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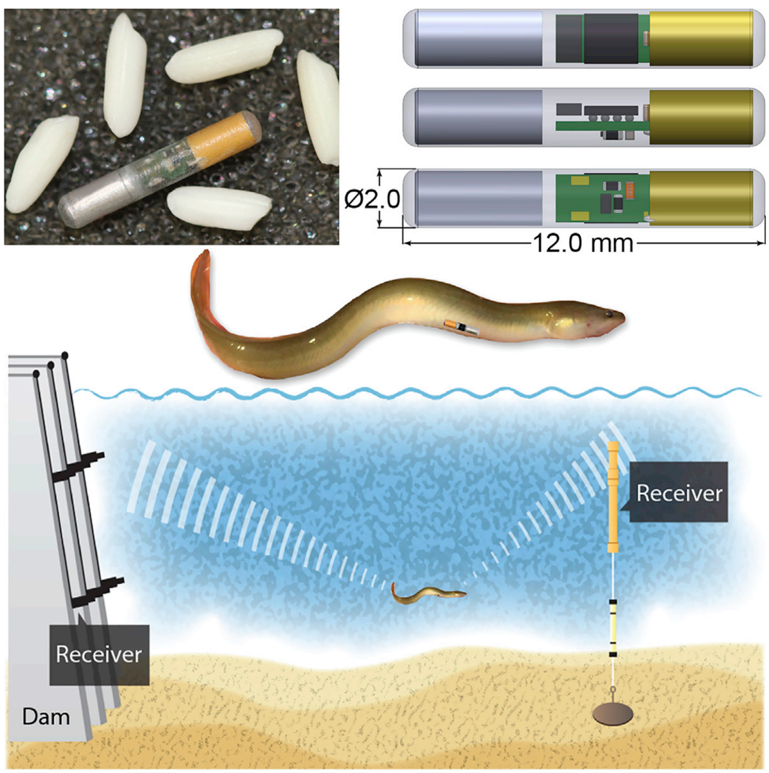

Deng et al. develop an acoustic transmitter through innovations in energy storage, materials science, and engineering design. The transmitter can be used to gain in-depth understanding of the movements and behaviors of small aquatic species. Information obtained using this new technology provides insights for ecological conservation and environmental mitigation.

Zhiqun D. Deng, Huidong Li, Jun Lu, ..., Jayson J. Martinez, Yuxing Wang, Jiguang Zhang

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Highlights

A new acoustic micro-transmitter is developed

A high-energy micro-battery based on Li/CFx chemistry powers the micro-transmitter

This technology provides insight for ecological conservation and mitigation

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An acoustic micro-transmitter enabling tracking of sensitive aquatic species in riverine and estuarine environments

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SUMMARY

Conservation of aquatic species requires in-depth understanding of their movement and behavior and their interactions with man-made hydraulic structures. Acoustic telemetry is a primary method to remotely track in 3 dimensions (3D) aquatic animals implanted with transmitters. The transmitter's weight and size are the major limiting factors because the transmitter should not affect the animals' natural behavior. Here, we present an acoustic micro-transmitter that weighs 0.08 g in air, only 1/3 that of existing technologies. The transmitter offers a source level of 148 dB (reference: 1 μPa at 1 m) and a service life of 30 days at a 5-s transmission rate. Nearly 100% of tagged fish were detected in field studies, demonstrating the viability of this technology for studying species of early life stages. Information resulting from the use of this technology provides valuable insight for ecological and environmental policy making and resource management worldwide.

INTRODUCTION

Conservation of aquatic species requires in-depth understanding of their spatial and temporal movements and behavior in natural water bodies or around man-made hydraulic structures.[1] Telemetry is the preferred approach because it allows aquatic species to be tracked remotely and individually by implanting them with an electronic transmitter that sends out a signal to be detected by receivers.[2] In addition, acoustic telemetry has become the primary telemetry method for aquatic environments because it has a long detection range, good performance in deep water, and an accurate three-dimensional (3D) tracking capability compared to other alternatives such as radio telemetry and passive integrated transponders (PITs).[3]

An acoustic telemetry system consists of acoustic transmitters, receivers, and data processing software.[4] The acoustic transmitters are typically implanted into the body cavity of aquatic species and intermittently produce encoded acoustic signals that are detected by receivers for identification and tracking. It is critical that the additional weight of the transmitters and implantation do not affect the species' natural behavior.[5] There have been many studies on the threshold of the tag burden (i.e., the ratio of transmitter weight to the fish weight), but no consensus has been reached in the scientific community.[6,7] As a general guideline, the American Fisheries Society recommends that "Fish generally should not be equipped with transmitters that weigh more than 1.25% in water or 2% in air of the fish's weight out of water."[8] Others have suggested a higher tag burden by showing that mortalities only occurred in fish having tag burdens >6%.[9] However, even with the relaxed

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guidance of 6% tag burden, many endangered species and critical life stages have not been studied because of technology limitations imposed by the size and weight of the transmitter. Therefore, despite the lack of a clearly established tag burden guidance, significant reductions in the available sizes and weights of acoustic transmitters would greatly enhance our ability to monitor and study larval or juvenile populations of fish species of interest, which would consequently benefit a wide range of fish conservation, environmental mitigation, and fishery management efforts. Several examples are included in Note S1.

The smallest acoustic transmitter commercially available is the injectable transmitter developed for the Juvenile Salmon Acoustic Telemetry System (JSATS),[10] a system that has been deployed throughout the Columbia River basin (Oregon, USA). JSATS was developed to evaluate the behavior and survival of juvenile salmonids migrating past dams, reservoirs, and the lower Columbia River estuary to the Pacific Ocean.[4] Considerable effort has been expended to understand the biological effects of surgical implantation of acoustic transmitters in yearling and sub-yearling Chinook salmon (*Oncorhynchus tshawytscha*). In a recent field study,[11] the injectable transmitter was found to substantially reduce the adverse effects of implantation for the tagged fish and significantly improve fish survival compared to larger commercially available transmitters. The use of this transmitter also reduces the time and cost of implantation, signaling that it will become a critical technology for studying migration behavior and survival of species and sizes of fish that have never been studied, leading to critical information for salmon recovery and the development of fish-friendly hydroelectric systems. However, the injectable transmitter, weighing 0.22 g in air, is still too heavy and large to be implanted in many sensitive species, as discussed earlier. Applying the 2% tag burden rule, this transmitter cannot be used in species that weigh <11 g. As a result, even smaller transmitters are needed to study these sensitive species.

Therefore, this study focused on designing, building, and evaluating a prototype acoustic micro-transmitter (AMT) that can be used to study the behavior and survival of aquatic animals weighing as little as 4 g. Juvenile American eels (*Anguilla rostrata*) and Pacific lampreys (*Entosphenus tridentatus*) were selected as the test species because they are ecologically and economically important and pose significant challenges for acoustic transmitter designs due to their thin and flexible bodies. Solving the challenges for juvenile American eels and Pacific lampreys will provide better transferability to other species. We developed the planned range of applications in collaboration with stakeholders; designed and demonstrated a state-of-the-art micro-battery technology for the AMT in terms of the battery dimensions and deliverable energy capacity; integrated the functionality of the AMT into a small, integrated circuit; optimized the transducer design by exploring different resonance modes; and designed the form factors that allow for optimum implantation methods to be developed for juvenile American eels and Pacific lampreys.

RESULTS AND DISCUSSION

AMT design

The AMT consists of 3 main components: (1) a lead zirconate titanate (PZT) ceramic tube transducer that emits acoustic signals, (2) an electronic circuit board that contains the power and control circuitry, and (3) a lithium/carbon fluoride (Li/CF_x) micro-battery as the power source. The AMT operates at 416.7 kHz, a frequency that was determined to be beyond the background noise in aquatic environments, especially in fast-moving waters or hydraulic structures.[12] The AMT is encapsulated by epoxy in a cylindrical body (Figure 1A).

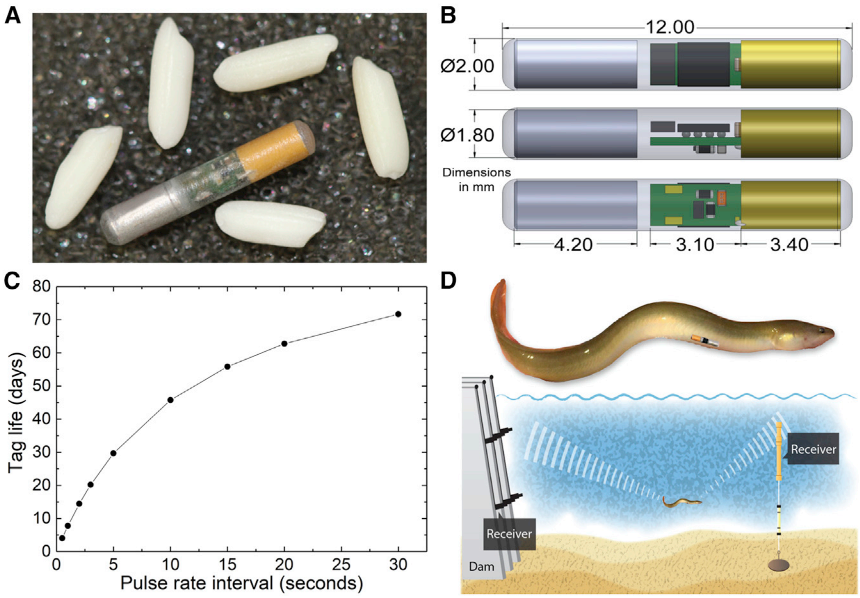

Figure 1. Overview of the AMT

(A) Photograph of the AMT next to a few grains of rice.

(B) Computer-assisted design drawing of the AMT.

(C) Operational life of the AMT as a function of the pulse rate interval.

(D) Illustration showing a use case of the acoustic transmitter (eel tracking near a hydroelectric dam). American eel photograph used with permission from Kevin Mayes, Texas Parks & Wildlife Department, Inland Fisheries Division.

One of the design goals of the AMT is to have a similar or smaller size and weight than those of the 12-mm PIT tag, which weighs ~0.10 g and has been successfully used in studying juvenile or small aquatic species.[13,14] Therefore, the overall form factor of the AMT has a cylindrical body similar to the PIT tag. The transmitter has a diameter of 2.0 mm and its overall length is 12.0 mm (Figure 1B), both of which are identical to those of the PIT tag. The average weight of the transmitter is 0.04 g in water and 0.08 g in air, which is 20% lighter than the PIT tag. Compared with the smallest acoustic transmitter commercially available, this new transmitter is merely 1/3 in weight and volume.[10] At a 5-s transmission rate, it has an operational life of 30 days (Figure 1C). It has a fixed source level of 148 dB (ref.: 1 μPa at 1 m), which results in an 80- to 140-m detection range in a realistic freshwater environment, depending on background noise levels. Figure 1D illustrates a use case of this technology in which it is used to track the movements of juvenile eels near a hydroelectric dam.

Electronics design

There were 3 major challenges for the electronics design to meet the overall AMT size and operational life requirements: (1) the total volume is reduced by 67% compared to the previous injectable transmitter[10]; (2) the battery capacity is reduced by ~80% (due to the volume reduction of the battery), while the required lifetime (number of transmissions) is only reduced by 40%; (3) the assembled circuit board must fit inside a 1.9-mm diameter cylinder to leave room for encapsulation and coating. These factors mandate a compact circuit design with high efficiency.

The AMT uses a small microcontroller (model EFM8SB10F8G "Sleepy Bee," 1.78 mm × 1.66 mm, Silicon Labs, Austin, TX, USA) to generate the modulated driving signals

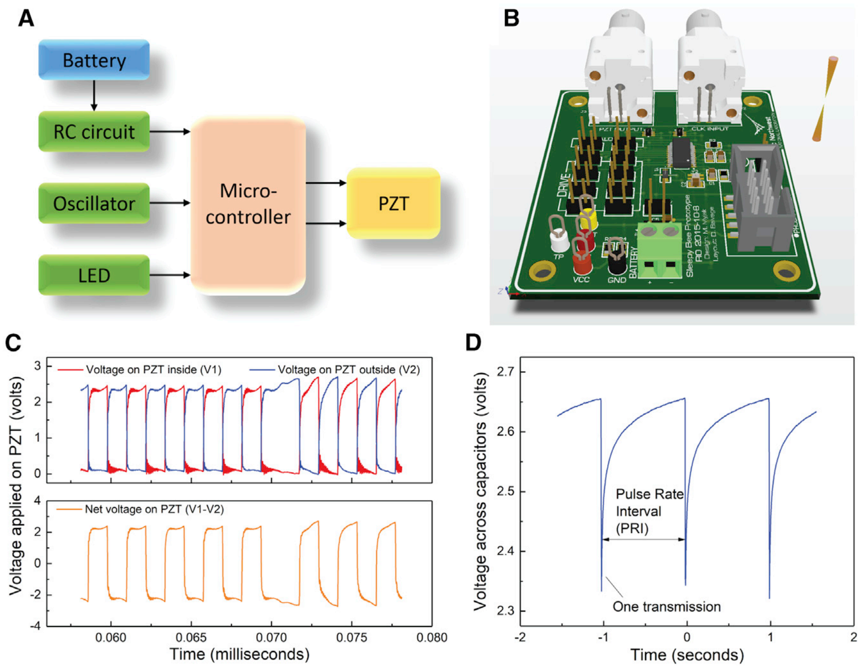

Figure 2. The AMT circuitry

(A) Circuit diagram of the AMT.

(B) Prototype AMT circuit board.

(C) PZT driving waveform.

(D) Energy consumption of acoustic transmission.

for the PZT at defined intervals. This microcontroller provides several features that are helpful for reducing the overall component count and power consumption. For instance, the microcontroller contains a linear regulator to reduce its internal supply voltage to 1.8 V. This feature not only reduces the energy consumption during signal transmission but also stabilizes the internal timers to prevent fluctuations in the battery voltage during signal transmission. With the help of a miniature external oscillator to calibrate the internal timers, the AMT can keep the modulation frequency within the required tolerance of $\pm$0.5% and the transmission rate fluctuation within $\pm$3%. During transmission, the microcontroller pulls energy from the battery and applies a 416.7-kHz voltage waveform directly on the PZT (Figure 2C) to induce a resonance. Based on the 350-mV voltage drop on the capacitors (Figure 2D) during the acoustic signal transmissions, the energy consumption for each transmission was calculated to be 26 μJ when the AMT was driven at 2.7 V, the operating voltage provided by the micro-battery. The light-emitting diode (LED) provides the transmitter with an optical means to receive configuration commands (including activation and deactivation of the transmitter) in the form of modulated ultraviolet light, which is emitted by a pen-styled activator. Figure 2A illustrates the overall electronic design. The components were evaluated on a prototype board (Figure 2B) and carefully chosen to achieve the best tradeoff between performance, power consumption, physical size and weight, and manufacturability.

The AMT can transmit 1 tag code or alternate between 2 tag codes. Each code is fully configurable and can be from 1 to 64 bits long. For backward compatibility with existing JSATS receivers, the typical tag code is 31 bits long, consisting of a 7-bit Barker code, a 16-bit tag number, and an 8-bit cyclic redundancy check. With 10 modulation cycles per bit, the tag code is 744 μs long.[12] Therefore, this new transmitter is fully compatible with the JSATS receivers available on the market from 2 fish tag vendors (Advanced Telemetry Systems and Lotek). Beyond these features, the microcontroller contains an on-board temperature sensor and can embed a 7-bit temperature reading into the tag code, if desired. A 128-byte lookup table specifies the correct cyclic redundancy check for a particular temperature reading.

Micro-battery

Because of the exceptionally small size and weight of the new AMT, no existing commercial battery on the market could fit into the small package and provide sufficient energy to support the service life needed. Therefore, a new micro-battery, based on the recently-developed MB306 micro-battery design,[15] was developed. Similar to the MB306, the new micro-battery (MB204) uses Li/CF_x chemistry and has a cylindrical shape. Details of the micro-battery design are included in Note S2.

The micro-battery has an outer diameter of 1.8 mm and a length of 4.2 mm (Figure 3). It weighs 20 mg and has a volume of 11 mm^3. The cell open-circuit voltage immediately after assembly was >3.3 V and the cell open-circuit voltage after 30 days was >2.9 V. Cell impedance R_{ct} was <4,000 Ω, the energy density was >300 Wh/L, and the specific energy was >160 Wh/kg. The as-prepared micro-batteries (inset of Figure 3A) successfully delivered the required capacity of 1.4 mAh (Figure 3A). After being stored at ambient conditions for 2 months, the capacity of the cell was still >1.4 mAh without any increase in cell polarization (Figure 3B), confirming the effectiveness of our sealing approach. The effects from temperature were evaluated at different high current rates. Acceptable capacities were delivered at 0°C, 10°C, and 24°C, which cover most of the operational temperature window the AMT is expected to encounter (Figure 3C).

Piezoelectric transducer design

The PZT tube transducer used in the transmitter has a length of 3.4 mm, an outer diameter of 1.8 mm, and an inner diameter of 1.4 mm. Its inner and outer wall surfaces are coated with gold as the electrodes. The length of the tube was carefully selected so the transducer operates in the length vibration mode (i.e., the tube resonates along its length direction at the 416.7-kHz operation frequency). Other vibration modes, such as the hoop mode and thickness mode, were not possible due to the small dimensions of the transmitter. Details on how the vibration mode and PZT tube dimensions were determined are included in Note S3.

Figure 4 demonstrates the 360° beam patterns of an AMT with the AMT oriented in the 3 orthogonal planes relative to the receiving hydrophone. For each beam pattern, the zero-degree direction and the direction in which the AMT rotated during the measurement are illustrated by the corresponding inset of the figure. Because the PZT tube resonates along the length of the tube, the source level in this direction should be the strongest. The measurement results demonstrated this (Figures 4A and 4B). The AMT was capable of producing a source level of 150 dB in this direction. As the PZT transducer rotated away from the hydrophone, the source level started to decrease because the resonance direction of the PZT was no longer pointed at the hydrophone. The source level decreased to ∼145–148 dB when the transmitter was facing sideways to the hydrophone (i.e., at 90° and 270°). As the transmitter continued to rotate, the source level increased again because the resonance direction (i.e., the length direction) started to move in line with the hydrophone. However, at orientations between 120° and 240°, the source level quickly dropped to as low as 136 dB (Figures 4A and 4B) because the acoustic signal was blocked by the circuit board and the micro-battery positioned behind the

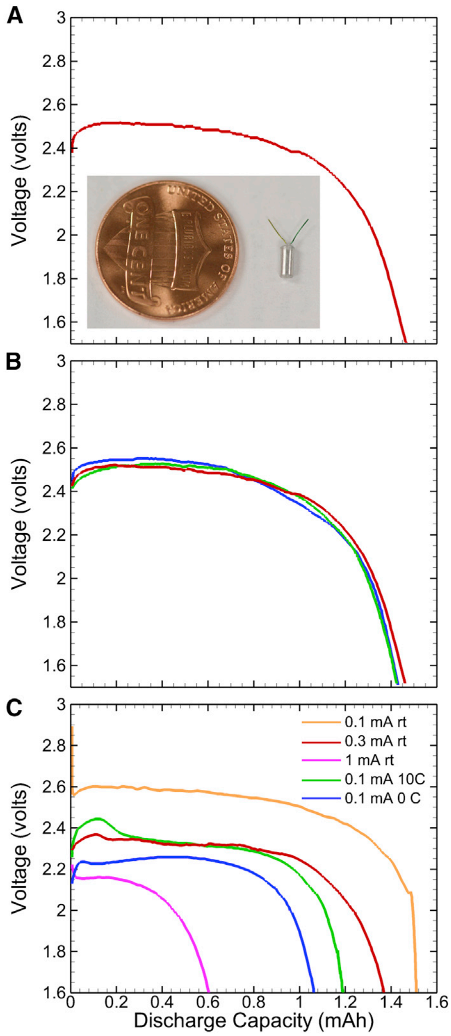

Figure 3. Micro-battery capacity evaluation results
(A) Discharge voltage profile of a typical micro-battery.
(B) Three micro-batteries discharged after 2 months of storage.
(C) Micro-battery discharge performance at high current rates and low temperatures.

PZT tube. This is a common phenomenon exhibited by acoustic transmitters. Compared to the typical beam pattern of an acoustic transmitter that uses the circumference resonance mode of the transducer, the beam pattern of the AMT was less uniform as the resonance of the transducer was along only 1 direction, rather than 360°. However, the source level difference between 0° and 90°/270° was merely $\leq$3 dB, most likely because of the relatively large surface area along the radial direction of the tube radiating the acoustic signal. The average source levels in the front-180° (i.e., the angular range in which the acoustic signal was not blocked by the transmitter body) for the 3 different AMT orientations were ~146–148 dB, which still corresponds to a detection range of ~80–140 m in fresh water, depending upon the noise level of the environment.

Tagging and field studies

We conducted a laboratory tagging study and developed a tagging procedure. A 2- to 3-mm incision was made to the base of the pectoral fin of a juvenile American eel

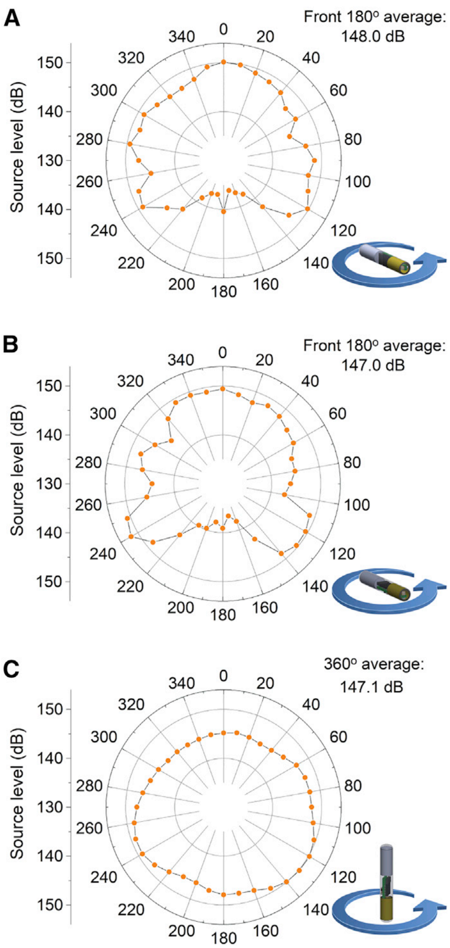

Figure 4. Acoustic beam patterns of the AMT

(A) When the axial direction of the cylindrical body of the AMT is oriented toward the hydrophone and the planar direction of circuit board within the AMT is oriented vertically relative to the surface of the water.

(B) When the axial direction of the cylindrical body of the AMT is oriented toward the hydrophone and the planar direction of circuit board within the AMT is oriented parallel to the water surface.

(C) When the axial direction of the cylindrical body of the AMT is oriented perpendicular to the water surface.

on the left lateral side (~1/3 of the total length of the eel) with a sterile 3.0-mm microsurgical scalpel. The AMT was then inserted by hand anteriorly into the body cavity. This tagging procedure took <60 s.[16] The biological testing results from implanting juvenile (yellow stage) American eels with the AMT showed that implantation is not likely to have an adverse effect on fish survival over a 38-day holding period. This finding is based on no fish mortalities observed during an extended holding study.[16] Only 2 of 26 tagged fish shed their transmitters during the first 20 days post-tagging—1 fish from the extended holding study and 1 fish from the sustained swimming study (after completing the sustained swimming test). The shedding most likely occurred through the incision, as that was the only possible exit point for the transmitter. The surgical procedure was effective in placing the transmitters within the body cavity without causing significant injury or infections at the tagging site. The sustained swimming tests showed no significant differences in swimming ability when comparing the implanted fish (n = 60) to the control fish (n = 60) for all of the size classes tested (113–175 mm).

Because of the unprecedentedly small size of this transmitter, its signal strength (source level) is ~10 dB lower than that of the smallest acoustic fish tag (the JSATS injectable transmitter) to date. As a result, its detection range in a freshwater environment is lower, ~80–140 m, compared to 200–300 m of the JSATS injectable transmitter. In marine environments, in theory, because of the stronger absorption of acoustic energy by seawater and the deeper water depths, which would lead to more spherical spreading of the acoustic signal, the detection range would be further reduced and thus limit the usefulness of this transmitter in vast and deep ocean waters. However, we believe that this technology can still be a great tool for studying sensitive species in estuaries where water passages are relatively confined and shallow.

During the summers of 2017 and 2018, >400 AMTs were manufactured at Pacific Northwest National Laboratory (PNNL) and implanted into fish for multiple field studies. The studies included different species in different environments: yellow-phase American eels in shallow water (<3 ft deep) in the Roanoke River (North Carolina) and in the tailrace below a dam in the Potomac River (Maryland) and juvenile Pacific lamprey in the fast-moving water in the Columbia River basin.[17] The results from both studies demonstrated that nearly 100% of the AMT-tagged individuals were successfully detected, and important behavioral information was successfully gathered. In the Columbia River basin study, the tagged juvenile Pacific lamprey weighed merely 4.6 g on average. This field study was the first time that acoustic telemetry was used in juvenile lampreys. Based on the study results, a research, monitoring, and evaluation plan was developed to inform the planning and prioritization for future juvenile lamprey dam passage investigations. Through the Roanoke River study, knowledge about the preferred migration routes of American eel was successfully obtained and consequently used to guide the construction of an eelway on the Potomac River (dam no. 4), which is ongoing. Overall, the field trials fully demonstrated the functionality of the AMT in challenging locations and the feasibility of studying sensitive species' behavior and survival using the AMT. Knowledge of eel and lamprey behavior and survival is critical for developing mitigation strategies for downstream and upstream passage, including the design of bypass systems at hydroelectric facilities and for irrigation diversion structures. Applying the guideline of the tag burden, the use of the AMT can be extended into studies of other sensitive species in many other regions, including the Amazon River basin (South America), the Mekong River basin (east and southeast Asia), and the Willamette River (Oregon, USA). For instance, recently, we also investigated the applicability of the new transmitter for juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and found that it may be used in juvenile salmon as small as 59 mm long.[18] It will also be a key tool for studying the early life stages of invasive species such as Asian carp and sea lamprey around the world.

In summary, we successfully designed and manufactured a revolutionary AMT by developing the smallest cylindrical battery in the world with a capacity of >1 mAh, a highly integrated circuit system that drives a PZT transducer directly without intervening components for acoustic signal generation and uses LEDs as the activation method, and a PZT tube transducer that unconventionally uses the length resonance mode for signal transmission rather than the circumference mode commonly used by existing acoustic transmitters. The AMT has an average source level of 148 dB, an operational life of 30 days at a 5-s transmission rate, and a dry weight of 0.08 g, which is ~1/3 the weight of the smallest commercially available acoustic transmitters.[10] The functionality of the AMT was fully demonstrated in real freshwater environments to study juvenile Pacific lamprey and American eels, which had never been studied with active telemetry methods before because of their thin flexible bodies and light weights. This technology enables the study of sensitive aquatic species and early life stages in riverine and estuarine environments, which are too small for the transmitters used currently. It provides critical information for ecological conservation and the development of environmentally friendly energy systems worldwide.

EXPERIMENTAL PROCEDURES

Resource availability

Lead contact

Further information and requests should be directed to the lead contact, Dr. Zhiqun D. Deng (zhiqun.deng@pnnl.gov).

Materials availability

This study did not generate new unique materials. Samples of the transmitter prototype may be available from the lead contact upon reasonable request.

Data and code availability

All of the data supporting this research have been shown in the article and supplemental information. Other related data are available from the lead contact upon reasonable request.

Battery fabrication

All of the prototype cells were assembled in an argon-filled glove box (MBraun, Stratham, NH, USA). Evaluations were conducted at room temperature, except for the temperature-variation experiments, which were performed in a temperature chamber (model TJR-A-WF4, Thermal Product Solutions, New Columbia, PA, USA). A Landt battery tester was used for the electrochemical tests of as-prepared micro-batteries at different rates. A CH Instruments (model 650D, CH Instruments, Austin, TX, USA) electrochemical analyzer was used for the impedance analysis and current measurement. The impedance analysis was performed over a frequency range from 0.01 Hz to 100 kHz with an amplitude of 5 mV.

Fabrication of the AMT prototypes

The fabrication steps of the AMT are illustrated in Figure S1. The firmware is first loaded into the microcontroller. The PZT transducer is then attached to the circuit board using a conductive silver epoxy (AA-DUCT 902, Atom Adhesives, Fort Lauderdale, FL, USA). A mixture of a non-conductive epoxy (EPO-TEK 301, Epoxy Technology, Billerica, MA, USA) and micron-sized glass bubbles (A16/500, 3M, St. Paul, MN, USA) with a mix ratio of 5:1 (epoxy:glass bubbles) is applied under a microscope to seal the 2 open ends of the PZT tube and left to cure overnight. To protect the PZT-circuit board assembly from moisture and any potential damage during subsequent fabrication steps, a 25-μm conformal layer of Parylene C is coated onto the board assembly. The micro-battery is then soldered onto the circuit board under a microscope. Individual transmitters are encapsulated in batches with the EPO-TEK 301 epoxy using an injection molding process. In this process, the transmitters are placed inside the individual cavities in a specifically designed plastic (Ultem 1000, SABIC, Pittsfield, MA, USA) mold. The epoxy then is injected through an inlet on the mold by an air sealant gun. As the epoxy is injected, it flows through all of the cavities and exits the mold through 2 outlets. The epoxy is kept flowing continually until no air bubbles are observed exiting the mold. After curing for 24 h, the transmitters are removed from the mold and polished to obtain a smooth finish so no tissue irritation could be caused to the tagged animal. Finally, another 25-μm layer of Parylene C is applied to the entire transmitter body as a coating so that the transmitter becomes waterproof and biocompatible.

Source level measurement

The source level measurements were conducted in freshwater within an acoustic water tank, which was 1.26 m long, 0.95 m wide, and 0.90 m deep. All of the interior surfaces of the tank and the bottom of the tank cover were covered with a 26-mm-thick anechoic layer (Aptflex F48, Precision Acoustics, Dorchester, UK). This layer is designed to minimize the interference of acoustic echoes and noises by significantly reducing the ultrasound reflections in the sub-megahertz frequency range. The AMT and a receiving hydrophone (model SC 001-2008-0404, Sonic Concepts, Bothell, WA, USA) were 1.0 m apart and 0.5 m below the surface of the water. An omnidirectional broadband projector hydrophone (Model TC-4034, Reson A/S, Slangerup, Denmark) was used to calibrate the receiving hydrophone before the measurements. The AMT was rotated by a motion control unit at a 10° interval and transmitted a 31-bit tag code at a pulse rate interval of 0.5 s.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrp.2021.100411.

ACKNOWLEDGMENTS

This study was funded by the US Army Corps of Engineers and the US Department of Energy Water Power Technologies Office. Eels and lamprey were handled in accordance with federal guidelines for the care and use of laboratory animals, and the protocols for our study were conducted in compliance with and approved by the PNNL Institutional Animal Care and Use Committee.

AUTHOR CONTRIBUTIONS

Z.D.D. conceived the project. All of the authors made significant contributions to development of the acoustic transmitter and writing of the manuscript and gave final approval for publication.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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