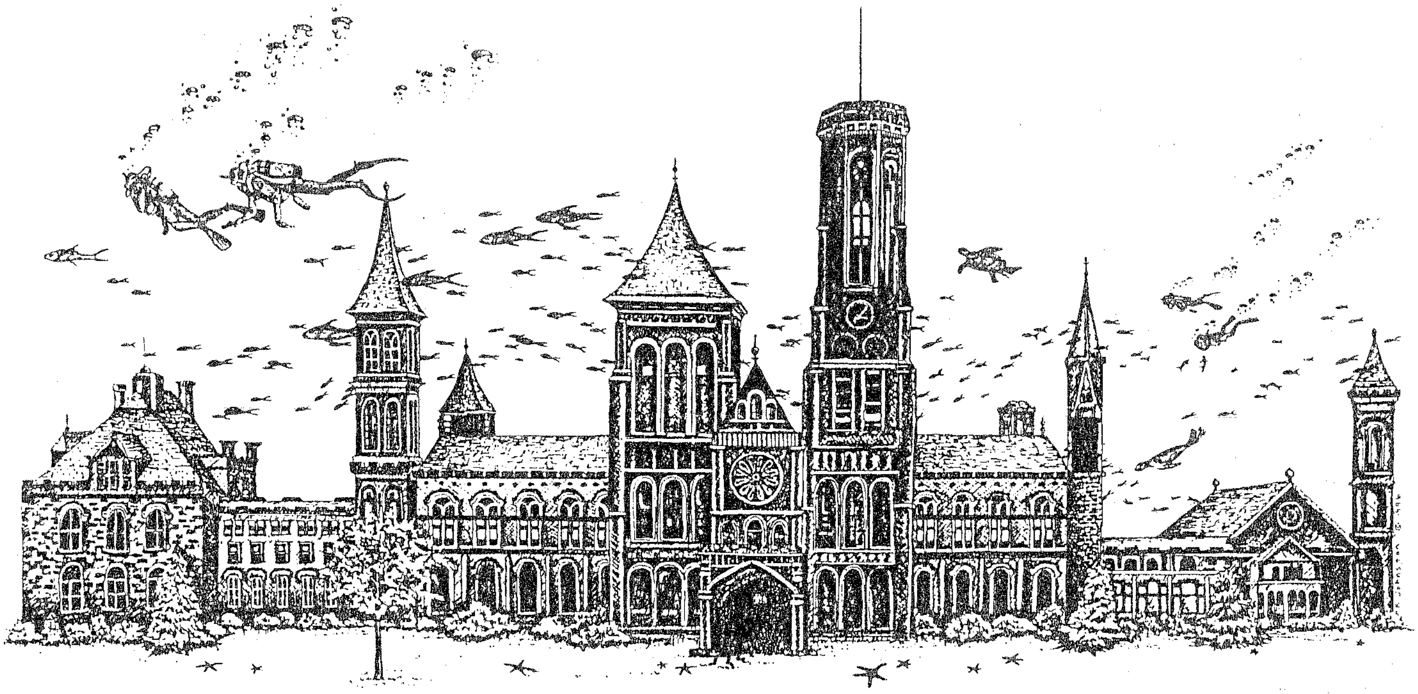
REPRINT

Lang, M.A. and C.C. Baldwin (Eds.). 1996. *Methods and Techniques of Underwater Research.*
Proceedings of the American Academy of Underwater Sciences Scientific Diving Symposium,
October 12-13, 1996, Smithsonian Institution, Washington DC. 236 pp.

METHODS AND TECHNIQUES OF UNDERWATER RESEARCH



PROCEEDINGS OF THE
AMERICAN ACADEMY OF UNDERWATER SCIENCES
1996 SCIENTIFIC DIVING SYMPOSIUM

SMITHSONIAN INSTITUTION

WASHINGTON, D.C., USA

ADVANCES IN ULTRASONIC BIOTELEMETRY FOR ANIMAL MOVEMENT AND BEHAVIOR: THE BLUE CRAB CASE STUDY

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Ultrasonic biotelemetry provides powerful new research opportunities for analyses of movement, habitat utilization, physiological function and behavior of marine organisms. Recent miniaturization of electronic components has resulted in small transmitters and data loggers, that can be applied to diverse species to telemeter not only location, but also environmental variables such as temperature and depth. An array of such tags are commercially available. Customized "smart" transmitters can be built to transmit physiological activities such as muscle contraction and EKG, as well as posture and orientation. As a case study illustrating the potential of ultrasonic telemetry, we explain how we applied "off-the-shelf" and innovative, customized tags to study blue crabs (Callinectes sapidus) in the murky waters of Chesapeake Bay. Our telemetry research is yielding new insights into habitat partitioning, feeding, agonistic interactions, molting, and mating to provide a detailed and comprehensive ecological analysis that would not be possible without this technology.

INTRODUCTION

Underwater telemetry has been used for over two decades (see review--Stasko & Pincock, 1977). In the last few years, burgeoning demand for small, battery-operated consumer electronics has stimulated development of a cornucopia of miniature, micropower electronic components. Unlike the early history of underwater telemetry, when it was technically difficult to do much more than ask "where is it?," now these components make it relatively easy to design "smart" transmitters that allow underwater scientists to ask much more complex, and at times more interesting, questions.

Simple position-finding, rather than telemetry of data, has been the dominant use of underwater electronics in the past. Locating beacons have been widely used to track animals' movements and migration and assess patterns of habitat use. In water, where low visibility often is a problem, they have been used to relocate instruments or sampling sites. Radio "beepers" have been the transmitters of choice in fresh (non-conductive) water because even very low-power ones with tiny batteries can reach distant receivers on land or aircraft (up to several km away), once the signal escapes the water. In conductive waters, radio waves are absorbed within a few cm, but sound propagates quite well (although not as well as radio waves through air). Consequently, in estuarine or marine waters ultrasonic pingers are the only practical tracking beacons, even though their transducers are heavier than radio antennas and they require larger battery capacity for comparable longevity. Their signals are received, and their position determined, by receivers coupled to underwater omni- or uni-directional hydrophones (Fig. 1). The radio and ultrasonic receivers typically operate on similar principles, converting the ultrasonic or radio frequency down to one in the audible range so the operator hears a series of "beeps". Range of ultrasonic systems, though, is much more variable than that of radio systems. One major factor that reduces range is refraction; in thermally stratified water, sound waves are "bent" toward the bottom and may completely bypass hydrophones in the resulting "shadow zones." The second major loss factor is absorption (attenuation) of ultrasonic energy by the water, suspended particles, solid objects like reefs, and especially gas bubbles (including those in or on tissues of submerged plants). Attenuation tends to increase as frequencies rise, creating trade-offs because the size of a transducer is inversely related to its resonant frequency. Thus, for small animals, operations usually are conducted between 50 and 100 MHz. On a calm day in unstratified water, such small transmitters typically can be heard to 1 km, but stratification can reduce range to < 50 m, and a bed of submerged aquatic vegetation can drop it to only a few meters.

Figure 1. Commercial (Sonotronics) ultrasonic receiver with headphones and directional hydrophone.

Systems more sophisticated than simple beacon transmitters have been utilized to provide more specific or detailed information (*e.g.*, transponders that allow sonar ranging to fish; low-frequency magnetically-coupled systems to monitor movements of lobsters fitted with antenna loops among reefs (Phillips *et al.*, 1984); or systems to telemeter physiological functions (Butler, 1989)). The challenge of making complex transmitters from pre-1980 electronic technology allowed few telemetric studies of behavior or physiology because the size and power constraints were so severe. Nevertheless, telemetry of measurements from otherwise unobservable animals provided unprecedented insights, and even revolutionized our understanding of fields such as the physiology of natural divers (seals, penguins) and the thermal biology of large endothermic fishes.

Contemporary electronic technology has opened up a host of new options in biotelemetry, allowing investigators to address an ever-expanding variety of questions. No longer are physiological or behavioral investigations limited to large animals; the abundance of tiny, micropower parts on the market allows construction of "smart" transmitters for quite small animals. Tiny discrete components (resistors, capacitors, transistors) are readily available now, as are integrated logic circuits, including families of microcontrollers (single-chip computers) as small as 200 mg.

The variety of data accessible by these "smart" transmitters also has expanded greatly with the development of small, low-power, inexpensive transducers. The following is by no means an exhaustive list of phenomena and how they may be measured by telemetry transmitters:

- biopotentials (EKG, muscle contraction, nerve firing): micropower amplifiers;
- temperature: thermistors, transistors, or digital, integrated circuits;

- posture/movement: externally by tiny switches or strain gauges or internally by biopotentials or impedance changes;
- sound/vibration: piezoceramic elements;
- light: resistive CdS cells, phototransistors, or integrated circuits;
- depth: micromachined silicon IC strain gauges;
- swim velocity: optically-read impellers, strain-gauge vanes, or voltages induced in magnetic fields; and,
- orientation: optically-scanned or fluxgate compasses.

The trend toward miniaturized parts has dramatically reduced the size of conventional "pingers" (Fig. 2). It also has made possible entirely new technologies. One example is passive implantable transponding (PIT) radio tags (Fig. 3) that provide identification at ranges of a few cm for mark-recapture studies or enumerating subjects as they traverse a narrow passage that brings them close to an energizing/receiving coil. Another is datalogging modules that memorize information for subsequent upload rather than transmitting it. These have proven particularly useful for widely-ranging animals like pinnipeds and penguins (*e.g.*, DeLong and Stewart, 1991).

Figure 2. A commercially-available miniature ultrasonic pinger producing a coded sequence of "beeps" at a crystal-controlled frequency in the vicinity of 75 KHz (Sonotronics, Inc., Tucson AZ).

A CASE STUDY: THE BLUE CRAB (*CALLINECTES SAPIDUS* RATHBUN)

Blue crabs are important predators in North American Atlantic and Gulf Coast estuaries. They are aggressive and cannibalistic, but vulnerable to predation immediately after molting, a process necessary for growth and mating.

We began using telemetry to study habitat use by blue crabs in the Rhode River (Chesapeake Bay) because the waters are too turbid for direct observations. Initially we used simple pingers from commercial sources or constructed in-house to track crabs in nature. Individuals were identified by transmitter frequency, pulse interval, or pulse-train coding. Tracking showed that blue crabs are highly mobile, and do not use various parts of the estuarine habitat randomly (Hines *et al.*, 1995). This led to the questions, "where do they forage?," "when do they show aggressive interactions (agonism)?," "where do they molt to avoid predation and cannibalism?," and "When/where do they mate?" To address these questions, we have progressively developed new capabilities in underwater biotelemetry.

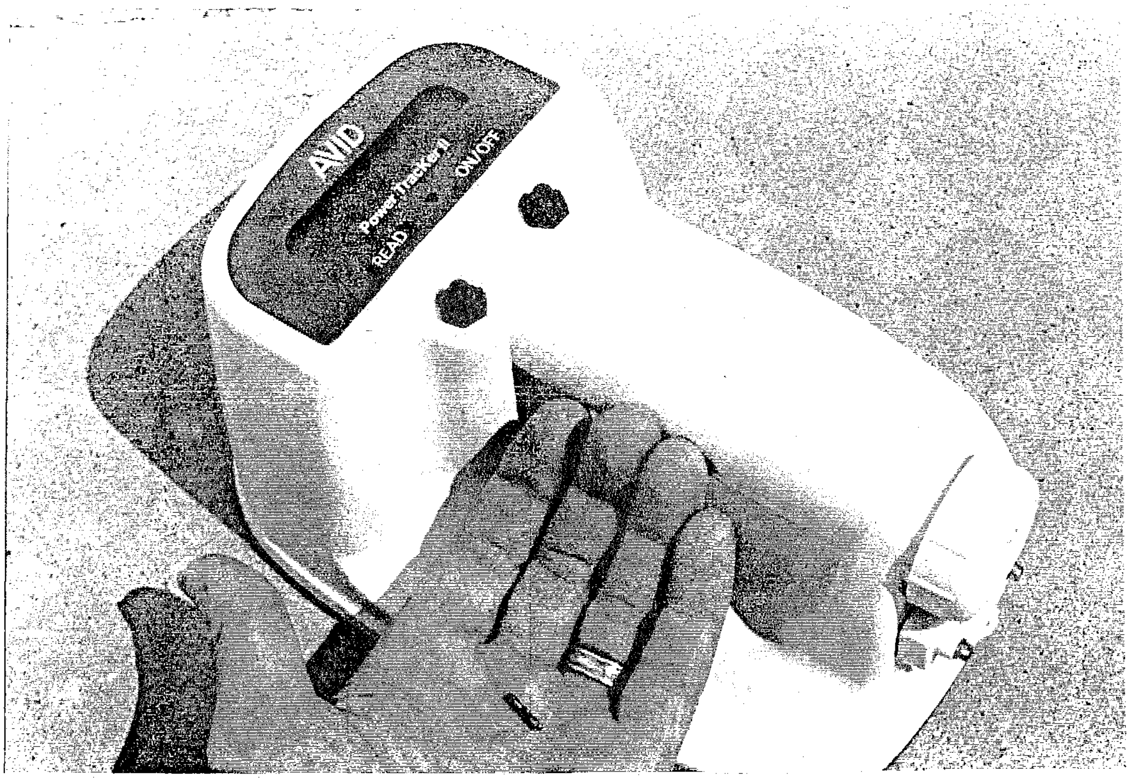

Figure 3. Passive Implantable Transponding (PIT) tag (in palm of hand) and the reader that induces power to the battery-less transmitter at one radio frequency, and reads the coded ID re-radiated by the transmitter at another frequency. The integrated circuit chip and its ferrite antenna are encapsulated in a 3 mm x 10 mm glass tube.

FORAGING

To detect where crabs forage in the field, we designed transmitters that provide short beeps for tracking and incorporate a biopotential preamplifier that prolongs the beep into a squeal whenever electrodes inserted through the carapace into the crab's mandible muscles detect the myopotentials of a contraction (Wolcott & Hines, 1989; Fig. 4).

These transmitters revealed that crabs often move rapidly for up to several km, but feed mostly while meandering in small areas of relatively high prey (clam) density, implying exploitation of patchy food resources. The number of bites during each feeding bout, calibrated by lab feedings, indicated that crabs consume mostly small and few large items. Some bouts also had complex structure, with groups of bites separated by pauses, raising the possibility that temporal structure might provide more information about food items taken in nature. Prey of known size and type were fed to the crabs in the lab and output from feeding transmitters recorded. Conversely, crabs with transmitters were released into the estuary on tethers and retrieved after a feeding bout was recorded, so stomach contents could be correlated with field and laboratory telemetry results. The results indicated that general inferences could be made about different prey types, but to do so with confidence would require precision unattainable by hand-recording (Nye, 1989), and we thus moved up to automatic logging of the telemetry data.

The patchiness of the food resources, and the high crab densities occurring in Chesapeake Bay (up to 1 crab/ m^2) raised questions about whether crabs aggregate onto prey patches. To address this, we created areas with abundant prey (clams, *Macoma balthica*) in large (400 m^2) field enclosures. Crabs with transmitters spent disproportionate amounts of time on patches, clearly aggregating to the resources.

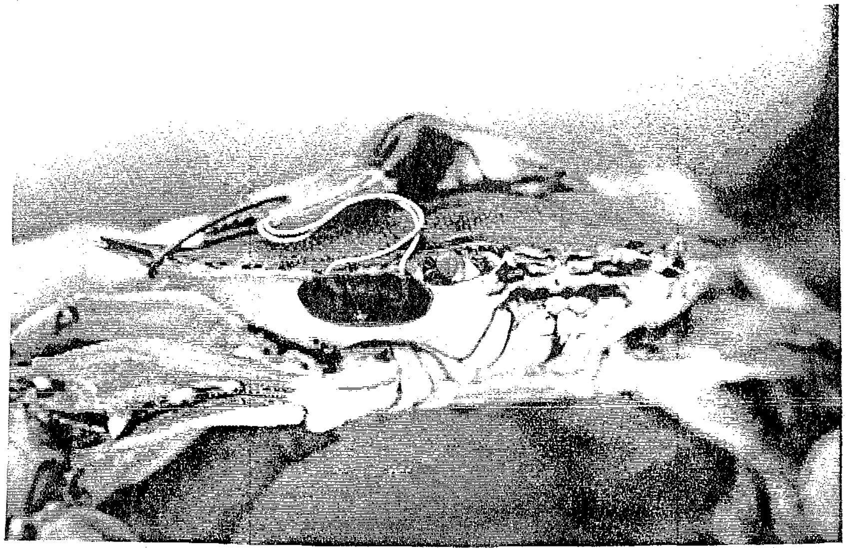

Figure 4. Blue crab fitted with a transmitter to telemeter foraging, by sensing the muscle potentials of "chewing". The electronics package is contoured to the crab's back and allows normal walking and swimming; the stainless steel electrodes are inserted a few mm into the end of the mandible muscles where they attach internally to the front of the carapace, and waterproofed with the patch of rubber from which the wires emerge.

AGONISM

Clams survive better at high versus low densities of blue crabs. This counterintuitive result led us to ask, "does aggression between crabs at high densities interfere with their ability to prey on clam patches?" To test for this phenomenon in crabs released in the field, we used transmitters incorporating a tracking beacon the pulse repetition rate of which was modulated by whether the crabs were performing a spread-claws threat display. Magnetic reed switches and magnets on the outside of the crabs' "elbows" were brought together as the claws hinged outward from the resting posture; when this turned on both switches (both claws spread), agonism was signaled. The peaks in aggressive displays occurred at or shortly after times of peak feeding, indicating that agonism and foraging are related.

To gain more detailed insight into the agonism/foraging relationship, we used more sophisticated transmitters, again in the controlled conditions of the field enclosures with introduced prey patches. Initially, we manually tracked crabs with transmitters that telemetered either foraging or threat displays. At the same time, we were developing transmitters that would telemeter multiple behaviors, in addition to individual identification, from several crabs at once (Fig. 5). These units, based on low-power microcontrollers, digitally telemeter 8 bits of data; some are used to signal on/off of behaviors, and others for individual identification. They revealed that peaks in agonism lag peaks in feeding, once again showing that agonism is associated with foraging, and suggesting that agonism could be interfering with foraging efficiency.

This result led to another question: "Is agonism associated spatially, as well as temporally, with foraging as crabs aggregate on patches?" To obtain detailed spatial information, we continued to use the digital transmitters, but installed an array of 4 microcontroller-based receivers at the corners of the field enclosure. The receivers communicated with a microcontroller-based master station that, by time-of-arrival of signals from the transmitters, determined where each crab was (within about 15 cm) and what behaviors it was exhibiting (feeding, threat display). If the position or behavior had changed, the master logged the data for subsequent upload to a laptop computer. A program on the latter allowed us to visualize the movements in accelerated time, distinguishing individual tracks and behaviors by color. This system showed that agonism decreases dramatically when there is more than one prey patch, and that foraging efficiency goes up as well. It appears that crabs will avoid each other to minimize combat damage, as long as they have alternative foraging sites.

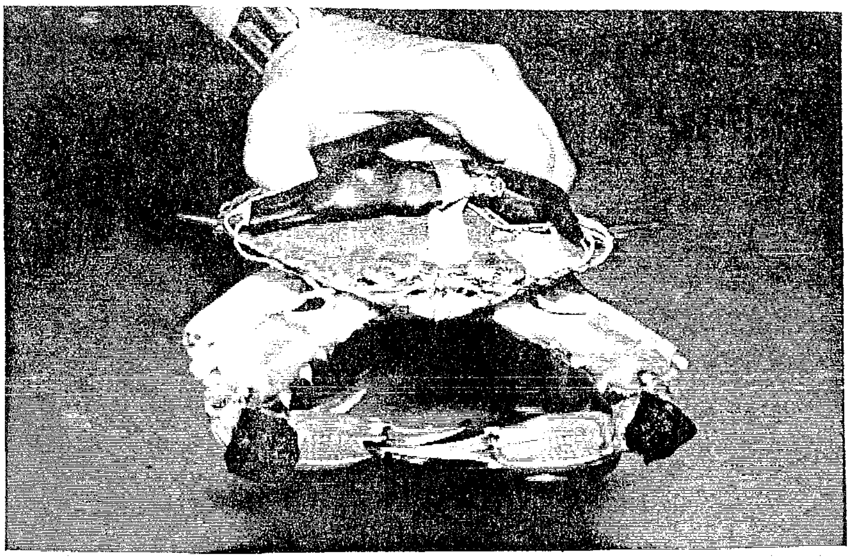

Figure 5. Blue crab fitted with multichannel digital transmitter. Electrodes for foraging behavior (mandibular biopotentials) are covered by a rubber patch on the crab's "cheek"; agonism is detected when magnets in blocks of foam rubber cemented to claws move close enough to close reed switches on the "elbows" as the claws are spread.

MOLTING BEHAVIOR AND HABITAT REQUIREMENTS

Molting crabs are very vulnerable to both predation and cannibalism, and we hypothesized that they require some protected habitat in which to molt. To determine what habitats they sample during premolt, and which they select as their molting site, we released crabs equipped with transmitters that would signal when the old shell split and pulled a magnet away from a reed switch (Fig. 6). These individuals were tracked manually until they molted, and the cast shell (with its transmitter) was then recovered at the molting site. The results indicate that gradients in predation pressure are important. In the Rhode River there is a sharp reduction in predation pressure up-estuary, and to molt

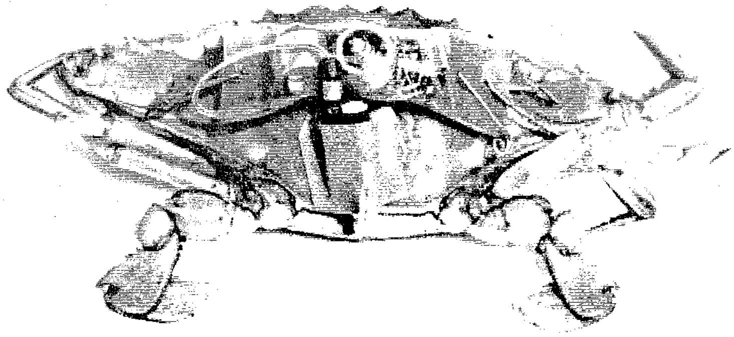

Figure 6. Shed shell of a blue crab, fitted with a transmitter that telemeters molting. As the crab lifted the dorsal carapace of the old shell and backed out, the magnet visible on the end of a monofilament "spring" was pulled free of a tube in which it was restrained next to a reed switch. The elastic monofilament swung it outward so that it would not re-close the switch after molting was complete and the dorsal carapace fell back onto the empty bottom of the exoskeleton.

adult males went up into the shallows (<15 cm) of tidal creeks (Wolcott & Hines, 1990). A more comprehensive study in the Pamlico Sound (NC), which seems to have a weaker predation gradient, showed both sexes molting over broader ranges in water averaging 1-2 m deep (Shirley & Wolcott, 1991). Both studies showed that crabs gradually reduce their mobility as they approach molt. About 3 days before molt there is a temporary dip in movement rates in areas with higher prey density than in sites ultimately chosen for molting. This may represent the animal's last chance to feed before muscle attachments become too weak.

MATING BEHAVIOR

To understand the temporal and spatial patterning of mating, our student (Matthew Kendall) has devised transmitters that telemeter time and place of pairing ("cradle carry" or "doubler" mate guarding), and copulation itself. He still is optimizing the "hug sensors" so they will not affect pairing latency or success.

LESSONS FOR POTENTIAL TELEMETRY USERS

Telemetry capability and hardware can be added to a research program incrementally if a modular plan is implemented. A quantum leap is not required. Much telemetry hardware is available off-the-shelf, including tracking beacons (some ID-coded, some available with automatic presence/absence autologging systems). Several firms offer temperature- or depth-sensitive transmitters, and a few biopotential transmitters have been marketed. Receivers that can be adapted to many kinds of data collection are readily available, and it is seldom worth the effort to design a custom unit.

To go beyond what is commercially available, researchers eventually must fabricate transmitters customized for their specific needs. The ultrasonic output stages are simple. Circuits for various "sensory" modules to modulate transmitters are available in the literature or, if not provided in papers, often can be obtained directly from the authors. Modest electronics skills are needed to adapt existing technology to new questions, and collaborators experienced in telemetry can be helpful, as can electronics engineers if they are able to embrace the telemetry ideals of "no parts, no power, no weight." The investment (especially of time) in a new transmitter type is not inordinate, but should not be expected to be trivial. Many potential users are discouraged by the notion that an underwater telemetry transmitter is a "use it <u>and</u> lose it" device. This need not be true if arrangements can be made to have transmitters fall off at the end of their useful life when they can be recovered with simple diver-operated directional hydrophones (Fig. 7). Our first such unit was constructed in a few hours, using a peanut butter jar as the housing, and successfully recovered a transmitter from the field.

Figure 7. Underwater diver-operated hydrophones for relocating ultrasonic transmitters. Both have light-bar displays that provide a simple indication of signal strength as the directional "funnel" is scanned back and forth; the larger unit has dual elements and provides visual indication of the side receiving the stronger signal. Similar devices, using underwater earphones to provide directional information to the diver, are commercially available.

Underwater telemetry, with ready access to such diverse building blocks, now presents many more options to researchers. Where direct observation is impossible or too intrusive, modern telemetry will allow us to "look" into many facets of aquatic animal ecology, physiology and behavior. The techniques presented above in our case study are not restricted to crabs. The ability to detect postures, or to sense biopotentials, is applicable to an enormous variety of animal activities and biological questions.

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