Neotrop. Ichthyol., 4(2):295-299, 2006

Scientific note

A portable bio-amplifier for electric fish research: design and construction

Jonathan K. Wells* and William G. R. Crampton**

The weak electric organ discharges (EODs) of gymnotiform knife fishes can be readily amplified with a wideband amplifier connected to submerged electrodes. The output from such an amplifier can then be monitored with audio speakers or digitized for subsequent analysis. Commercially available devices are expensive and usually require mains electrical power. Here we provide design notes and instructions for the construction and calibration of a cheap, portable, battery-powered, AC-coupled wideband bio-amplifier. This device was designed to allow electric fishes to be located in the field, and also to permit the relatively noise-free acquisition of EOD waveforms from specimens held in temporary captivity. This contribution is intended to encourage students of neotropical ichthyology to explore electric signaling in gymnotiform fishes.

As fracas descargas dos órgãos elétricos (EODs) dos gimnotiformes podem ser facilmente amplificadas com um amplificador de banda larga conectado a eletrodos submersos. A saída do amplificador pode ser monitorada com auto-falantes ou digitalizada para análise subsequente. Equipamentos disponíveis comercialmente são caros e requerem fontes elétricas potentes. Nós fornecemos aqui o esquema e as instruções para a construção e calibragem de um bio-amplificador barato, portátil, energizado à bateria recarregável. Este equipamento foi desenhado para permitir a localização de peixes elétricos no campo, além de permitir a aquisiçào relativamente sem ruídos de ondas de EODs de espécimes mantidos temporariamente em cativeiro. Esta contribuição pretende encorajar estudantes de Ictiologia Neotropical a explorar sinais elétricos em peixes gymnotiformes.

Key words: Communication, Electric Organ Discharge, Gymnotiformes, Knife fishes, Method.

The Neotropical gymnotiform knifefishes generate continuous stereotyped electric organ discharges (EODs) for object localization and social communication (review in Bullock *et al*. 2005). In addition to mediating short-term communication, such as courtship and territorial contests, there is now overwhelming evidence that gymnotiform EODs serve as species-specific mate-attraction signals, and as such, often unambiguously discriminate closely related species that co-occur in ecological assemblages. EODs can therefore serve as diagnostic taxonomic characters for discriminating cryptic species (Crampton & Albert, 2005).

Electric fish EODs can be detected with submerged electrodes, amplified, and converted to digital waveforms using an analog-digital (A-D) acquisition device. Amplification is necessary because gymnotiform EODs never exceed more than a few hundred millivolts, except in the strongly electric eel *Electrophorus electricus* (L.). By placing electrodes posterior and anterior to a fish held in temporary captivity, the waveform and repetition rate of its EOD can be amplified and acquired in a standardized manner that permits comparison among individuals, populations or species. Following amplification, the electrical voltages of EODs can also be converted to sound in a loudspeaker. This allows electric fish to be located in the field, and permits non-invasive monitoring of their distributions.

Until recently the most technically challenging aspect of working with electric signals under field conditions was digitizing them to a computer. However, with recent advances in audio recording technology, cheap A-D acquisition devices are available for personal computers. These typically permit AC-coupled acquisition at sampling rates of up to 96 kSs (S = samples), and at resolutions of up to 24-bits. Most have very

*University of Florida, Department of Electrical and Computer Engineering, 216 Larson Hall, PO Box 116200, Gainesville, FL, 32611.
*University of Florida, Florida Museum of Natural History, PO Box 117800, Gainesville, FL, 32611. (current address) Department of Life Sciences, University of Toronto at Scarborough, 1265 Military Trail, Toronto, Ontario, M1C 1A4. E-mail: crampton@utsc.utoronto.ca

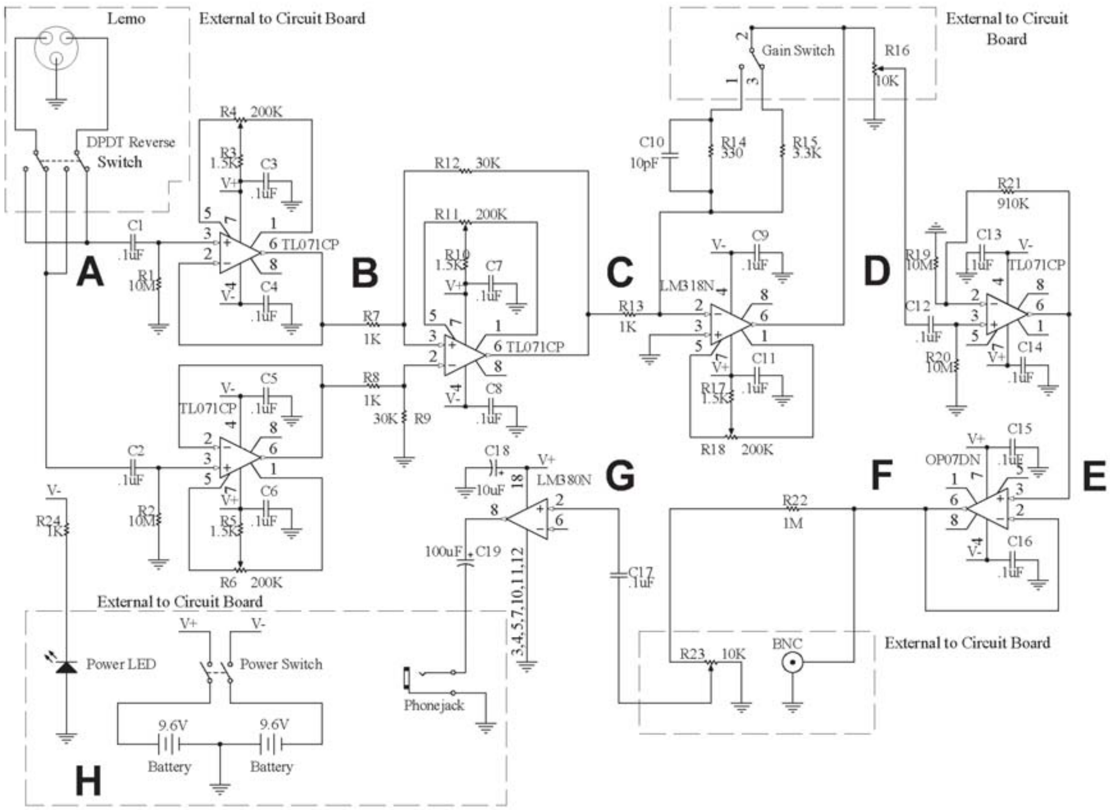

Fig. 1. Circuit plan for bio-amplifier. For stages A - H refer to text. Boxes with dashed lines refer to components external to circuit board. Symbols follow universal engineering protocol.

low signal-noise input ratios and can be used to make excellent EOD recordings. Many models can also either take power from a portable computer or from portable rechargeable batteries (e.g. the Edirol UA-5). These devices also have drivers that allow simple (un-triggered) waveform acquisition with commercial sound programs (e.g. Audition, Adobe Systems Inc.) or bioacoustics software (e.g. SAS-LAB, Avisoft Inc., Germany).

Amplification of EODs with the maximum signal-to-noise ratio (i.e. minimum relative noise levels) requires differential amplification, and also sensitivity to the full bandwidth of frequencies in the signal. For gymnotiform fishes this corresponds to 0 Hz (i.e. direct current, DC) to approximately 24 kHz. These are the minimum and maximum frequency components in any known species. Specialized bio-amplifiers with these specifications are available, and most have extra options such as the ability to filter unwanted frequencies, or the ability to record in DC-coupled mode in which any natural DC-offset to the signal is preserved. Nonetheless, these instruments are expensive. Also, few are designed for battery use. Exceptions are the BMA-200 preamplifier (CWE inc.) and the SR-5113 amplifier (Advanced Measurement Technology, Inc.).

We designed a portable wide-band AC coupled bio-amplifier for recording electric fishes in field conditions. This paper describes the design of this instrument and presents instructions for its construction and calibration. The unit is several times cheaper than commercial options and can be built with simple electronics tools and a standard electronics guide (e.g. Sinclair, 2005). This paper also describes the construction of dipole electrodes for use with this instrument.

Specifications

The bio-amplifier is an AC-coupled analog amplifier designed to accept signals from a submerged, grounded dipole electrode via a differential input. The AC-coupled circuitry rejects any DC offset (deviation from 0V) in a signal that may be caused by subtle differences in the construction properties of the two electrode poles. This ensures that the signal is always centered at zero volts. The frequency response of the device is approximately 0.1 Hz to 110 kHz at -3dB with low gain settings, and around 0.2 Hz to 30 kHz at -3dB with high gain settings. The gain is adjustable from 0 to 1,000 in two positions (each 0 to x10 gain), providing a maximum peak-to-peak output of approximately 19 V. The common mode reject ratio of the device is approximately 85 dB. Noise levels approximate an equivalent input of 12 mV RMS. The device includes a switch to reverse input polarity. In addition to a BNC output (to an oscilloscope or A-D device), the bio-amplifier includes a separate audio amplification circuit, with an independent gain control for headphones or an external speaker. Power is provided by two 9.6V rechargeable batteries, allowing the unit to operate continuously for approximately 36 hours with full gain and headphone settings, or approximately 72 hours with medium gain settings, and the headphone circuit switched off. The unit weighs 0.7 kg (1.1 kg with batteries) and is splash-resistant.

Circuit Design

The circuit plan is illustrated in Fig. 1, with stages A - H (listed below) annotated. A list of components is provided in Table 1. The signal enters the amplifier via the grounded dipole electrode input. We utilized a 3-pin Lemo input for its rugged construction, but a 3-pin microphone socket would

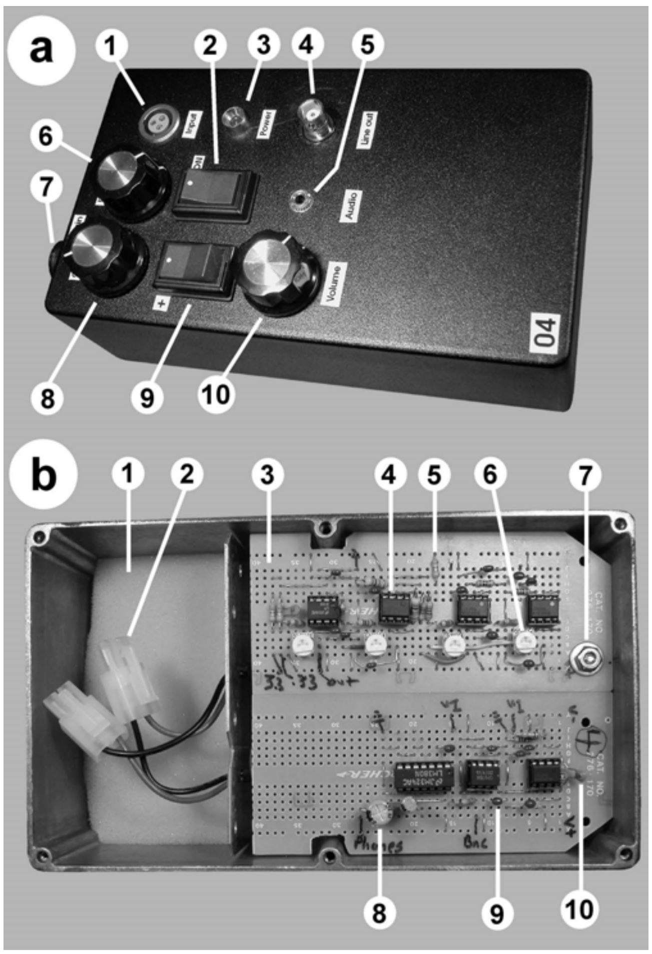

Fig. 2. External (a) and internal (b) views of the bio-amplifier. a: 1, dipole input; 2, power switch; 3, power LED; 4, BNC output to oscilloscope/digitizer; 5, earphone jack; 6, fine gain control; 7, covered ground stub; 8, coarse gain switch; 9, dipole reversal switch; 10, earphone volume control. b: 1, battery bay (rechargeable batteries removed); 2, battery terminals; 3, circuit board; 4, operational amplifier; 5, resistor; 6, trimmer potentiometer; 7, mount to bracket; 8, electrolytic capacitor; 9, ceramic disk capacitor; 10, 4pF ceramic disk capacitor across R20 as optional high-frequency attenuator.

Table 1. List of components for the construction of a bio-amplifier and electrode. Qt. = quantity. IC = integrated circuit. [1]for optional replacement of one of the 1 kΩ resistors in stage A. [2]optional across resistor R-20 (Fig. 1) for high frequency noise attenuation (see Fig. 2). [3]specifications for 'laboratory' model (see Fig. 3) with 200 mm positive/negative pole separation. For field electrode replace cable with robust microphone cable, and eliminate smaller PVC tube.

Component	Qt.	Part	Manufacturer/ Supplier
Circuit board			
PC board	2	276-0168	Radio Shack
Operational amplifier 8-pin IC	4	TL071CP	Texas Instruments
Operational amplifier 8-pin IC	1	LM318N	National Semiconductor
Operational amplifier 8-pin IC	1	OP07DN	American Microsemi-conductor
IC socket: 8-pin retention contact	6	276-1995	Radio Shack
Operational amplifier 14-pin IC	1	LM380N-ND	National Semiconductor
IC socket: 14-pin retention contact		276-1999	Radio Shack
Potentiometer (linear taper): 10 kΩ	2	271-1715	Radio Shack
Potentiometer (trimmer): 200 kΩ	4	TP-200K	Radio Shack
Potentiometer (trimmer): 1 kΩ	1[1]	271-280	Radio Shack
Resistor: 10 MΩ	4	271-0312	Radio Shack
Resistor: 1 MΩ	1	271-0312	Radio Shack
Resistor: 910 kΩ	1	271-0312	Radio Shack
Resistor: 30 kΩ	2	271-0312	Radio Shack
Resistor: 3.3 kΩ	1	271-0312	Radio Shack
Resistor: 1.5 kΩ	4	271-0312	Radio Shack
Resistor: 1 kΩ	4	271-0312	Radio Shack
Resistor: 330 Ω	1	271-0312	Radio Shack
Capacitor (electrolytic): 100 μF	1	272-1028	Radio Shack
Capacitor (electrolytic): 10 μF	1	272-1025	Radio Shack
Capacitor (ceramic disk): 0.1 μF	16	272-0109	Radio Shack
Capacitor (ceramic disk): 10 pF	1	272-0809	Radio Shack
Capacitor (ceramic disk): 4 pF	1[2]	272-0809	Radio Shack
External to board			
3-pin socket (dipole input)	1	EGG.2B.303.CLL	Lemo USA
Bulkhead BNC connector	1	530-CP-1094-AST	Delta Components
Earphone jack	1	274-0248	Radio Shack
Switch: 2 pole rotary	1	690-C5P0112N-A	Mouser Electronics
Switch: DPDT rocker	2	275-0691	Radio Shack
Knob for rotary switch/linear taper	3	274-0416	Radio Shack
LED holder	1	276-0080	Radio Shack
LED 5 mm green	1	276-0022	Radio Shack
Battery connector	2	23-445	Radio Shack
Rechargeable battery: 9.6 V	2	23-331	Radio Shack
Casing			
Aluminum box	1	546-1590WR1BK	Mouser
Metal bracket	1	A23Z	Simpson Strong-Tie
Metal bracket	2	A21Z	Simpson Strong-Tie
Screw cover (for ground stub)	1	HNG-180	Pro-Dec Products
Rubber cushion feet	4	64-2346	Radio Shack
Electrode [3]			
3-pin plug (dipole input)	1	FGG.2B.303.CLA D527	Lemo USA
Cable strain relief for plug	1	GMA.2B.050.DN	Lemo USA
Shielded stereo mic. cable (m)	2	Flex / 24 AWG EZID	Ramtech Inc.
PVC pipe - main casing (mm)	210	Schedule 40 - 1/4"	Harvel Inc.
PVC pipe – cable input (mm)	70	Schedule 40 - 1/8"	Harvel Inc.
0.625 mm pure silver wire(mm)	150	SFW 22a	CC Silver & Gold.

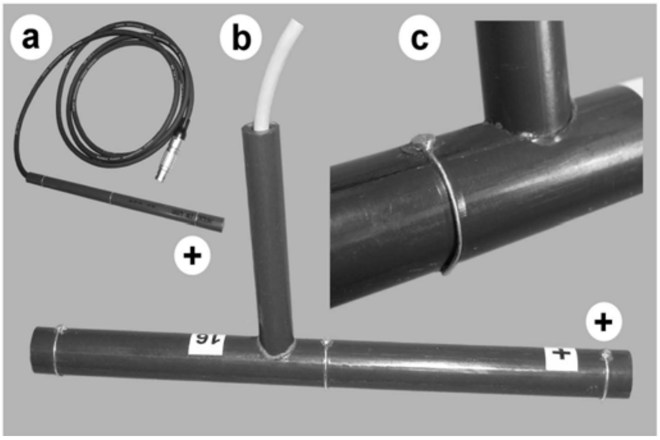

Fig. 3. Dipole electrodes designed for field (a) and laboratory (b, c) use. For each, positive pole (silver ring) is marked +, with ground pole in middle and negative pole at opposite end. The ground pole from b is magnified in c. Note more robust cable in the field electrode.

suffice. At input there is a double-pole-double-throw (DPDT) switch to reverse the polarity of the signal if desired. The signal is first differentially amplified in stage A, and then converted to a single ended output and amplified further in stage B. Because resistors exhibit manufacturing variation in resistance, it is imperative that R7 and R8 are selected so that their resistance values lie within 1% (10 Ω) of each other (it does not matter if that value is not precisely 1 kΩ). If this is not possible, one of these resistors can be replaced with a 1 kΩ trimmer-potentiometer and its resistance varied until it exactly matches that of the other resistor. After stage B, the signal comes to the variable gain stages of the amplifier. The 2-way coarse-gain switch amplifies the signal an additional 10 x (if selected) in stage C. A linear taper potentiometer then controls the fine-gain amplification in stage D. There is then a final amplification stage, E, before the signal is sent to the output buffers (stage F). At stage D, depending on the noise environment, a simple high-frequency noise filter can be added by placing a 4pF capacitor across R20 (see Fig. 2). This attenuates most noise over 30 kHz, has only a slight effect on frequencies in the 24-30 kHz range, and allows all frequencies below 24 kHz to pass. This ensures that no electric fish signal frequencies are attenuated. Stage F of the circuit buffers the signal and drives the line-out to the BNC jack. Following this is a final amplification stage (G) that buffers and drives the headphone jack. Here, a 10 kΩ linear taper potentiometer allows the output volume to be adjusted. External to the signal path are the power supply and power LED (stage H). Two rechargeable 1600 mA 9.6 V NimH batteries, in series, drive the amplifier with the node between them used for reference (ground). This provides a maximum V+ and V- of 9.6 V, and a maximum peak-to-peak output of 19.2V. The LED is tied to the V- supply and ground in series with a 1KΩ resistor. This lights the LED when the batteries are charged and the unit is switched on.

Construction

The device is encased in an aluminum box with an external black polymer coating (Fig. 2). Metal brackets are glued with silica rubber to provide the battery-bay walls and to provide a support onto which the main circuit board is bolted. The black finish on the aluminum box is not conductive so a small ground stub is inserted through a hole drilled in the casing allowing a direct ground connection from the internal surface of the casing to an external ground connection (e.g. alligator clip attached to a wire to true ground). All components can be soldered to the main circuit board using silver solder flux. An alternative approach is to have the circuit board professionally etched. This allows several boards to be made automatically, and reduces assembly effort, but does not result in significantly improved performance. An optional addition is to bolt lanyards attachments to the box for carrying straps.

Calibration

The design of the bio-amplifier includes four trimmer potentiometers (R4, R6, R10 and R17 in Fig. 1) that can be adjusted to provide maximum signal quality. These are calibrated as follows: first, each input to the amplifier should be tied to ground by connecting the three inputs of the 3-pin input plug with a short segment of copper wire; next, with the amplifier switched on, use a multi-meter to measure the voltage difference between the output (pin 6) of each operational amplifier and ground. Each potentiometer (beginning with R4 and continuing in numerical order) should then be adjusted until the voltage difference between the output (pin 6) of its corresponding operational amplifier and ground is as close to 0V as possible.

Electrode Design and Construction

The dipole electrode comprises a negative and positive pole from which the signal is differentially amplified, and a central ground pole (Fig. 3). These poles are connected to the amplifier by shielded two-channel audio cable (in which shield is the ground) terminating with a three-pin plug. To reduce interference from external noise, and to achieve the best signal-to-noise ratio, requires attention to four points. First, the wires to the electrodes should be separated near the poles, and not near the amplifier. Second, the overall length of the cable from poles to 3-pin plug should not be excessive. A reasonable working length is around 1.5 – 2 m. Third, the electrode poles should be made of a highly conductive material such as pure silver. Finally, because dissimilar metals in water can generate small DC potentials (causing an overall DC bias between the poles), the copper wire-silver junction between the wires in the cable and the poles should not be exposed to the water.

The electrodes illustrated in Fig. 3 meets these requirements. The main casing is made of PVC piping of 14 mm diameter, with an internal bore of 9 mm. Three 2 mm holes are drilled through the casing at equidistant spacing. These correspond to the positive and negative poles, and to the central ground. A 1 mm wide and deep grove is scored around the outside of the pipe at the position of each hole. The two cable wires (and the twisted ground shield in the middle) are passed through each of these and soldered to a length of pure silver wire. The soldered joint is then fed back through the hole, and the silver wire wrapped once around the pipe and soldered to itself with silver solder flux to form a single robust ring. The wires and ground meet at the end of the intact portion of the audio cable either at the end of the pipe (field electrode, fig. 3a), or inside a second smaller section of PVC piping which is 10 mm in diameter with an internal bore of 6 mm (laboratory electrode, Fig. 3 b, c). In the latter case, the smaller pipe is inserted into a hole of slightly less than 10 mm diameter near the center of the main pipe, twisted into position and glued. Finally, the entire electrode casing is filled with clear silicone rubber to isolate the junctions between the silver wire and the cable wires/ground. By constructing laboratory electrodes of various sizes it is possible to record fishes of varying total length. Useful lengths are 10, 20, 30 and 50 cm between poles.

To increase the sensitivity of the electrodes and reduce their tendency to generate DC potentials we electroplated them with silver chloride (AgCl). When the electrode unit is built and the silicone rubber completely cured, it is placed in a saturated solution of NaCl (table salt). Each pole is then in turn connected to the positive terminal of a fully charged 12V automotive battery. A wire from the negative terminal of the battery should be placed in the water to act as the cathode. The positive pole (anode) will attract Cl- ions and react with the Ag+ ions to form a AgCl coating, which is black. Reversing the polarity cleans the electrode (and generates bubbles of hydrogen).

Acknowledgements

We thank Carl Hopkins, Charles Smyth, and Max Westby for technical advice. JKW received funding from the University of Florida Undergraduate Scholar's Program. WGRC was funded by the National Science Foundation grants DEB 0084704, 0102593, and 0138633.

Literature Cited

Bullock, T. H., C. D. Hopkins, A. N. Popper & R. R. Fay. 2005. Electroreception. New York: Springer.

Crampton, W. G. R. & J. S. Albert. 2006. Evolution of electric signal diversity in gymnotiform fishes. Pp. 647-731. *In*: Ladich, F., S.P. Collin, P. Moller & B.G Kapoor (Eds.). Communication in Fishes. Enfield, N.H. Science Publishers Inc.

Sinclair, I. R. 2005. Practical Electronics Handbook. Oxford: Newnes Press.

Received December 2005
Accepted May 2006