented on the wireless board. The ability to easily replace the communications device if needed is very desirable since technological advances constantly provide new wireless devices with improved performance and lower power consumption. Customer requirements can also dictate what communication scheme is necessary as tradeoffs between range, data rates, power consumption, ease of software implementation, etc. need to be considered.
Figure 13. A photograph of the two arrangements of the biotag. The “A” prototype is a single stack of the circuit boards and measures 50 x 25 x 25 mm and “B” is a multi-column stack measuring 85 x 25 x 15 mm.

Sensor Description. The penguin’s location while on foraging missions will be tracked using a GPS receiver with helical antenna. The advantage of using the helical antenna is that it could be connected directly to the GPS input without the need of an impedance matching network, which is generally required for chip antennas. The operating frequency of chip antennas also tends to suffer from detuning issues when covered with potting material. There was no noticeable change in GPS performance with a potted helical antenna when compared to an unpotted version during testing; cold/warm/hot starts were virtually identical. The antenna is placed at the top of the stack with a clear view to the sky to make sure that a GPS signal is found and locked on as fast as possible whenever the penguin surfaces in between foraging dives.

To further help understand penguin behavior while on diving missions, a three axis digital compass, a three axis digital accelerometer, and a light sensor
have been implemented in the biotag. These sensors can provide information on trajectory and diving behaviors while the animal under test is foraging for prey.

Temperature, pressure, and conductivity sensors will be used to collect data for salinity calculations. Salinity is an important parameter that has been shown to influence ocean dynamics and many physical ocean processes and therefore might also have an effect on penguin foraging behavior. All three sensors were placed in close proximity on the CTDB to provide in-situ temperature, pressure and conductivity readings. The close proximity assures that accurate salinity profiles can be attained.

Temperature is measured using a miniature surface mount thermistor (Murata NCP21XW223J03RA). A thermistor was chosen for its long-term stability, so that re-calibration due to drift would not be regularly required. Thermistors are resistive in nature, where the resistance changes inversely with temperature and require a bias voltage or current to operate. A four wire impedance measurement technique is used to monitor changes in voltage due to changes in temperature, after a bias signal is applied. The four wire circuit, meaning separate bias and sense lines are used, assures that any effects of parasitic lead resistance are eliminated during measurement. Since the resistance to temperature relationship of thermistors is inherently very non-linear, a resistor is also placed in parallel to provide improved linearity in the expected temperature range (-5 to 35 C).

The pressure sensing component consists of a miniature piezoresistive sensor cell (Keller Series 1 TAB) which provides an output voltage as a function
of absolute pressure and has a 200 bar maximum pressure range to correspond to the maximum expected operating depth of the biotag instrument (2000 m). This sensor also requires a bias signal for operation. The sensor cell uses its piezoresistive sensor elements in a Wheatstone bridge configuration to monitor changes in pressure. An excitation voltage is required to read the pressure variations seen by the device. The Keller pressure sensor also has the advantage that it is easily attached to the CTDB by surface mount soldering.

The conductivity cell is made up of four electrodes, and as was the case for the thermistor circuit, was designed to be measured using a four wire impedance measurement technique, consisting of two drive electrodes and two sense electrodes. A four-wire connection was particularly important in this application, since the c-cell board was connected to the signal conditioning board by a zero-insertion force connector, and not permanently connected to the analog board. The resistance at the connector could possibly change each time the device was assembled, and also could change over time (for example, as the potting material used to encapsulate the biotag became saturated with water).

Finally, a wet-dry sensor provides a binary signal telling the system whether it is in or out of the water to assure that any unneeded sensors such as the CTD are not consuming power when the penguin is not foraging for food under water. It should be noted that both the wet-dry sensor and the conductivity cell electrodes will not be covered with potting material during encapsulation since they need to make contact with the sea-water to operate properly.
Conductivity Circuit. While the CTDB’s thermistor and the pressure sensor only require a simple DC bias to operate, the conductivity cell demands circuitry that provides a bipolar alternating drive signal to the conductivity sensor. A bipolar signal assures that the sensor does not see a DC offset in the drive signal since a constant drive voltage or current can cause electrolysis at the electrodes which could possibly damage the conductors and introduce conductance measurement errors over long term operation.

An alternating signal with a sufficiently high frequency is required to overcome the presence of an electrolytic double layer capacitance (DLC) that appears at the electrode-to-water interface when the conductivity cell is subjected to a electric current or voltage. But the signal frequency needs to be low enough as not to cause impedance errors due to electrode-to-electrode parasitic capacitances. We found that using a 10 to 100 kHz drive signal assured that the measured impedance is dominated by the actual seawater conductivity. An operating frequency of 16 kHz was selected for the device, as this was low enough to allow a relatively low sample rate A/D converter to be used and is easily generated by the system microcontroller board.

For this prototype design we chose to use an alternating square wave for the conductivity cell’s drive signal. An accurate conductivity measurement can be made by measuring the high and low portions of the bipolar square wave signal at the sense electrodes, taking the difference and converting the result to a conductivity value. The sensed voltage levels of this type of signal can then be accurately sampled with the system’s A/D converter as shown in figure 14. An
advantage of this differential measurement technique is that any common mode voltages (noise, DC offset, drift, etc.) are cancelled out. It is advisable that the drive electrodes do not see voltage levels during the high and low phases of the square wave signal that are large enough to possibly cause conductor damage due to electrolytic effects.

![Diagram of Current and Voltage](image)

**Figure 14.** The conductivity cell’s drive current, which is a bipolar square wave signal, produces a square wave voltage at the output which can be sampled at the high and low phases.

So as not to overdrive the sensor, we decided to use a square wave drive signal with a fixed current magnitude since we know the expected conductivity range encountered during the logging mission. This expected range falls between approximately 2 to 70 mS/cm which corresponds to a measured resistance range of 16 to 554 ohms. We wanted to keep the drive voltage well below 50mV but high enough to overcome system noise at the higher
conductance levels. We therefore chose a drive current of 0.53 mA for the square wave amplitude. The conductivity cell’s square wave drive signal with fixed current magnitude is produced using the circuit shown in figure 15.

![Diagram of drive and readout mechanism](image)

**Figure 15. Simplified diagram of the drive and readout mechanism used for accurate conductivity measurements.**

The Linear Technology LT3092 programmable 2-terminal current source is used to provide the square wave’s fixed drive signal. As stated before, the output was set for a current of 0.53 mA. The bipolar square wave is produced by feeding the DC current into the inputs of a Texas Instruments TS3A24159 dual,
single-pull double-throw (SPDT) analog switch. This type of switch can be described as two 2:1 multiplexers in a single package that switch simultaneously when subjected to a control signal.

Each switch has two inputs with only one connected to their corresponding output at a time. The input lines can be toggled to the output using the control signal. The inputs of each switch are either labeled NO, meaning that the line is not connected to the output with no control signal, or NC, meaning that this line is connected to the output line also with no control signal present. By inserting the high side of the drive signal into the NO side of one switch and at the same time into the NC side of the other switch and simultaneously feeding the low side into the opposite inputs of each switch, and driving the switching device with a square wave control signal of desired frequency, a bipolar square wave with constant current amplitude can be created. The control source is a 16 kHz clock signal generated by the PMB’s RTC clock circuit. The switching mechanism is shown in detail in figure 16.

The output of the switching circuit is directed to the drive electrodes of the conductivity cell. The corresponding generated voltage at the sense electrodes due to the sea-water impedance is then buffered before it is read using a high precision instrumentation amplifier. Buffering of the sense lines is required to assure that the instrumentation amplifier does not cause any loading effects; i.e. no current is drawn from the sense electrodes. Besides providing gain to the low level sensed signal, the instrumentation amplifier also converts the bipolar signal to an alternating square wave centered at 1.25V, which is the required voltage
offset to assure that the A/D input signal is always centered within the converter's specified range, which is 0 to 2.5 V.

![Diagram of alternating bipolar drive signal](image1)

Figure 16. Illustration of how the alternating bipolar drive signal is produced by the conductivity sensor’s drive and readout circuit. Alternating the direction of the constant drive current though the use of the dual switches, produces a bipolar voltage across the sea-water resistance (which is the inverse of the measured conductance). This is represented by the output resistor in the diagram. The sensed voltage can then be read and sampled.

CTD Characterization

In previous work the conductivity cell, thermistor and pressure sensor were tested individually to determine their initial feasibility (Broadbent et al.,
The results demonstrated 95% confidence intervals for the conductivity, temperature and pressure sensors of ±0.083 mS/cm, 0.01 °C, and ±0.135 dbar, respectively. Presented here is the evaluation of the 4-electrode conductivity circuit designed for the low-cost conductivity cell and the soft potting gel used to encapsulate the thermistor and the pressure sensor.

**Conductivity Cell.** A conductivity curve was established for a fabricated conductivity cell using the 4-point circuit described in the conductivity circuit section of this document. The conductivity cell was calibrated using a standard seawater sample with a known salinity of 34.996 (IAPSO). The conductivity cell and a calibrated thermistor were submerged in the salinity standard and placed in a water bath where the temperature was varied from 32 to 2°C, respectively. For each temperature increment, 2500 conductivity voltages were measured, recorded and averaged. This was performed five times with a 10 second interval. The conductivity (mS/cm) at each temperature was calculated using the Electrical Conductivity Method formula (Clesceri et al., 1998). The five repeated conductance measurements were plotted against the corresponding conductivity (Figure 17).

The calculated coefficient of determination or R-square value of the averaged data points (0.9998) indicates good linear correlation between the measured (conductance) and predicted variable (conductivity). The repeatability of the conductivity cell was assessed by calculating the standard error for the five repeated measurements. The standard errors ranged from 0.0012 to 0.015 mS/cm. Assuming the distribution being measured is Gaussian, this would imply
that the 95% confidence interval of measurements was ±0.0024 mS/cm and ±0.029 mS/cm for the range of 30 to 60 mS/cm.

Figure 17. The conductance vs. conductivity of the conductivity cell for five replicate measurements.

Table 1 shows the errors in salinity measurement which would be expected to result from the observed errors in conductivity and temperature measurements. The table shows the salinity error for the extremes of temperature and conductivity measurable by the device, as well as for ‘typical’ 42mS/cm and 15C seawater. The errors range in magnitude from 0.014 to 0.089 salinity units, with the error for ‘typical’ seawater about 0.04 salinity units. This
error is an order of magnitude worse than that of a dedicated CTD probe, but we believe that this accuracy is unmatched for a miniaturized biologger device.

Table 2. Error from 95% CI Deviations in Temperature and Conductivity.

<table>
<thead>
<tr>
<th>Conductivity (mS/cm)</th>
<th>Temperature (°C)</th>
<th>Actual Salinity</th>
<th>Error in Measured Salinity</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>1.8497</td>
<td>0.0292</td>
<td>1.58%</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>0.9173</td>
<td>0.0142</td>
<td>1.55%</td>
</tr>
<tr>
<td>70</td>
<td>2</td>
<td>89.5461</td>
<td>0.0891</td>
<td>0.10%</td>
</tr>
<tr>
<td>70</td>
<td>30</td>
<td>43.0255</td>
<td>0.0338</td>
<td>0.08%</td>
</tr>
<tr>
<td>42</td>
<td>15</td>
<td>34.1675</td>
<td>0.0395</td>
<td>0.12%</td>
</tr>
</tbody>
</table>

Temperature Sensor. The CTD thermistor that was tested in the previous work was potted using a high gel re-enterable encapsulant (3M 8882) and then re-tested. The CTD board with the potted linearized thermistor was placed in a deionized water container in a water bath chamber and resistance measurements were acquired from 0 to 35 °C using a 6½ digit multimeter (Agilent 34410A). The water temperature was measured using a calibrated
thermistor with an accuracy of ±0.01 (US Sensor USP3201). Five roughly simultaneous readings (each reading being the average of 300 samples) were taken from the calibrated thermistor and the potted CTD thermistor at each temperature increment. A regression was performed on the potted CTD thermistor and 5 calibrated thermistor average readings were obtained at each temperature setting. The standard error (SEM) and 95% confidence interval (CI) for the repeatability of the thermistor (unpotted and potted) were calculated for each temperature and shown in Figure. 18. The achievable precision is expected to be somewhat better than the confidence interval measured for 35 ºC (0.052 ºC) for the potted because the rest of the readings indicate that a standard error of 0.008 ºC or better is achievable, giving a 95% confidence interval of about 0.015 ºC.
Figure 18. The calculated standard errors for the thermistor (unpotted and potted).

**Pressure Sensor.** A pressure sensor was potted using the same high gel re-enterable encapsulant (3M 8882) as the thermistor. The potted pressure sensor was tested identically to the previous experiments with the unpotted pressure sensor, where the tests were conducted using a small pressure chamber, where the internal air pressure was monitored with a highly accurate pressure gauge (±0.25%, 0 to 75 psi). The voltage measurements for 0 to 50 dbar were recorded and graphed along with previous data from the unpotted pressure sensor (Figure 19). The linear regression data showed excellent correlation between the measured (mV) and the predicted (dbar) variable with an R-square value of 0.9996 for the potted and 0.9999 for the unpotted. The sensor
sensitivity was slightly changed by the high gel encapsulant from 1.9318 to 1.9192 mV/dbar for the range of 0 to 50 dbar.

![Graph of measured voltage vs pressure](image)

**Figure 19.** The linear regression of the unpotted and potted pressure sensors with trendlines.

**Conclusions**

In this work we continue to develop and test a small, low-cost CTD biotag for mid-sized marine animals. The biotag consists of modular circuit boards that can be reconfigured into several packaging schemes and two different layouts were presented in this paper. Also described in this paper was the 4-electrode conductivity cell circuit which uses a bipolar square wave signal that produces a voltage output.
Preliminary individual sensor results have verified that the sensors performed as intended. The conductivity cell, using the designed conductivity circuit, performed better than the preliminary tests using electronic instruments to generate the square wave functions. The initial 95% confidence interval was ±0.083 mS/cm and the circuit generated one was ±0.029 mS/cm.

Initial laboratory experiments showed that the high gel re-enterable encapsulant used to protect the thermistor and pressure sensor from saltwater performed as intended. The results for the thermistor were relatively the same for both tests, unpotted and potted, where the 95% confidence intervals were 0.01 °C and 0.015 °C, respectively. The pressure sensors (unpotted vs. potted) were compared by their individual sensitivities (gradients) which were highly consistent at 1.9318 to 1.9192 mV/dbar, respectively.

The CTD sensor package with the high gel encapsulant would offer useful, precise CTD and salinity information for marine biogger applications. Future work intended includes continued sensor characterizations, determination of salinity measurements, instrument underwater packaging and field tests.
Chapter 4: A Miniaturized CTD-tag for Evaluating Use of Physical Structure by Mid-sized Marine Predators

Abstract

Physical characteristics of the ocean such as salinity and temperature, and biological information such as foraging depths and times, have been collected by using animal-borne data loggers mounted on large marine predators. However, salinity measurements have yet to be incorporated into smaller tags for mid-sized predators. We have developed a small, low-cost instrument that is capable of measuring conductivity, temperature, and depth along with behavioral and geo-location data during foraging trips. The design of the instrument has been optimized for deployments on medium-sized marine predators; its dimensions are 100 mm x 40 mm x 20 mm. In this paper we discuss one design of the new biotag including conductivity, temperature, and depth calibrations, laboratory comparison tests, and initial field demonstrations. Calibration data and laboratory tests showed that the device is capable of measuring salinity accurately, with precision similar to commercial oceanographic instruments. Comparisons of data generated by the leading oceanographic CTD with a CTD profile generated by the new biotag showed similar conductivity and depth; however there was a delay associated with the packaged thermistor data, a problem that can be addressed by repackaging. Animal-borne field trials were
conducted on loggerhead sea turtles (*Caretta caretta*). Overall, the initial proof-of-concept work described here is quite encouraging. It shows that with slight modifications, the recently developed miniaturized, inexpensive biotag will be able to capture oceanographic and biological data while attached to marine predators in the size range of many diving seabirds.
Introduction

The study of fine-scale linkages between foraging behavior and the physical microstructure of the marine habitat is necessary for understanding the effects of environmental change on marine predators. Large diving marine predators have been used to collect data on ocean conductivity, temperature and depth to determine such links, but smaller animals have been largely overlooked (Lydersen et al., 2002, Hooker and Boyd, 2003, Bailleul et al., Biuw et al., 2007, Charrassin et al., Costa et al., Boehme et al., 2008). The use of CTD data storage tags for smaller marine predators has been limited by several factors including substantial size, high cost, reduced salinity range and/or low accuracy (Fedak, 2004, Curtis and McGaw, 2007, Birkelund et al., 2011). In this work we demonstrate the capabilities of a miniaturized CTD sensor system that can be used on mid-sized marine predators such as turtles, fish, and seabirds. The multi-sensor system, or biotag, measures conductivity, temperature, pressure, light, acceleration, magnetic fields, wet/dry, and GPS location. It is also equipped with a wireless module for communication and a rechargeable battery for multiple deployments. To minimize cost and maximize salinity accuracy, a CTD sensor board was fabricated using printed circuit board (PCB) techniques on a liquid crystal polymer substrate (LCP). The sensor board consists of a novel conductivity cell, thermistor, piezoresistive pressure module and a wet/dry sensor. However, the circuit boards are of a modular design so that prototypes for different marine species can be packaged. In this work we focused on conductivity, temperature and depth measurements and GPS location. Biotag
design, calibration, sensor characterization and CTD field trials of these systems are described here.

We describe field deployments of the biotags on loggerhead sea turtles \textit{(Caretta caretta)} at Casey Key, Florida. Female loggerhead sea turtles breed and nest in the vicinity of their natal beach between May and August along the western coast of Florida. During this time the females will come ashore for approximately 1 hour to lay eggs and then return to the sea for a period of 14 days, repeating this cycle 3 to 7 times over the summer. Instruments can be attached and recovered from these animals to record at-sea data during internesting periods (periods between nesting events in a single season). These animals are ideal for new instrument testing because nesting occurs on highly accessible beaches, internesting periods last days, females return to predictable locations, and several instruments can be attached, providing comparison data. Previously developed data-storage tags data have captured the diving and surfacing behavior of female loggerhead sea turtles during their inter-nesting period (Houghton, 2002, Hays et al., 2007, Sobin and Tucker, 2008) with some studies suggesting that immature loggerhead turtles are limited to water masses with certain physical characteristics (Carreras et al., 2006, Revelles et al., 2008) and that water column profiles influence juvenile diving behavior (Howell et al., 2010). However, none of the previous studies have instrumented loggerhead turtles with a CTD device to measure ocean salinity.
Methods and Materials

CTD-tag Packaging. The biotag was designed to acquire physical and biological data while attached to mid-sized marine predators. It was equipped with a conductivity cell, thermistor (NCP21XW223J03RA, Murata Manufacturing Co., China), pressure sensor (Series 1 TAB, Keller America, USA), 3-axis digital accelerometer (ADXL345, Analog Devices, USA), 3-axis digital compass (HMC5843, Honeywell, USA), light sensor (ISL29003, Intersil, USA), wet/dry sensor, GPS receiver (MN5010HS, Micro Modular Technologies, Singapore) and helical antenna (SL1300, Sarantel, UK). In previous works we described the fabrication of a novel single substrate, miniature, low-cost conductivity, temperature, depth sensor board (CTDB) with interconnects, the conductivity cell’s drive circuit, the system boards description and initial soft-gel potting of the thermistor and pressure sensor (Broadbent et al., 2010a and b). In this work we describe the initial underwater packaging concept of the system for animal-based deployments (Figure 20).

Figure 20. A schematic representation of the biotag showing the streamlined and smoothed contour along with the internal circuit board layout. The circuit boards were arranged in a multi-stack elongated configuration.
A compact, hydrodynamic and robust packaging scheme that allowed for direct exposure to the surrounding environment for the conductivity cell while isolating the internal system was needed. We used two packaging strategies for the initial prototype that included a soft-gel (8882 High Gel Re-enterable Encapsulant, 3M, USA) and a urethane designed for low moisture sensitivity (80A Liquid Urethane, Forsch Polymer Corp., USA). Since salinity determinations require very accurate and highly sensitive measurements, the soft-gel potting material was used on the CTDB to expose the conductivity cell to the surrounding seawater while protecting the adjacent thermistor and pressure sensors. An O-ring was used on the potted CTDB to ensure soft gel isolation and a secure fit in the mold. Once potted with the soft gel the flexible CTDB was connected to the analog signal conditioning board (ASCB). The system boards were then placed in an aluminum mold which was filled with the liquid urethane material.

In aquatic animals, the mass of the device is considered less important than its fluid dynamics (Wilson et al., 1991). In order to reduce tag–induced turbulence and drag on the marine predator the shape of the biotag was hydrodynamically streamlined (Bannasch et al., 1994). The internal circuit boards were arranged in an elongated, multi-column stack to decrease the height of the device. The area where the internal helical GPS antenna was situated was rounded and tapered and the contour was smoothed and minimized by tapering the front end using a glass bead-filled urethane. The terminal end was
shaped with a relatively sharp edge. The overall dimensions of the packaged biotag were 100 mm x 43 mm x 24 mm and it weighed 104 g (Figure 21).

Figure 21. A photo of a packaged biotag with the front end tapered for increased hydrodynamic effect. Visible within the O-ring is the CTD sensors encapsulated in the soft-gel material.

**Calibration.** Once the biotag was packaged, the conductivity, temperature and pressure sensors were calibrated independently. Conductivity calibration tests were conducted in a refrigerated bath/circulator (NESLAB RTE 7, Neslab Instruments Inc., USA) using an International Association for the Physical Sciences of the Oceans (IAPSO) standard seawater sample (Ocean Scientific International Limited, England). The conductivity calibration procedure for a packaged biotag entailed taking 300 repeated measurements of the standard seawater sample (S = 34.995, K_{15} = 0.99987) at eight different temperatures (32 to 0 °C) in order to vary its conductivity. Temperature measurements were determined using a calibrated thermistor probe (USP3021, U.S. Sensor Corp, USA) with an accuracy of ±0.01°C and a range of -20 to 70
°C. The conductivity (mS/cm) at each temperature was calculated using the Electrical Conductivity Method formula and plotted against the average measured conductance (mS) of the conductivity cell for all replicates. Temperature calibrations were conducted using the same circulating refrigerated water bath and thermistor probe by taking 300 repeated measurements at six different temperatures (35, 28, 21, 14, 7, 0 °C). Pressure calibrations were performed in a water pressure chamber with a calibrated digital pressure gauge with an accuracy of ±0.25% (MG-9V, SSI Technologies, USA). Pressure measurements were taken every 3 seconds for 2 minutes at nine different pressures (0 to 160 psi).

**Laboratory Tests.** Salinity comparison tests were conducted after calibrations against a calibrated 5-electrode conductivity cell with thermometer (Conductivity/Salinity Adapter CSA-1250 and Automatic Thermometer Bridge ATB-1250, Neil Brown Instrument Systems, USA) and an inductive style conductivity cell with thermistor (XR-420 CTD, RBR Ltd, USA). Comparison tests were conducted in plastic tanks equipped with stirrers containing water at five different salinities (approximately 8.96, 16.82, 23.89, 31.40, 40.32) at room temperature. Instruments were equilibrated to the salinity for 15 minutes then approximately simultaneous measurements of conductivity and temperature were recorded from all three instruments. The biotag was programmed to average 300 samples per measurement. Salinity was determined using the Practical Salinity Scale 1978 (Lewis, 1980).
Field Trials

CTD profile Tests. Trial CTD and GPS location deployments of the biotag were conducted in the DeSoto Canyon, Gulf of Mexico (28° 40’23.51”N, 87° 43’50.18”W) aboard the RV Weatherbird II during September 2011. Five vertical water column CTD profiles to 100 meters of depth were collected during the cruise. The biotag was attached to a rosette sampler which was equipped with a commercial CTD (Sealogger CTD SBE 25, Sea-Bird Electronics, Inc., USA) for comparison. The biotag was programmed to acquire GPS location when dry every 30 minutes and CTD measurements every 5 seconds. The Sealogger CTD sampling rate was every 0.25 seconds. The rosette was lowered to a depth of 100 meters at a rate of approximately 20 meters per minute and either returned immediately to the surface (4 times) or stopped at depth for 5 minutes (1 time) to acquire a steady comparable CTD profile. Data sets from the biotag and CTD instrument were analyzed using Microsoft Excel.

Turtle Deployments. Conductivity, temperature, depth and daily activity patterns were documented by deploying biotags on nesting female loggerhead turtles. These tags were programmed to record conductivity, temperature, depth and GPS location. The objective of the turtle study was to evaluate the robustness of the biotag while attached to a marine predator and to capture the correlation, if any, between daily activities and physical microstructure that might influence surface duration and/or timing. Additional commercial instruments were attached to the turtles and included a data storage tag (DST Milli-F, Star-Oddi, Iceland), GPS datalogger (Model F1G, Sirtrack, New
Zealand), an ARGOS transmitter (Kwisat, Sirtrack, New Zealand) and a solar powered geomagnetic tag (Sea Tag Geo, Desert Star, USA). Furthermore, a Coastal Ocean Monitoring and Prediction System (COMPS) offshore buoy maintained by University of South Florida was stationed near the deployment site (27° 10' 1.40" N, 82° 55' 2.00" W), providing baseline data with which to compare salinity results obtained from the turtles. The buoy was equipped with a Seabird Electronics, SBE-37SM (RS-485) MicroCat that was positioned at 1 meter of depth deep.

Deployments were conducted on two female loggerhead sea turtles (Mishell2 and Wiblet4) at Casey Key (27° 08’54.06” N, 82° 28’35.66” W), a barrier island on Florida’s southwestern coast, during June 2011. Loggerhead turtles were selected for the biotag deployments because of high instrument recovery opportunities. Nightly tagging patrols (conducted by Mote Marine Laboratory Sea Turtle Conservation and Research Program) using VHF radio transmitters and satellite tag data were used to locate the turtles on shore. Selection of females was based on prior known nesting history and a history of site fidelity to the respective island. After oviposition was completed, turtles were held temporarily in a portable plywood corral to facilitate the instrument attachments. The carapace was cleaned of epibiota and wiped with alternating washes of fresh water and alcohol to ensure dryness. The ARGOS transmitter was affixed to the carapace with two part epoxy (Powers) that was smoothed into a hydrodynamic shape. The DST, GPS datalogger, geomagnetic tag and biotag were attached to the carapace with epoxy putty (EP-200, RectorSeal, USA) for easier removal.
The application process took 1-2 hr to complete. The box was removed and the turtle resumed its departure to the sea.

The biotag was programmed to record GPS position data when out of water every 30 minutes and conductivity, temperature, and depth readings every 5 seconds when in water. In addition, the biotag was equipped with an additional external battery pack that would allow for a 16 day sampling period. The time stamp on the biotag was taken from the initial GPS location lock from the satellites in UTC and then converted to Eastern Standard Time (EST). Raw CTD data acquired from the biotag (mS, kΩ, V, respectively) were converted to conductivity (mS/cm), temperature (°C) and depth (m) using calibration equations. Data sets from the biotag and DST were analyzed with Matlab and Microsoft Excel. GPS and ARGOS locations were plotted using Google Earth.

Results

**CT and Salinity Comparison Tests.** Accuracies of the conductivity and temperature instruments used in comparison tests were ±0.0025 mS/cm and ±0.0025 °C (5-electrode conductivity cell with thermometer) and ±0.003 mS/cm and ±0.002 °C (inductive conductivity cell with thermistor). Conductivity, temperature and salinity comparisons between the biotag and the comparison instruments were recorded and calculated as percent difference and then averaged to indicate the overall accuracy or mean percent difference of the biotag with respect to the commercial instruments. The mean percent differences between the biotag and the commercial instruments (Neil Brown and RBR) were 0.38% and 0.47% for conductivity, 1.24% and 1.17% for temperature
and 1.06% and 0.78% for salinity, respectively. The percent differences in salinity measurements between the biotag and the commercial instruments ranged from 2.12% at 8.96 to 0.13% at 40.32 (Table 3). The statistical data showed that the miniature, inexpensive 4-electrode conductivity cell performed closely to the more expensive commercial 5-electrode and inductive conductivity sensors, but the performance of the soft-gel thermistor was not as close to the commercial temperature sensors. This result will be addressed further in the discussion section. The overall results of the salinity measurements indicated that the biotag performed well when compared to the more expensive commercial instruments over the entire salinity range.

Table 3. Percent Differences Between the Biotag and Commercial Instruments at Several Salinities.

<table>
<thead>
<tr>
<th>Salinity (measured by Neil Brown)</th>
<th>Percent Difference (%) between Biotag and Neil Brown</th>
<th>Percent Difference (%) between Biotag and RBR</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.960</td>
<td>2.12</td>
<td>1.86</td>
</tr>
<tr>
<td>16.818</td>
<td>0.79</td>
<td>0.45</td>
</tr>
<tr>
<td>23.888</td>
<td>1.05</td>
<td>0.80</td>
</tr>
<tr>
<td>31.397</td>
<td>0.93</td>
<td>0.63</td>
</tr>
<tr>
<td>40.316</td>
<td>0.40</td>
<td>0.13</td>
</tr>
</tbody>
</table>
**CTD Profile Deployments.** Conductivity, temperature, and depth profiles were recorded for the biotag and the Sealogger CTD SBE 25 instrument. GPS coordinates from the sampling sites were captured from the biotag and agreed precisely with the ship’s GPS instrument. The compared conductivity and depth profiles showed highly consistent trends in measurements between the two instruments (figures 22a and 22c). The temperature profiles showed a significant thermal delay of approximately 150 seconds in the soft-gel potted thermistor when compared to the commercial instrument (Figure 22b).

Pre and post-deployment comparisons between the biotag and an RBR CTD instrument were performed using a seawater sample and the absolute differences in salinity were recorded as 0.34 and 0.20, respectively.
Figure 22. Conductivity (a), temperature (b) and depth (c) profiles recorded in the Gulf of Mexico September 2011. The blue solid line represents measurements from the Sealogger CTD SBE 25 instrument and the red dotted line represents the biotag measurements.
**CTDs on Loggerhead Turtles.** The two biotags were recovered from the instrumented loggerhead turtles after time periods of 12 and 22 days. The latter tag remained on the turtle (Mishell2) for two consecutive internesting periods and had exhausted its battery capacity. The first tag recovered (Wiblet4) performed well in terms of power consumption, but suffered damage to the soft-gel that encapsulated the CTD flexible board. A measurable slit was detected in the soft-gel material which allowed seawater contact with the conductivity, temperature and depth electric leads. The second tag had observable biofouling on the CTD sensors due to the loss of battery power and lengthened deployment period. CTD and GPS data were recovered from both tags, but no post hoc calibrations could be performed due to the wear and tear on the sensors; therefore the CTD values presented are absolute.

The instrumented turtles made many dives during the data sampling period, demonstrating the ability of the biotag to record CTD profiles. GPS locations from the biotag were captured only when the turtles were on the beach; except for one from Mishell2 (27º 07’59.04” N, 82º 28’51.18” W) 1 hour 8 minutes after leaving the beach (Figure 23, labeled M2_3). The tag with the damaged soft-gel had approximately 12 hours of CTD profile data before the seawater caused interference with the electrical connections and caused erratic depth recordings.
Figure 23. Satellite track from the ARGOS transmitter of the two turtles tagged (courtesy of Dr. Tony Tucker, Mote Marine Laboratory). Mishell2 is shown in Red and Wiblet4 is green. The orange and green markers represent GPS locations acquired by the GPS unit on the biotags. The location of the COMPS offshore buoy used for baseline comparison CTD data is shown by the yellow marker.

The conductivity cell and thermistor performed well for approximately 4 days. The second tag had suffered damage to the internal flash memory chip, but data were recovered by extracting and re-wiring the chip to a new circuit board. The data recovered showed CTD profiles for the first 8 hours of the deployment and then intermittently saved data for 8 additional days. The data retrieved from the turtles showed GPS coordinates of where the turtles were tagged, individual diving and surfacing behaviors, as well as ocean conductivity and temperature. The two CTD data sets collected revealed that each turtle exhibited an individual and different dive profiles (Figure 24a and 24b), even though their number of dives were almost identical. During the first 24 hours
both turtles were most active within the first four hours of leaving the beach and then began surfacing only once or twice an hour (Figure 25).

Salinity data from the COMPS offshore buoy were reported hourly and conditioned using a 3h low-pass filter. Random daily salinity measurements from the biotag were compared to the COMPS buoy and resulted in a 0.62 average salinity difference.

Salinity and temperature measurements at depths greater than 1.5 meters were reported for the first four days of Wiblet4’s internesting period (Table 4). The data showed that the ocean salinity and temperature experienced by the turtle varied from 30.13 to 35.39 and 25.57 to 31.27 °C, respectively. The depth-salinity plot showed that the turtle spent time throughout the water column with a maximum depth of 20.44 meters; days 4 and 5 were spent mostly at depth (Figure 26). Investigations of the physical characteristics experienced by the turtle showed that the salinity structure of the water column was not as well mixed as expected. On days 4 and 5 (June 7 and 8) the turtle experienced a broad range of salinities (30 to 34) at depth (Figure 27), even though she was located at different coordinates during that time period (Figure 28). The salinity range experienced by the individual turtle could be due to a near shore salinity front formed by a recent rain event (2.30 inches within 2 weeks) or by upwellings (i.e. underground springs) mixing freshwater into the seawater column.
Figure 24. Conductivity, temperature and depth data acquired by loggerhead turtles. (a) Shows the first 12 hours after Wiblet4 left the beach. (b) Shows the first 8 hours after Mishell2 left the beach.
Figure 25. Time course of diving activity after leaving the nesting site.

Table 4. Field Deployment of a Biotag on a Loggerhead Turtle (Wiblet4) June 2011.

<table>
<thead>
<tr>
<th>Date</th>
<th>Number of dives</th>
<th>Maximum depth (m)</th>
<th>Distance from beach (km)</th>
<th>Temp range (°C)</th>
<th>Salinity range</th>
</tr>
</thead>
<tbody>
<tr>
<td>05 Jun</td>
<td>87</td>
<td>14.26</td>
<td>15.78</td>
<td>26.40-29.86</td>
<td>30.84-35.17</td>
</tr>
<tr>
<td>07 Jun</td>
<td>16</td>
<td>20.45</td>
<td>30.60</td>
<td>25.57-30.66</td>
<td>30.62-35.28</td>
</tr>
<tr>
<td>08 Jun</td>
<td>18</td>
<td>20.44</td>
<td>26.55</td>
<td>26.28-31.27</td>
<td>30.16-34.92</td>
</tr>
</tbody>
</table>

Number of dives were calculated using TDR and biotag conductivity data. Maximum depths were acquired from TDR data. Distance from beach is the straightline distances acquired from ARGOS tag. Temperature and salinity ranges were from >1.5 meters depth.
Figure 26. Salinity vs depth for a four day period recorded from a loggerhead turtle (Wiblet4).

Figure 27. Time measurement of the salinity encountered by the turtle from June 5-8th.
Figure 28. ARGOS data locations of the turtle for the 4 day period (June 5-8th). Courtesy of Dr. Tony Tucker, Mote Marine.
Discussion

The data storage tag described here is the first to incorporate an effective miniature low-cost conductivity cell with commercially available transducers (thermistor and pressure sensor) to capture oceanographic and biological data while attached to marine predators. The conductivity cell and pressure sensor performed well in laboratory tests and field trials when compared to more expensive commercial instruments. The thermistor performed well in laboratory tests, but exhibited a slow response time during field trials. The delayed temperature response relative to that of the conductivity cell can cause incorrect calculations of salinity. In addition, during animal-borne deployments packaging issues caused sensor and electronic component failures. The work demonstrates that the packaging scheme requires further modification.

The problem with thermistor response time is readily improved by packaging the thermistor in a denser or thinner material that exhibits a higher thermal conductivity ($\kappa$) than the soft-gel. The soft-gel applied to the thermistor in the present packaging scheme was approximately 2.5 mm in thickness and acted as a thermal insulator that dampened the system, thus delaying equilibration.

The instrument packaging material, a urethane, caused electronic component failures. The internal flash memory chip was placed on the circuit board using a ball grid array (BGA) and due to the elasticity of the urethane the solder balls flexed when mechanical stress (pressure) was applied and released. This mechanical stress issue can be overcome by using a harder packaging material such as an epoxy. Virtually all of the packaging problems may be
resolved by constructing a mold that segregates the conductivity cell and pressure sensor while encasing all other components within a hard epoxy.

Despite the shortcomings generated by the present packaging strategy, the biotag was able to capture several days of oceanographic and biological data. The data showed that the turtles were most active during the first 4 hours after leaving the nesting site and became less active as they traveled in the coastal waters. During days 3 and 4 the turtle exhibited only one individual dive per hour with most of the time spent at a depth of 20 meters. The salinity data collected showed some highly interesting physical water mass features encountered at depth by the turtle, although it was difficult to determine if there was a correlation between daily activity patterns and the ocean’s physical characteristics. However we have shown that salinity data collected with the biotag have the potential to be a beneficial tool for loggerhead turtle research. Several studies have suggested that physical features such as frontal systems, mesoscale eddies, and prevailing currents, can influence juvenile turtle behavior (Carreras et al., 2006, Revelles et al., 2008, Howell et al., 2010). This biotag, when attached to smaller, juvenile turtles has the potential for yielding important oceanographic and biological data.

Currently only two CTD data storage tags are available commercially, CTD tag (SMRU Instrumentation, UK) and DST CTD (Star-Oddi, Iceland). The size of the CTD tag is 120 mm x 85 mm x 60 mm (excluding antenna) with a weight of 600g. It utilizes a commercial inductive conductivity device, which can be limited by near-field effects causing erratic measurements. The DST CTD
dimensions are 15 mm x 46 mm (diameter x length) and weighs 21g. It has a two electrode cell conductivity sensor which is not highly sensitive or stable over time. The biotag discussed in this paper is 100 mm x 43 mm x 24 mm and weighs 104g. It has a four electrode conductivity cell which is highly stable and sensitive. Additionally incorporated within the tag are several digital sensors; an accelerometer, a compass and a light sensor, which were not utilized for this paper. In sum, the new biotag has the potential to provide valuable environmental data to both oceanographers and biologists that other tags cannot, either because of size, cost, or sensor limitations.

Conclusions

We have shown that the newly developed multi-sensor system described here has the potential to collect conductivity, temperature, depth and salinity measurements during animal-borne deployments. In addition, we have shown how important individual sensor packaging is to the overall integrity of the system. Comparisons with current commercial CTD tags, suggest that there is a need for a smaller, low-cost instrument as an additional tool suited for the collection of environmental data. Through experimentation and loggerhead turtle deployments we have shown that the miniaturized system described here has the potential to collect valuable physical and biological information while attached to smaller, mid-sized marine predators.
Summary

The ability to instrument mid-sized marine predators and actively record biological and oceanographic data requires technological advancements in miniaturization and sensor fabrication. Biologging instruments have become reliable and useful tools for large marine predator research, but not for smaller animals such as seabirds. Therefore a miniaturized multi-sensor biotag was developed at the University of South Florida to determine physical ocean structure by mid-sized marine predators. This work produced a novel miniature rigid/flex salinity measurement device which was integrated with additional sensor to construct the multi-sensor biotag. Sensor characterizations and biotag evaluations were conducted in the Gulf of Mexico and enabled some of the first CTD observations while attached to loggerhead turtles.

The multi-sensor biotag discussed in this dissertation is the first to incorporate an effective miniature low-cost conductivity cell coupled with inexpensive commercially available transducers to capture oceanographic and biological data while attached to marine predators. Despite the packaging shortcomings of this initial proof- of-concept prototype this work is encouraging and with future refinement has the potential to become a useful tool for biologists and oceanographers.
References Cited


Hunt, R., 1995. How to increase the accuracy of solution conductivity measurements, Sensor Development Inc.


Appendices
Appendix 1: Biotag System Specifications

Table A1. Biotag Characteristics and Specifications.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conductivity</td>
<td>Range: 0-70 mS/cm</td>
</tr>
<tr>
<td></td>
<td>Accuracy: ~ 0.0161 mS/cm</td>
</tr>
<tr>
<td>Temperature</td>
<td>Range: 0-35°C</td>
</tr>
<tr>
<td></td>
<td>Accuracy: ~ 0.012 °C</td>
</tr>
<tr>
<td>Pressure</td>
<td>Range: 0-110 dbar</td>
</tr>
<tr>
<td></td>
<td>Accuracy: ~ 2.0%</td>
</tr>
<tr>
<td>size</td>
<td>100 mm x 43 mm x 24 mm</td>
</tr>
<tr>
<td>Weight</td>
<td>~ 104 g</td>
</tr>
<tr>
<td>Additional Sensors</td>
<td>3-axis accelerometer, 3-axis compass, light, wet/dry, GPS</td>
</tr>
<tr>
<td>Battery</td>
<td>Rechargeable: 5-10 days</td>
</tr>
</tbody>
</table>

Figure A1. Image of the packaged biotag with sensors labeled.
Appendix 2: Biotag Response to Biofouling

Biofouling is one of the primary limiting factors in terms of sensor measurement accuracy and deployment longevity for autonomous oceanographic instruments. Once an instrument, including biologging tools, is immersed in a marine environment biological growth begins. Therefore, an experiment to determine the effect of biofouling on the performance of the exposed conductivity cell was performed.

Two biotags were deployed in Bayboro Harbor, St. Petersburg, Florida for a period of 14 days. One biotag was used as a control (turned off) and another was recording CTD data every 5 seconds. The biotags were monitored daily for biological growth via photographs. The conductivity and temperature sensors were calibrated prior to deployment and salinity measurements were taken after the field trial using an IAPSO standard seawater sample (S= 34.995).

As indicated by figures A2 and A3, biological growth occurred on the control conductivity electrodes; whereas no growth was observed on the active (electrified every 5 seconds) conductivity electrodes. The post- deployment salinity measurements remained accurate with a difference of 0.3 from the standard.

As a preliminary test it has been shown that taking a conductivity cell measurement every 5 seconds (release of short electrical pulses across the electrodes) can reduce the amount of biofouling to an exposed sensor in a marine environment. This application has been shown to immobilize nuisance
species as a method to prevent biofouling (Abou-Ghazala and Shoenbach, 2000).

Figure A2. An image of the control conductivity cell with biological growth after a 14 day deployment.

Figure A3. An image of the experimental conductivity cell after a 14 day deployment. The image shows that very little growth accumulated on the biotag and no growth was seen on the conductivity electrodes.
Appendix 3: Practical Salinity Scale 1978

(Lewis, 1980)

\[ S = a_0 + a_1 R_{t}^{1/2} + a_2 R_{t} + a_3 R_{t}^{3/2} + a_4 R_{t}^2 + a_5 R_{t}^{5/2} + \Delta S \]

Where \( \Delta S \) is given by

\[ \Delta S = [t - 15/ 1 + 0.0162 (t-15)] (b_0 + b_1 R_{t}^{1/2} + b_2 R_{t} + b_3 R_{t}^{3/2} + b_4 R_{t}^2 + b_5 R_{t}^{5/2}) \]

And:

\[
\begin{align*}
    a_0 &= 0.0080 \\
    a_1 &= -0.1692 \\
    a_2 &= 25.3851 \\
    a_3 &= 14.0941 \\
    a_4 &= -7.0261 \\
    a_5 &= 2.7081 \\
    b_0 &= 0.0005 \\
    b_1 &= -0.0056 \\
    b_2 &= -0.0066 \\
    b_3 &= -0.0375 \\
    b_4 &= 0.0636 \\
    b_5 &= -0.0144
\end{align*}
\]

Valid from \( S = 2 \) to \( 42 \), where:

\( R = C \text{ (Sample at } t) / C \text{ (KCl solution at } t) \)
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About the Author

Heather A. Broadbent grew up in Michigan, Florida and Tennessee. She earned a B.S. degree in Biology from Eastern Michigan University and then moved back to the “Sunshine State” in 1995. In 2000 Heather began working at the University of South Florida’s Center for Ocean Technology developing miniature ocean sensors. She greatly enjoyed combining technology with oceanography and received a M.S. in Marine Science from USF in 2005.

Heather began developing biotags for marine predators in 2007 and entered the Ph.D. program in 2008. During her academic pursuits, Heather has been supported financially by the Office of Naval Research, C.W. “Bill” Young Fellowship and the Sanibel-Captiva Shell Club Mary & Al Bridell Memorial Fellowship. These academic awards have afforded Heather many valuable professional opportunities such as domestic and international conferences, publications, penguin field research in Argentina and a research cruise in the Gulf of Mexico.