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In Situ Aquatic Biomonitoring Platform

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13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

Nekton research is developing a biological sensor bay (BioBay) for use on the Ranger AUV. Transporting living biosensors on an AUV opens up new opportunities for environmental monitoring. Biological sensors provide a better indication of overall water quality because organisms react to a variety of different pollutants instead of being chemical specific. Measuring the extent to which a clam is open or closed (gape) has been shown to correlate with the presence of pollutants in the water. In order to account for intra-population statistical fluctuations the BioBay is capable of transporting approximately 16 clams each with a dedicated gape sensor.

Through this design and research effort we have created several BioBay prototypes, and assessed their impact on Ranger performance. Clam gape sensing circuitry has been designed and integrated with Ranger's data logging capabilities. Open water field tests have been conducted with the BioBay mounted on Ranger.

Results from the BioBay design project are discussed in this report along with descriptions of the BioBay designs and details about test procedures.

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Introduction

The goal of this project was to design and build a prototype mobile Biological Early Warning System (BEWS) platform for rapid detection and profiling of toxicants in water. The prototype is an external payload bay, called BioBay, for Nekton's Ranger micro-UUV. Ranger is an autonomous submersible vehicle that is 91 cm long and weighs approximately 5 kg including BioBay. BioBay was designed to continuously monitor shell gape in 16 living bivalved mollusks. Our principal goals were to determine the navigational performance of Ranger outfitted with BioBay, characterize water flow around clam shells mounted in BioBay, and to select a clam gape sensor.

The main parameters we addressed were:

- Vehicle speed and efficiency, stability and handling performance
- Uniformity of exposure for each clam, and flow regime, laminar vs. turbulent
- Type and accuracy of clam gape sensor.

Body: Statement of Work with Detailed Tasks

Task 1. Create BioBay mockups of several external module designs

a. Determine what size clams would be accommodated
b. Specify a gape sensor
c. Design and fabricate electronics for monitoring clam gape
d. Detailed mechanical design of BioBay mockups
e. Fabricate the BioBay mockups and Ranger interface

Task 2. Test BioBay Ranger-mounted mockups in flow tank

a. Design load cell fixture for Ranger
b. Establish Coefficient of Drag baseline for Ranger
c. Evaluate effect on drag of each BioBay mockup
d. Implement flow visualization technique on flow tank
e. Use flow visualization to ensure mockups have adequate flow through clam locations

Task 3. Conduct field trials of Ranger BioBay with one mock up design

a. Demonstrate in situ operation of Ranger configured with a mockup
    1) Install mockup BioBay on Ranger
    2) Implement RS-232 data logging for BioBay sensor
    3) Trim vehicle for BioBay payload
    4) Operate vehicle with BioBay in an open water field test
b. Assess operating performance of Ranger so configured.
    1) Program Ranger to execute standard behaviors
    2) Monitor/correct any control problems

Task 4. Specify BioBay for Phase II development

a. Establish relationships with potential Phase II collaborators.
b. Propose a BioBay design for further work during Phase II
c. Identify problems with BioBay mockups and methods to address them.
d. Identify further tests that need to be run in order to characterize clam or sensor reaction to Ranger platform.

Task 1: Create BioBay mockups of several external module designs

a.) <u>Determine What Size Clams Would be Accommodated</u>

Corbicula fluminea was selected as the clam to use in BioBay based on discussions with Dr. Jay Levine, Clam Pathologist at North Carolina State University School of Veterinary Medicine and based on its use by Dr. Joel Allen in static River Biomonitoring devices.

The appropriate size of C. fluminea for BioBay was then selected based on the dimensions of the Ranger vehicle and the number of specimens needed (16) to provide reliable statistical results and comparison to results obtained by others using statically situated clam BEWS. Another constraint was sufficient room for clam gape excursions and the sensor monitoring clam gape. A third constraint was the decision to glue one shell of a specimen to an anchor post and to monitor excursion of the other valve. Based on these constraints, we obtained specimens having a nominal width of 1.25cm.

b.) <u>Specify a Gape Sensor</u>

Accurate and reliable sensing of clam gape during autonomous operations was another key design constraint. In order to address this constraint we assessed the inductive proximity sensors used in the static clam BEWS as well as optical and Hall Effect sensors.

First, we evaluated optical sensing using an LED-photo receptor combination, with a beam-blocking flag affixed to the movable valve. It became clear that such a system would be poorly quantitative of shell gape changes for several reasons. First, valve motions are small, i.e. fractions of a millimeter. This small amount of movement is insufficient to block the LED light source and cause the sensor to signal a change in state. Second, changes in ambient light, turbidity and marine fouling would have a substantial impact on sensor performance.

Second we assessed the passive eddy current sensors used in other Clam BEWS. These sensors are large and expensive compared to both Hall Effect sensors and optical sensors. These sensors require extensive analog signal processing circuitry. The advantage with these sensors is that they generate a linear analog output proportional to the field sources distance from the sensor. Both the Hall Effect sensor and the optical sensor only provide a change in state at a predetermined magnitude sensor reading (on or off, open or closed). The Inductive proximity sensors were too large, too expensive, and required too much signal processing for use on the BioBay.

Next we assessed Hall Effect sensors and selected them (Figure 1) for use in the prototype BioBay. Hall Effect sensors are very small, inexpensive, and easy to waterproof. They provide a well defined 5V-0V transition when placed in the immediate vicinity of a strong magnetic field. This signal change is easy to interpret with a microprocessor. A magnet is glued to the top half of the clam's shell which moves with respect to the Hall Effect sensor. As the magnet/clam shell moves, the strength of the magnetic field varies causing the Hall Effect sensor to transition in and out of saturation. These sensors transition from 5V to 0V with very little movement from the magnet. This makes them well suited for monitoring clam gape. They work regardless of light level, or the amount of particulate in the water. They are resistant to marine fouling and contamination.

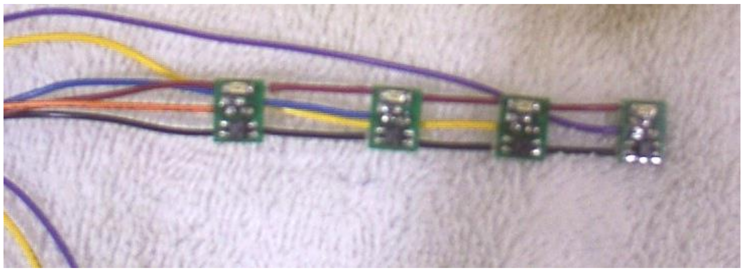


Figure 1: Hall Effect Sensor Board

c.) Design Electronics for Monitoring Clam Gape

Once the Hall Effect sensors had been identified, a circuit board was designed to interpret signals from 16 Hall Effect sensors. The circuit board has 16 analog to digital inputs. This was done so linear analog Hall Effect clam gape sensors could be used later if needed. The sensor board provides the array of Hall Effect sensors with 5V. It reads the signals from the Hall Effect sensors and outputs an RS-232 data stream which is interpreted and logged by the processor onboard Ranger. A custom circuit board was designed and fabricated for this application.

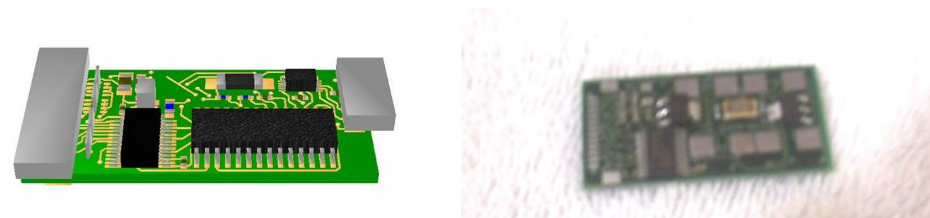


Figure 2: Main sensor board prototype design, and populated PCB

Once the board was designed and fabricated, software was written to log the clam gape data onto Ranger. The sensor board was assembled into BioBay and tested in the shop. After some modifications were made to the sensor board, BioBay was tested in open water.

d.) Detail Mechanical Design of BioBay Mockups

Nekton's Ranger AUV is a 5kg, 36" long 3.5"Ø AUV, so sensor payloads must be small, and preferably neutrally buoyant. Minimizing drag on the Ranger platform is a key issue as well. Each design housed multiple clams and clam sensors. The designs also allow for a YSI conductivity and temperature sensor to be carried. Three BioBay concepts were created.

The first design is the nose mounted BioBay. Clams are arranged in four rows of four clams that surround the sensor board pressure vessel in the center of the vehicle. This design was fabricated and developed into a working prototype. It is a compact design that is easy to integrate onto the vehicle, does not increase the vehicle's frontal area, and allows space for several YSI sensors.

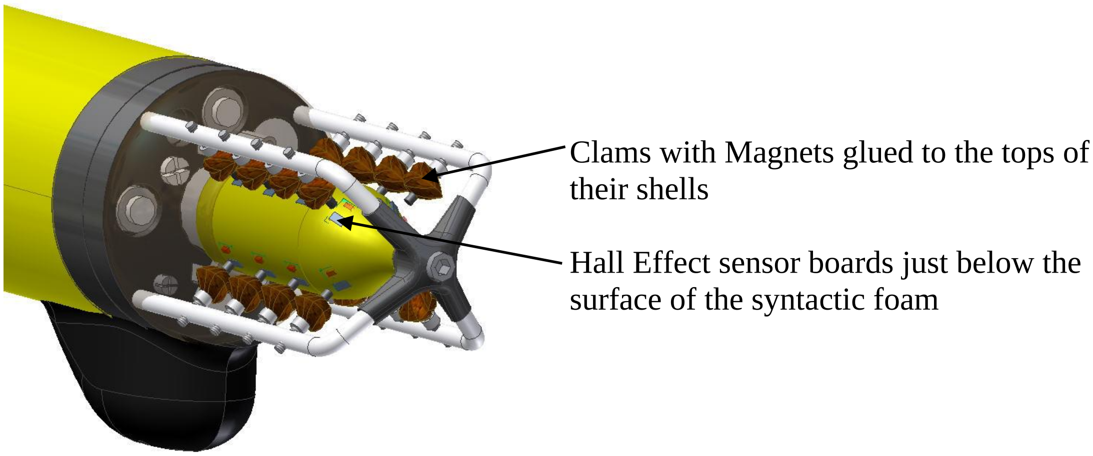


Figure 3: Nose Mounted BioBay

The next design had clams located in pods that hung below the vehicle. The main sensor board was located in a pressure vessel underneath a flow form on the front of the vehicle. The advantage of this design was that it allowed more room to carry additional YSI sensors on Ranger. The drawback was that it significantly increased the frontal area of the vehicle and greatly increased the drag of the vehicle.

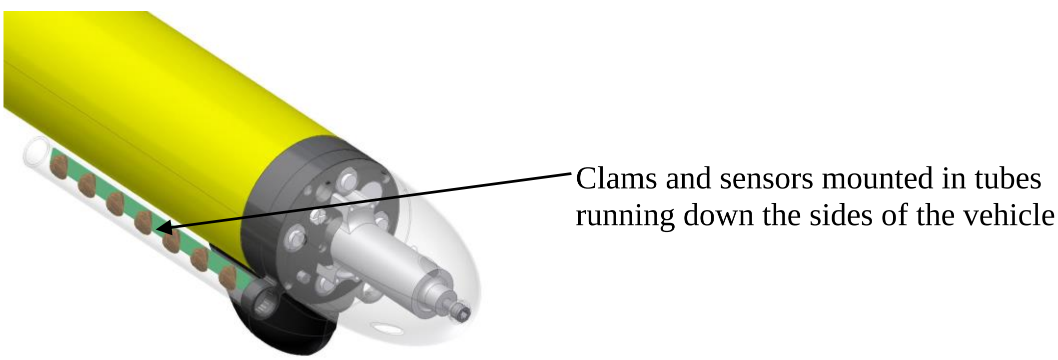


Figure 4: Clam Pod BioBay

The third design was created after the first design had been developed into a working prototype. Therefore, this design took into account the lessons learned from the previous two. The design is a low drag shape that allows water to flow in over the top of the clams. The disadvantage of this design is that it holds a maximum of eight clams. A nose shape similar to this was flow-tested to determine drag, but a working prototype of this BioBay was not developed under the Phase I contract.

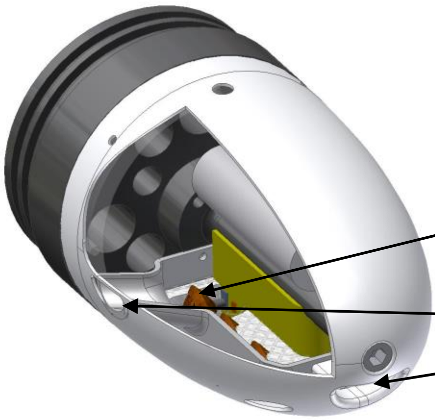


Figure 5: Low Drag BioBay

e.) **Fabricate the BioBay mockups and Ranger interface**

The nose mounted BioBay was designed to have the sensor board pressure vessel mounted to the center of the front end cap. The outside wall of the pressure vessel is lined with four rows of four Hall Effect sensor boards. The Hall Effect sensor boards are then epoxied to the pressure vessel. This entire unit is submerged in a mold that contains syntactic foam. The foam flows in and around the pressure vessel and the Hall Effect sensor boards, encapsulating everything in water tight insulation.

The mold shapes the syntactic foam into a hydrodynamic flow form. This unit is surrounded by four posts that wrap around and meet at the front of the vehicle. An elbow fitting allows the power and RS-232 communication wires to go back into the main vehicle without going through a connector. Clams are mounted to BioBay by super gluing a nylon screw to the bottom half of their shell. The screws thread into one of the four posts. The entire BioBay unit then attaches to the front of Ranger. The BioBay is a modular sensor payload, which can be easily removed and replaced with another payload.

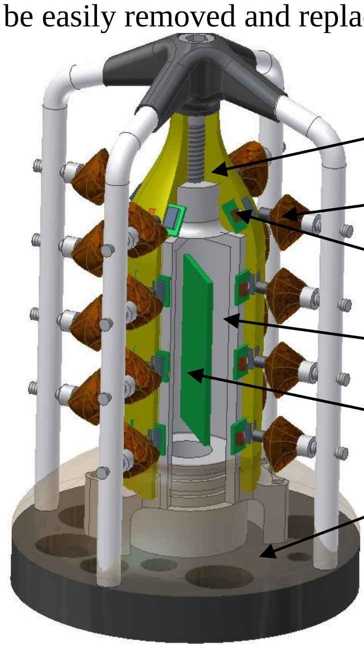


Figure 6: Working BioBay prototype design

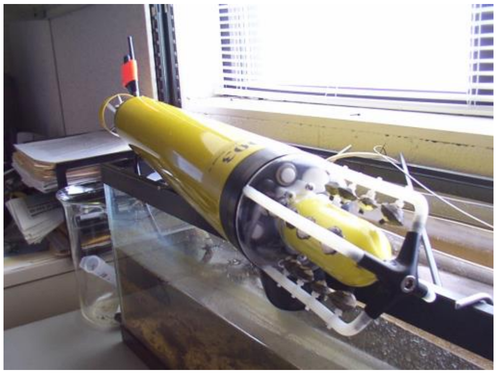
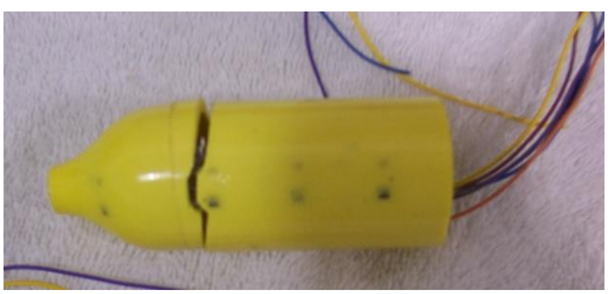


Figure 7: BioBay installed on Ranger

Problems encountered during BioBay Fabrication

The main problem we encountered during the construction of the BioBay, was assembling and casting the main pressure vessel flow form. The main pressure vessel has 16 Hall Effect sensor boards epoxied to its circumference. These are arranged in 4 rows of 4 boards (one of the rows is shown in Figure 1). The wires from these boards pass through holes in the top of the pressure vessel, and terminate into the main sensor board. The Hall Effect sensor boards and wires are difficult to insert into the mold, and even more difficult to inject the liquid syntactic foam such that it fills in around these boards and wires without any air bubbles. It was also difficult to remove the finished casting from the mold. Figure 8 shows a casting that fractured while we were trying to remove it from the mold.

After this first failed attempt, the air bubbles were eliminated by painting the surface of the pressure vessel before it was placed in the mold. The problem with removing the part from the mold was solved by making a fixture that allowed the finished part to be gently pressed out of the mold with a bearing press.



Fractured syntactic foam casting

Figure 8: Example of Fabrication Problem

Task 2: Test BioBay Ranger mounted mockups in flow tank

One goal of this project was to determine what effect the BioBay had on the performance of the Ranger AUV. In order to do this, the Ranger AUV was mounted in Nekton's flow tank.

A baseline coefficient of drag was determined by running the standard Ranger nose shape at different flow speeds and looking at the force produced on the six-axis load cell. The coefficient of drag for the BioBay Ranger is 90% greater than the coefficient of drag for the standard nose configuration. The Phase I BioBay design has proven to be a successful proof of concept. Future designs will eliminate this increase in drag by incorporating low drag features similar to those shown in Figure 16. The increase in coefficient of drag will greatly decrease mission duration.

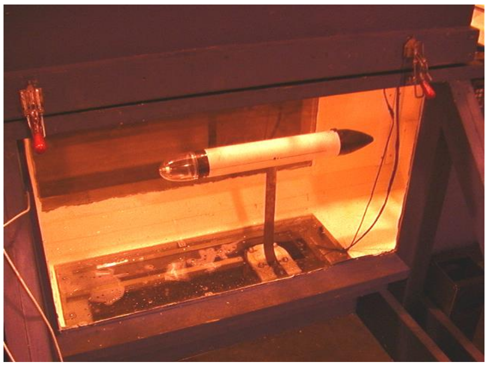


Figure 9: Ranger mock up with standard nose configuration used for obtaining coefficient of drag base line

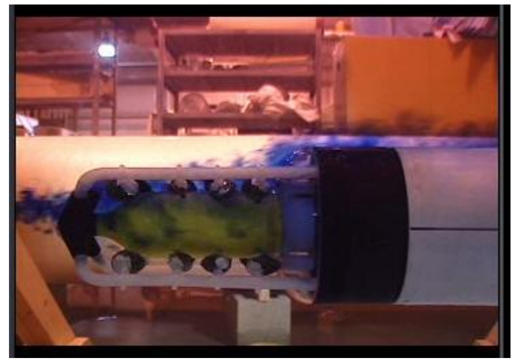


Figure 10: Ranger in Flow Tank with BioBay undergoing flow visualization testing

Flow visualization (Figure 10) was also used to make sure each clam received adequate flow. For this test, 16 unoccupied clam shells were installed in the BioBay. BioBay was installed on Ranger, and Ranger was installed in the flow tank. Blue dye was used to make sure that water was passing by each of the clam positions. This test verified that all clams were receiving adequate flow and that the water passing by each clam was moving at close to the same speed as the water passing over the rest of the vehicle. This ensures that there is very little latency between when the vehicle drives through a packet of water, and when the clam is exposed to the toxins that may or may not be present in that packet of water.

Task 3: Conduct field trials of Ranger with one mockup design

a.) <u>Demonstrate in situ operation of Ranger configured with a mockup</u>

The first living clam test of BioBay was conducted in water by gluing one valve of *Corbicula fluminea* to a nylon screw. The screw was threaded into one of the four posts that surrounded the main sensor board pressure vessel. A magnet was glued to the top half of the clam shell. The screw and the magnet were glued to the clam using a jig to hold the screw and the magnet in radial alignment with each other. This enabled us to adjust for variation in clam size by screwing the post in or out in order to provide consistent initial sensor values. Screwing the clam in and out adjusts the magnet's distance from the Hall Effect sensor, and allows the open/shut threshold to be set. The screw and magnet were glued to the clam using high strength super glue that cures underwater. This allowed the clam, the screw and the magnet to be loaded into the jig, apply the glue, and then place the entire jig back in the water to minimize the length of time the clam is out of the water.

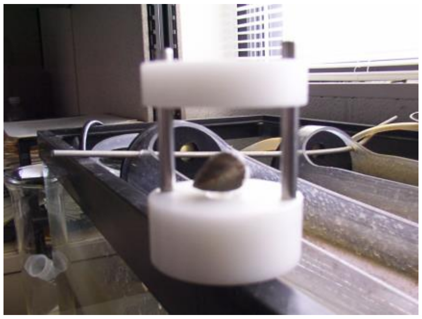


Figure 11: Jig for gluing screws and magnets to clams

BioBay was placed in a beaker of fresh water and the sensor board was turned on. The open/shut threshold was then set by turning the mounting screw. The function of the LED used as visual indicators of shell opening and closing was then checked. This allows the user to easily adjust the open/shut threshold without the main sensor board sending data to HyperTerminal. The live clam was observed as it opened and started siphoning water. The main sensor board registered an open condition. A syringe of saline was then discharged near the clam to simulate swimming through a region of contaminated water. The clam closed its shell; the main sensor board registered a closed condition. This validated that BioBay worked in a controlled environment, and that the Hall Effect sensors and magnets had been matched correctly.

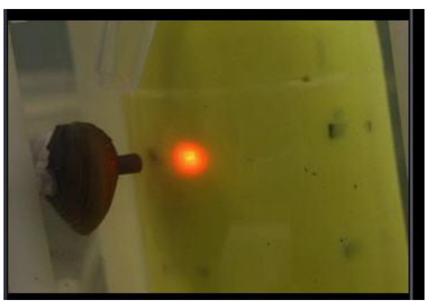


Figure 12: Initial test of BioBay with a live clam

b.) <u>Assess operating performance of Ranger so configured</u>

Open water testing Ranger performance with an attached BioBay was conducted at Blue Stone Quarry using empty shells and living claims. Empty shells were used first to reproduce the drag induced by living specimens. In this test, we assessed the effect of BioBay on Ranger's drag coefficient, center of pressure and, thereby, navigational controller performance. This test demonstrated clearly that vehicle functionality was not adversely affected by BioBay induced drag.

Next, open water tests were conducted with 3 living clams and 13 empty shells (Figure 13). During this test, the three live clams were visually observed to open and close a number of times, and extend their siphons. Data was not recorded in these first tests so no post-mission statistical analysis was conducted.

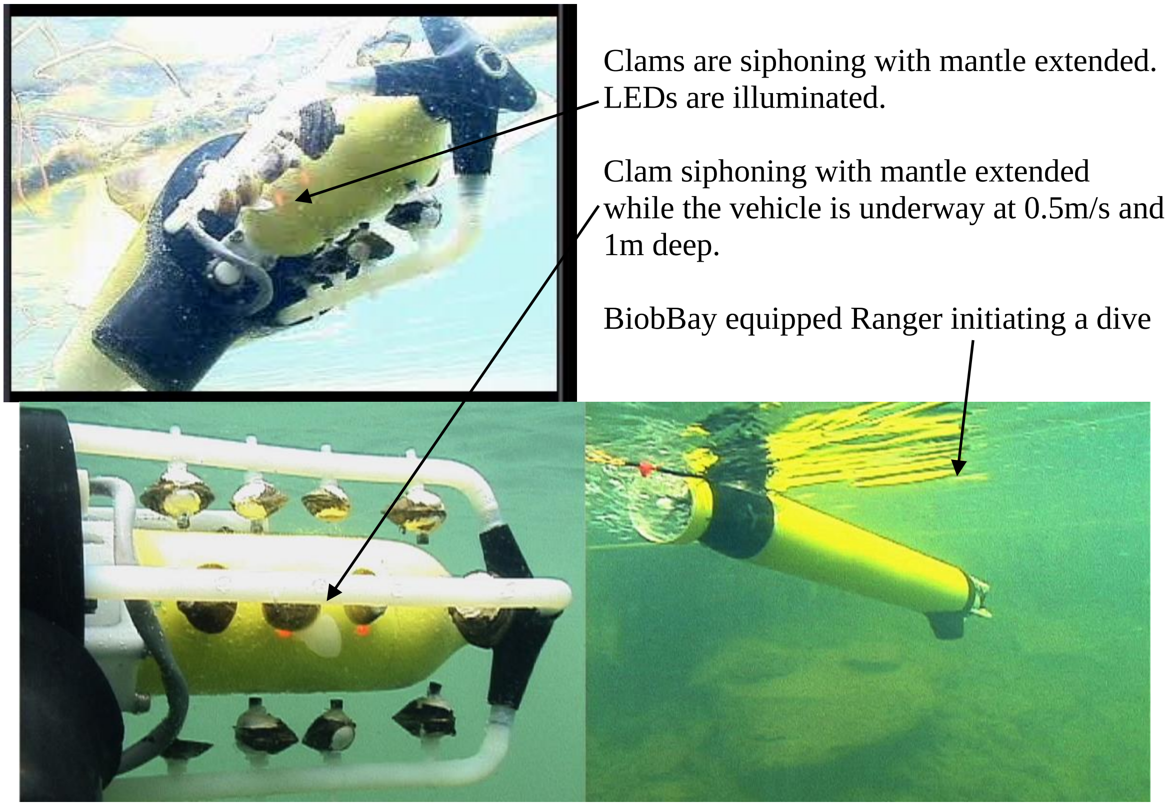


Figure 13: BioBay with clams

Live tests were subsequently run at Lake Elton. In these tests, data was recorded at 1 second intervals for clam state (open or shut), conductivity, temperature, vehicle depth, GPS position and elapsed mission run time. For each of these missions, the vehicle would transit from one end to the other of a set course, with GPS waypoints at both ends. The vehicle would dive to one meter deep for 30 seconds, then come to the surface to get a GPS fix. The vehicle would correct heading based on the GPS fix and then dive again. Ranger was released from the pier and ran back and forth between the outer waypoint and the pier, three times. The clam state data and the GPS position data from Lake Elton are shown in Figure 14 and 15.

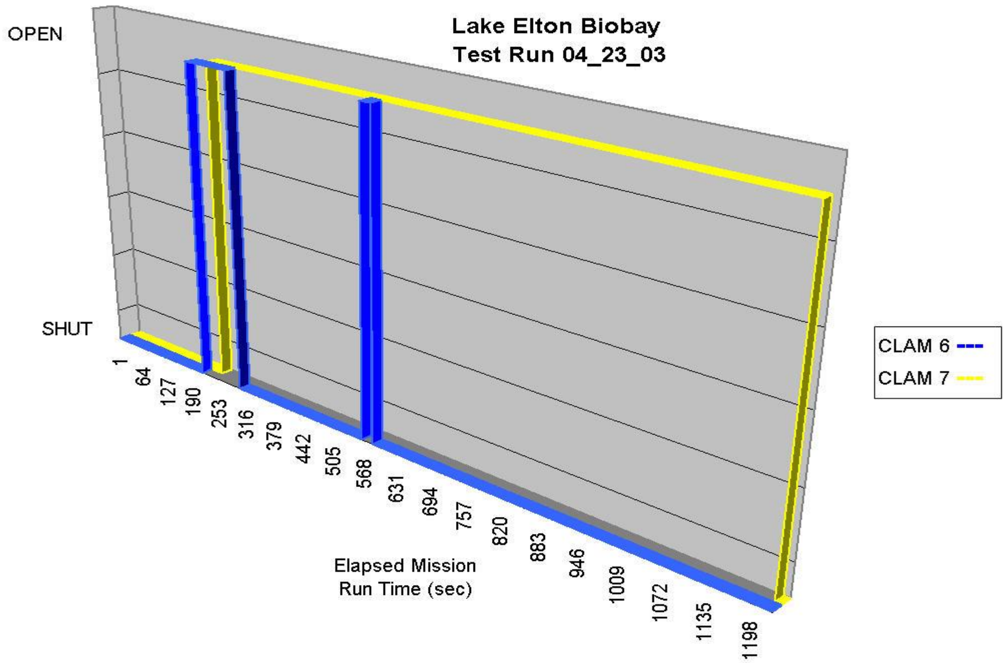


Figure 14: Clam open/shut data recorded during the Lake Elton mission

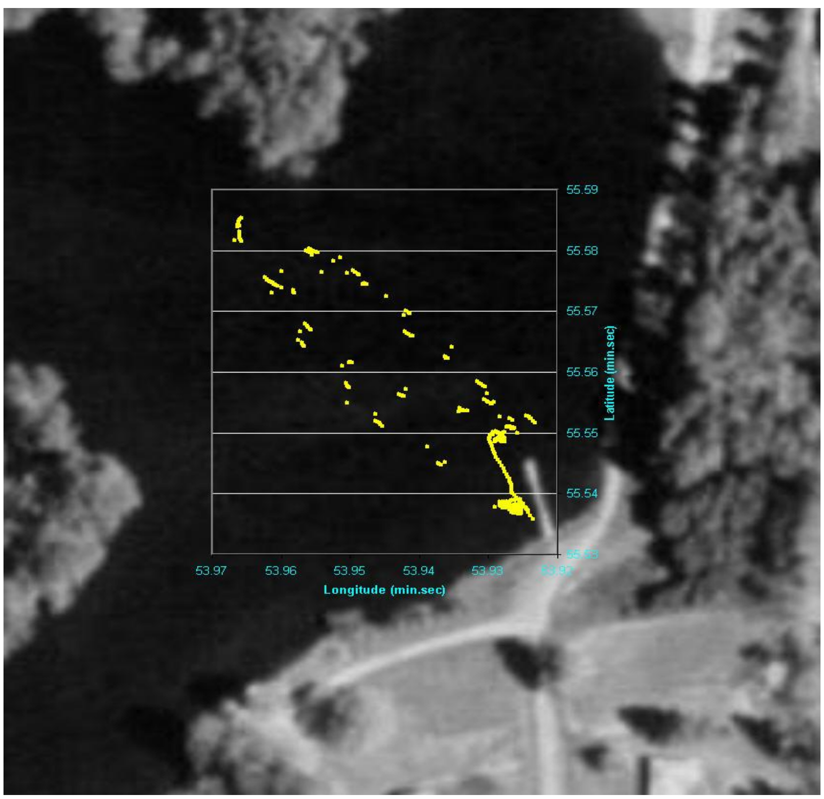


Figure 15: GPS position fixes while Ranger runs out and back away from the pier on Lake Elton. Ranger was launched and retrieved from the pier

Task 4.) Specify BioBay for Phase II development

Throughout the Phase 1 contract we have been talking with people in the field of Biomonitoring. We have established prospective Phase II partners at the NCSU School of Veterinary Medicine. We have also established contacts with experts in the areas of tissue / cell based biosentinels, chromatophore based biosentinels, as well as miniature mass spectrometers. We believe that eventually these technologies will outperform whole organism biosensors, and when they do we will be ready to incorporate them into a BEWS.

At the beginning of Phase II we plan to identify chemicals and respective concentration levels that clams respond to consistently. We also plan to conduct an in depth analysis of which Ranger specific irritants (vibration, acceleration, flow velocity, pressure shock, temperature shock, etc.) illicit a close shell response from the clams. Armed with this information, we would begin the detailed mechanical design of a refined BioBay. Please see the previously submitted Phase II proposal for a detailed discussion of the proposed phase II effort.

The Biobay design that we would probably pursue during a Phase II is the design shown in figure 16. This is a low drag BioBay that fully encloses the clams. We propose this concept design based on our experience from Phase I. However, more clam research has to be conducted in order to pursue this design with certainty. This design solves some problems that we had during Phase I. The first problem that this design solves is the 90% increase in coefficient of drag. This design has very low drag compared to the Phase I working prototype. The other problem the low drag BioBay solves is the issue of enclosing the clams. The Phase I prototype design has the clams attached to struts that are subject to bumping and brushing up against things. The low drag BioBay encloses the clams and only exposes them to the water passing through the outer shell. We would also like to address the problem of attaching the clams to the BioBay. Currently this is done by screwing the clams into the struts. This works well, but it is time consuming and possibly traumatic for the clams. We would like to investigate an adjustable quick release setup during Phase II.

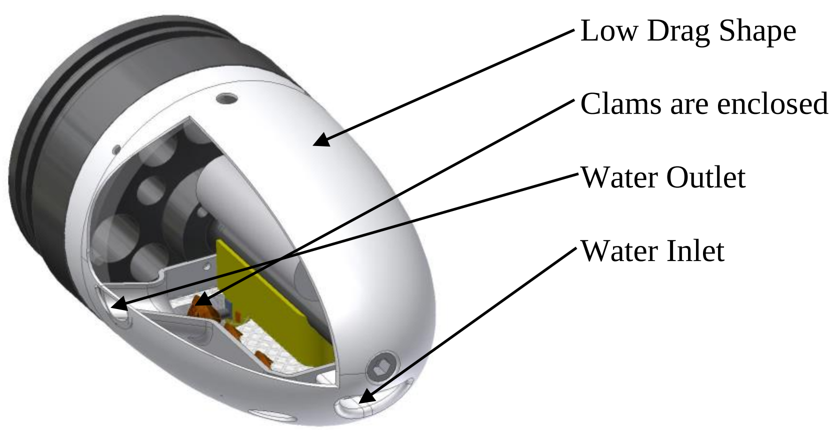


Figure 16: Low Drag BioBay

Along with the low drag BioBay, we recommend incorporating more environmental monitoring sensors onto Ranger. This would include, pH, dissolved oxygen, fluorometer, and turbidity sensors. These sensors would be used in conjunction with the biosentinel to obtain a higher level of certainty from biosentinel sensor readings.

Key Research Accomplishments

Phase I Objectives	**Phase I Accomplishments**
1.) Create mockups of several BioBay external module designs	Nekton designed three BioBay variations: one BioBay design was developed into a working prototype; one was built as a flow tank test mockup.
2.) Test Ranger-mounted mockups in the flow tank	Two designs were tested in the flow tank: the working prototype and the mockup
3a.) Demonstrate in-situ operation of Ranger with a mockup	Accomplished in open water and video taped
3b.) Assess operating performance of Ranger with a BioBay mockup	90% increase in coefficient of drag
4.) Research alternative sensing technologies	Tissue-based biosensors recommended and pursued as alternate sensors. Evaluated biosensor technologies to integrate into BioBay during Phase II
5.) Specify Biobay for Phase II development	Recommended a concept design for a clam based BioBay to be investigated during Phase II.
	Additional Accomplishments
	Designed and implemented a clam gape sensing method on the working prototype
	Designed and built a working prototype
	Designed and built the necessary circuitry to interpret the clam gape signals
	Logged Clam gape data on board Ranger
	Demonstrated the BioBay working with live clams in an open water environment

Reportable Outcomes

1.) We successfully designed, fabricated a prototype BioBay, and tested its functionality with living clams.

2.) We tested a mockup of the low drag BioBay in the flow tank.

3.) A publication has been accepted for presentation at the Oceans 2003 international conference (Paper #184 – see Appendix C).

> Abstract - Nekton research is developing a biological sensor bay (BioBay) for use on the Ranger AUV. Transporting living biosensors on an AUV opens up new opportunities for environmental monitoring. Biological sensors provide a better indication of overall water quality because organisms

react to a variety of different pollutants instead of being chemical specific. Measuring the extent to which a clam is open or closed (gape) has been shown to correlate with the presence of pollutants in the water. In order to account for intra-population statistical fluctuations, BioBay is capable of transporting approximately 16 clams each with a dedicated gape sensor.

Through this design and research effort we have created several BioBay prototypes, and assessed their impact on Ranger performance. Clam gape sensing circuitry has been designed and integrated with Ranger's data logging capabilities. Open water field tests have been conducted with the BioBay mounted on Ranger.

Results from the BioBay design project are discussed in this report along with descriptions of the designs and details about test procedures.

4.) Submitted a journal article for publication in the proceedings of the 2003 Unmanned Underwater Submersibles Technology Conference. Same abstract and manuscript as above.

5.) We applied for Phase II Army SBIR funding based on the results of Phase I and the further investigation of this technology that we would like to pursue. (Proposal # A2-1141, submitted in response to Army SBIR A02-185.)

6.) Collected clam gape data along with several other channels of data. The raw data can be found in the appendix.

Conclusions

Results of Research

We successfully demonstrated in Phase I that a Ranger micro-UUV equipped with a BioBay can operate as a mobile BEWS, using clams. We conclude that Ranger can perform a valuable fast detect & profile mobile BEWS function with clams of an appropriate size and, presumably, any other living biosensor of a compatible size and readily detectable behavior.

We determined the drag penalty associated with carrying BioBay, developed a method for sensing clam gape which we incorporated into the design of the functioning BioBay prototype, and tested BioBay with live clams, observing their response to a stimulant in the lab. We then tested BioBay with live clams carried through the water by Ranger logging clam gape data with several other channels of data.

Importance of Research, "So What?"

The results of our Phase I effort demonstrate clearly that clams can be used as a biosentinel on a mobile underwater platform. Factors associated with a mobile platform on a vehicle were rapidly accommodated by the living clams. The clams remained open while the vehicle was underway during various navigational maneuvers, enabling monitoring of that behavior for toxicant detection without vehicle related artifacts. More research is required to characterize quantitative response to specific pollutants and to further investigate possible vehicle sources of data artifacts. In summary, use of clams as a mobile biosentinel has clear merit and is worth pursuing.

Another important result from Phase I is the clam gape sensing method. The Hall Effect sensors are much smaller than the inductive proximity sensors used traditionally in gape sensing. They are easy to waterproof, have low power consumption and are a small fraction of the price of inductive proximity sensors.

Recommended Changes and New Directions for Future Research

Future research should investigate further effects of general environmental conditions as well as response to specific toxicants. It should also encompass assessment of other biosentinel technologies as they are developed, such as tissue-, cell-, and chromatophore-based sensors as they are developed in formats appropriate for a small autonomous vehicle. We also think that the Biosensor platform should be equipped with more standard environmental parameter sensors such as a fluorometer, dissolved oxygen, pH and turbidity which would provide data for more sophisticated parametric modeling and, therefore, better assessments of toxicant conditions.
Based upon what we learned during Phase I, we feel the following is a list of requirements that a field- ready biosensor must comply with.

1.) Detect broadband waterborne toxins
2.) Fit on Ranger (nominally 3.5”Ø x 7” length maximum)
3.) Not degrade Ranger’s performance any more than the Phase I design
4.) Have a response time of 1-2 seconds or less
5.) Be able to reset to a “no toxins present” state
6.) Be field transportable by one person
7.) Require very little support equipment
8.) Must not introduce harmful chemicals or organisms into the water that’s being tested; must not risk introducing invasive species into an area.

While this is a challenging list, achieving the goals would be of real value in toxicant monitoring for military and homeland defense applications.

References

{1} Allen, HJ, KL Dickson, H Martin, KA Thesen and WT Waller. 2002. Monitoring Watersheds: Biomonitors and Other Measures.

{2} William H. van der Schalie, Tommy R. Shedd, Paul L. Knechtges, Mark W. Widder. 2001. Using Higher Organisms in Biological Early Warning Systems for Real-Time Toxicity Detection

{3} Robert W. Fox, Alan T. McDonald. 1992. Introduction to Fluid Mechanics,

Appendices

A) Sample Data from Lake Elton BioBay Testing. Entire file available upon request.

B) List of Personnel receiving pay from research project

C) Oceans 2003 Paper: "Development of a Biological Sensor Bay for the Ranger AUV"

Appendix A: Sample Data from Lake Elton BioBay Testing

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
1	elapsed seconds	Hour	Minute	Second	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	Latitude (deg)	Latitude (min)	Longitude (deg)	Longitude (min)	Conductivity (microsiemens)	Temperature (deg C)	Depth(m)	Motor PWM, count out of 255
2	1	10	8	20	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9291	0.061	18	0.031	0
3	2	10	8	21	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.55	78	53.9258	0.061	18	0.031	0
4	3	10	8	22	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9258	0.061	18	0.031	0
5	4	10	8	23	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9258	0.063	17.99	0.033	0
6	5	10	8	24	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9257	0.061	17.99	0.03	0
7	6	10	8	25	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9257	0.061	17.99	0.03	0
8	7	10	8	26	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9257	0.061	17.99	0.031	0
9	8	10	8	27	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9256	0.061	17.98	0.033	0
10	9	10	8	28	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9256	0.061	17.99	0.03	0
11	10	10	8	29	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9256	0.061	18.01	0.029	0
12	11	10	8	30	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9255	0.061	18.06	0.031	0
13	12	10	8	31	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9255	0.061	18.08	0.031	0
14	13	10	8	32	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9255	0.061	18.09	0.03	0
15	14	10	8	33	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9254	0.061	18.08	0.03	0
16	15	10	8	34	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9254	0.061	18.06	0.03	0
17	16	10	8	35	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9254	0.061	18.04	0.031	0
18	17	10	8	36	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9254	0.061	18.03	0.03	0
19	18	10	8	37	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9254	0.061	18.02	0.031	0
20	19	10	8	38	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9254	0.061	18.02	0.031	0
21	20	10	8	39	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9254	0.061	18.01	0.031	0
22	21	10	8	40	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9254	0.061	18.01	0.03	0
23	22	10	8	41	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9254	0.061	18.02	0.029	0
24	23	10	8	42	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9254	0.061	18.04	0.028	0
25	24	10	8	43	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.537	78	53.9255	0.061	18.07	0.03	0
26	25	10	8	44	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9255	0.061	18.12	0.029	0
27	26	10	8	45	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9256	0.061	18.17	0.03	0
28	27	10	8	46	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9256	0.061	18.21	0.03	0
29	28	10	8	47	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9257	0.061	18.24	0.03	0
30	29	10	8	48	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9257	0.061	18.27	0.029	0
31	30	10	8	49	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9257	0.061	18.29	0.03	0
32	31	10	8	50	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9257	0.061	18.3	0.029	0
33	32	10	8	51	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9257	0.061	18.31	0.03	0
34	33	10	8	52	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9257	0.061	18.31	0.031	0
35	34	10	8	53	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9257	0.061	18.31	0.029	0
36	35	10	8	54	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9257	0.061	18.3	0.029	0
37	36	10	8	55	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9257	0.061	18.29	0.029	0
38	37	10	8	56	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9258	0.061	18.3	0.03	0
39	38	10	8	57	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9258	0.061	18.27	0.029	0
40	39	10	8	58	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9258	0.061	18.22	0.029	0
41	40	10	8	59	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9259	0.061	18.16	0.028	0
42	41	10	9	0	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9259	0.061	18.11	0.03	0
43	42	10	9	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9259	0.061	18.06	0.03	0
44	43	10	9	2	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	35	55.538	78	53.9259	0.061	18.02	0.03	0

Appendix B: List of Personnel receiving pay from research project

Ryan Moody, Principal Investigator
Brett Hobson, Director, Ocean Engineering
Mathieu Kemp, Director, Physics Group
Chuck Pell, Director, Science and Technology
Bryan Schulz, Senior Electrical Engineer
Heather Pinnix, Software Engineer
Jim Meyer, Mechanical Technician

Appendix C

Development of a Biological Sensor Bay for the Ranger AUV

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Abstract - Nekton Research is developing a biological sensor bay (BioBay) for use on the Ranger AUV. Transporting living biosensors on an AUV opens up new opportunities for environmental monitoring. Biological sensors provide an indication of overall water quality because organisms react to a broad variety of different pollutants. Measuring the extent to which a clam is open or closed (gape) has been shown to correlate with the presence of pollutants in the water.[1] In order to account for intra-population statistical fluctuations, BioBay is capable of transporting 16 clams, each with a dedicated gape sensor.

Through this design and research effort we have created several BioBay prototypes and assessed their impact on Ranger's performance. Clam gape sensing circuitry has been designed and integrated with Ranger's data logging capabilities. Open water field tests have been conducted with BioBays mounted on Ranger.

Results from the BioBay design project are discussed in this report along with descriptions of the designs and details about test procedures.

Introduction and Background Information:

This research and development effort has been carried out as a Phase I SBIR under the auspices of the U.S. Army Center for Environmental Health Research. The goal of this project was to design and build an external payload bay for carrying a Sentinel Bivalve Biological Early Warning System (BioBay). The prototype BioBay has been designed to be carried by Nekton's Ranger AUV. By implementing BioBay on Ranger, we have explored the feasibility of carrying a Biosensor on a small AUV. The performance of the Ranger AUV has been evaluated before and after the addition of BioBay. The main concerns we have addressed are:

1.) Does BioBay hinder vehicle speed, stability, or handling performance?
2.) Will the clams be exposed to uniform flow, and is the water passing over them representative of the water that the vehicle is driving through at the same instant?
3.) Can clam gape be accurately sensed in BioBay?

A wide range of work has been done using whole organism bio-sentinels. Whole organisms are able to detect a wide variety of pollutants, many of which are toxic to humans. Dr. Joel Allen, formerly at the University of North Texas and now with U.S. EPA, has been working with bivalve biosensors. The designs that we have developed for this mobile effort are similar to the designs used by Dr. Allen.

Progress to Date:

1.) <u>Evaluated Technologies for Sensing Clam Gape:</u>

In order to extract information from a Bio-sentinel, one has to be able to sense its behavior. Clam behavior generally involves a shell open - shell shut response to a stimulant. The shell is hinged in one place, so the open condition and shut condition is easy to identify. Past work in this area used inductive proximity sensors to sense when the clam was open or shut. For this effort, we evaluated several sensing technologies and settled on using magnets and Hall Effect sensors. Hall Effect sensors are very small, inexpensive, and easy to waterproof. They provide a well defined 5V-0V transition when placed in the immediate vicinity of a strong magnetic field. This signal change is easy to interpret with a microprocessor. A magnet is glued to the top half of the clam's shell which moves with respect to the Hall Effect sensor. As the magnet/clam shell moves, the magnetic field varies causing the Hall Effect sensor to transition in and out of saturation.

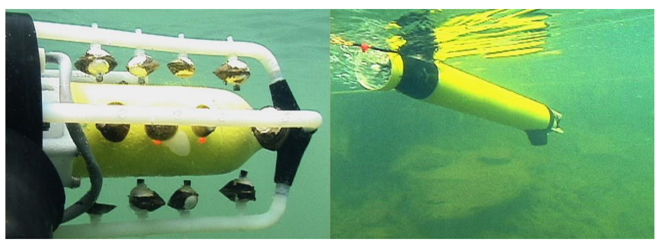

Figure 1: Ranger AUV with the nose mounted clam-based biosensor.

2.) <u>Designed, Fabricated and Tested Clam Sensing Circuitry:</u>

Once the Hall Effect sensors had been identified, a circuit board was designed to interpret signals from 16 Hall Effect sensors. The circuit board has 16 analog to digital inputs. This was done so analog clam gape sensors could be used later if needed. The sensor board reads the signals from the Hall Effect sensors and outputs an RS-232 data stream which is interpreted and logged by the processor onboard Ranger. A custom circuit board was designed and fabricated for this application.

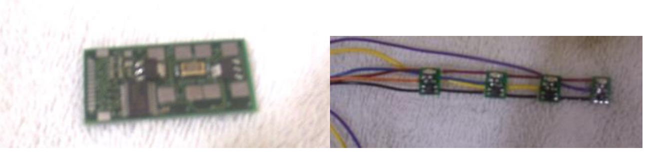

Figure 2: Main sensor board and Hall Effect sensor assembly

Once the board was designed and fabricated, software was written to log the clam gape data onto Ranger. The sensor board was assembled into BioBay and tested in the shop. After some modifications were made to the sensor board, BioBay was tested in open water. The sensor board is capable of reading 16 analog channels for future clam sensing work.

3.) Designed Three BioBay Configurations:

Nekton's Ranger AUV is a 5kg, 36" long 3.5"Ø AUV, so sensor payloads must be small, and preferably neutrally buoyant. Minimizing drag on the Ranger platform is a key issue as well. Each design housed multiple clams and clam sensors. It also allowed for a YSI, conductivity and temperature sensor to be carried. Three BioBay concepts were created.

The first design is the nose mounted BioBay. Clams are arranged in four rows of four clams that surround the sensor board pressure vessel in the center of the vehicle. This design was fabricated and developed into a working prototype. It is a compact design that is easy to integrate onto the vehicle, does not increase the vehicle's frontal area, and allows space for several YSI sensors.

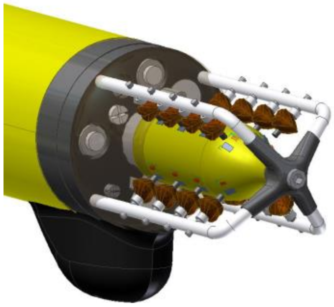

Figure 3: Nose Mounted BioBay

The next design had clams located in pods that hung below the vehicle. The main sensor board was located in a pressure vessel underneath a flow form on the front of the vehicle. The advantage of this design was that it allowed more room to carry additional YSI sensors on Ranger. The drawback was that it significantly increased the frontal area of the vehicle and greatly increased the drag of the vehicle.

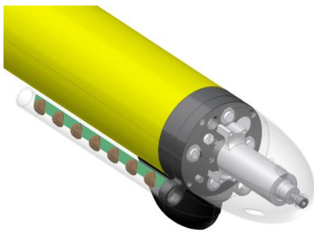

Figure 4: Clam Pod BioBay

The third design was created after the first design had been developed into a working prototype. Therefore, this design took into account the lessons learned from the previous two. The design is a low drag shape that allows water to flow in over the top of the clams. The disadvantage of this design is that it holds a maximum of eight clams. A nose shape similar to this was flow tested to determine drag, but a working prototype was not developed under the Phase I contract.

Figure 5: Low Drag Biobay

4.) Fabricating BioBays:

The nose mounted BioBay was designed to have the sensor board pressure vessel mounted to the center of the front end cap. The outside wall of the pressure vessel is lined with four rows of four Hall Effect sensor boards. The Hall Effect sensor boards are then epoxied to the pressure vessel. This entire unit is submerged in a mold that contains syntactic foam. The foam flows in and around the pressure vessel and the Hall Effect sensor boards, encapsulating everything in water tight insulation.

The mold shapes the syntactic foam into a hydrodynamic flow form. This unit is surrounded by four posts that wrap around and meet at the front of the vehicle.

An elbow fitting allows the power and RS-232 communication wires to go back into the main vehicle without going through a connector.

Clams are mounted to BioBay by super gluing a nylon screw to the bottom half of their shell. The screw threads into one of the four posts. The entire BioBay unit then attaches to the front of Ranger. The BioBay is a modular sensor payload, which can be easily removed and replaced with another payload.

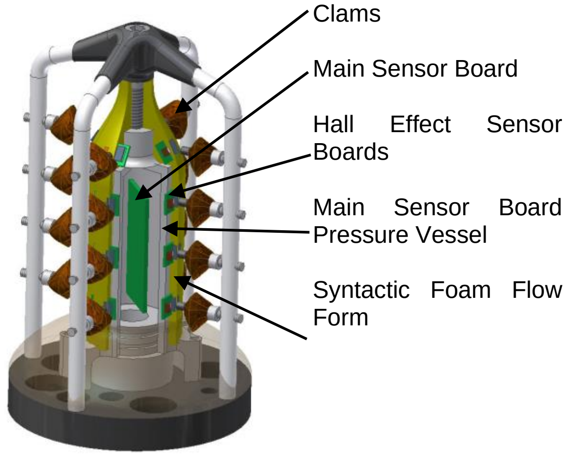

Figure 6: BioBay Assembly

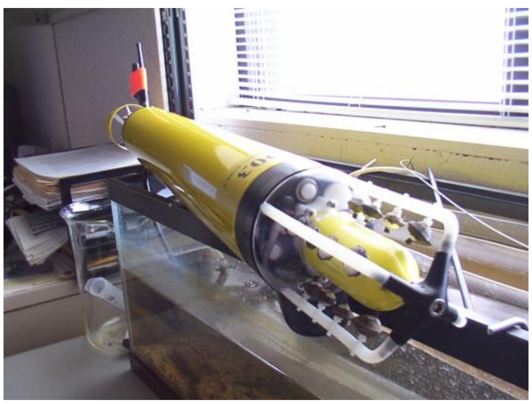

Figure 7: Finished Biobay Installed on Ranger

5.) Flow Tank Testing:

One of the goals of this project was to determine what effect the Biobay had on the performance of the Ranger AUV. In order to do this, the Ranger AUV was mounted in Nekton's flow tank. A baseline coefficient of drag was determined by running the standard Ranger nose shape at different flow speeds and looking at the force produced on the six-axis load cell. The coefficient of drag for the BioBay Ranger is 90% greater than the coefficient of drag for the standard nose configuration. The Phase I BioBay effort is a proof of concept, but in the future, this will have to be addressed. This increase in coefficient of drag will greatly decrease mission duration.

Figure 8: Ranger mockup mounted on six-axis loadcell in flow tank test section

Flow visualization was also used to make sure each clam received adequate flow. For this test, 16 unoccupied clam shells were installed in the BioBay. BioBay was installed on Ranger, and Ranger was installed in the flow tank. Blue dye was used to make sure that water was passing by each of the clam positions.

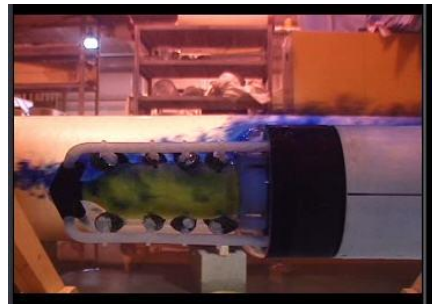

Figure 9: Flow visualization around the BioBay

6.) Testing Biobay with Live Clams:

The first clam test involved installing one live clam on BioBay and testing it in a beaker of fresh water. The clams used for this study are *Corbicula fluminea*. These clams are a widespread introduced species that are easy to find and easy to keep alive. We used *C. fluminea* where it was already present. Clams were installed on BioBay by gluing a nylon screw to the bottom half of their shell. The screw threaded into one of the four posts that surrounded the main sensor board pressure vessel. A magnet was glued to the top half of the clam shell. The screw and the magnet were glued to the clam using a jig to hold the screw and the magnet in radial alignment with each other. This allowed the clam to thread in and out on the post without varying the strength of the magnetic field radially. Screwing the clam in and out adjusts the magnet's distance from the Hall Effect sensor, and allows the open/shut threshold to be set. The screw and magnet were glued to the clam using high strength super glue that cures underwater. This allowed the clam, the screw and the magnet to be loaded into the jig, apply the glue, and then place the entire jig back in the water to minimize the length of time the clam is out of the water.

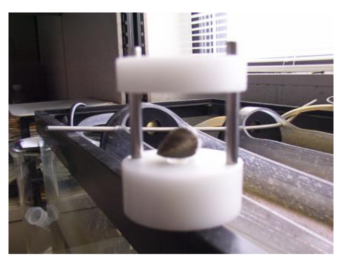

Figure 10: Jig for gluing screws and magnets to clams

BioBay was placed in a beaker of fresh water with the main sensor board powered up. The open/shut threshold for the clam was adjusted by rotating the clam to the necessary position. The Hall Effect sensor boards that are embedded on the outside of the main sensor board pressure vessel also have LEDs that light up when the clam opens. This allows the user to easily adjust the open/shut threshold without the main sensor board sending data to HyperTerminal. The live clam was observed as it opened and started siphoning water. The main sensor board registered an open condition. A syringe of saline was then discharged near the clam to simulate swimming through a region of contaminated water. The clam closed its shell; the main sensor board registered a closed condition. This validated that BioBay worked in a controlled environment, and that the Hall Effect sensors and magnets had been matched correctly.

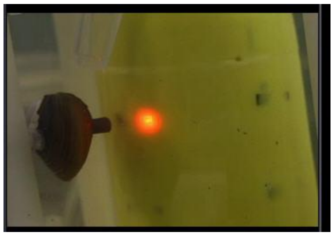

Figure 11: Initial test of BioBay with a live clam

7.) **Testing BioBay in Open Water:**

The first open water test for BioBay was to make sure that the vehicle could still be controlled. Increasing the vehicle's coefficient of drag by adding drag to the front of the vehicle could bring the center of pressure so far forward that the vehicle's controllers could no longer keep it flying in a stable condition for a given speed. Fortunately, this was not the case. The pitch and yaw controllers worked flawlessly even with the increased drag.

After the vehicle passed this test, three of the unoccupied clams were removed and replaced with live clams. Ranger was run in this configuration while recording clam open/shut, conductivity, temperature, depth, GPS position and elapsed mission run time.

Figure 12: Open water testing. Clams are siphoning with mantle extended; LEDs are on

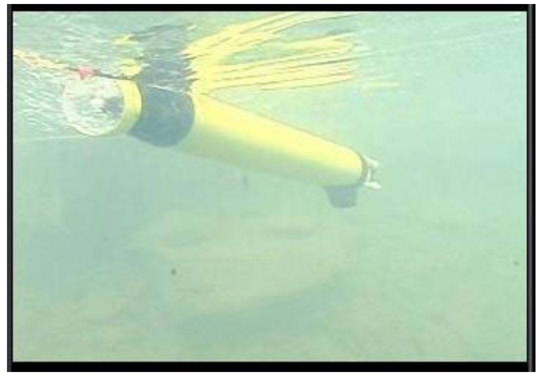

Figure 13: Ranger initiating a dive with BioBay

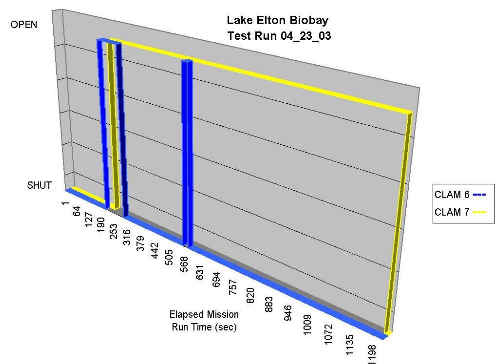

Figure 14: Clam open/shut data recorded during the Lake Elton mission

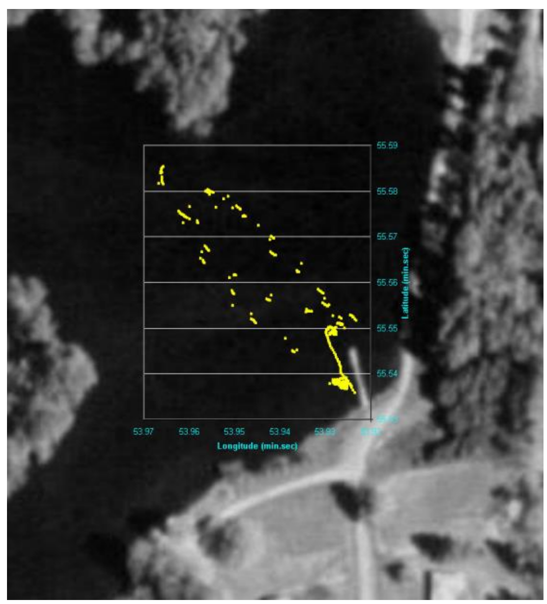

Figure 15: GPS position fixes while Ranger runs out and back away from the pier on Lake Elton. Ranger was launched and retrieved from the pier

Conclusions:

The Phase I BioBay design has worked well enough that we would like to develop it further. We were able to log clam gape data on Ranger while operating the vehicle in an open water environment. Ranger is able to swim in a controlled fashion even with the increase in drag. It's definitely possible to operate this sensor on Ranger. We hope to learn exactly what the clams can tell us, and what other Biosensors we can integrate in future work.

Proposed Future Efforts:

Nekton has submitted a Phase II proposal for funding to continue development of the BioBay. Under Phase II, we would like to characterize the conditions that cause clams to respond to irritants other than water-borne toxins. We are concerned about flow velocity, temperature and depth changes, and vibration and acceleration experienced by the clams while enroute on Ranger. We would also like to evaluate concentration levels that evoke a repeatable response from the clams. We also wish to pursue other biosensor technologies. We are looking for biosensors that fulfill the following criteria:.

9.) Detect broadband water-borne toxins
10.) Fit on Ranger (nominally 3.5"Ø x 7" length maximum)
11.) Do not degrade Ranger's performance any more than the Phase I design
12.) Have a response time of 1-2 seconds or less
13.) Be able to reset to a "no toxins present" state
14.) Be field transportable by one person
15.) Require very little support equipment
16.) Introduce no harmful chemicals or organisms into the areas where they do not already occur

We are interested in variety of different manifestations such as cell based, tissue based, or whole organism based. If they meet the criteria, or come close to meeting them, we would appreciate you contacting us.

Another area we would like to investigate is statistical sensor fusion to validate or dismiss the data coming from the clams or other biosensor. Ranger has the ability to carry a suite of water quality sensors in addition to BioBay. These sensors can be monitored to see if they reporting similar conditions as the clams. This data can be statistically processed in near real time to aid in interpreting the data from the biosensor. We foresee several areas where a Biosensor-equipped Ranger may be useful. One is monitoring water quality and early pre-intake detection of contaminants in drinking water sources for forward deployed troops; another is monitoring reservoirs for homeland security.

Acknowledgements:
We would like to thank Tom Shedd and the U.S. Army Center for Environmental Health Research, Phase I SBIR Sponsor as well as Dr. Joel Allen U.S. EPA. Co-Authors at Nekton Research include: Brett Hobson, Mathieu Kemp, Chuck Pell, Heather Pinnix, and Bryan Schulz.

References:
[1} Allen, HJ, KL Dickson, H Martin, KA Thesen and WT Waller. 2002. Monitoring Watersheds: Biomonitors and Other Measures.