Invertebrate Biology 126(3): 279–286.

DOI: 10.1111/j.1744-7410.2007.00098.x

A novel device to monitor small changes in underwater distances

Theodore A. Uyeno[1,a] and Henry S. Hsiao[2]

[1] Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599-3280, USA
[2] Department of Biomedical Research, University of North Carolina, Chapel Hill, North Carolina 27599-7575, USA

Abstract. This study describes a technique to record small (1 cm) distance changes or movements occurring in seawater using a simple electronic circuit and miniature ball electrodes. This low-cost technique was designed to directly measure such changes in a broad range of experiments involving small marine invertebrates while minimizing the mechanical loading of the structures of interest. The circuit detects and amplifies small changes in the resistance of seawater between two electrodes as the distance between them varies; these are then converted to voltage changes. After calibrating the output of the device with known distance measurements, it was evaluated using a test organism by monitoring and recording the body flushing behavior of the sea squirt, *Styela plicata*. Electrodes were sewn to the tunic at the base of the atrial siphon and changes in its diameter were recorded >24-h periods. Using ~3-cm-tall sea squirts, the distance between the electrodes expanded and contracted with a range of 5.5 mm during rhythmic flushings. Flushes occurred on average every 4.1 min and showed a rapid initial contraction, followed by a slower expansion while refilling. Attaching electrodes to the tunic had little mechanical or behavioral effect; the untouched control specimen had similar flushing rates. The movement monitor circuit is stable, sensitive, and performed well in full-strength seawater experiments where good spatial and high temporal resolution, low inertial loading, and low noise were required. The technique, as shown here, can be used to record a wide range of animal movements and further suggested modifications of the circuit may suit a broad range of other experimental situations.

Additional key words: measurement, movement, behavior, electronic distance

A frequent difficulty in testing biomechanical functions in marine invertebrates is the accurate and precise measurement of small linear distances over time. Here, we present an electronic circuit design for recording such data with low construction costs and good spatial and temporal resolution. We tested this technique by monitoring the flushing behavior of the leathery (or pleated, or rough) sea squirt, *Styela plicata* LESUEUR 1823, as quantified by measured changes through time in the base diameter of the atrial siphon (Fig. 1).

Directly monitoring changes in distance in seawater can be technically challenging. The chosen technique must be compatible with the high conductivity of seawater. It must minimize the mechanical loading of structures of often small or soft-bodied invertebrates. In addition, it should not interfere with other techniques such as electromyography or pressure measurements. Previously used techniques include a number of methods: sonomicrometric, magnetic, optic, mechanical, and electric. As reviewed below, each has limitations.

Ultrasonomicrometry is a common technique that uses piezoelectric crystal transducers. Distance is measured by recording the time taken by ultrasonic waves to travel between crystals. This technique offers high temporal and spatial resolution, is commercially available (e.g., Sonometrics Inc., London, ON, Canada), and may be configured to record from multiple probes. However, the transducers are often large and require special placement and difficult directional positioning. In addition, commercial systems are expensive and their compatibility with other instrumentation, especially electromyography, must be well understood. Platt et al. (1998) noted that the amplitude and frequency of the signal emitted by the

[a] Author for correspondence.
E-mail: ted.uyeno@nau.edu

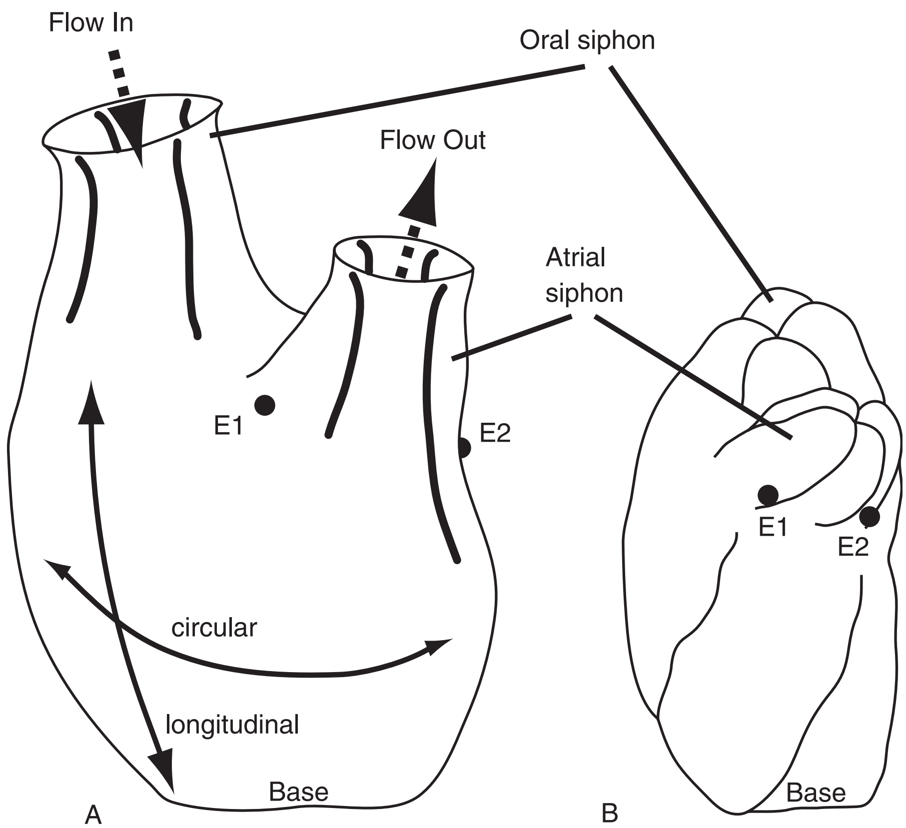

Fig. 1. Diagram of *Styela plicata*. **A.** Expanded and feeding position. **B.** Fully contracted state during a spontaneous body flushing. E1 and E2 represent the location where the two electrodes were attached to the tunic. The circular and longitudinal muscle fiber orientations are indicated.

probes, as well as their harmonics, have many characteristics of high-frequency electrical noise. Additionally, the ultrasound interference signals may introduce alias artifacts because of the relatively low sampling rates used in electromyography.

The voltage of a Hall-effect transducer varies with changes in magnetic flux. Thus, voltage output may be calibrated to changes in distance between the transducer and a small magnet. Eapen (1997) used this technique to record the opening and closing of clam valves in response to various chemicals. It is a simple and cost-effective technique. To avoid the complications of waterproofing and recalibration for an aquatic medium, Eapen did not immerse the Hall-effect probe or the magnet. Instead, they were mounted on supports that extended out of the water. Thus, the mechanical loading of the specimens by the supports and the probe and magnet potentially interfered with the natural movements of the animal.

Optical methods are conceptually simple and do not load the structures of interest. Modern digital cameras offer increasingly better temporal and spatial resolution and post-processing software may automate frame-by-frame calculations of changes in length. However, the initial cost may be substantial and there are often technical complications; the water must be clear and calm, aquaria must be built for visibility, the consistent tracking of specific reference points is often difficult, and the camera positions, lens characteristics, field of view, corrections for parallax errors, and, in the case of three-dimensional specimens, depth of focus must be appropriate.

Direct recording of movement or force may be the oldest technique. If one portion of a structure is stationary, the movable portion may be tied to the lever arm of a force transducer or even a smoke drum kymograph (Hoyle 1953). The difficulty associated with this method is the mechanical loading of the structure of interest. Thus, natural movement and range of motion are impeded.

Our technique is one of several that sense changes in electrical properties between electrodes as the distance between them varies. Sandeman (1968) recorded changes in capacitance detected by a receiver electrode moving in a field generated by two additional electrodes. Marrelli & Hsiao (1976) developed a method of detecting the angle of a crustacean joint, with two miniature (0.25 mm) ball electrodes that generated current (and voltage) fields about the joint. A sampling electrode mounted on the moving limb recorded changes in resistance as the joint angle varied. Kier (1992) further modified this circuit to measure the linear oscillatory motion of a cuttlefish fin (Kier et al. 1989). Vertical position is monitored by generating a linear current field between electrode plates above and below a sensing electrode attached to the fin.

The circuit described here was designed to reduce mechanical loading and facilitate the instrumentation of small marine invertebrates using only two electrodes. Here, the voltage output of the circuit varies with the resistance between the electrodes and is correlated with the distance between them.

We tested this two-electrode movement monitoring circuit by recording the body flushing, or squirting, of the leathery or pleated sea squirt, *S. plicata* (Subphylum Urochordata, Class Ascidiacea). The following is a description of the structural and behavioral characteristics of *S. plicata* that were of importance in the preparation and testing of the movement monitoring technique.

The sessile adults can grow to ~10 cm in height. They attach either individually or in small clumps to solid substrates in protected areas with good water movement off the east coast of North America (Ruppert & Fox 1988). The tan-colored, bag-shaped body of *S. plicata* has two muscular siphons with diagnostic four dark brown stripes (Fig. 1A). The oral (or buccal, or branchial, or incurrent) siphon is located opposite the base and allows water and food, mostly invertebrate larvae (Bingham & Walters 1989), to enter the pharyngeal basket for filtration (Bone et al. 2003). Water exits through the more basally positioned atrial (or excurrent) siphon. The feeding current is generated by cilia on the pharyngeal basket (Riisgård 1988). As indicated by their common name "squirt," tunicates may expel much of this internal water as a result of a spontaneous muscular body wall and siphonal contractions, thereby causing the animal to decrease in volume (Kott 1989). In this respect, members of the genus *Styela* are among the most active tunicates (Charriaud 1982). Hoyle (1953) reviewed the general function and characteristics of the squirting behavior: it has been variously thought to eject foreign particles, feces, or sexual products; to aid respiration; to be homologous with locomotory pulsations of presumably planktonic ancestors; or to be a defensive response to external stimuli. Hecht (1918) noted that these external stimuli included mechanical, light, temperature, and a variety of chemical stimulations.

The surface epidermal cells of the body wall secrete the leathery tunic (or test) that gives rise to the common name of this class: "tunicate." As styelid tunicates possess the toughest and most fibrous tunics (Kott 1989), we attached the miniature ball electrodes to this thick and convoluted tissue layer. The tunic is used by the animal primarily in mechanical protection (Kott 1989) and chemical defense (Pisut & Pawlik 2002) and is unique because it is partly composed of a fibrous native cellulose known as tunicin (Sugiyama et al. 1991). It has a complex construction with a thin sinuous cuticle containing small papilliform projections and a deep layer of ground substance composed of tunicin fibrils, a number of different granulocytes, blood vessels, and amoeboid cells (Mishra & Colvin 1969; Smith & Dehnel 1971; Lunetta 1983; Di Bella et al. 1998).

In this study, we describe the design, construction, and calibration of a circuit that was developed to be of broad use in precision measurement and recording of small distance changes under seawater conditions. A novel design feature is the reduction of the electrodes in both size and in number in order to reduce mechanical loading. As such, the circuit may be especially useful in experimental preparations involving smaller marine invertebrates. As a test of this, we use the circuit to directly record aperture diameter changes of the base of the siphon in the sea squirt, *S. plicata,* and then analyze the results.

Methods

Design of movement monitoring circuit

The first stage of the circuit is an isolation transformer (Fig. 2). The electrodes are connected to the input of a high-frequency response 1:1 transformer to help isolate circuit ground from the electrode reference ground. This transformer allows other voltage measurements, such as electromyography, to be made without artifacts and in isolation, because the electrical ground reference is independent. There are some considerations pertaining to the supply of power to this stage: in this circuit, we use the low-noise, high-input impedance TL-082 general-purpose operational amplifier as its maximal differential input voltages are $\pm$15 V. The use of a conventional 24-V AC power supply that we modified to provide $\pm$12 V (Fig. 2) provided an adequate voltage range to ensure sufficient resolution. An AC/DC power supply using the L7824 positive voltage regulator integrated circuit ensures clean and stable power. Alternatively, portability may be achieved using a low-current 24-V battery, or the equivalent number of lower voltage batteries connected in series. Such a setup may also be useful to experimentalists who are also using other electrical signal recording techniques, such as electromyography.

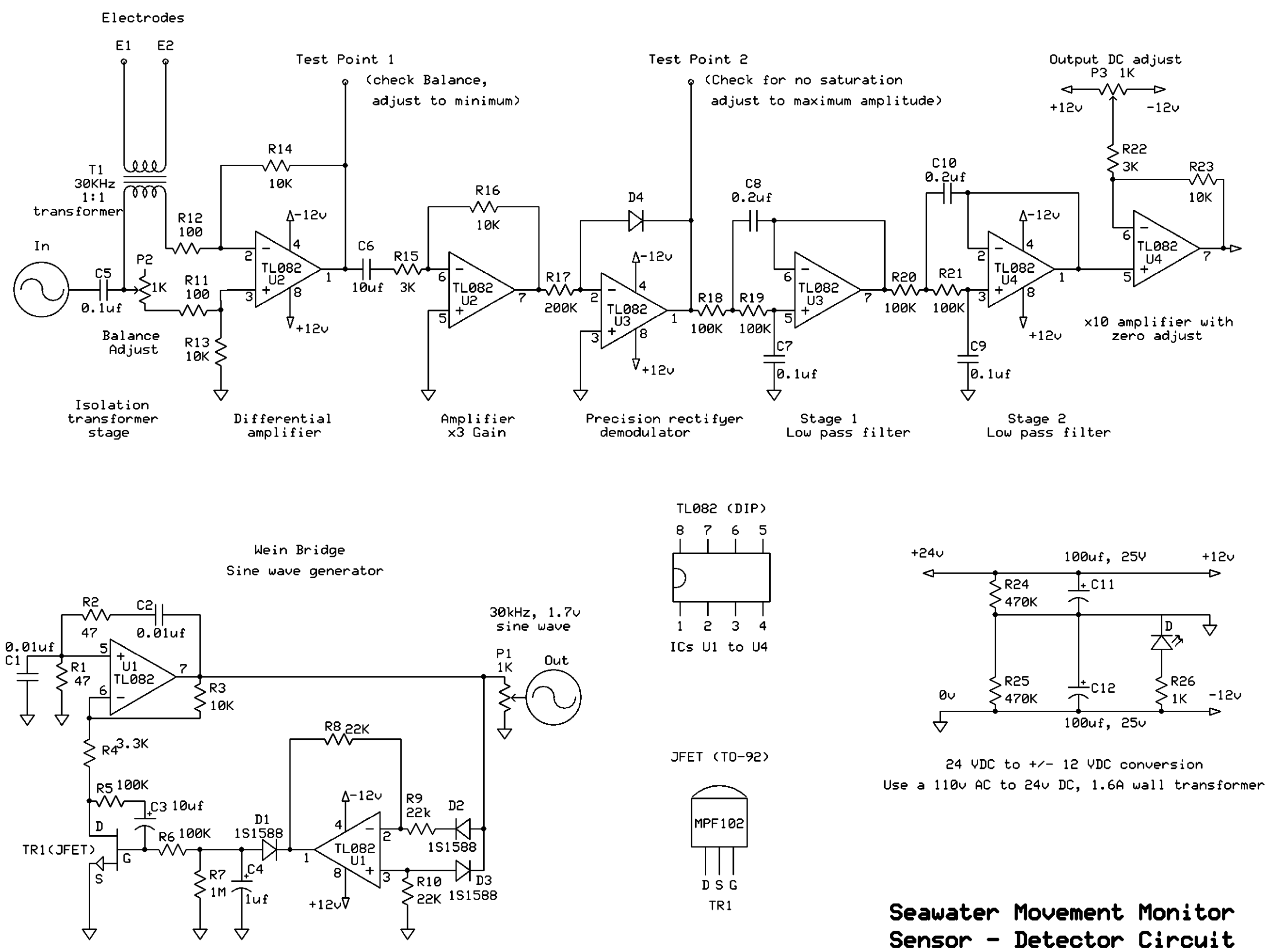

Fig. 2. Movement monitor circuit schematic.

The movement monitor circuit uses a front-end, 30-kHz, sine-wave generator with variable amplitude to generate the carrier frequency. This frequency was selected as it is well above the movement frequency, or even any muscle activation frequencies, of the sea squirt. In this way, the circuit does not add lower frequency noise. A Wein bridge oscillator circuit was specifically selected because of its stability, exceptional low noise, and low distortion characteristics. In this bridge circuit, the equivalent resistance of the electrodes is balanced using a potentiometer to nullify a reference voltage. Furthermore, adjusting the reference voltage in this bridge circuit effectively nulls out baseline resistance and the desired motion signal can be highly amplified without increasing the artifacts due to the electrode properties.

Next, the signal is sent to a series of conditioning stages. First, the signal is amplified at high gain and demodulated. Demodulation is performed with a rectifier and two low-pass filter stages (80 dB decade^{-1}). Finally, in the last stage, the extracted signal is amplified and the baseline is adjusted to zero for display and recording or digitizing.

The unique features of this circuit are the initial ground isolation in combination with a modified bridge circuit and a carrier frequency generator. These combine to give a stable, low-drift, and low-noise distance measurement.

Considerations in electrode design

The circuit uses a low-current AC signal to avoid the polarization and plating of the sensor/detector electrodes. To limit lower frequency noise, these silver/silver chloride electrodes were made of the shortest possible lengths of a 0.2-mm-diameter wire insulated from the base to almost the tip. For best linearity, the uninsulated tips are melted into matched 0.5-mm balls using a propane torch. However, simply stripping insulation from a small portion

(0.2–0.5 mm) of the end also provides satisfactory results. The electrodes were then "chlorided" to stabilize the electrode potential (Geddes 1972). This was accomplished by electrically connecting the electrodes and soaking the tips in chloriding solution (200 mL distilled H_2O, 100 gm $FeCl_3$, 100 mL HCl) until a dull dark finish appeared on the exposed tips (~10 min). After initial chloriding, we maintained the electrodes between experiments by immersing the tips in full-strength commercial bleach (6% sodium hypochlorite by mass). To minimize the use of expensive silver wire, one may solder it to a lead of a different metal. However, it is important to insulate and not immerse this connection in water, as it would generate current and artifactual noise.

System setup, calibration, and testing

The output signal of the circuit was recorded on a computer hard drive using an analog-to-digital converter card. The output was also monitored on an oscilloscope. When using this system with other electrical systems, such as electromyography, it is important to ground each independently so as not to introduce ground loop artifacts.

While simple detection of motion requires only the balancing of the electrode resistance to nullify the reference voltage within the bridge stage, accurate calibration allows the measurement of actual distances. Changes in the electrode tip shape and the surface area as well as changes in the salinity of the water require that the circuit be calibrated before each experiment. Using calipers attached to a micromanipulator, we measured the distance between the electrodes immersed in full-strength seawater (35‰) while recording the voltage output (Fig. 3). The relationship between voltage and distance was described by computer fitting the appropriate power function using the statistical software R (R Development Core Team 2006). The amount of drift shown by the circuit was also assessed by allowing the circuit to record >10 h. As resistance varies with salinity, the water was tested at the beginning and end using a calibrated refractometer.

Organismal preparations

Five small adult specimens of *Styela plicata* (representing an average expanded height of ~3 cm) were collected from pilings at the Institute of Marine Sciences of the University of North Carolina in Morehead City, NC. They were maintained and tested in aquaria of constant temperature (21°C) and salinity (35‰). Electrodes were attached on either side of the

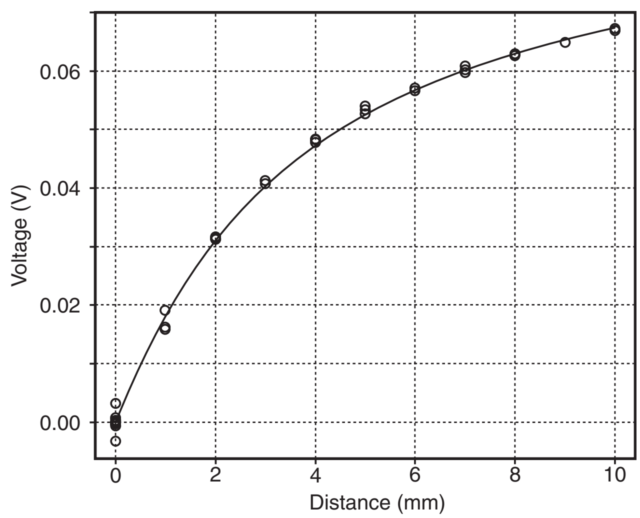

Fig. 3. Movement monitor calibration correlating voltage output (V) to distance (mm) between electrodes. Open circles represent voltage recordings at particular known inter-electrode distances, and the line of best fit is described by Eq. (1).

base of the apical siphons of these individuals using 5-0 gauge black braided silk sutures. The knot was held in place with cyanoacrylate glue. The base of the siphon was chosen because it represented a section of the body wall that was easily identifiable in contracted individuals and was covered by a thick tunic to which electrodes could be sewn. The specimens were tested for a duration of 24 h (12 h light:12 h dark) with a sampling rate of 500 Hz. One specimen did not have electrodes attached and instead was videotaped for 3 h during dark (using infrared light) and light periods. This was done in order to control for the effect of the electrode attachment.

Results

Calibration

The circuit was designed to be as linear as possible within a 1 cm range. However, the two-electrode design cannot be entirely linear as the field strength decreases and resistance increases with increases in distance. A test calibration documented the extent of this effect. Using two electrodes with spherical tips of a diameter of 0.5 mm, immersed in seawater of 35‰ and 21°C, and altering the distance between the tips 0–10 mm, we generated the graph in Fig. 3. The relationship between voltage and distance can be described by a simple asymptotic function:

$$V = \frac{0.094D}{3.977 + D} \tag{1}$$

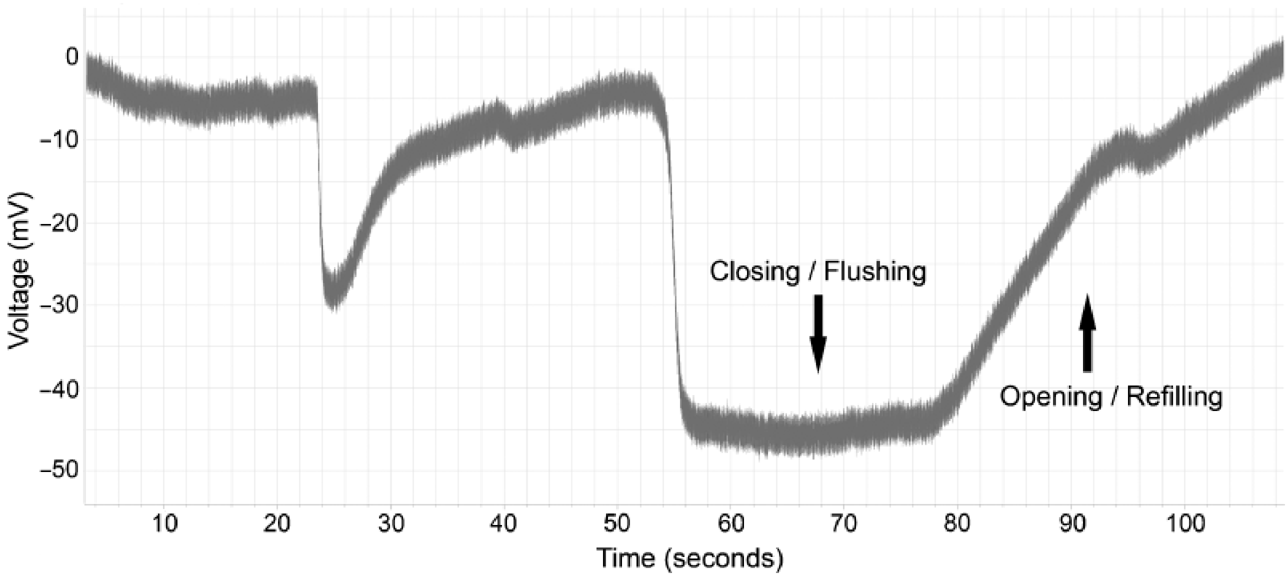

Fig. 4. Raw data trace from the movement monitor showing two typical body flushings or squirts. See text for details.

where D is the distance (mm) and V is the voltage (V). Here, it is important to either carefully adjust the DC offset (1 kΩ potentiometer "P3" in Fig. 2) to zero, or standardize the voltage values after recording, such that both D and V are initially equal to zero.

If evaporation cannot be controlled, a significant amount of drift occurs. Ten-hour recordings from electrodes separated by 1 cm showed a drift of 9.1 mV h^{-1}. During this time, salinity changed 35–41‰. When the test was performed again in a covered container, the drift was negligible over the same period. Finally, we found that warm-up period of ~4–8 min was necessary. The initial voltage output was low and increased to a stable reading before the end of that period.

Movement recordings

The overall average time between flushings was 248 s (4.13 min). However, individual average durations were in the range of 159–304 s. Video analysis of the inter-flushing time of a specimen without attached electrodes was, on average, 282 s. These inter-flushing durations, however, were highly variable: they could occur so rapidly that complete refilling was not possible or they could be as long as 6030 s. Often, the initial flushing periodicity was higher than later periods and, in one case, this was correlated with the light/dark phase. This individual, with an overall average inter-flushing duration of 304 s, showed less activity (a longer duration) in the dark phase (an average of 660 s) and a shorter duration in the bright phase (290 s). None of the other specimens, including the individual videotaped using infrared lighting, showed diurnal behavior.

Using a calibration curve generated before each trial, we calculated the diameter of the base of the apical siphon. As an example, we generated the calibration curve in Fig. 3 before testing a specimen that was 32 mm in height when fully extended. The apical siphon of this individual opened by 4.0 mm as indicated by a change in output of 45 mV (Fig. 4). All five specimens were ~30 mm in height and had an average total range of movement of 5.5 mm.

The movement monitor trace in Fig. 4 shows two flushing movements, with contraction represented by a decrease in voltage. Both flushings showed rapid contraction of the body wall, followed by a slow and gradual refilling phase. In the first flushing, contraction stopped at ~1.3 mm and refilling began immediately. In the second, the siphonal lumen neared complete contraction at ~4.0 mm and was held at that diameter for 22 s before beginning to refill.

Discussion

The electrode and circuit showed excellent temporal stability and spatial resolution. It has been used in a subsequent study using electromyography to correlate muscle activation patterns with the movement of beaks in the octopus buccal mass (Uyeno & Kier 2007). Our calibrations showed a change of ~8 mV mm^{-1} within our 1 cm working range. The signal within this range, however, required linearization.

Theoretically, as a ball electrode is a point source for the electric field, the intensity of the field is inversely proportional to the square of the distance. In practice, this relationship between distance and voltage output is calculated for each setup to compensate for different electrode shapes and variations in the conductivity of the electrodes, water, and animals. Fortunately, during the experiment this relationship appeared to be stable when drift due to evaporation was controlled.

There are a number of possible modifications that may improve specific performance characteristics when adapting this technique. First, the best linearity arises from a matched pair of spherical silver/silver chloride (ball) electrodes. However, if recording the occurrence of movement is all that is required, the shape of the conductive tip becomes less important; simply removing the insulation from the end of any conductive metal wire or using a screw head as the electrode tip also functions well. If linearity is required, and the additional field electrodes do not pose technical problems, the three-electrode technique described by Kier (1992) may be more appropriate. Second, distances >1 cm may be measured by increasing the signal voltage, by decreasing the signal frequency, and by increasing the electrode tip size. Third, if the circuit is to be used as a micrometer, spatial resolution may be raised by making moderate increases in the gain of the output amplification stage. However, careful balancing of the system, the use of precisely spherical silver/silver chloride electrodes, the use of a notch filter or Faraday cage to reduce noise (especially 50 or 60 cycle interference), and perhaps even additional or different filter stages may be required. Finally, it should be possible to calculate velocity and accelerations for movements recorded with this device.

With some modification, there is potential for many additional uses of this technique. If such a circuit is used in conjunction with an RF transmitter to telemetrically broadcast the output signal, then the entire circuit may be packaged to be completely autonomous and attached to free-swimming animals. Second, measurements in air are possible using larger, flat plate electrodes and by modifying the circuit to measure capacitance. With air as the dielectric medium, capacitance may be measured by using a much higher driver frequency. This technique was used in the musical devices of Leon Theremin (Glinsky 2000). The 1920s instrument had two antennae that controlled pitch and volume: by adjusting the distances between his hands and the antennae, the player uses his body capacitance to modulate the heterodyned output of two inductance-capacitance tank oscillators, resulting in a sound reminiscent of eerie 1950s science fiction movies. If the medium is fresh water, the resistance between the electrodes is higher and so smaller driving currents and smaller amplifications would be required. Finally, the circuit may be used in a feedback control arrangement with electric movement generators (motors, hydraulic or pneumatic pumps, electroactive polymer, or nitinol wire artificial muscles, etc.) in order to maintain a stationary position or control movement in a predetermined pattern. In this arrangement, the output of the circuit is used to control the position.

In conclusion, the direct recording of the diameter changes in the base of the siphon of *S. plicata* allowed us to characterize movement profiles relating to the flushing and refilling behavior. Such recordings allowed a test of a new technique to record changes economically and simply in underwater distances over time between two small and easily attachable electrodes. The technique was found to be quite serviceable over a period of days and offered useful temporal and spatial resolution over its 1 cm range. A number of suggested modifications of the circuit may allow the technique to be adaptable to a number of different uses by many invertebrate biologists.

Acknowledgments. We would like to thank the following people: UNC-CH undergraduate student, Ms. Michelle Lee, help with the recording procedures; Mrs. A. Uyeno was of great help in collecting animals; Dr. R.T. Jobe provided thoughtful aid in the analysis of the calibration trials; Drs. W.M. Kier and T.T. Uyeno provided helpful comments on the manuscript; and Mr. M.S. Stella helped maintain the animals. This project was funded by the Department of Biology, University of North Carolina, 2005 Wilson Award to T.A.U. and a DARPA grant (N66001-03-R-8043) to Dr. W.M.K.

References

Bingham BL & Walters LJ 1989. Solitary ascidians as predators of invertebrate larvae: evidence from gut analyses and plankton samples. J. Exp. Mar. Biol. Ecol. 131: 147–159.

Bone Q, Carré C, & Chang P 2003. Tunicate feeding filters. J. Mar. Biol. Assoc. UK 83: 907–919.

Charriaud E 1982. Direct measurements of velocity profiles and fluxes at the cloacal siphon of the ascidian *Ascidiella aspersa*. Mar. Biol. 70: 35–40.

Di Bella MA, Cassarà G, Russo D, & De Leo G 1998. Cellular components and tunic architecture of the solitary ascidian *Styela canopus* (Stolidobranchiata, Styelidae). Tissue Cell 30: 352–359.

Eapen JT 1997. An electronic device to record behavioural activity of bivalves. Indian J. Exp. Biol. 35: 663–664.

Geddes LA 1972. Electrodes and the Measurement of Bioelectric Events. John Wiley & Sons Inc., New York. 364 pp.

Glinsky A 2000. Theremin: Ether Music and Espionage. University of Illinois Press, Urbana. 403 pp.

Hecht S 1918. The physiology of *Ascidia atra* Leseur. J. Exp. Zool. 20: 429–434.

Hoyle G 1953. Spontaneous squirting of an ascidian *Phallusia mammillata* Cuvier. J. Mar. Biol. Assoc. 31: 541–562.

Kier WM 1992. Hydrostatic skeletons and muscular hydrostats. In: Biomechanics (Structures and Systems): A Practical Approach. Biewener AA, ed., pp. 205–231. IRL Press at Oxford University Press, New York.

Kier WM, Smith KK, & Miyan JA 1989. Electromyography of the fin musculature of the cuttlefish *Sepia officinalis*. J. Exp. Biol. 143: 17–31.

Kott P 1989. Form and function in the Ascidiacea. Bull. Mar. Sci. 45: 253–276.

Lunetta GD 1983. Comparative study of the tunics of two ascidians: *Mogula impura* and *Styela partita*. Acta Embryol. Morphol. Exp. 4: 137–149.

Marrelli JD & Hsiao HS 1976. Miniature angle transducer for marine arthropods. Comp. Biochem. Physiol. 54A: 121–123.

Mishra AK & Colvin JR 1969. The microscopic and submicroscopic structure of the tunic of two ascidians *Boltenia* and *Mogula*. Can. J. Zool. 47: 659–663.

Pisut DP & Pawlik JR 2002. Anti-predatory chemical defenses of ascidians: secondary metabolites or inorganic acids? J. Exp. Mar. Biol. Ecol. 270: 203–214.

Platt RS, Kieser TM, & Easton PA 1998. Eliminating ultrasonic interference from respiratory muscle EMG. Resp. Physiol. 112: 203–213.

R Development Core Team 2006. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN: 3-900051-07-0. http://www.R-project.org.

Riisgård HU 1988. The ascidian pump: properties and energy cost. Mar. Ecol. Prog. Ser. 47: 129–134.

Ruppert EE & Fox RS 1988. Seashore Animals of the Southeast. University of South Carolina Press, Columbia, SC. 429 pp.

Sandeman DC 1968. A sensitive position measuring device for biological systems. Comp. Biochem. Physiol. 24: 635–638.

Smith MJ & Dehnel PA 1971. The composition of tunic from four species of ascidians. Comp. Biochem. Physiol. 40B: 615–622.

Sugiyama J, Persson J, & Ghanzy H 1991. Combined infrared and electron diffraction study of the polymorphism of native celluloses. Macromolecules 24: 2461–2466.

Uyeno TA & Kier WM 2007. Electromyography of the buccal musculature of octopus (*Octopus bimaculoides*): a test of the function of the muscle articulation in support and movement. J. Exp. Biol. 210: 118–128.