

Revised 5-6-99

Development and Field Testing Rapid Diagnostic Test Kits for Paralytic Shellfish Poisoning and Amnesic Shellfish Poisoning
"Submitted under the BAA"

Project Number:

00482-BAA

Restoration Category:

Proposer: Jellett Biotek Limited

Lead Trustee Agency: ADEC

Cooperating Agencies:

Alaska SeaLife Centre: NO

Duration: 1st year, 3 year project

Cost FY 00: \$294,000

Cost FY 01: \$ 50,000

Cost FY 02: \$ 30,000

Geographic Area Prince William Sound, Kodiak

Injured Resource/Service: Clams, Mussels, Subtidal communities

ABSTRACT

This project involves the development and testing of rapid screening tests to detect two marine biotoxins that affect the Alaskan shellfishery, amnesic shellfish poisoning (ASP) and paralytic shellfish poisoning (PSP). These toxins can cause sickness and even death in individuals who consume contaminated shellfish. With a reliable field testing method, coastal communities and shellfisheries will be able to ensure shellfish is safe to eat before harvesting. This will lead to safer subsistence harvesting of shellfish which can replace the lost or decreased availability of injured resources such as harbour seals, sea lions, herring and ducks. Jellett Biotek has a working prototype of a rapid test for PSP and have the antibodies for an ASP test. We have applied to the ASTF for funding to help further develop and optimize these tests, thus making them available for field trials. The feasibility of establishing ongoing beach monitoring will be assessed.

INTRODUCTION

This project is being proposed to help ensure that shellfish is safe to harvest in communities within the Prince William Sound and Kodiak areas. The shellfishery can be a valuable food resource from a subsistence harvest perspective, as a replacement for other food sources that were affected by the oil spill. These species include harbour seals, sea lions, ducks and herring. The Prince William Sound and Kodiak Island areas suffer from serious outbreaks of paralytic shellfish poisoning (PSP) and occasionally amnesic shellfish poisoning (ASP). This project plans to develop and field test state of the art field screening tests for these two marine biotoxins. Marine biotoxins occur naturally when, under certain conditions, specific species of algae "bloom". These occurrences are frequently known as "red tides". Bivalves such as mussels and clams are filter feeders and will ingest this toxic algae and become toxic for human consumption. Harvesting and consuming shellfish at these times can cause illness and death to those who consume them, but the shellfish will subsequently clear the toxins and become safe to eat over time.

During the past Alaskan toxin season from April 1998 to December 1998, Jellett Biotek Limited received funding from the Alaska Science and Technology Foundation to field trial our current cell based test kits for PSP. This trial was very successful, particularly in laboratory settings, but pointed to the need for an easy to use, single use field test for PSP.

The rapid tests to be developed can be performed by relatively unskilled individuals and will provide visual qualitative (yes/no) results in less than 20 minutes.

Jellett Biotek is providing in kind contribution to this project of specialized antibodies to detect the two toxins, as well as substantial research and intellectual property to date. We in fact have a working prototype for the PSP rapid test and are confident that it can be optimized for the profile of toxin analogues found in Alaskan waters. We are also confident we will develop the rapid test for ASP by January 00, well before the expected field trial date of April 2000.

This proposal is broken into two components, the development of these tests and subsequent field trials of the rapid tests in communities within the Prince William Sound and Kodiak Island areas.

This project may be directly linked to the proposed Youth Intern Research Project (Youth Area Watch) for Kodiak Island and indirectly linked to clam bed restoration project.

NEED FOR PROJECT

A. Statement of the Problem

There has been a loss or reduction in available subsistence food resources in the oil spill affected areas with species such as harbour seals, sea lions, ducks and herring. With the availability of a cost effective, simple marine biotoxin testing technology, coastal communities can test to ensure the safety of shellfish beds, thus opening up this resource for safe subsistence harvesting.

Current shellfish testing for all of Alaska is performed at the Alaska Department of Environmental Conservation (ADEC) regulatory lab in Palmer. This involves collecting shellfish samples, shipping them to the lab, where the toxin extracted from the shellfish tissue is injected into live mice. The amount of time it takes for the mouse to die from respiratory paralysis is an indication of the amount of PSP toxicity present in the shellfish. The current regulatory limit is 80ug of saxitoxin per 100 grams of shellfish tissue. In the case of amnesic shellfish poisoning, or ASP, HPLC tests (high performance liquid chromatography) tests are done at the ADEC lab to ensure the shellfish samples are free of domoic acid, which causes ASP. Samples from commercial shellfisheries receive priority at the ADEC lab for testing under the state program, leaving many beaches and recreational shellfish areas without monitoring for PSP or ASP. The cost for having a PSP test performed by the ADEC lab is \$125 per sample, if the sample is not covered under the state regulatory testing program. An ASP test is \$100 per sample.

Jellett Biotek Limited plans to develop simple- to- use immunochromatographic tests (similar to home pregnancy tests) that will be used to screen for PSP and ASP within 20 minutes. These will be simple yes/no tests to indicate whether or not the shellfish is affected by these two marine biotoxins. These tests will not provide a quantitative result, and are not meant to replace the regulatory test for the commercial shellfishery, but will be a reliable initial screen for toxicity, at a projected cost of about one tenth the cost of the mouse bioassay and HPLC tests. Jellett Biotek plans to have these tests approved by global regulatory authorities (USFDA in the US) as a pre-screen for ASP and PSP.

B. Rationale/Link to Restoration

This project should be undertaken to help validate a rapid, inexpensive test for monitoring for marine biotoxins that affect the shellfishery in the Prince William Sound and Kodiak areas. By testing the shellfish beds in the area, windows of harvest opportunity may be found, making the harvesting of shellfish for subsistence or recreational purposes much safer. This may eventually lead to a cost effective, broadly based biotoxin monitoring program in areas currently not monitored for toxicity and permit access to the shellfish resource that is currently not available. The access to the shellfishery will help replace subsistence food resources lost or limited as a consequence of the oil spill in the affected areas. Examples of the affected species include harbour seals, sea lions, ducks and herring.

Enhanced public safety may result as individual harvesters will have access to a screening methodology that will help protect against harvesting contaminated product, leading to potential economic development in tourism or a commercial shellfishery in the restoration area.

The proposed field trials are an integral part of the development of the rapid tests as they will be tested in actual conditions by relatively inexperienced individuals. Jellett Biotek wants to demonstrate that these tests are simple, robust and effective at screening for PSP and ASP and protecting communities and individuals from harvesting contaminated shellfish.

The proposed trials will compare the efficacy of the rapid tests to the current regulatory testing methods that are recognized by the Alaska State government as being effective public health screens for these toxins.

C. Location

We have requested the assistance of the Ouzinkie Tribal Council, the Tatitlek IRA Council as well as the Kodiak Tribal Council in providing the field sites for these tests. We also have letters of support to participate in the trial from the Alaska Native Tribal Health Consortium, Community Environmental Services. We will select representative shellfish sites in the Prince William Sound and Kodiak Island. All coastal communities with potential shellfisheries may be affected by the results of these trials.

Community Involvement and Traditional Knowledge

This project will rely heavily on local knowledge of the tribal councils for identifying potentially important shellfish areas and for developing sample collection plans. This project will coordinate with the proposed Kodiak Youth Area Watch. Students in Kodiak Island will be trained to collect shellfish samples and perform the test procedures related to the project.

We will focus our field trial efforts on areas that are large subsistence consumers of shellfish. Our tests will be designed to be extremely easy to use and we will provide a training session for the students and supervisors at field site locations.

Upon completion of the project Jellett Biotek will provide an easy to understand report on the efficacy of the field tests and their economics and ease of use. We will also assess the merits and costs involved in developing an ongoing shellfish monitoring program which incorporates this technology. This report will be widely distributed to interested community groups. We will develop commercialization strategies to ensure the tests are available in Alaska once the trial has been completed. Jellett Biotek will also make the trial results available on our web site and we will collaborate with the Kodiak Island Borough Community Development Department in promoting shellfish safety through the school system and other public awareness opportunities. We will arrange for at least one community seminar to discuss the trial results and the implications to the coastal communities and shellfisheries. We will attempt to organize ongoing shellfish monitoring programs using the assistance of tribal councils and other community groups. Hugh Short, Spill Area Wide Community Involvement Coordinator, has assisted in providing contacts to several tribal councils and economic development organizations in the affected communities.

PROJECT DESIGN

A. Objectives

The objective of the project is to develop, then demonstrate the efficacy, ease of use and cost effectiveness of the rapid screening tests for ASP and PSP during the FY 00. In the longer term

this will enable shellfish resources in the affected communities to be monitored on an ongoing basis for health safety. It is expected that this will lead to the ability to exploit the resource for subsistence and recreational purposes. The shellfisheries may be a potential economic generator in the region.

B. Methods

Jellett Biotek has already begun development of the rapid tests and will increase the pace of development once our proposal is approved. We will test the prototype tests in house with Alaskan shellfish samples that we have gathered from this year's trial of the MIST[®] kits in Alaska, as well as archived shellfish samples from this year's biotoxin monitoring program at the DEC lab. We will then manufacture enough prototype tests to supply the field trials, which are expected to begin in April 00 and continue until September 00. Jellett Biotek will then analyze the field trial data and prepare a report by November 2000(FY01). A community seminar will be provided on the trials prior to May 01, as well as recommendations on a beach monitoring program for the 2001 toxin season.

We are requesting EVOS funding of the final aspects of the rapid test development to commence in October 99, and when we will prepare the protocol and training materials for the field participant training session, that will occur in April or May 2000. The rapid tests will be manufactured between January 00 and April 00, in preparation for the trials.

During the field trials, shellfish samples will be collected in the affected region, the samples split with one half of the sample going to the ADEC regulatory lab in Palmer for regulatory tests, and the other half of the shellfish sample tested in the field using the rapid tests. The results of the field tests will be compared to those obtained by the ADEC lab (mouse bioassay for PSP and HPLC test for ASP).

To demonstrate the robustness and reproducibility of the field tests, we have selected 4 field sites within the affected region. Each field site will perform a minimum of 100 field tests (50 shellfish samples, testing for both ASP and PSP) using the new rapid tests over the normal toxicity season of April to September. We will use a sampling procedure that will collect shellfish samples ensuring geographical distribution of the samples as well as diversity in shellfish species. Jellett Biotek will provide a training session to all participants to ensure they understand how to collect shellfish samples, perform the tests and report the results correctly.

An alternative to this approach would be to collect samples over the toxin season and "archive" the samples to be tested at a later date. Although more convenient and cost effective to do this, there are concerns that the toxin profile found in the shellfish may change over time. The data will be much better if the samples are tested by the ADEC lab and the field site at relatively the same time to ensure comparability in the data of the different test methods. The proposed approach worked very well in a previous trial using our cell based MIST[™] kits for detecting paralytic shellfish poisoning.

C. Cooperating Agencies, Contracts and other Agency Assistance

We are collaborating with the Alaska Department of Environmental Conservation Regulatory Lab in Palmer on validating the results of the field trial. We are also collaborating with the Alaska Native Tribal Health Consortium, Community Environmental Services for field trials. It was decided to apply for assistance from the Exxon Valdez Oil Spill Trust Fund (EVOS) for the field trial portion of the project due the high level of community involvement that is required, and the fact that the affected areas have some of the highest levels of PSP found in the world. There appears to be a real need for a reliable, cost effective monitoring program at the community or even individual level.

The private sector partners in this project will include Jellett Biotek, who will provide the rapid diagnostic tests, a training program in their use, technical and analytical support to project management and test kit commercialization strategies. The collaborating tribal councils, Alaska Native Tribal Health Consortium, and trained members of the Kodiak Youth Area Watch will collect shellfish samples, homogenize and split the samples, sending half into the ADEC lab in Palmer, and performing the rapid tests on the other half of the sample. They will also be responsible for reporting results to Jellett Biotek.

The ADEC lab in Palmer is a very important government partner in this project. They will perform the corroborative mouse bioassays, HPLC analysis and rapid tests on shellfish samples against which the field tests will be compared. Jellett Biotek worked closely with the ADEC last year in the field trials for our cell based MIST™ kits.

Schedule

Measurable Project Tasks for FY 00 (October 1, 1999 - September 30, 2000)

The following is a summary of the major tasks to be completed

- | | |
|--------------------------|---|
| October99 - December 99: | Final development of rapid tests for ASP and PSP
Develop trial protocol and training manuals for all field testing sites |
| January 00 -April 00: | JBL will validate rapid tests using Alaskan shellfish samples
JBL to produce sufficient rapid tests for trials (about 1000 tests) |
| April 00-May 00: | JBL will provide a training course for all trial participants in the shellfish sample collection and rapid test procedures. We will review popular harvesting sites and develop a shellfish sampling plan. |
| May 00 - September 00: | All trial participants will collect approximately 50 shellfish samples at each site, homogenate the tissue and send half of the tissue to the ADEC lab in Palmer. The site participant will perform both ASP and PSP rapid tests on each sample. Site |

participants will report test results to JBL. Jellett Biotek will coordinate testing with the DEC lab.

01 Fiscal year (October 1- September 30)

October 00- November 00: Jellett Biotek will analyze the data and prepare a report on the trial results and recommending future monitoring strategies
December 00 - April 01 : Jellett Biotek will provide a seminar to all trial participants and interested communities on trial results and ramifications.
April 15 01 Report to EVOS
April 01 International Shellfish Toxicity conference - Presentation of Data
April 30 Submit articles to journals
April to June Public Awareness Seminars - Alaska
April to May Potential to begin beach monitoring program
March 02 Final report on pilot monitoring program

B. Project Milestones and Endpoints

The following are milestone dates and deliverables by that date:

<u>Date</u>	<u>Deliverable</u>
December 31, 99	Prototype rapid tests for ASP and PSP complete written protocol for the trial, copies delivered to trial participants
April 30, 00	a minimum of 1000 spot tests manufactured and pre-tested, monthly delivery/testing schedule developed for all sites.
May 30,00	Training course for all sites completed and shipping schedule begun
September 30,00	All field testing will be completed and data analysis begun
FY01	
December 31,00	Report on trial completed and distributed to EVOS and trial partners
April 15,01	Annual report due to the Trustee Council
April 30,01	Seminar on trials completed, recommendations for future actions regarding beach monitoring This seminar may be held in conjunction with a hazardous algal bloom conference held by the ASTF during this time period. Public awareness program for schools completed
May 31,01	Potential start date for pilot community beach monitoring program
December 31, 01	Completion of pilot monitoring program
March 31,02	Final report on monitoring program

C. Completion Date

All project objectives will be complete by March 31, 2002
Publications and Reports

There will be no manuscripts submitted for publication in FY00. The data from the trial will be ready for submission to TOXICON or the Journal of Association of Official Analytical Chemists by April 30, 2001.

Professional Conferences

We plan to present this data at a Harmful Algal Bloom Conference in Alaska. We also plan to present the data at one other major international conference in the year 2001.

Coordination and Integration of Restoration Effort

We will attempt to coordinate the sample collection procedures with other activities occurring in the area. We have asked Hugh Short, Spill Area Wide Community Involvement Coordinator, to assist us with this. Both the Ouzinkie and Tatitlek tribal councils have agreed to participate and we hope to work with the Kodiak Youth Area Watch to collect samples and perform the tests. Discussions have been held with the Kodiak Area Native Association (Frank Peterson) and the Kodiak Island Borough Community Development Department to collaborate on public information programs.

We will coordinate this trial with the ongoing biotoxin monitoring activities of the DEC lab to as great an extent possible to minimize the costs of the project.

Jellett Biotek has included substantial in-kind contribution to the project through the provision of our specialized antibodies for test development, extensive staff training in immuno-assay development, as well as intellectual property already developed for the immunochromatographs. In addition, there may be considerable in-kind contribution from the trial sites in obtaining the shellfish samples and conducting the test.

Proposed Principal Investigator

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Principal Investigator

Dr. Joanne F. Jellett is a professional marine microbiologist and president of Jellett Biotek Limited. She is an entrepreneur and developer of the Maritime In Vitro Shellfish Test (MIST™) cell based bioassays for paralytic shellfish poisoning.

Dr. Jellett has conducted considerable research in marine invertebrate immunology and is a well known international expert and consultant in marine biotoxins. She has numerous scientific publications to her credit.

Dr. Jellett has completed specialized training in lateral flow immunoassay development and will be the technical leader in developing the rapid tests for ASP and PSP. Projects objectives to be met by Dr. Jellett include the development, testing and manufacture of prototype rapid tests for ASP and PSP. She will also provide technical advice to the field trials and be responsible for data analysis, report production and submission of articles for peer review and subsequent journal publication.

Other Key Personnel

Mr. Raymond Roberts will be the overall project manager and will be responsible for all economic analysis and business issues related to the trial and report and recommendations.

Nancy Morse will be the trial manager and responsible for trial protocol, training materials, and trial data management.

2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

Budget Category:	Authorized FY 1999	Proposed FY 2000	LONG RANGE FUNDING REQUIREMENTS	
			Estimated FY 2001	Estimated FY 2002
Personnel		\$72.8		
Travel		\$31.7		
Contractual		\$85.0		
Commodities		\$21.5		
Equipment		\$47.6		
Subtotal	\$0.0	\$258.6		
Indirect		\$35.4		
Project Total	\$0.0	\$294.0	\$50.0	\$30.0
Full-time Equivalents (FTE)		1.7		
Other Resources	JBL		\$294.0	In-Kind \$600.0

Dollar amounts are shown in thousands of dollars.

Comments:
 The FY2000 budget reflects the development and establishment of trials of rapid tests for ASP and PSP.
 Jellet Biotek is contributing our specialized antibodies and substantial research and development expertise. Long range funding requirements represent the potential for a community marine biotoxin beach monitoring program to be implemented, April 2001 to December 2001, and have the carry over of \$30K in FY2002. Savings in labour may be possible by using students or volunteers to collect shellfish samples and perform the tests. We have substantial community support for this project.

FORM 4A
Non-Trustee
SUMMARY

Project Number:
Project Title: Development and Field Testing of Rapid Diagnostic Test Kits for Paralytic Shellfish Poisoning and Amnesic Shellfish Poisoning
Name: Raymond L. Roberts, Jellet Biotek Limited

FY00

Prepared: May 6/99

2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

Personnel Costs:		Position Description	Months Budgeted	Monthly Costs	Overtime	Proposed FY 2000	
Name							
J. Jellet		Principal Investigator	2.5	6000.0		15,000.0	
R. Roberts		Project Manager	2.0	6000.0		12,000.0	
N. Morse		Data Manager	0.3	4800.0		1,440.0	
E. Belland		R&D Manager/Training	1.0	4000.0		4,000.0	
M. Laycock		Antibody Expert	1.0	5000.0		5,000.0	
Vacant		ADEC technical Support	3.0	3000.0		9,000.0	
P. Panamarioff		Ouzinkie Trial Site	1.0	4000.0		4,000.0	
G. Kompkoff		Tatitlek Trial Site	1.0	4000.0		4,000.0	
Kodiak Youth Area Watch		Kodiak Youth Area Watch	1.0	4000.0		4,000.0	
A. MacMillan		Accounting/admin support	1.0	2400.0		2,400.0	
P. Bishop		Immuno-assay Technician	2.0	2000.0		4,000.0	
R. MacQuarrie		Immuno-assay Technician	4.0	2000.0		8,000.0	
		Subtotal	19.8	47200.0	0.0		
		Personnel Total				\$72,840.0	
Travel Costs:		Description	Ticket Price	Round Trips	Total Days	Daily Per Diem	Proposed FY 2000
		Aifare - Halifax to Anchorage - Workshop Jellet/Roberts	2480.3	2	10	200.0	6,960.6
		Aifare - Halifax to Anchorage - Training session Jellet/Robert	961.0	2	10	200.0	3,922.0
		Trial site travel costs for training to Palmer	5000.0	1	12	200.0	7,400.0
		Miscellaneous travel for sample collection					2,000.0
		Car Rental (Jellet/Roberts)					1,000.0
		Aifare to Kodiak, Prince William Sound Destinations	1000.0	2	12	200.0	4,400.0
		Aifare/per diem to visit suppliers/consultants					6,000.0
							0.0
							0.0
							0.0
							0.0
							0.0
							0.0
		Travel Total					\$31,682.6

Project Number:
 Project Title: Development and Testing Rapid Diagnostic Test Kits for Paralytic Shellfish Poisoning and Amnesic Shellfish Poisoning
 Name: Raymond L. Roberts, Jellet Biotek Limited

FORM 4B
 Personnel & Travel
 DETAIL

FY00

Prepared: May 6/99

2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET
 October 1, 1999 - September 30, 2000

Contractual Costs:		Proposed FY 2000
Description		
Mouse Bioassay / PSP control - ADEC lab 200 samples @ \$125/sample		25.0
HPLC tests for ASP control - ADEC lab 200 samples @ 100/sample		20.0
Analytical support for both ASP and PSP samples		5.0
Immunoassay consulting assistance		12.0
Antibody Development		20.0
Equipment set up and training		3.0
	Contractual Total	\$85.0
Commodities Costs:		Proposed FY 2000
Description		
Courier costs for shipping samples/test kits		5.0
Immuno-chromatographic membranes		4.0
Conjugate pads, reagents		6.0
Standards		2.0
Plastic casings for membranes		1.5
Foil Packs and dessicants		1.0
Other Miscellaneous		2.0
	Commodities Total	\$21.5

FORM 4B
 Contractual &
 Commodities
 DETAIL

Project Number:
 Project Title: Development and Field Testing Rapid Diagnostic Kits
 for Paralytic Shellfish Poisoning and Amnesic Shellfish Poisoning
 Name: Raymond L. Roberts, Jelllett Biotek Limited

FY00

Prepared: May 6/99

2000 EXXON VALDEZ TRUSTEE COUNCIL PROJECT BUDGET

October 1, 1999 - September 30, 2000

New Equipment Purchases:		Number of Units	Unit Price	Proposed FY 2000
Description				
Extraction kits for shellfish tissue	4	0.1	0.5	
Refills for extraction kits	8	20.0	0.2	
Shipping vials (for DEC lab)	200	2.0	0.4	
Prototyping equipment:			0.0	
IVEK Microstriper II	1	20.1	20.1	
Vacuum Pump	1	0.5	0.5	
Kinematic Matrix 1201 Membrane cutter	1	3.4	3.4	
Kinematic Matrix Programable shear	1	13.0	13.0	
Kinematic Matrix 2210 Universal Laminator	1	5.0	5.0	
Track Key for Matrix 2210	1	0.3	0.3	
Foil Packaging Equipment	1	1.2	1.2	
Contingency for duties, taxes and shipping		3.0	3.0	
			0.0	
Those purchases associated with replacement equipment should be indicated by placement of an R.			New Equipment Total	\$47.6
Existing Equipment Usage:		Number of Units		
Description				

Project Number:
Project Title: Development and Field Testing of Rapid Diagnostic Test Kits for Paralytic Shellfish Poisoning and Amnesic Shellfish Poisoning
Name: Raymond L. Roberts, Jelllett Biotek Limited

FORM 4B
 Equipment
 DETAIL

FY00

Prepared: May 6/99