Technical Report 0001

Biointerfaces for Two-way Communication to Assess Hazards in the Aquatic Environment

U.S. Army Center for Environmental Health Research
Fort Detrick, MD 21702-5010

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U.S. Army Medical Research & Materiel Command
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Workshop Report:
Biointerfaces for Two-way Communication to Assess Hazards in the Aquatic Environment

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13. ABSTRACT *(Maximum 200 words)*

In October 1998, a workshop sponsored by the Defense Advanced Research Program Agency (DARPA) convened to discuss the potential for using instrumented aquatic sentinel species to transmit or bring back information relevant to environmental and human health hazards in geographical regions of concern. Workshop participants, including experts in the fields of ecology, toxicology, telemetry, animal behavior, and sensor technology, discussed research on tracking free-ranging aquatic organisms and receiving telemetered information on movement, behavior, and physiological condition. Monitoring systems now functional or under development can monitor physiological parameters such as activity and swimming speed, movement patterns, heart rate, cardiac output, and ventilation rate. Increasingly sophisticated approaches are being developed for interacting with free-ranging aquatic species, and future possibilities include the use of various devices to direct the movements of aquatic organisms to specific areas of concern. Considering advances in technology that are now or will soon be available, workshop participants discussed how best to implement the use of living environmental biomonitors. Workshop participants were enthusiastic about the future potential for using aquatic organisms to provide timely information on contaminants in freshwater and marine environments.

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ACRONYMS

APL	Applied Physics Laboratory (Johns Hopkins University)
AUV	Autonomous Underwater Vehicle
CHAT	Communications History Acoustic Transponders
CNS	Central Nervous System
DARPA	Defense Advanced Research Projects Agency
DoD	Department of Defense
DSP	Discrete Spectrum Processor
EKG	Electrocardiogram
EMG	Electromyogram
FINS	Fish Inertial Navigational System
GPS	Global Positioning System
MEMS	Microelectromechanical Systems
NINA	Norwegian Institute for Nature Research
RF	Radio Frequency
SAV	Submersible Assessment Vehicle
USACEHR	U.S. Army Center for Environmental Health Research
XMTR	Transmitter

WORKSHOP HIGHLIGHTS

In October 1998, a workshop sponsored by the Defense Advanced Research Projects Agency (DARPA) convened to discuss the potential for using instrumented aquatic sentinel species to transmit or bring back information relevant to environmental and human health hazards in geographical regions of concern.

Workshop participants included national and international experts in the fields of ecology, toxicology, telemetry, animal behavior, and sensor technology. Several experts presented their research efforts on tracking free-ranging aquatic organisms and receiving telemetered information on movement, behavior, and physiological condition. Species monitored ranged from invertebrates such as lobsters to different species of fish. Systems now functional or under development can monitor physiological parameters such as activity and swimming speed, movement patterns, heart rate, cardiac output, and ventilation rate. Increasingly sophisticated approaches are being developed for interacting with free-ranging aquatic species, and future possibilities include the use of various devices to direct the movements of aquatic organisms to specific areas of concern.

Considering advances in technology that are now or will soon be available, workshop participants discussed how best to implement the use of living environmental biomonitors. They proposed conceptual approaches under two different scenarios: a "Blue-Sky" approach with no preconceived configurations or limitations and a Near-Term approach incorporating existing or developing technology. Examples of "Blue-Sky" concepts included:

- Send a few fish with limited instrumentation into an area to evaluate general hazard. If a hazard is found, send in additional fish equipped with a complete sensor package plus a submersible assessment vehicle (SAV). The SAV will provide additional sensors and serve as a communication platform to satellites through buoys. A variation on this approach proposed that fish would rise to the surface to facilitate communication rather than using an SAV.
- Use multiple fish species outfitted with sensors to monitor activity, stress, and chemical-specific responses. A collective network system would be used to share information among fish; each fish would have the capability for on-board processing and self-calibration. Fish would be guided using a Fish Inertial Navigational System. A drone fish or autonomous underwater vehicle (AUV) would be used for outside communication.

Examples of Near-Term concepts are:

- Use a number of free-roaming fish with location sensors and a few with physiological and chemical sensors. If abnormal conditions are indicated, a technician or SAV with samplers and additional sensors would enter the area and sample
- Deploy caged fish to areas of concern along a gradient including cages in reference (clean water) areas. Cages would include physicochemical sensors plus communication technology.

- Use multiple species and sensors. Data would be gathered by an AUV and transmitted to the user through buoys.
- Use oceanographic platforms equipped with physicochemical sensors. Monitor fish that are either caged or free-swimming. If an event were noted, investigators could analyze water collected by water samplers on the platform.

Workshop participants were enthusiastic about the future potential for using aquatic organisms to provide timely information on contaminants in freshwater and marine environments. Some of the research challenges that lie ahead in the further development of this promising area include:

- Developing techniques to control the movement of free-ranging aquatic organisms through techniques such as genetic engineering, associative learning, lateral line stimulation, or olfactory stimulation.
- Designing signal acquisition and processing systems that maximize the range for tracking and monitoring aquatic organisms while minimizing the need for intrusion into monitored areas.
- Distinguishing physiological/behavioral patterns indicative of toxic contaminants from those caused by other environmental and biological variables.
- Miniaturizing components to minimize impact on aquatic organisms and on power requirements.

1. INTRODUCTION

In October 1998, a workshop sponsored by the Defense Advanced Research Projects Agency (DARPA) convened to discuss the possibilities of developing controlled biological systems that could assist the military in assessing environmental quality concerns. The concept of using aquatic species for military purposes has already been applied in the Navy's highly successful marine mammal program. Monitoring systems now exist which give real-time information on the physical condition of mammals or fish housed in pens or tanks. The larger question is whether such an instrumented sentinel species released in an area of concern can transmit or bring back information relevant to environmental and human health hazards.

National and international specialists in the fields of ecology, toxicology, telemetry, animal behavior and sensors met to explore and discuss the possible applications of aquatic organisms as living sensors and gathers of environmental information (see Agenda, Appendix A). Presentations were given by attending scientists summarizing their research experiences and areas of expertise. The participants were then tasked with developing practical approaches to using living environmental biomonitors under two different scenarios: a "Blue-Sky" approach with no preconceived configurations or limitations and a second approach incorporating existing or near-term technology. For this purpose, the participants were assigned according to areas of expertise to one of three groups, in order to maintain a diversity of disciplines in each group. The concepts, approaches and findings of each group are summarized and evaluated in this report.

2. SYNOPSIS OF PRESENTATIONS

Most of the first day of the workshop featured presentations by experts on a range of topics relevant to controlled biological systems in aquatic environments. Subjects discussed included:

- Telemetered physiological data from free-ranging fish (section 2.1, McKinley)
- Tracking and receiving telemetered information on the physiology and behavior of aquatic organisms (section 2.2, Wolcott)
- Using telemetered data to determine fish movement and behavior patterns in response to environmental factors (section 2.3, Moser)
- Tracking the behavior, activity (EMG) and physiology of free-ranging fish species (section 2.4, Økland)
- Behavioral assays to assess the effects of neurotoxic contaminants and other stressors on aquatic organisms (section 2.5, Little)
- Aquatic toxicology of military unique compounds and other materials (section 2.6, Burton)
- Developing and applying sensors, processing and analyzing signals, and telemetering/communicating data in the marine environment (section 2.7, Sarabun)
- Sensors and the "electronic canary" (section 2.8, Knechtges)

- Tracking and physiological monitoring systems for free-ranging aquatic organisms (section 2.9, Voegeli)
- Animal telemetry systems (section 2.10, Anson)
- Real-time automated biomonitoring (section 2.11, Shedd)
- Behavioral responses of fish to low frequency acoustic patterns (section 2.12, Brown)
- Biotelemetry and behavioral ecology of lobsters (section 2.13, Watson)
- Real-time biomonitoring of bees to assess environmental contamination (section 2.14, Bromenshenk)

These papers provided valuable background information for the subsequent breakout group discussions of the Near-Term and "Blue-Sky" possibilities for using free ranging aquatic animals to provide information on potential human health or environmental threats.

2.1. Research and Development in Fish Physiological Telemetry. Presented by: Dr. R. Scott McKinley, Professor and Industrial Research Chair in Biotelemetry, Department of Biology, University of Waterloo, Waterloo, Ontario, Canada.

The overall goal of Dr. McKinley's research is to assess environmental change from the perspective of the free ranging animal. To accomplish this goal Dr. McKinley has employed a number of physiological telemetry procedures to remotely monitor the "well-being" of the animal in the wild. Measures of "well-being" include cardiac output (stroke volume X heart rate), electromyogram activity of various muscles and heart rate. These telemetered signals can be detected up to 1 km in freshwater. To date, these physiological parameters have been measured on a number of fish species. He is currently involved in the development and testing of biochemical micro-probes (0.3 mm diameter) to monitor "stress levels" in fish. In addition, Dr. McKinley simultaneously monitors environmental conditions surrounding individual fish. These include water depth, water temperature, conductivity and light incidence (geo-positioning). Future environmental sensors will include ammonia and pH. Dr. McKinley is presently conducting studies to assess the effects of physical (water flow/temperature/pH) and chemical (chlorine, ammonia, tetrachlorophenols) stressors on free-ranging fish. Dr. McKinley's research seeks to address the following questions:

- Can we accurately reflect metabolic changes in free swimming fish?
- How does metabolic rate vary response to acute/chronic environmental changes?
- How does acclimation influence recovery?
- Can we assign a metabolic cost to environmental change?

2.2. Behavioral Ecology/Telemetry. Presented by: Dr. Thomas Wolcott, Professor, Marine, Earth and Atmospheric Sciences, North Carolina State University.

Dr. Wolcott has worked in terrestrial systems, investigating the role of land crabs in their ecosystems by using radio and optical transmitters to track individuals and monitor temperature and heart rate. He has collaborated with avian biologists and veterinarians by designing radio systems to telemeter heart rate or multiple internal temperatures. For aquatic systems he has developed ultrasonic transmitters that permit tracking individual animals or re-locating instruments, as well as systems that telemeter various kinds of information from free-ranging animals. Examples include individual identification codes, molting, feeding, swimming, depth and aggressive displays in blue crabs, and tailbeat frequency as an estimator of metabolic cost in young sharks. In large field enclosures, an array of hydrophones was used to continuously log behavior, position and interactions of multiple animals. His recent transmitter designs telemeter accumulated data summaries to minimize manpower requirements and missed data. He also has developed hardware and software for a microcontroller-based, autonomous vertically-migrating drifter instrument capable of responding to environmental variables and mimicking movements of living larvae to elucidate how clouds of larvae (or pollutant plumes) might be transported by small-scale current regimes. This instrument is being upgraded by addition of an on-board GPS receiver so it can log its own tracks.

2.3. Research and Development in Fish Behavior. Presented by: Dr. Mary Moser, Research Associate, Center for Marine Science Research, University of North Carolina at Wilmington.

Dr. Moser has been studying fish physiology and behavior since 1982. Experimental work has included laboratory testing of salinity and dissolved oxygen effects on respiration. Field observations of behavior have included the use of sonic and radio telemetry, and underwater camcorders. Many transmitters have been tried by her team including monitors by buccal attachment. Research has been conducted on a variety of species; marine, estuarine and anadromous fishes. Research objectives have included experiments to aid upstream passage of anadromous alosids. Field studies were designed to direct anadromous alosids into a lock chamber using current velocity changes and into road culverts using light. Passage was increased by 30-65% using these attraction methods. Her current research program involves prevention of Shortnose and Atlantic sturgeon from entering blasting zones. This research demands that cues to fish behavioral changes be identified along with telemetry and acoustic profiling to assess individual and group behaviors. She has found that sturgeon do not avoid dam turbines and may even be attracted because of bottom disturbance. She has also observed shad to be very sensitive to environmental changes, i.e., light intensity or cars passing on a bridge can affect their behavior. Dr. Moser's interest include controlling fish direction and determining how water currents affects fish. She has used acoustic devices to assess position, and light and vibration to produce fish movement. She is particularly interested in whether it is possible to use hormones to elicit migrational responses.

2.4. Applied Research Using Physiological Telemetry. Presented by: Dr. Finn Økland, Coordinator of Aquatic Biotelemetry Studies, Norwegian Institute for Nature Research (NINA), Trondheim, Norway.

Dr. Okland has developed different methods for attachment of radio transmitters, acoustic transmitters, and physiological telemetry tags and sampling techniques for manual and automatic tracking. These techniques have been used in several projects, mainly focusing on recording the behavior, migratory pattern and swimming speed of adult salmonids in rivers in Denmark, Iceland, Finland, Norway, Russia and Sweden. Other projects, including several Danish studies, using miniature radio tags surgically implanted into the body cavity to follow the movement and estimate the predation of Atlantic salmon (*Salmo salar*) smolts. Transmitters have also been implanted into Northern pike (*Esox lucius*) and pike perch (*Stizostedion lucioperca*) to monitor the home range during a one year period in two Danish lakes.

In 1993, investigations to develop a technique to record spawning behavior in Atlantic salmon started. First a method was developed to identify spawning behavior from other types of activity by use of Eiler activity tags. This method was used to successfully record spawning behavior in wild and farmed salmon. In 1994, Dr. Okland participated in developing a surgical technique for implanting EMG radio tags in Atlantic salmon in Canada. By recording the bioelectrical activity directly from the axial swimming muscle this method can more accurately record both swimming and spawning related behaviors. He has used this technique to correlate the EMG signals to swimming speed in Atlantic salmon, brown trout and lake trout using a swim speed chamber. The accuracy of this technique has also been studied in Atlantic salmon by comparing the EMGs recorded with the actual behavior of the animal. He has also participated in the development of surgical procedures to implant heart rate transmitters in wild Atlantic salmon in Canada.

In Norway, investigations includes recording swimming performance of wild and farmed Atlantic salmon just prior to spawning and swimming performance of Adult salmon carrying external and implanted telemetry transmitters. At sea, wild salmon have been followed during the last stage of return migration by use of coded acoustic transmitters; and their movements recorded using a newly designed dual hydrophone system for automatic determination of direction. Work in Italy involved surgically implanted coded acoustic transmitters in Dusky groupers (*Epinephelus marginatus*) to record homing, site fidelity when released into a marine reserve. Since 1991, Dr. Okland has been involved in approximately 40 different research projects with aquatic telemetry having ongoing collaboration with scientists in several countries. His speciality is bridging the gap between the biological and technical requirements of the many different methodologies used in telemetry.

Studying fish in rivers and oceans required various monitoring techniques, with tradeoffs between longevity, signal strengths and tag size and weight. Since tags now can be programmed to turn on and off during the project, the longevity in smaller tags can be significantly increased. Equipment for telemetry is developing rapidly and Dr. Okland is particularly interested in the development within two areas. The first is the possibility to automatically record the position of animals in three dimensions over larger areas using acoustic techniques. Having this possibility, he would like to record more than just the location. Therefore, the second area is the development within physiological tags, particularly EMG and heart rate (or better cardiac output). Reliable estimates of energy expenditure (EMG) and physiological state (heart rate, cardiac output) can be used to increase the biological knowledge of fish behavior and to identify and assess environmental hazards.

2.5. Fish Neuro-behavior. Presented by: Dr. Ed Little, Columbia Environmental Research Center, USGS, Biological Resources Division.

Dr. Little is a behavioral toxicologist whose interests include looking at ways to translate behavior into a population-level effects. His research focuses on consequences of altered habitats from environmental contamination, global change, and physical habitat disruptions relative to survival and viability of aquatic organisms. Basic and applied laboratory and field research are conducted to determine: 1) the influence of sublethal contaminant exposure on the behavioral function of aquatic organisms, 2) the impacts of ultraviolet radiation on amphibians and aquatic species and understanding the biotic and environmental factors affecting UV-induced injury, and 3) the effects of physical habitat alterations on habitat use and distribution of sturgeon in the Missouri River. The goal of these efforts is to provide scientific understanding and technologies needed to support sound management and conservation of natural resources. Through his work he has developed a suite of behavioral assays measuring predator/prey interaction, locomotory responses, competition, contaminant avoidance, and physiological correlates of behavioral neurotoxicity. Dr. Little utilizes computer-assisted analysis of locomotory behavior to measure speed, distance traveled and tortuosity of path and considers locomotory behavior a good candidate for monitoring neurotoxins. Avoidance of metals-contaminated plumes is presently being evaluated in Chinook salmon at the Hanford Nuclear Reservation on the Columbia River. Dr. Little has deployed sonic buoys to monitor the movements of Pallid sturgeon over 125 miles of the Missouri River.

2.6. Aquatic Toxicology. Presented by: Dr. Dennis Burton, Senior Research Scientist, Wye River Research and Education Center, University of Maryland at College Park.

Dr. Burton studies the aquatic toxicology of military unique compounds which include chemical warfare agents and their degradation products, munitions, explosives and propellants. He is interested in finding out whether sensors on fish can detect these agents. Current environmental dilemmas faced by the military are submerged ordinance with casings that are corroding over time, hazardous waste dumps, and non-point contamination sources. Contamination is also associated with older military industrial practices requiring contamination mapping, spills and leaks from equipment and weapon systems, and transportation accidents. Submerged pipeline leakage of oil and gas has released hydrocarbons and heavy metals. Releases of contaminants also can occur from deployment activities such as the intentional releases by oil fires experienced during the Gulf War. Intentional poisoning of resources by the enemy are other concerns that the military faces in deployment situations. Treaty information may also require toxicological data verification.

2.7. Biological Signal Acquisition and Interpretation. Presented by: Dr. Charles Sarabun, Senior Research Scientist, Applied Physics Lab, Johns Hopkins University.

Dr. Sarabun has been involved with numerous projects concerned with measurement and analysis of physical oceanographic, electric, electromagnetic, and acoustic data from the marine environment. He has collaborated with U.S. Army Center for Environmental Health Research (USACEHR) in modifying the fish ventilatory monitoring electrodes/signal conditioning for operation in high salinity water, improving the algorithms used for data analysis and the measurement of nutrients (nitrogen, phosphorus) and chlorophyll-a fluorescence. The APL is involved with numerous research, development, testing, and evaluation programs with the Department of Defense (DoD) (air defense, submarine technology, space applications, strategic systems, and power projection systems) and medical applications in science and technology. Areas of expertise include: 1) sensor fabrication, modification, and deployment applications; 2) signal processing and analysis; and 3) telemetry and communications applications. Examples of sensor fabrication/application include: 1) miniaturization of Time of Flight Mass Spectrometer to suitcase size, FTIR-SERS (Fourier Transform Infrared Surface Enhanced Raman Spectrometer), and Mass-Spectrometer Threat Identification System for biological warfare agents; 2) fabrication of templated polymer using ligands for molecule specific recognition; and 3) micro mechanical and composites design and fabrication (MEMS). Signal processing/analysis capabilities include 1) application of processing technologies (filtering) to acoustic surveillance in "noisy" environments; 2) MRAL (Multiple Return Association and Localization) implementation and Alpha-Beta tracking to improve location recognition and tracking of mobile "targets"; 3) use of information processing chain (clustered threshold - M-of-N - feature discrimination - time evolution discrimination) for discriminate analysis of sonar signals; and 4) application of these technologies in environmental use (bluegill ventilatory signal processing using time/frequency/time-frequency domain processing). Research/expertise in the area of telemetry and communications include high frequency ground wave (data/voice from submerged platform via buoyant cable), RF (buoys, satellite communications, balloon-borne devices), biomedical inductive link, infrared links, and antenna/antenna systems development.

2.8. Biosensors. Presented by: CDR Paul L. Knechtges, Program Officer, Office of Naval Research, and Deputy Director, U.S. Army Center for Environmental Health Research.

Commander Knechtges has surveyed emerging sensor technologies and disciplines with interest in development of the "electronic canary" for use during military deployments. He has visited many universities, federal laboratories, and private companies to learn more about these new technologies. CDR Knechtges summarized key technologies and disciplines that may someday become the building blocks of the ideal risk analysis tool. These technologies and disciplines include advanced materials chemistry, microelectromechanical systems (MEMS), cybernetics, biomimicry, tissue-based sensors, and advanced signal processing, including artificial intelligence. Advances in materials chemistry are most notable in the field of polymer chemistry; scientists and engineers can now custom-build polymers with special properties and selective affinities for substances. Some of these polymers can incorporate biomolecules or their analogs in the polymer structure, making them act as bioreceptors. Such advanced materials could also be incorporated into MEMS, which are receiving widespread interest from the military and private sectors. MEMS incorporate the functions of computing, communication, and power with the ability to sense, actuate, and control for larger systems. Some of the more interesting MEMS include electo-optical devices that can sense chemicals with remarkable response times.

Scientists and engineers in the fields of cybernetics and biomimicry will lead the way in linking these technologies to living organisms and interpreting the resulting data flow. Some promising near-term advances in this area include in situ physiological monitoring of whole animals and tissue-based sensors.

2.9. Research and Development in Aquatic Transmitters. Presented by: Fred Voegeli, Founder/President VEMCO Limited, Canada.

Mr. Voegeli is an electrical engineer with twenty-five years in the field of bio-telemetry. VEMCO Limited specializes in sensors and ultrasonic telemetry and tracking systems with research scientists clients in marine biology and fisheries in 42 countries. With collaboration from Dalhousie University Biology Department, they have successfully developed three dimensional radio linked ultrasonic positioning systems and a number of novel sensor systems for fish and cephalopod physiological monitoring. Existing technology for gathering data from sensors on and in underwater animals and transmitting the data to the surface via acoustic telemetry includes sensors for measuring depth, temperature (internal and external), swimming speed, heart rate, EMG, mortality, and differential pressure. New sensors under development are cardiac output (ultrasonic Doppler blood flow), respiration (ventilatory pressure), conductivity, tail beat dynamics, geolocation using light sensor forming sextant and geolocation using magnetic flux measurements. Transmitters include simple position tags, data telemetry tags, data storage and retrieval tags (archival tags) and Communications History Acoustic Transponders (CHAT) tags transmitters which store data and download data with two-way acoustic telemetry.

Receivers that have been developed are VR60 basic telemetry receiver with connection to a personal computer for data gathering and GPS position, VR28 four channel system with 360 degree horizontal scan showing direction to fish, VR170 seventeen element receiver system for 3D positioning and tracking, VRAP-BUOY system for fine scale positioning and data telemetry with 1 meter resolution, and VR20 automated fully submerged download site for CHAT tag data.

Future goals are building on their strength in physiological monitoring. Current work in miniaturization of the Doppler blood flow probe is only the first step in designing tools for energetics and stress measurement. The necessity of innovative sensors has been recognized to qualify the stress measurement so that normal reactions to predators, prey, and reproduction activities can be identified. It is intended to develop sensors which are less invasive and can be deployed quickly and consistently with minimum of handling of the animal. VEMCO has made significant advances in Tail beat dynamics sensors which measure the total output power of a fish. This technique is far better than electromyogram because it does not limit the user to one of red or white muscle, does not suffer from delicate and unreliable electrode placements and is fast and repeatable.

2.10. Research and Development in Aquatic Transmitters/Sensors. Presented by: Mr. Peter Anson, Vice-President of Technology, LOTEK Engineering, Inc., Canada.

LOTEK is an electronic (hardware/software) engineering company specializing in animal information systems. Mr. Anson has been with the company since inception (1984) and has been actively involved in the development of most of its technical products. He is the conceptual originator of the MAP 500 spread spectrum acoustic telemetry system. He was the chief architect and sole software developer for the DSP 500 digital radio receiver. He engineered the first application of algebraic coding to wildlife telemetry. He provided overall project management for the world's first GPS animal location system, including an object-oriented real-time operating system and a layered communication subsystem using HDLC and Reed-Solomon coding. He also supervised hardware and software development of the SRX 400 VHF receiver/data logger, and led a research and development group working on the development of wireless LAN technology. Mr. Anson's special interests are in the area of the digital and statistical signal processing and coding.

Lotek has been supplying radio telemetry equipment to the wildlife research community for 15 years. Products include a VHF receiver/data logger, a digital radio receiver/coprocessor for real time signal detection and frequency analysis, antenna switching and frequency conversion peripherals, animal GPS systems, and many varieties of radio transmitters (including EMG, temperature, pressure, and heart rate sensing systems). In the last four years, we have been working on advanced acoustic systems, and we are field testing an acoustic spread system (Code Division Multiple Access - CDMA) 3-D positioning system. Other marine products include data storage tags for open ocean applications, depth sensors and combined acoustic/radio telemetry systems. Lotek has core competence in radio and acoustic design, microcontrollers, GPS, low power system design and circuit miniaturization, and digital signal processing.

2.11. Introduction: Biological Controls. Presented by: Mr. Tommy R. Shedd, U.S. Army Center for Environmental Health Research.

Mr. Shedd has been involved with the development of new acute and chronic aquatic toxicity test methods. Recent research has focused on the development of test methods that provide real-time data for rapid aquatic hazard detection and evaluation. The USACEHR mission is to direct and conduct research, development, testing, and validation in the areas of medical environmental surveillance and environmental health in support of force medical protection. The USACEHR is investigating the use of sentinel biological systems for early detection of toxic hazards in the environment. The bluegill sunfish and the honey bee sentinel systems under development have shown promise in the early detection of acutely toxic conditions.

The automated fish biomonitoring system continuously monitors the ventilation and movement patterns of the bluegill (*Lepomis macrochirus*). Physiological stress to the bluegills, characterized by changes in fish ventilation and movement patterns, is used as an early warning to identify developing acute toxicity of a treated groundwater (effluent) discharge at Old O-Field, Aberdeen Proving Ground, MD. A personal computer continuously monitors and records ventilatory rate, ventilation depth, cough rate, and whole body movement of up to 32 fish simultaneously. When the monitoring system identifies a potentially toxic event, a sample is automatically collected for chemical analysis, a remote monitor in the treatment facility control room identifies the problem to the facility operators, and if necessary, the discharge is diverted to storage tanks until the problem is resolved. The USACEHR has funded a number of studies using sentinel species to monitor toxic hazards in the environment in and around military facilities (e.g., honey bees; see section 2.14).

2.12. Fish Behavior/Lateral Line Response. Presented by: Dr. Neil A. Brown, the University of New Orleans (currently with NAB&Associates, Inc., Lexington, MA).

Dr. Brown is a naval architect involved with hydrodynamics, underwater acoustics, ship and weapon silencing. While involved in a project attempting (with little success) to acoustically deter fish from fatal entrainment into hydro turbines and cooling water intakes, he learned of Norwegian research that showed juvenile salmon to be deterred by infrasonic (10 Hz.) pulsations in shallow water. Ruling out "hunger" and "lust" as exploitable drivers for fish deterrence, Dr. Brown hypothesized that the operative driver for this observed response was "fear" evoked by the perception of predator attack. Examining this "Predator Hypothesis," the water flow field before an attacking fish was studied analytically, yielding, first, that the associated time scale was indeed commensurate with the period of a signal with frequency 10 Hz. Next, and most convincing, was the finding that the spatial scale of the gradient of water particle hydrodynamic motion was quite precisely matched to the length of the lateral line of a small fish (salmon smolt). Acoustic signals were ruled out as stimuli, being orders of magnitude too long in wavelength to match the lateral line. The hydrodynamic near-field of a monopole, however, has the appropriate length scale. The lateral line of the prey-fish, in conjunction with the ears, which are highly sensitive transducers of whole-body motion, is therefore indicated as the sensor for the transient hydrodynamic signal of an attacking predator fish. It is suspected that, when appropriately stimulated, this sensor system triggers an involuntary flight reaction; perhaps involving the Mauthner cells - but this is a speculation outside Dr. Brown's competence.

Together with personnel of Alden Research Laboratory, Holden, MA, Dr. Brown developed and patented a fieldable, high-powered system called the Particle Motion Generator (PMG). Direct measurements of the hydrodynamic field confirmed its similarity to that of the Norwegian laboratory device, but with adjustably larger amplitude as well as selectable frequency. Field trials against caged fish, however, yielded inconclusive results. Was the field too strong? Were the fish being rapidly habituated? Was the confinement of the cage a factor? Did strong harmonics in the high level signal overwhelm the fish's discrimination ability? These and other questions must be resolved in order to effectively utilize hydrodynamic signals for communication with fish.

2.13. Physiological Ecology. Presented by: Dr. Windsor Watson, Professor of Zoology and Director of the Center for Marine Biology, University of New Hampshire.

Dr. Watson's expertise lies in the general areas of neuroethology and physiological ecology. He has worked primarily with marine invertebrates, but also has experience with primates, fish, and other vertebrates. Two current areas of research particularly relevant to this workshop is bio-telemetry and behavioral ecology of lobsters. He has used telemetry to track the movements of lobsters and horseshoe crabs in estuaries, and has developed systems to transmit physiological data, such as EMGs and EKGs . His goal is to be able to obtain relevant behavioral and physiological data from freely moving animals in their natural habitat. The advantage of using large mobile invertebrates is unimpeded behavior while equipped with transmitter backpacks, relatively slow movement which enhances tracking capabilities, and stereotypical behaviors that enable easier translation of transmitted data into actual behaviors and responses. Recent work includes both field and laboratory studies to investigate the behavioral responses of lobsters to temperature and salinity. Field studies indicated that lobsters, like many mobile estuarine species, move up and down the estuary in response to changing gradients of temperature and salinity. Sensitivity studies are underway to determine lobsters' responses to changes in temperature and salinity and how lobsters translate sensory input into appropriate movements. His team has developed a cardiac assay useful for indicating when lobsters detect a change in their environment and they have been using it to tell if they sense very small changes in temperature, salinity and light levels. This very sensitive assay is believed to be adaptable to detection of contaminants in the water. Also designed and employed is avoidance/attraction behavioral assays to determine the responses of lobsters to temperature and salinity. These assays have given insight into how these animals respond to certain stimuli. This knowledge will help in investigations of the learning capabilities of lobsters and the possible control of their movements using operant conditioning paradigms combined with advanced electronics. Another system being developed is the use of lobsters, clams and fish to detect contaminants in estuaries, taking advantage of current technologies and their most recent findings with lobsters. This self-contained system will be designed to be deployed in a number of sites within an estuary. The goal is to determine if such a technique will provide a sensitive indicator of water contamination, and whether it is possible to tease apart biological responses to contaminants from responses to normal fluctuations in light levels, currents and water chemistry.

2.14. Biomonitoring Systems Using Bees. Presented by: Dr. Jerry Bromenshenk, Adjunct Professor, Division of Biological Sciences, University of Montana-Missoula.

Dr. Bromenshenk's defense-funded research program brings together an interdisciplinary team to provide analytical and engineering tools to optimize real-time, telemetric extraction of information from biological systems. These studies include identification of spatiotemporal codes of information at the cellular through the population level that can be correlated to the performance dynamics and behavior of bees and their colonies. Also included are real-time chemical sampling and analysis systems that document the immediate responses of bees to exposures to toxic chemicals. The objective is to provide new technologies and associated applications that are enabled by real-time, field-based, one- and two-way communication with biological systems. Applications encompass a greatly improved knowledge of the basic functioning and control systems employed by these highly social insect systems, new approaches 'to biological research and educational models, environmental monitoring, enhanced pollination strategies, improved screening of semiochemicals, and use of bees by DoD as controlled biological systems; i.e., detection of the presence of chemical and biological agents of harm (see http://www.biology.umt.edu/bees). This program utilizes smart hives fitted with extensive arrays of sensors that gather data at rates up to 1/200 of a second, digital weather stations, and real-time chemical sampling and analysis instrumentation. Additional systems track bees with harmonic radar; time/date stamp the activities of microchip tagged bees, and even monitor for fluorescence of bee-carried, bioengineered bacteria. Thirty of these hives are deployed at field sites in Maryland, Montana, and New Mexico. Another 20 are planned for deployment in Tennessee, California, Michigan, and Washington. All data is transmitted to University of Montana via telephone, wireless modem, and fiber optic connections through the Internet. At the University of Montana, parallel processing computers analyze the incoming data using Artificial Neural Networks and provide feedback commands to trigger field-based devices such as air sampling pumps. Some of these hives are being used for basic research and development. Others are deployed as part of a sophisticated biomonitoring program at a military installation.

3. SYNOPSIS OF BREAKOUT SESSIONS

Following the individual presentations, workshop participants met in three breakout groups to consider possible approaches for using instrumented, free-ranging aquatic species to transmit or bring back information relevant to environmental and human health hazards. Group participants were selected across disciplines to strengthen collective input (see Appendix A for breakout group assignments). Each group was asked to construct two types of concept plans: "Blue-Sky" and Near-Term. "Blue Sky" concept instructions were open-ended, allowing the freedom to explore any and all possibilities. No limits were put on the type of system to be designed by the group, and cost was not considered an impediment. Near-Term concepts were limited to those systems that could be developed over the next several years in light of expected technology advancements during that period. Following the breakout group sessions, all participants met in plenary session to review and discuss the merits of the each group's concepts. Some general conclusions from the plenary discussion are provided in section 3.4.

3.1. Group I Concept: Caged And Uncaged Test Organisms

Blue-Sky Solution

Two projects were proposed to address the "Blue-Sky" scenario. Project I would monitor the aquatic environment using several fish that report one data type (i.e., location upon a toxic encounter). Upon sensing a "danger", the fish would communicate location through interactive GPS transmitters. A reporter (Judas) fish or group of fish would then be deployed into the affected area. This/these fish would be equipped with a complete suite of sensors and communication abilities. A Submersible Assessment Vehicle (SAV) with a full suite of state-of-the-art biochemical physiological/environmental sensors would be available to interface with biosensors deployed at points of interest. Communications would be maintained using satellite uplinks and/or downloaded to communicator buoys. The SAV would serve as a platform for the release of a stimulus or pheromone for control of the animals.

Project II would monitor the environment with a combination of free-swimming animals equipped with various biosensors interfacing with a mobile/submersible communication buoy. Animals would be controlled using various methods: genetic engineering, associative learning, stimulation of the lateral line system, or stimulation of olfaction through the use of pheromones or other stimulants.

Technological challenges that would be faced include communication and data analysis, signal detection/ processing, sensor design (neurological and physiological), attachment, miniaturization, and biological control.

Near-Term Solution

Project I would use a large number of free-roaming fish outfitted with location sensors. Some of these individuals would be outfitted with other physiological/chemical sensors. If avoidance behavior by location or physiological sensors were "tripped," a technician or SAV outfitted with sampling equipment and chemical sensors would enter the suspected area and sample the environment.

Project II would deploy groups of caged fish in bodies of water which could possibly be a conduit for hazardous chemicals. Caged fish would be positioned to form an assessment gradient with a reference cage in an unaffected area of the same water. Baselines and control levels would be established so that out of control response (toxicosis) would be detected with satellite reporting. Cages would be outfitted with environmental sensors (i.e., water quality, current, light monitors) and specific (i.e., malathion) or group (i.e., organophosphate) chemical sensors - possibly using microcantilever technology. Cages would be equipped with video capability to monitor startle responses due to the presence of outside stressors (i.e., predator, aggressive individual). Cages would also serve as a communication platform for a GPS beacon, satellite uplink, and remote sensing of assessment organisms with archival and transmittal capability. Cages would be large enough to contain sufficient individuals for statistical analysis and made of material (i.e., plastic) which would be resistant to breakdown and difficult to detect. Individual fish would be outfitted with physiological sensors - cardiac output, EMG, electroencephalogram, and blood analytes.

The use of caged fish reduces some of the positioning and communication problems associated with free-ranging fish. On the other hand, free-ranging fish have mobility and a search ability not possible for caged fish, and caged fish require more intrusion into the area to be studied.

Benefits associated with these models include: early warning potential, direct measure of changing water quality/fish physiological state, and impacts on commercially important fish. Less research is required for the Near-Term concepts, and technology is already available for some of the physiological parameters.

3.2. Group II Concept: Multiple Species

Blue-Sky Solution

A variety of species would be used as high gain amplifiers or indicators of toxic environmental hazards. Overall activity and stress (locomotion/acceleration, ventilation rate, cardiac output, blood chemistry) would be measured. Biosensors would be developed to detect or become sensitized to specific chemical compounds (possible utilization of fish conditioned with a hypersensitive immune system). A CNS-Collective Network System would be deployed where all organisms locally share information. This network would allow for lower energy needs per individual due to shorter distances of transmission and relay of information to the user. Organisms would have onboard preprocessing to reduce the amount of information transmitted and be "self-calibrating." Self calibration would help determine background levels of response in a natural environment. A "drone" would be utilized to mimic and follow organisms and be able to communicate with organisms and the host user. The drone could be an organism or autonomous underwater vehicle (AUV).

Organisms would act as a "cruise missile" and be directed to an area of interest to begin a search. The search mode would allow behavioral avoidance to be assessed along with physiological information. Operant conditioning, lateral line stimulation or a direct stimulus would be used to guide the fish in a specific direction. A Fish Inertial Navigational System (FINS, Figure 1) would guide organisms with a preset or controllable mapping system. An internal device would determine the organism's position and would "steer" the organism based upon the mapping system. Energy storage/generation would be provided by a Piezo energy source, propeller (dynamo) or chemical energy (food harvesting-ATP battery). Sensors would utilize MEMS, and color indicators or patches to reduce the use of invasive technologies.

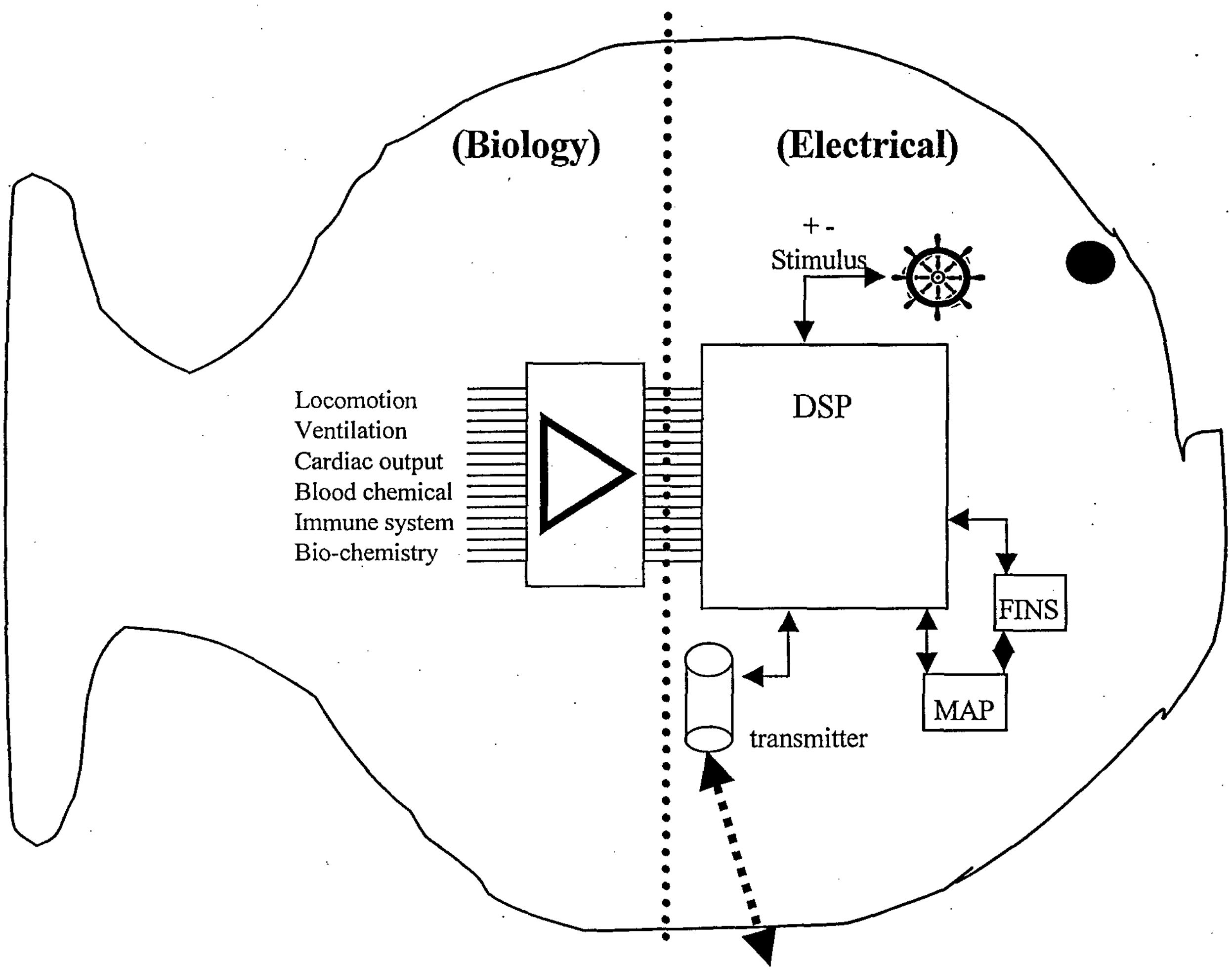

Figure 1. Fish Inertial Navigational System (FINS)

Some of the benefits associated with this approach are the use of a variety of species and endpoints, a staged response to toxicant detection, the use of combined robotic and biological components, and data and communications support. Research challenges include development of FINS, the complexities of interfacing with biological systems, associating fish responses with specific stimuli, and communication and power requirements.

Near-Term Solution

This system would utilize fish school position architecture (free swimming fish/lobster) and fixed sonar buoys. A Holographic Communication Network (Figure 2) would collect information from all organisms via sonar buoys to get an overall picture of the environmental surroundings. FINS would only provide position and time. The organism's physiological state (i.e., EMG, tail beat, ventilation rate, mortality) and environmental parameters (i.e., temperature, pH, conductivity, dissolved oxygen, etc.) would be assessed.

An AUV would be developed to provide a platform (and power requirements) to transmit information from the sonar buoys to the user. The vehicle would provide signal processing capabilities of information received. The vehicle would be instrumented with organisms (fish, crabs, clams, etc.) for direct physiological monitoring (ventilation rate, EMG, blood volume, etc.). The AUV would investigate areas of interest based upon information gathered from the local area positioning system.

This system would provide a flexible approach capable of using several species and multiple biosensors while utilizing many elements of existing technology. Use of the AUV to pre-process data would help minimize data transmission bandwidth. Technical challenges and limitations include the use of a fixed buoy system, power and range considerations, expense and time to development, and the development of FINS.

3.3. Group III Concept: Fixed Location

Blue-Sky Solution

This group's "Blue-Sky" scenario called for a hardy organism, potentially with genetically-enhanced sensors for target molecules, cloned to minimize variation, with identified neural nodes for detecting sensory input and for introducing control signals (Figure 3). A typical deployment would consist of a vanguard of minimally-instrumented animals; if they showed a stressor avoidance response, a second wave of animals instrumented for the expected pollutant(s) at the "alarm" site would be released. An electronic/program control would maintain the organism's vertical position in the water column to allow RF communication. The programmed fish would rise to the surface where communication would be achieved via buoy system.

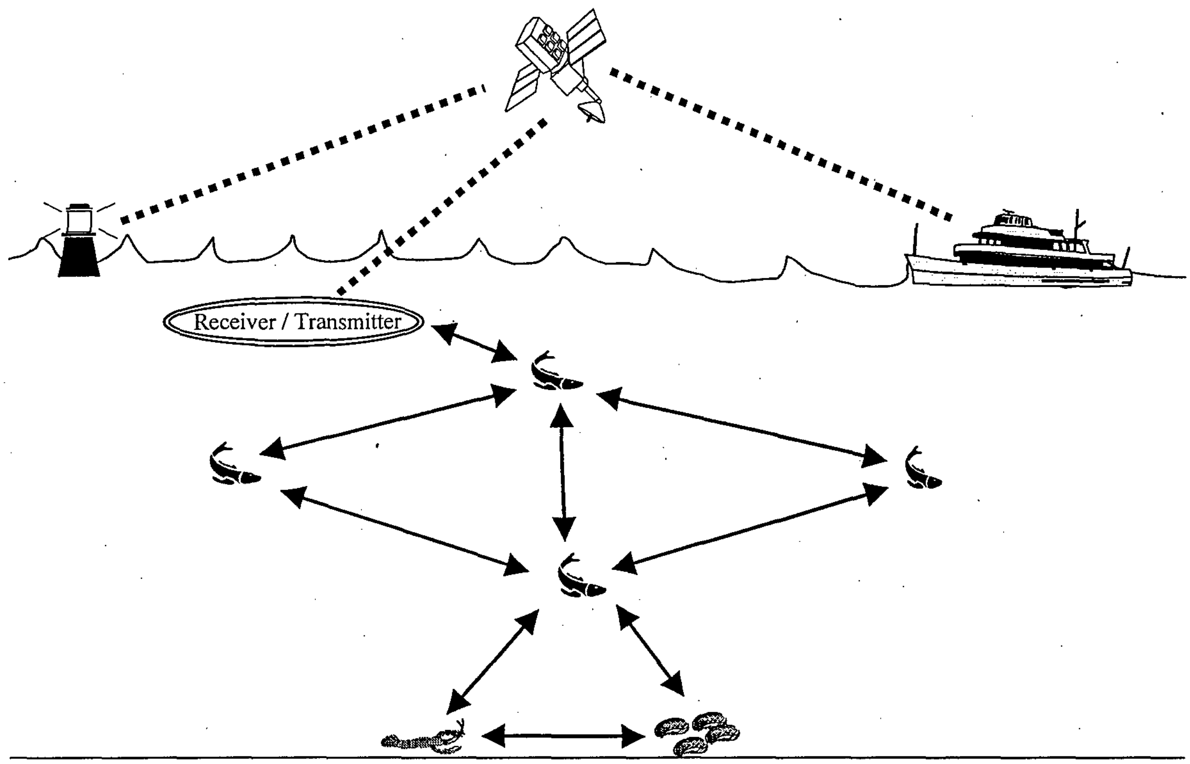

Figure 2. Network Communication System

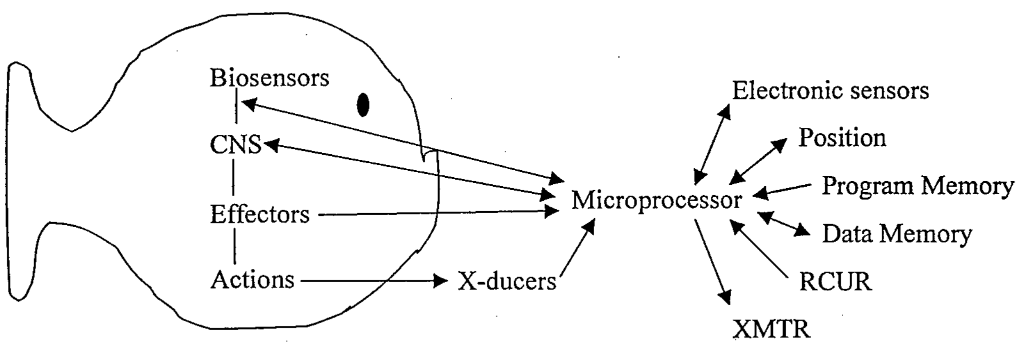

Figure 3. Group III's Organism

Some of the suggested strengths of this idea include the tie-in to the CNS to increase stimulus/response information, minimizing variation through the use of cloned fish, and control over the fish. Challenges include neural data interpretation, implementing genetic engineering approaches, power considerations, the need for fish to rise to the surface to communicate with buoys, and application in varying environments.

Near-Term Solution

Group III's Near-Term scenario involved platforms with standard oceanographic sensors (e.g., temperature, salinity, water level, currents, fluorescence, dissolved oxygen) and water samples. These would be linked to instrumented organisms, either penned or in a bounded system, or free-ranging. If the organisms signaled a pollution event, the platform would obtain a water sample to allow investigators to identify the causal agent upon arrival on-site. Platform sensors would provide data surrounding onset of the event and vectors indicating the origin of the water parcel and where it was predicted to go. Real-time physical and biological data (i.e, ventilation rate, heart rate, cardiac output, tail beat frequency) would be relayed by packet radio, cell phone, or other appropriate carrier.

Some benefits identified for this approach include the use of existing technology, further investigation of fish responses through water sampling and chemical analyses, use of caged animals, and favorable power considerations.

Critical research areas include:

- Identify suitable species (tough, smart, trainable, with plastic pre-existing behaviors that provide useful indications of exposure to target agents).
- Elucidate "normal" patterns of response to pollutants for each candidate species, identify other relevant measurements (e.g., blood chemistry, neurotransmitters, oxygenation, integument colors).
- Identify plastic and potentially useful behaviors, and their modulators (e.g., hormones for migration, circadian clocks for vertical migration).
- Identify neural pathways/patterns for specific sensory stimuli, and for controlling specific behaviors (e.g., locomotion, turning, ascent/descent).

3.4. Evaluation of Breakout Group Concepts

Each proposed concept presented by the three groups during the general assembly was rated using an evaluation form that considered six criteria: the ability to meet requirements, practically, validity, cost, time to field, and risk of investing.

The "Blue-Sky" approach allowed participants to develop programs with no preconceived configurations or limitations. Given that "anything was possible", most workshop participants rated all three groups "acceptable" in meeting the six criteria using the "Blue-Sky" approach.

To evaluate Near-Term approaches that incorporate existing or near-term technologies, workshop participants considered feasibility of implementation over the next several years. Groups II's Multiple Species Concept was rated the highest by workshop participants in terms of meeting the requirements of the controlled biological system program, but participants concluded that Group II's concept would require higher costs, longer time to field, and a greater risk of investing as compared to other proposed concepts.

4. CONCLUSIONS

Scientists from around the world met to discuss the possible applications of aquatic organisms as living sensors and gatherers of environmental information. Several fascinating concepts were proposed to address the development of an aquatic biological controlled system to detect environmental hazards. A proposal using multiple species or organisms linked to an AUV was rated the most favorable by the general assembly in meeting the program's objectives. This vehicle would serve as a platform for the transportation, communication and physiological monitoring of the organisms. The general assembly concluded that with the current advances in the field of technology it would be possible to develop an instrumented free-swimming organism that could monitor and transmit relevant information concerning environmental and human health hazards. Many scientists departed the meeting with new ideas for future research projects in the field of controlled biological systems.

APPENDIX A

WORKSHOP AGENDA

October 22, 1998

0830 - 0840	Welcome: Administrative Remarks and USACEHR Overview	Dr. Hank Gardner	USACEHR
0840 - 0900	Controlled Biological Systems Overview	Dr. Alan Rudolph	DARPA
0900 - 0915	Workshop Concept	Mr. Tom Shedd	USACEHR
0915 - 0920	R&D in Fish Physiological Telemetry	Dr. R. Scott McKinley	Waterloo Biotelemetry Inst. Univ. of Waterloo, CAN
0920 - 0940	Behavioral Ecology/Telemetry	Dr. Thomas Wolcott	NC State Univ.
0940 - 0950	R&D in Fish Behavior	Dr. Mary Moser	Center for Marine Science Resource & Univ. of NC, Wilmington
1000 - 1030	Break		
1030 - 1040	Applied Research Using Physiological Telemetry	Dr. Finn Økland	NINA/NIKU, Norway
1040 - 1050	Fish Neuro-behavior	Dr. Edward Little	USGS-Midwest Sci. Ctr.
1050 - 1100	Aquatic Toxicology	Dr. Dennis Burton	Univ. of MD
1100 - 1105	Introduction: Data Analysis/ Sensor/Communications	Mr. Tom Shedd	USACEHR
1105 - 1135	Biological Signal Acquisition and Interpretation	Dr. Charles Sarabun	Johns Hopkins Univ. Applied Physics Lab
1135 - 1145	Biosensors	CDR. Paul Knechtges	USACEHR
1145 - 1300	Lunch		
1300 - 1310	R&D in Aquatic Transmitters (Ultrasound Telemetry)	Mr. Fred Voegeli	Vemco Ltd.,CAN
1310 - 1320	R&D in Aquatic Transmitters/Sensors	Mr. Peter Anson	Lotek Eng.,Inc., CAN

1320 - 1325	Introduction: Biological Controls	Mr. Tom Shedd USACEHR
1325 - 1340	Fish Behavior/Lateral Line Response	Dr. Neal Brown Univ. of New Orleans
1340 - 1350	Physiological Ecology	Dr. Windsor Watson,III Univ. of NH
1350 - 1515	Breakout Session 1: Systems Development	
1515 - 1530	Break	
1530 - 1700	Breakout Session 1: Group Presentations/Evaluations/Discussion	
1700	Administrative Remarks	

October 23, 1998

0830 - 1000	Breakout Session 2: System Refinement	
1000 - 1030	Break	
1030 - 1200	Breakout Session 2: Group Presentations/Evaluations/Discussion	
1200 - 1300	Lunch	
1300 - 1400	Breakout Session 3: Technology Requirements	
1430 - 1500	Closing Remarks	COL. David Danley USACEHR

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BREAK OUT GROUP ASSIGNMENTS

Sessions I & II
October 22, 1998

Group I	Group II	Group III
Dr. R. Scott McKinley (Chair)	Dr. Mary Moser (Chair)	Dr. Thomas Wolcott (Chair)
CDR Paul Knechtges	Mr. Peter Anson	Dr. Neal Brown
Dr. Edward Little	Dr. Dennis Burton	Dr. Morris London
Dr. Daniel Ondercin	Dr. Charles Sarabun	Dr. Finn Økland
Dr. Jerry Bromenshenk	Dr. Windsor Watson, III	Mr. Fred Voegeli
Dr. Jennifer Brower	Mr. Tom Shedd	Dr. Alan Rudolph
COL David Danley	LTC Karl Friedl	Dr. Robert Finch
Dr. William van der Schalie	Dr. Hank Gardner	Mr. Mark Sunkel
Notetaker: Mr. Joseph Beaman	Notetaker: Mr. Mark Widder	Notetaker: Ms. Marianne Curry

Session III
October 23, 1998

Hardware/Software	Behavior	Hazard Assessment
Mr. Peter Anson (Chair)	Dr. Neal Brown (Chair)	Dr. Dennis Burton (Chair)
Dr. Morris London	Dr. Jerry Bromenshenk	CDR Paul Knechtges
Dr. Finn Økland	Dr. Edward Little	Dr. R. Scott McKinley
Dr. Daniel Ondercin	Dr. Mary Moser	Mr. Tom Shedd
Dr. Charles Sarabun	Dr. Tom Wolcott	Dr. William van der Schalie
Mr. Fred Voegeli	Dr. Windsor Watson, III	Mr. Mark Sunkel
Dr. Jennifer Brower		COL David Danley
LTC Karl Friedl		Dr. Robert Finch
Dr. Alan Rudolph		Dr. Hank Gardner
Notetaker: Mr. Mark Widder	Notetaker: Ms. Marianne Curry	Notetaker: Mr. Joseph Beaman