

REPORT DOCUMENTATION PAGE

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1. REPORT DATE (DD-MM-YYYY) 21/12/2007		2. REPORT TYPE Final Report		3. DATES COVERED (From - To) 01/01/06-06/30/07	
4. TITLE AND SUBTITLE Investigation of Neural-Immune Profiling, Transcriptomics and Proteomics and Clinical Tools in Assessing Navy Dolphin Health				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER N00014-06-1-0297	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Tracy Romano				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Mystic Aquarium & Institute for Exploration 55 Coogan Blvd. Mystic, CT 06355				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research 800 N. Quincy St. Arlington, VA 22217-5000				10. SPONSOR/MONITOR'S ACRONYM(S) ONR	
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12. DISTRIBUTION / AVAILABILITY STATEMENT Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The United States Navy maintains and employs approximately 70 bottlenose dolphins (<i>Tursiops truncatus</i>), and 25 sea lions (<i>Zalophus californianus</i>). These marine mammals are deployed all over the world and are specially trained to carry out specific missions and to provide Fleet support for the U.S. Navy. Given the value of these animals to the U.S. Navy, health maintenance and disease prevention for these animals are vital to the U.S. Navy Marine Mammal Program's (NMMP) mission and focus. In order to maintain health and disease prevention in these animals it is critical to have the tools to assess health status before, during and after deployment as well as assess efficacy of preventative measures such as vaccines and immunostimulants. This proposal represents the continued development and initial evaluation of three merging technologies that have been focused on advancing our understanding of marine mammal health and disease. These technologies include immune profiling with the advent of dolphin-specific reagents and assays, functional genomics utilizing a dolphin microarray and proteomics. These technologies will continued to be developed, and the technologies will be applied to a subset of archived dolphin samples including both healthy and ill dolphins. Results from these technologies will be analyzed in conjunction with available clinical data to evaluate the potential in determining and assessing dolphin health.					
15. SUBJECT TERMS Immune function, Immune System, Dolphin, Stress, Neural-Immune					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unlimited	18. NUMBER OF PAGES 5	19a. NAME OF RESPONSIBLE PERSON Tracy Romano
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code) 860-572-5955 Ext. 102

FINAL REPORT

GRANT #:N000140610297

PRINCIPAL INVESTIGATOR: Tracy Romano

INSTITUTION: Mystic Aquarium & Institute for Exploration (MAIFE)

GRANT TITLE: Investigation of Neural-Immune Profiling, Transcriptomics
Proteomics as Clinical Tools in Assessing Navy Dolphin Health

AWARD PERIOD: 1 January 2006 - 30 June 2007

OBJECTIVE(S): The overall objective of this proposal is to evaluate immune profiling, transcriptomics and proteomics in relation to clinical measurements used in evaluating dolphin health. The specific objectives for the reporting period are: 1) To continue the development of dolphin-specific reagents including the cloning of immune response genes, assays to quantify hormones and immune status, and methodologies to measure gene and protein expression 2) To examine a subset of archived dolphin samples to determine quality of RNA and suitability for microarray analysis, proteomics and additional immunological profiling 3) To evaluate the immune profile data gathered and carried out so far on the archived dolphin samples in conjunction with clinical data 4) To add additional immune profile data, microarray and proteomic data on the subset of archived samples and to determine correlative relationships (if any) among the immune profile data, transcriptomics and proteomics

APPROACH: The cloning of dolphin immune response genes e.g. CD8 α and β , CD56 was carried out by extracting RNA from dolphin peripheral white blood cells, obtaining cDNAs by reverse transcription, and using primers that have been designed based on homologous regions of known CD8 sequences from other species to amplify dolphin CD8 α and β . CD8 α and β were then cloned into mammalian expression vectors either as full-length clones or as extracellular domains only. Both were then transfected into Chinese hamster ovary (CHO) cells and selected via drug selection. Secreted protein was then purified and quantified and will be used in subsequent monoclonal antibody production. Anti-peptide antibodies were generated against CD8 β in collaboration with Pfizer, Inc. Quantification of catecholamines was carried out via High Performance Liquid Chromatography with Electrochemical Detection. Methodologies for human and other mammal samples were compared and optimized for measuring dolphin catecholamines. Assays for immune function were adapted or developed specifically for dolphins. For example, an assay from Chemicon, Inc. which utilizes BrdU, a thymidine analog, was optimized for dolphin cells in our laboratory. Mitogen incubation time, cell number and mitogen concentration were determined for optimal and suboptimal dolphin lymphocyte proliferation. A subset of archived dolphin blood cell samples (from both healthy and ill dolphins) preserved in RNazol was sent to MUSC for RNA extraction to determine quality and quantity of RNA and suitability for application to the dolphin microarray. Neural-immune data gathered thus far on archived dolphin samples was analyzed in conjunction with clinical information available on the same dolphin set as well as the dolphin microarray samples sent to MUSC.

ACCOMPLISHMENTS:

1) *The development of dolphin-specific reagents including the cloning of immune response genes, assays to quantify hormones and immune status, and methodologies to measure gene and protein expression*

a. Cloning of immune response genes The CD8 α and β and CD56 genes were cloned from dolphin peripheral blood lymphocytes and adrenal gland and the sequences analyzed. Histidine tags were added to both full-length CD8 α and β as well as to the extracellular domains only, utilizing PCR. Chinese hamster ovary (CHO) cells were transfected with expression vectors containing full-length CD8 α or β individually, or co-transfected with both expression vectors, in order to make cell lines expressing individual subunits or the entire CD8 α,β heterodimer, respectively. To make secreted forms of the proteins, CHO cells were transfected with expression vectors containing truncated CD8 α or β . All transfections resulted in selection of drug resistant cells, except for the co-transfection with both full-length genes. CHO cells already expressing full-length CD8 α were transfected with the full-length CD8 β expression vector, and resulted in cells resistant to both selection drugs. Flow cytometry of permeabilized CHO cells co-transfected with full-length CD8 α and β expression vectors vs. untransfected cells, using anti-histidine tag antibody did not reveal clear results. However, flow cytometry utilizing the CD8 β peptide antibodies that we generated in this effort (described below), revealed increased fluorescence (38% - 79%) when compared with untransfected cells, for both permeabilized and unpermeabilized transfected cells, suggesting that CD8 β was being synthesized and expressed on the cell surface. CHO cell supernatants from transfections with truncated CD8 α or β were analyzed by Western blot. Results revealed a single band just under 30kDa for truncated CD8 β (expected protein size 20kDa). For truncated CD8 α (expected size 25kDa), several bands were seen, but the dominant band was 40kDa. Both molecules contain multiple glycosylation sites, which may account for the size discrepancies. Further analyses need to be carried out in this regard. Simultaneously, in collaboration with Pfizer, Inc., anti-peptide antibodies have been generated to CD8 β . Five of the anti-peptide antibodies to dolphin CD8 β that were positive by flow cytometry have been subcloned to the tertiary stage, grown-up and purified.

b. Assays to quantify hormones and immune status The BrdU lymphocyte proliferation assay has been optimized utilizing dolphin and beluga cells. For the dolphin, incubation times tested were 72, 96 and 128 hours with 72 hours showing the optimal response. Doses of T cell mitogen (Con A) and B cell mitogen (LPS) tested ranged from 0.05 - 5 μ g/ml and 0.625 - 10 μ g/ml. Peak stimulation for T cells was achieved with 1 - 2.5 μ g/ml Con A depending on the animal, while maximal stimulation of B cells occurred between 2.5 - 5 μ g/ml LPS. In addition, a Natural Killer cell functional assay using flow cytometry was further optimized for both dolphin and beluga. Effector cell to target ratios ranging from 200:1 - 6.25:1 have been tested with incubation times of 2, 4, and 6 hours. Both human (K562) and mouse (Yac-1) cell lines have been tested as target cells and different purification techniques tested. The optimal results were with Yac-1 as targets, with an incubation time of 2 hours and purification with a double density gradient. High Performance Liquid Chromatography technology has successfully been utilized to measure dolphin catecholamines in our laboratory. After troubleshooting, it was determined that the newer more efficient extraction methods do not give

the sensitivity needed to detect the low plasma catecholamine concentrations in dolphin plasma.

c. Methodologies to measure gene and protein expression (See Report of Greg Warr, MUSC)

2) *Examination of a subset of archived dolphin samples to determine quality of RNA and suitability for microarray analysis, proteomics and additional immunological profiling* A subset of archived dolphin frozen buffy coats (from both healthy and ill dolphins) preserved with RNazol were sent to MUSC for RNA extraction to determine quality and yield of RNA and if suitable, application to the dolphin microarray (See Report of Greg Warr, MUSC). Cells were also thawed to determine viability for select assays. The degree of viability varied among samples although a subset of samples with the same approximate percentage of viable cells was determined.

3) *Evaluation of immune profile data gathered and carried out so far on the archived dolphin samples in conjunction with clinical data* The dolphin database that we created with the results of the neural-immune measures and clinical information has been analyzed. The criteria for healthy dolphin vs. and ill dolphin were established in collaboration with veterinarians from the U.S. Navy Marine Mammal Program.

Descriptive statistics were obtained for normal healthy dolphins based on sex and older vs. younger dolphins for the following neural-immune measures: ACTH, cortisol, aldosterone, norepinephrine, dopamine, total lymphocytes, white blood cells, T cells, B cells, T helper cells, class II⁺ cells. The categories evaluated for ill dolphins included WBC>11,000, lymphocytes<10%, GGT>100, Inappetance, Medications, ESR>50, Fe<100, Fe>400. The hormones such as ACTH showed a significant difference between male and female dolphins. Lymphocyte subsets showed significant differences between age groups (e.g. T, B, T helper, class II⁺ and total lymphocytes). Ill animals showed statistically significant increases in white blood cells, epinephrine and norepinephrine levels, while dopamine was significantly lower in ill dolphins.

4) *Evaluation of immune profile data, microarray and proteomic data on the subset of archived samples* Neural-immune measures, clinical information and whether the animal was considered ill or healthy was sent to MUSC with the corresponding RNA samples that were applied to the dolphin microarray to integrate with the microarray results (See Report of Greg Warr, MUSC).

CONCLUSIONS: The assays that we have developed and or adapted are all feasible assays to use to assess health given the constraints of obtaining blood samples from cetaceans (both captive and wild). The molecular techniques used to obtain specific immunological markers are often difficult and tedious, but worth the focused effort in obtaining the molecule of choice vs. more classical methodologies which do not guarantee success in obtaining the targeted molecule. Sex and age need to be taken into consideration when evaluating the dolphin immune system and dolphin health since in normal healthy dolphins neural-immune measures (T, B, T helper, class II⁺ and total lymphocytes) were significantly different depending on the age group, and hormones were different for healthy males vs. females. This warrants further investigation in more defined and specific sampling of age categories since the samples obtained opportunistically only allowed for division into 2 major age categories. The neural-immune measures that were significant in ill dolphins were white blood cells, epinephrine, norepinephrine and dopamine. These results indicate that increases in

catcholamines may be indicators of illness in addition to white blood cells. Further investigation of catecholamines and white blood cells in disease processes is warranted focusing on time course of events and specific illness and/or disease. (See Report of Greg Warr, MUSC for conclusions of genomics and proteomics).

SIGNIFICANCE: The neural-immune tests developed and adapted for bottlenose dolphins and analyzed with the clinical information available, do show changes in both captive and wild animals depending on stress (as shown in prior ONR supported studies), age and disease state. These tests will be useful for monitoring health in both captive and wild dolphins as well as useful in assessing efficacy of vaccines, and the monitoring of the impact of the environment and various stressors on dolphin health. (See Report of Greg Warr, MUSC for significance of genomics and proteomics).

PATENT INFORMATION: No patents have been filed.

AWARD INFORMATION:

Student Travel Award for IAAAM 2007, Orlando, FL and first place for Overall Student Presentation Award in the resident/postdoc category for IAAAM 2007, Orlando, FL. (Brucella tests- disease and dolphins)

Student Travel Award for IAAAM 2006, Nassau, Bahamas and second place for Overall Student Presentation Award in the resident/postdoc category at IAAAM 2006, Nassau, Bahamas. (Brucella tests- disease and dolphins)

PUBLICATIONS AND ABSTRACTS:

Mancia, A., Lundquist, M., Romano, T., Peden-Adams, M., Fair, P., Kindy, M., Ellis, B., Gattoni-Celli, S., McKillen, D., Trent, H., Chen, Y., Almeida, J., Gross, P., Chapman, R., and Warr, G. 2007. A Dolphin Peripheral Blood Leukocyte cDNA Microarray for Studies of Immune Function and Stress Reactions. *Developmental and Comparative Immunology*. 31(5):520-9.

Mancia, A., Romano, T.A., Gefroh, H.A., Chapman, R.W., Middleton, D.L. Warr, G.W. and Lundquist, M. 2007. Characterization of the Immunoglobulin A Heavy Chain gene of the Atlantic Bottlenosed Dolphin (*Tursiops truncatus*). *Vet. Immunol. Immunopathol.* 118:304-309.

Romano, T., Fair, P.A., Bossart, G.D., Mitchum, G., Rice, C., Peden-Adams, M. An Overview of Immune Assessment in Bottlenose Dolphins During Capture-Release Studies from the HERA Project. In: Harbor Branch Oceanographic Institution, Inc. Technical Report No. 93, p.25-26, 2006.

Peden-Adams, M.M., Romano, T.A., EuDaly, J.G., Muir, D.C., Houde, M., Bossart, G.D., Fair, P.A., Keil, D.E. Comparison of immune modulation in two mammalian species in relation to PFOS exposure. Perfluoroalkyl Acids and Related Chemistries: Toxicokinetics and Mode-of-Action Workshop. Society of Toxicology - Current Concepts in Toxicology. February 2007; Arlington, VA.

Meegan, J., Smith, C., Wong, S., Jensen, E., Van Bonn, W., Romano, T., Sidor, I., Dunn, L. Brucella sp. infected bottlenose dolphin (*Tursiops truncatus*) cases in two populations: serologic and clinical evaluations.

In: Proceedings of the 38th Annual Conference of the International Association for Aquatic Animal Medicine. 2007; Vol. 38; 111-113.

Field, C., Meegan, J., Goldstein, T., Gulland, F., Goertz, C., Tuomi, P., Jessup, D., Miller, M., Doroff, A., Gill, V., Romano, T., Dunn, L. A comparison of marine origin *Brucella* seroprevalence between three populations of sea otters (*Enhydra lutris*). In: Proceedings of the 38th Annual Conference of the International Association for Aquatic Animal Medicine. 2007; Vol. 38; 134-135.

Mancia, A., Lundquist, M., Romano, T., Peden-Adams, M., Fair, P., Kindy, M., Ellis, B., Gattoni-Celli, S., McKillen, D., Trent, H., Chen, Y., Almeida, J., Gross, P., Chapman, R., Warr, G. Monitoring stress reaction and immune function in wild dolphins *Tursiops truncatus* using a dolphin peripheral blood leukocyte cDNA microarray. In: Proceedings of the 10th International Congress International Society of Developmental and Comparative Immunology. 2006; Vol. 10; 94.

Stuckey, J., Romano, T., Rice, C.D., EuDaly, J.G., Mitchum, G., Bossart, G., Fair, P., Peden-Adams, M. Evaluation of brominated flame retardants in relationship to bottlenose dolphin immunity. *The Toxicologist* (Supplement to Toxicological Sciences) 2006; 90(S-1).

Peden-Adams, M., Rice, C., Muir, D., Houde, M., Mitchum, G., Bossart, G., Fair, P., Romano, T. Development and utilization of a standardized suite of immune assays in healthy and non-healthy bottlenose dolphins. In: Proceedings of the 10th International Congress International Society of Developmental and Comparative Immunology. 2006; Vol. 10:194.

Meegan, J., Romano, T., Mazzaro, L., Sidor, I., and Dunn, J. 2006. Serosurveillance of Wild Bottlenose Dolphins (*Tursiops Truncatus*) and Evaluation of Both Newly Developed Marine-Specific and Commercially Available *Brucella Abortus* Serologic Tests For The Detection of Antibodies To Marine-Origin *Brucella*. In: *Proceedings of the 37th Annual Conference of the International Association for Aquatic Animal Medicine*. Vol. 37, pp. 65-66 (Abstract and Presentation)

Wise, J., Wise, S., Kelly, R., Throckmorton, H., Kraus, S., O'Hara, T., Romano, T., 2006. The Effect of Environmental Contaminants: Chromium, Mercury, and Tributyltin, on Northern Atlantic Right Whale Cells. In: *Proceedings of the 37th Annual Conference of the International Association for Aquatic Animal Medicine*. Vol. 37 p. 110 (Abstract and Presentation)

Mancia, A., Lundqvist, M., Romano, T., Peden-Adams, M., Fair, P., Kindy, M., Ellis, B., Gattoni-Celli, S., McKillen, D., Trent, H., Chen, Y., Almeida, J., Gross, P., Chapman, R., and Warr, G., 2006. Monitoring Stress Reaction and Immune Function in Wild Dolphins *Tursiops Truncatus* Using a Dolphin Peripheral Blood Leukocyte cDNA Microarray. In: *Proceedings of the 10th International Congress International Society of Developmental and Comparative Immunology*. Vol. 10 p.94 (Abstract and Presentation)

Peden-Adams, M., Rice, C., Muir, D., Houde, M., Mitchum, G., Bossart, G., Fair, P., Romano, T. 2006. Development and Utilization of a Standardized Suite of Immune Assays in Healthy and Non-Healthy Bottlenose Dolphins. In: *Proceedings of the 10th International Congress International Society of Developmental and Comparative Immunology*. Vol. 10 p. 194 (Abstract and Presentation)

FINANCIAL STATUS REPORT
(Long Form)

(Follow instructions on the back)

1. Federal Agency and Organizational Element to Which Report is Submitted Office of Naval Research		2. Federal Grant or Other Identifying Number Assigned By Federal Agency N00014-06-1-0297		OMB Approval No. 0348-0039	Page of 1 pages
3. Recipient Organization (Name and complete address, including ZIP code) Sea Research Foundation 55 Coogan Blvd, Mystic, CT 06355					
4. Employer Identification Number 061480300		5. Recipient Account Number or Identifying Number		6. Final Report ☒ Yes ☐ No	
7. Basis ☐ Cash ☒ Accrual					
8. Funding/Grant Period (See instructions) From: (Month, Day, Year) 1/1/2006		To: (Month, Day, Year) 6/30/2007		9. Period Covered by this Report From: (Month, Day, Year) 1/1/2006 To: (Month, Day, Year) 6/30/2007	
10. Transactions:		I Previously Reported		II This Period	
				III Cumulative	
a. Total outlays		0.00		125,000.00	
b. Refunds, rebates, etc.				0.00	
c. Program income used in accordance with the deduction alternative				0.00	
d. Net outlays (Line a, less the sum of lines b and c)		0.00		125,000.00	
Recipient's share of net outlays, consisting of:				0.00	
e. Third party (in-kind) contributions				0.00	
f. Other Federal awards authorized to be used to match this award				0.00	
g. Program income used in accordance with the matching or cost sharing alternative				0.00	
h. All other recipient outlays not shown on lines e, f or g				0.00	
i. Total recipient share of net outlays (Sum of lines e, f, g and h)		0.00		0.00	
j. Federal share of net outlays (line d less line i)		0.00		125,000.00	
k. Total unliquidated obligations					
l. Recipient's share of unliquidated obligations					
m. Federal share of unliquidated obligations					
n. Total Federal share (sum of lines j and m)				125,000.00	
o. Total Federal funds authorized for this funding period				125,000.00	
p. Unobligated balance of Federal funds (Line o minus line n)				0.00	
Program income, consisting of:					
q. Disbursed program income shown on lines c and/or g above					
r. Disbursed program income using the addition alternative					
s. Undisbursed program income					
t. Total program income realized (Sum of lines q, r and s)				0.00	
11. Indirect Expense		a. Type of Rate (Place "X" in appropriate box) ☒ Provisional ☐ Predetermined ☐ Final ☐ Fixed			
		b. Rate		c. Base	
		d. Total Amount		e. Federal Share	
12. Remarks: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation.					
13. Certification: I certify to the best of my knowledge and belief that this report is correct and complete and that all outlays and unliquidated obligations are for the purposes set forth in the award documents.					
Typed or Printed Name and Title <i>Denise H. Armstrong Chief Financial Officer</i>				Telephone (Area code, number and extension) <i>860 572 5955 x 302</i>	
Signature of Authorized Certifying Official <i>[Signature]</i>				Date Report Submitted August 10, 2007	

REPORT OF INVENTIONS AND SUBCONTRACTS

(Pursuant to "Patent Rights" Contract Clause) (See Instructions on back)

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1. a. NAME OF CONTRACTOR/SUBCONTRACTOR Mystic Aquarium Institute For Exploration		c. CONTRACT NUMBER N000140610297		2. a. NAME OF GOVERNMENT PRIME CONTRACTOR	
b. ADDRESS (Include ZIP Code) 55 Coogan Boulevard Mystic, CT 06355		d. AWARD DATE (YYYYMMDD) 20060101	b. ADDRESS (Include ZIP Code)		d. AWARD DATE (YYYYMMDD)
				4. REPORTING PERIOD (YYYYMMDD) a. FROM 20060101 b. TO 20070630	

SECTION I - SUBJECT INVENTIONS

5. "SUBJECT INVENTIONS" REQUIRED TO BE REPORTED BY CONTRACTOR/SUBCONTRACTOR (If "None," so state)	NAME(S) OF INVENTOR(S) (Last, First, Middle Initial)	TITLE OF INVENTION(S)	DISCLOSURE NUMBER, PATENT APPLICATION SERIAL NUMBER OR PATENT NUMBER	ELECTION TO FILE PATENT APPLICATIONS (X)				CONFIRMATORY INSTRUMENT OR ASSIGNMENT FORWARDED TO CONTRACTING OFFICER (X)	
				(1) UNITED STATES		(2) FOREIGN			
	a.	b.	c.	(a) YES	(b) NO	(a) YES	(b) NO	(a) YES	(b) NO
#5 (a-g) Not Applicable									

f. EMPLOYER OF INVENTOR(S) NOT EMPLOYED BY CONTRACTOR/SUBCONTRACTOR		g. ELECTED FOREIGN COUNTRIES IN WHICH A PATENT APPLICATION WILL BE FILED	
(1) (a) NAME OF INVENTOR (Last, First, Middle Initial)	(2) (a) NAME OF INVENTOR (Last, First, Middle Initial)	(1) TITLE OF INVENTION	
(b) NAME OF EMPLOYER	(b) NAME OF EMPLOYER	(2) FOREIGN COUNTRIES OF PATENT APPLICATION	
(c) ADDRESS OF EMPLOYER (Include ZIP Code)	(c) ADDRESS OF EMPLOYER (Include ZIP Code)		

SECTION II - SUBCONTRACTS (Containing a "Patent Rights" clause)

6. SUBCONTRACTS AWARDED BY CONTRACTOR/SUBCONTRACTOR (If "None," so state)					
NAME OF SUBCONTRACTOR(S)	ADDRESS (Include ZIP Code)	SUBCONTRACT NUMBER(S)	FAR "PATENT RIGHTS"		DESCRIPTION OF WORK TO BE PERFORMED UNDER SUBCONTRACT(S)
			(1) CLAUSE NUMBER	(2) DATE (YYYYMM)	
#6 (a-f) Not Applicable					

SECTION III - CERTIFICATION

7. CERTIFICATION OF REPORT BY CONTRACTOR/SUBCONTRACTOR (Not required if: (X) as appropriate)	SMALL BUSINESS or	☒	NONPROFIT ORGANIZATION
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I certify that the reporting party has procedures for prompt identification and timely disclosure of "Subject Inventions," that such procedures have been followed and that all "Subject Inventions" have been reported.

a. NAME OF AUTHORIZED CONTRACTOR/SUBCONTRACTOR OFFICIAL (Last, First, Middle Initial) Tract Romano, PhD	b. TITLE Senior Vice President Of Research & Zoological Operations	c. SIGNATURE 	d. DATE SIGNED 20071221
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