CRC Travel Award

Training in *Vibrio* Methods and Risk Management

(1-22nd November 2010, USA)

Judith Fernandez-Piquer

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

Project No. 2010/761

ISBN: 978-1-925982-01-5

NON-TECHNICAL SUMMARY

PROJECT NO: 2010/761 Training in *Vibrio* Methods and Risk Management

PRINCIPAL INVESTIGATOR: Judith Fernandez-Piquer

ADDRESS: Private Bag 54, University of Tasmania, Sandy Bay 7001, Tasmania
Judith.Fernandez@utas.edu.au

(PROJECT) OBJECTIVES OF RESEARCH TRAVEL GRANT/ INDUSTRY BURSARY

Aims of the trip:

1) To demonstrate to an internationally audience involving researchers and policy makers that *Vibrio parahaemolyticus* (Vp) grows differently depending on the oyster species, including showing that SRO does not support Vp growth at storage temperatures as high as 28°C.
2) To present the Vp model developed in Australian oysters at the international Vibrios in the Environment (VE2010) conference and at the FDA Gulf Coast Seafood Laboratory (GCSL)
3) To receive training on the use of a risk management tool to predict Vp densities at harvest sites using satellite remote sensors at the Gulf Coast Research Laboratory (GCRL)
4) To obtain feedback from international experts and knowledge of other international studies for improvement of the discussion of the CRC Vibrio project (2007/719)
5) To get training on methodologies for Vibrio detection and enumeration at the GCRL and at the GCSL
6) To learn about the management strategies used for the BP oil spill in Gulf of Mexico.
7) To receive an update about the USA oyster industry

NON TECHNICAL SUMMARY:

During the month of November 2010 a series of training activities for improved knowledge of the Vibrio monitoring and risk management in oysters were conducted in Ocean Springs-Biloxi, Mississippi and Dauphin Island, Alabama, USA.

Communicating to an international group of researchers, industry and legislators that Sydney Rock oysters (SRO) do not support Vp growth at storage temperatures as high as 28ºC resulted in high impact. WHO/FAO is considering this information for the review of the quantitative risk assessment on Vp in raw oysters. Shellfish growers and researchers from USA are willing to collaborate on projects to test the fate of Vp in SRO when grown in USA waters.

Industry and legislators showed interest in the Vp model developed for Australian Pacific oysters, and requested further details about the Vp tool. Industry sees the software as a high value educational tool and would like to access to the online version once available to the public. Legislators need the scientific work involved in the development of the models to be published.

Steps for the implementation of the Vibrio remote sensing technique in Australia have been learned. This tool provides useful information for real-time Vp monitoring at oyster harvest sites although it will require time for development and optimisation. Research in the USA started in 2003 and they still collecting Vibrio data regularly in seawater, oysters and sediments in different

geographic locations, looking at factors that influence the presence of the bacteria and improving image resolution and mathematical equations.

Suggestions, comments and feedback from Vibrio experts regarding the results of the CRC project (2007/719) will improve the quality of Results and Discussion sections of Judith Fernandez-Piquer's thesis. Dr Angelo DePaola has been identified as a possible referee for reviewing the thesis.

Molecular techniques for the enumeration of three Vibrio species related to seafood illnesses in shellfish (Vp, *V. cholerae* (Vc) and *V. vulnificus* (Vv)) were learned. All are DNA-based tests, and are cost effective when many samples need to be analyzed. Therefore they would be useful to implement in accredited laboratories dedicated to Vibrio monitoring.

Due to confidentiality reasons, the management plan used for the BP oil spill in the Gulf Coast is not completely known to the public. However, actions taken during the management, considerations for opening shellfish growing areas and part of the research performed in different laboratories as a result of the spill were learned.

The oyster industry in southern USA is mainly wild; therefore only one experimental oyster farm and an experimental hatchery were visited. At the moment, most of their oyster research aims to test different production technologies to observe which is the most adequate for their environment. Some oyster farms are expected to open next year. Researchers involved in these oyster projects are seeking collaborations with universities in Australia.

In conclusion, this trip has been a great opportunity for learning about new Vibrio methodologies and techniques that are not available in Australia at the moment but which could be implemented to improve the management of Vibrio in oysters. Extra information about oil spill management strategies and an update about the oyster industry in USA has added value to the research trip. Communicating Seafood CRC Vibrio research with the Australian oyster industry to an international audience has provided a better understanding about need for different risk management polices depending on country and also the oyster specie. The overall feedback about the presented Vp research is positive and new projects involving SRO are proposed.

OUTCOMES ACHIEVED TO DATE

- Knowledge about a USA Vibrio remote sensing tool that can be used to enhance Vp risk management in the Australian shellfish industry
- International recognition about differences in Vp growth in Australian oysters. These data have enhanced appreciation for how oyster species and geographical location need to be factored into international risk assessment.
- Information has been gained about strategies used to manage harvest of oysters affected by the Gulf of Mexico oil spill.
- Protocols and skills have been acquired for enumerating different Vibrio species using rapid real-time PCR.

(PROJECT) OUTPUTS DEVELOPED AS RESULT OF TRAVEL GRANT/ INDUSTRY BURSARY:

1) To demonstrate to an internationally audience involving researchers and policy makers that *Vibrio parahaemolyticus* (Vp) grows differently depending on the oyster species, including showing that SRO does not support Vp growth at storage temperatures as high as 28°C.

The Vp model for Australian Pacific oysters was presented last year at the International Conference in Molluscan Shellfish Safety ICMSS2009 in France (Fernandez et al., 2009). Its performance was validated during two summer samples of oysters containing natural population of Vp in NSW Pacific and Sydney Rock oysters. Results show that the model is fail-safe for Vp prediction and that SRO does not support Vp growth even at the highest temperature tested (28°C) (Fernandez et al., 2010).

Validation studies of the Vp model for Australian oysters was presented during visits to the FDA GCSL, Alabama on 18th November 2010 and during the VE2010 conference from 7 to 12th November. Differences in Vp growth in SRO versus PO were discussed. Members of the FDA and USA shellfish growers were interested in learning more about SRO. Dr Angelo DePaola of the FDA proposed a possible project in their lab to observe if SRO shows the same behaviour with Vibrio species in the USA. Bill Dewey, a shellfish grower in Shelton WA who works as well on public policy and communications, would be interested in growing SRO in his oyster farm as a trial to observe if there is a difference in Vp growth among SRO and American oysters (AO).

Dr Iddya Karunasagar, senior fishery officer at the WHO/FAO, presented the quantitative risk assessment on Vp in raw oysters (QRVP) on 10th November 2010 at the VE2010. He explained that risk assessments are the basis for determining appropriate levels of human protection and those measures need to be standardised by CODEX for international acceptance. Among the different considerations to be taken into account for the review of the QRVP, is the lack of growth observed for Vp in SRO. This is a result of the data which the CRC submitted to WHO/FAO and a WHO/FAO meeting that Dr Tom Ross attended where the Australian SRO data were discussed.

The USA is proposing several changes in oyster storage conditions and time-to- refrigeration for Gulf oysters, specifically reducing the latter to <10h as part of the Vibrio control plan. At the moment, Australia has different storage conditions requirements (≤10C after 24h). It is important that WHO/FAO is aware of differences in Vp growth observed in two species of Australian oysters as a first step in recognising that risk management practices need to consider different oyster species.

2) Presenting the Vp model developed in Australian oysters at the international Vibrios in the Environment (VE2010) conference and at the FDA Gulf Coast Seafood Laboratory (GCSL)

The Australian Vp model predicts different Vp growth rates than the model currently proposed by the FDA and WHO/FAO risk assessments. Acceptance of the Australian Vp model would imply that policies need to take into account species-geographical differences.

A poster showing validation of the Vp model in Australian oysters was presented at the VE2010 conference (Appendix 1) for international recognition of the need to apply Vp polices depending on geographical location. Dr Yukiko Hara-Kudo, chief researcher of the division of microbiology at the National Institute of Health Sciences in Japan expressed interest in more information about

the model so that it can be considered for Vp risk assessment in raw oysters. This could be important for Australian exporters that need to reach Japan's markets.

The Vp predictive tool was introduced to USA shellfish growers which found it to be a useful educational tool. They would seek more information about it and its future availability online. Researchers in the field also expressed interested in knowing more details about the Vp tool.

3) Training on the use of a risk management tool to predict Vp densities at harvest sites using satellite remote sensors at the Gulf Coast Research Laboratory (GCRL)

The first week of training was at the Gulf Coast Research Lab (GCRL) (Appendix 2). This included meeting Dr Jay Grimes, one of the principal investigators on the research project "The use of remote sensing and molecular detection to predict the risk of infection by *Vibrio parahaemolyticus".* The project is funded by the Oceans and Human Health Initiative of the National Oceanic and Atmospheric Administration, which started in 2003.

The goal of the project is to provide maps that show estimates of Vp densities in different geographical harvest locations. Satellite temperature data are acquired from NASA's MODerate resolution Imaging Spectroradiometer (MODIS) satellite sensor. They then use the data collected at the John C. Stennis Space Center, Mississippi to generate sea surface temperature maps (Figure 1). These maps illustrate water temperatures by using a range of colours. The next step is to apply a FDA formula which predicts levels of Vp depending on temperature in order to generate maps that show predicted densities of Vp (Figure 2). An environmental sampling study is conducted in parallel to ground-test predictions versus observations.

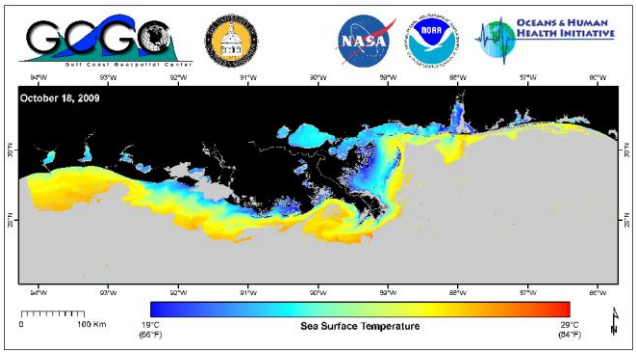

Fig 1. Temperatures image (12-Nov-2009 04:55) (Grimes et al., 2008)

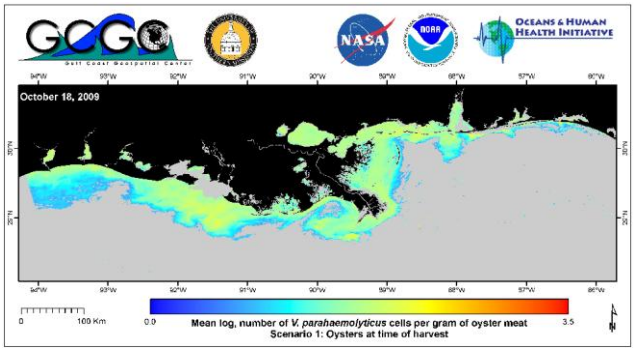

Fig 2. Mean log Vp levels (12-Nov-2009 05 :03) (Grimes et al., 2008)

In 2007, the correlation between remotely sensed sea surface temperature and *in situ* measurement was found to be good (R^2=0.86), thereby it was concluded that remotely sensed sea surface temperature data are a sufficiently accurate substitute for direct measurement (Phillips et al., 2007). In the same publication, it was suggested that environmental factors other than

temperature may further explain Vp density variability and can be used for revision of the current FDA risk assessment model based solely on water temperature. In fact 3 years later, in 2010, the relationship between environmental factors (sea surface temperature, chlorophyll a and turbidity) and pathogenic Vibrios in the northern Gulf of Mexico was published (Johnson et al., 2010) and a positive correlation was found between turbidity and Vp levels in the overlying waters and oysters. They concluded that incorporation of the data on the relationship between turbidity and total or pathogenic Vp into risk assessment models would improve accuracy. Thereby, the ability to measure turbidity, temperature, and other relevant environmental factors on a global scale using remote sensing technology would be beneficial. At present, one of Dr Grimes'PhD students is examining the relationship between Vp and plankton levels. Research related to parameters that influence Vp growth are also being performed in other states (Louisiana and California) to overcome any possible geographical variances. Most of the studies involve data gathering (water temperature, salinity) during oyster collection and Vibrio testing in seawater, oyster and sediment.

The maps, when available, will provide an important tool for real-time Vp monitoring in order to minimise public health risk. In this respect, they can be use to aid in making informed and timely decisions for intervention when the predicted risk may be high. It also can be used as a marketing tool to educate consumers, maintain consumer confidence and further enhance the already well-established reputation of the seafood industry.

Several disadvantages in this technique have been identified. One is legal issues that include rights of images. Another disadvantage is the cost of work involved in validation and maintenance of the software. The resolution that they are able to offer at the moment is $1km^2$. That magnitude is not very accurate when information is needed for smaller areas. An additional consideration is if images can be obtained in real time. Technical issues also include image problems in the presence of clouds or surface reflection. At the moment, Vp prediction is based on the tlh gene, a species-specific marker. Thus it does not predict risk due to the presence of specific pathogenic strains which represent a small percentage of the total population (DePaola et al, 2003; Parveen et al, 2008).

The recommended steps necessary to implement remote sensing in Australia are:
- Determine the availability of services in Australia that provide satellite water temperature data.
- Validate that the temperatures predicted by the satellite are the same as *in situ* - Develop software that can convert satellite temperature profiles into Vp level (e.g. via colour) by using a predictive formula that calculates levels of Vp depending on temperatures (the FDA formula could be applied as a reference but likely needs to be modified to take into account geographical differences)
- Validate that Vp levels predicted by remote sensing are similar to levels observed in harvest/grow waters, including seasonal effects.
- Determine if other environmental factors need to be included in the formula for Vp prediction (e.g. including salinity effects)

<u>4) Feedback from international experts and knowledge of other international studies for improvement of the discussion of the CRC Vibrio project (2007/719)</u>

Presentation of the Vp model at the FDA GCSL and during the VE2010 conference produced comments and suggestions from the audience. Some points to consider for the content in the preparation of a scientific paper are:

- Validation studies involved only five challenge tests for each oyster species and there are no replications for test temperature. This means that there is the possibility of variability if there are

more trials performed in a future. In the defense of the Vibrio project, we can state that the model has been developed with more tests than other publications in which a model was developed with only three challenge tests (Yoon et al., 2008). Moreover, the validation of the Vp model has added ten additional challenge tests.
- Initial environmental levels of Vp in some SRO validation tests were relatively high (4 logs). Scientists at the FDA stated that they observe that Vp counts in American Oysters may not increase higher than 4 logs. Therefore, it could be that there was no growth in SROs because maximum Vp levels were already reached at the beginning of the storage trials. In the defense of the Vp model, it was observed that some challenge tests started with Vp levels of 2.5 logs and they did not get to reach the 4 logs.

Future publications produced from the Vp CRC project will include discussion about differences in methodologies used in the USA and those used in CRC/UTAS studies, as well as the importance of predictive models for post-harvest management in Australia and the USA.

5) Training on methodologies for Vibrio detection and enumeration at the GCRL and at the GCSL

The visit to the GCRL and FDA GCSL resulted in new information about Vibrio methodologies (Appendices 2 and 3). This included hands-on practiced with colony hybridization and real-time PCR (Appendix 4).

Colony hybridization was performed to enumerate Vp and Vv in oysters, seawater and sediment. A DVD that includes all the steps during this assay is available. Real-time PCR from MPN tubes for enumeration of *V. cholerae* (*ctx* gene), Vp (*tlh*, *trh*, *tdh* genes), *V. vulnificus* (*vvha* gene), or a multiplex approach that detected several species at the same time: Vc+Vv+Vp (commercial DuPont kit) were also performed during the training. Protocols for each assay are available (Appendix 2).

The use of different methodologies was discussed during training as well as at the roundtable during the VE2010 conference. It was suggested that different methods are needed depending on the oyster bacterial load as well as the geographical location. The specificity of the primers to use in PCR assay was also discussed. Some researchers in Chile (Dr Romilio Espejo) and also in France (Dr Dominique Hervio-Heath) have found positive amplification of *tlh* in other *Vibrio* spp. that are not *V. parahaemolyticus*. This observation depends on the primer sequence and also on the PCR conditions, which may need to be optimized. The specificity for the *tlh* primers that we used at UTAS for Vp model development has been previously studied showing no amplification of other Vibrio spp. (Nordstrom et al., 2007).

The FDA labs are opting for the use of PCR because this method is sensitive and robust. Other methods recommended in the BAM can be still be used as some labs and countries do not have adequate technology and funds to implement a molecular-based approach. A significant disadvantage of DNA tests is that isolates can not be readily obtained because bacteria are not cultured.

During the VE2010 conference, Muhammad Islam (PhD student) gave a talk about the use of a new technique for genotypic studies of *V.cholerae* strains present in the aquatic environment of Sydney, Australia. He would be a useful contact for information regarding this specie.

6) Management strategies used for the BP oil spill in Gulf of Mexico

Oyster management processes used in the USA following the 20th April 2010 BP Gulf of Mexico oil spill were learned during this trip. Similar strategies might be used for management of oil spills along the Australian coast.

The priority in the management plan was aimed at maintaining a clean shoreline. The precaution measures taken were closure of fishing activities and monitoring of fish. The re-opening protocol was based on no appearance of oil and seafood testing (chemical and sensory analysis). The surveillance included air, water, dockside and seafood processing facilities. A map showing the opened and closed areas was developed. At the moment, a small part of the Gulf of Mexico remains closed. Evaluation of long-term effects on fish/shellfish is not known and may take 2-3 growing seasons. It was highlighted that seafood is tested now more than ever in the USA. However, it will be necessary to work on increasing consumer trust. For this, marketing surveys are being performed and a marketing program is going to be running for 1-2 years.

The FDA labs have been working in some projects with BP. The experiments are mostly done to observe the accumulation of oil in fish and shellfish tissues after artificial contamination. Sensory and chemical analyses are usually performed. The FDA has a website with diverse information (reopening of closed waters, monitoring and surveillance, etc.) about the oil spill available to the public (US FDA 2010).

The GCRL has also been involved in projects with BP. They have been profiling microbial communities to look at the effect of oil by sampling water and sediments in impacted and non-impacted areas. They have been also performing microscopic studies to observe accumulation of bacteria in oil droplets. Studies of the occurrence of dioxigenases (required for aromatic ring diffusion to break the structure of the petroleum) in different *Vibrio* spp. have also been done.

Other knowledge in oil spills discussed during the VE2010 conference included the use of dispersants and fertilizers to increase the rates of biodegradation, and the increase in hydrocarbon-degradating bacteria in seawaters. It has been mentioned that more than 75 different bacteria are able to degrade hydrocarbons.

7) Update on the USA oyster industry

During the VE2010 conference, the CRC Vp model software was presented to Bill Dewey (a shellfish grower in Shelton WA who works as well on public policy and communications). He is very interested in further information about the model as well as when it will be available on-line. He is also interested in the possibility of growing SRO. Bill Dewey has donated a DVD titled "farming the tidelands" that shows the oyster farming in Western USA.

All oyster production in the south of the USA is wild-caught. An experimental oyster farm in Bayou le Batre, Louisiana was visited. They are testing different production methods towards future oyster farming in the area. Some of the gear used is imported from Australia.

An experimental hatchery was also visited on Dauphin Island, Alabama. At the moment, the hatchery is only used for research proposes. They conduct projects for oyster breeding and reef restoration.
The experimental oyster farm and the hatchery are run by researchers from Auburn University (Appendix 5). The main responsible is Bill Walton and the operators are Scott Rikard and Glen Chaplin. They would be interested in collaborations between their university and universities in Australia.

ABOUT THE PROJECT/ACTIVITY

BACKGROUND AND NEED

Vibrio spp. are naturally-occurring in seawater and accumulate in oysters due to filter feeding activity. Some Vibrio spp. (mostly *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae*) can cause human disease if accumulated in oysters that are eaten raw or undercooked. Improvement in shellfish monitoring is necessary to decrease outbreaks and not loose consumer trust gained by the Australian oyster industry. Predictive tools can be used to enumerate Vibrio in oysters and will be helpful to monitor and design cold supply chains, as well as address potential regulatory issues such as for importing countries. This CRC-sponsored travel was necessary to gain more knowledge about other Vibrio management tools, including new remote sensing predictive tools and molecular techniques used by accredited laboratories in the USA. This trip also provided an opportunity to defend part of the research findings in the Vibrio project in front of an international audience of experts.

RESULTS

As a result of the travel, steps needed to implement Vibrio remote sensing in Australia and the protocols-skills for molecular Vibrio analyses have been learned. CRC Vibrio research was presented and it will assist risk assessors in considering differences among oyster species and geographical locations. Knowledge on oil spill strategies used in USA was gained and can support environmental management in Australia. Future collaborative oyster projects may include studies of Vp accumulation in SRO grown in USA waters and evaluation of Vibrio remote sensing techniques.

INDUSTRY IMPACT

ANTICIPATED PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

Different potential outcomes can be identified from this project; most of them are classified as not immediate changes for industry.

Collaborations for SRO studies between USA shellfish growers and Australian researchers have been created. There is a potential for involving regulatory agencies e.g. US FDA and NSW Food Safety Authority. There is also a possible link for sharing experiences between oyster farming procedures and possible market for oyster farming materials.

The Vibrio remote sensing training has provided the industry with another proactive method for evaluating Vp levels at harvest sites. Industry can use this tool for monitoring initial Vp levels and also for selecting oyster harvest sites where there are lower levels of Vp without need of sampling and on real-time using satellites.

Oil spill management protocols used in US Gulf of Mexico can be considered in Australia by regulatory agencies. Some of the benefits would be a decrease in the uncertainty of regulators facing a similar incident and it also would help oyster industry as a good oil spill management would involve less impact on oyster production.

FDA has been working on developing a “universal” Vp method to be used in all countries. It has proposed to use real-time PCR because it is a sensitive and robust assay. During this training,

protocols and skills to perform the technique have been learned. Companies that want market access to the USA will have an available method recognized by their regulatory agencies for *Vibrio* spp. testing.

SUMMARY OF CHANGE IN INDUSTRY
(What immediate changes might be expected for business/industry?)

There are not many immediate changes for business/industry. Networking opportunities between oyster growers in Washington (Bill Dewey) and primary industries science and research in NSW (Wayne O'connor) for SRO studies have been created. Possible collaborations between universities, e.g. Auburn University and Australian Maritime College at University of Tasmania, have been proposed. A protocol for *V. vulnificus* detection using real time PCR has been sent to Tom Madigan at the South Australian Research and Development Institute (SARDI) as a possible methodology to use in some of their current projects. The remaining Vibrio methodologies will be sent shortly to Dr Cath McLeod who is the senior research scientist working on shellfish food safety at SARDI. The knowledge regarding oil spill management will be sent shortly to Anthony Zammit who is the Shellfish Safety scientist for the NSW Food Authority.

WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?
(What will be the impact?)

An adoption of the Vibrio remote sensing technique in Australia will provide an important tool for real-time Vp monitoring in order to minimise public health risk. In this respect, it can be used to aid in making decisions for intervention when the risk may be high. It also can be used as a marketing tool to educate consumers, maintain consumer confidence and further enhance the already well-established reputation of the seafood industry,

Taking into account the oil spill management protocols used in USA Gulf of Mexico by Australian regulatory agencies may decrease the impact that oil spills have on oyster farming areas and thereby reduce oyster production rejections.

Implementation of the Vibrio testing protocols that are used in USA in Australian laboratories will allow to any oyster grower or regulatory agency to perform this test. Oysters tested using recognized methodologies will be easier to approve for export to USA.

WHAT BARRIERS ARE THERE FOR CHANGES TO OCCUR?

The consideration of the Vp model developed in Australia by international policy makers will depend on the acceptance of the Vp scientific publication.

A project needs to be developed for validation of the Vibrio remote sensing technique in Australia. For this to happen, a commercial company who can supply satellite information needs to be contacted for pricing. The success on this will be if the funds necessary for the project are evaluated positively by the oyster industry.

Implementation of Vibrio testing using USA protocols will depend on the infrastructure available in Australia. Shellfish research laboratories (e.g. SARDI) need to be approached for their acceptance.

IF NOT ALREADY HAPPENING, WHEN WILL THE CHANGES OCCUR?
(e.g. 2 businesses will adopt project findings and two more are expected to adopt findings within 12 months)

The author of the report can not give a detailed schedule for the changes. It is suggested that at least one company in Australia will be interested in the Vibrio remote sensing technique; at least one laboratory will adopt the Vibrio methodologies and all states will possibly consider the recommendations regarding oil spill management within the next 12 months.

WHAT IS THE LIKELIHOOD THAT THESE CHANGES WILL OCCUR?
(e.g. 50% chance that four businesses will adopt project findings)?

The author of the report can not give a detailed likelihood of the changes. It is suggested that there is a high probability that WHO/FAO will accept the data regarding the Vp model, there is a medium level of likelihood for the adoption of the Vibrio remote sensing and the Vibrio methodologies in Australia. The possibility of the acceptance of the oil spill management used in USA is difficult to estimate as it depends on regulatory policies in different State.

WHAT BARRIERS ARE THERE TO ADOPTION OF THESE CHANGES AND WHAT ACTION COULD BE TAKEN TO OVERCOME THESE?

(e.g. to adopt project findings will require group training/sharing equipment/invest additional capital etc.)

The consideration of the Vp model by legislators will depend on the acceptance of the Vp work for publication in a scientific journal.

The use of the Vibrio remote sensing technique is Australia will require project investment, and development and integration of the model into a software application.

The adoption of the Vibrio testing protocols used in the USA will require the testing laboratory to invest in the necessary equipment for performing the assays.

COMMUNICATION OF PROJECT/EXTENSION ACTIVITIES

WHAT IS THE INFORMATION THAT NEEDS TO BE COMMUNICATED?

1) New Vp management tool for oyster growers: recommendations for Vibrio remote sensing technology in Australia.

The aim of the Vibrio remote sensing technology is to provide maps that show estimates of Vp densities in different geographical locations by using satellite data. The maps when available will provide an important tool for real-time Vp monitoring at harvest sites in order to mantain public health. In this respect, it can be used as an aid in making informed and timely decisions for opening-closing harvest areas. The steps necessaries for the implementation of this technique in Australia have been identified following the plan used in US as an example. It is proposed to

create a new project to work on this in order to provide a tool for real-time Vp monitoring in oyster harvest sites without the need for sampling.

2) Methodologies for Vibrio monitoring in shellfish

Vibrio methodologies were learnt at the GCRL and FDA GCSL. Techniques performed included molecular assays: colony hybridization and real-time PCR. Colony hybridization was performed for enumeration of Vp and Vv from oysters, seawater and sediment. A DVD that includes all the steps performed during this assay is available. Real-time PCR from MPN tubes for enumeration of Vc (*ctx* gene), Vp (*tl, trh, tdh* genes), Vv (*vvha* gene) or multiplex species at the same time: Vc+Vv+Vp (commercial DuPont kit) were performed. Protocols for each assay are available to anyone interested. It is proposed their implementation in Australian laboratories as it can help to access US markets.

3) Oil spill management: strategies used in USA to manage the effects of the Gulf oil spill.

Management processes used in the BP oil spill occurred on the 20th April in USA were learned during this trip. They can be considered for regulatory entities in Australia. The priority in the management plan aimed at maintaining a clean shoreline. The precaution measures taken were closure for fishing activities and monitoring of the caught fish. The re-opening protocol was based on no appearance of oil and seafood testing (chemical and sensory analysis). Further details can be obtained from http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/Seafood/ucm210970.htm. The actual situation is that most of the oyster harvest sites are opened and the seafood tested for market is considered safe. However, it has been necessary to work on increasing the consumer trust. Oil spill management protocols used in US Gulf of Mexico can be considered in Australia by regulatory agencies. Some of the benefits would be a decrease in the uncertainty of regulators facing a similar incident and it also would help oyster industry reducing the impact of oil on oyster production.

4) Information about the oyster farming in USA

The oyster industry in the USA southern states is predominantly wild while some oyster farms are located in the west coast. A DVD showing the west coast shellfish industry entitled "farming in the tidelands" has been kindly donated by one of the shellfish growers in Washington and it is available for anyone interested by contacting the SeafoodCRC. An experimental oyster farm and a hatchery located in the south were visited. At the moment, they are only used for research proposes. There is the possibility that three new oyster farms will be under development next year. Auburn University in the USA is seeking collaborations with Australian universities.

5) SRO does not support Vp growth at storage temperatures as high as 28°C.

The senior fishery officer at the WHO/FAO, presented the quantitative risk assessment on Vp in raw oysters (QRVP) on 10th November 2010 at the VE2010. He explained that risk assessments are the basis for determining appropriate levels of human protection and those measures need to be standardised by CODEX for international acceptance. Among the different considerations to be taken into account for the review of the QRVP, is the lack of growth observed for Vp in SRO. This is a result of the data which the CRC submitted to WHO/FAO and a WHO/FAO meeting that Dr Tom Ross attended where the Australian SRO data were discussed. Members of the FDA have proposed a possible project in their lab to observe if SRO shows the same behaviour with Vibrio species in the USA. Shellfish growers in Shelton WA would be interested in growing SRO on their oyster farms as a trial to observe if there is a difference in Vp growth among SRO and AO.

WHO IS/ARE THE TARGET AUDIENCE/S?

Oyster industry
Shellfish regulators
Researchers working on shellfish projects (including Universities and Research centres)

WHAT ARE THE KEY MESSAGES?

1) The FAO has recognized differences in Vp growth among oyster species.
2) Vibrio remote sensing is a useful management tool that could be implemented in Australia for knowledge of the Vp levels at harvest.
3) No observed Vp growth in SRO has produce high interest among the oyster industry, regulators and researchers. In addition, persons in the USA are interested in testing for the effect by growing SRO in USA waters.
4) New molecular methodologies are used in the USA for Vibrio enumeration in shellfish. They have been shown to be sensitive and robust for use among different laboratories and could be used for Vibrio monitoring in Australia.
5) The oil spill management used in USA has been successful because most of the affected areas are now open for harvesting oysters. Some of the strategies used in USA could be considered for management in Australia.

WHAT IS THE CALL TO ACTION?

(What is it you want people to do once you communicate the key message to them –i.e. what change of behaviour or action do you want them to take?)

1) Develop a new project to produce a Vibrio remote sensing tool for Australia. For this, it is necessary to partner with a company that can integrate the Vp model into their service.
2) For companies that wish to export to international markets, regulators will recognize US FDA Vibrio testing protocols.
3) Regulators in Australia will consider oil spill management practices that have been successful in the USA.
4) SRO growers will develop successful collaborations with growers in the USA.

COMMUNICATION CHANNELS

(How can these messages be communicated and by who?):

Channel	*Who by*	*When*
Seafood CRC magazine	***Emily-Judith***	***2011***
Email	***Judith***	***2011***
Workshops/meetings	***Researchers/ Regulatory agencies***	***2011***

LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS

WHAT IS YOUR FEEDBACK?

(e.g. *What difficulties were experienced in undertaking this research and how did this affect the project, what improvements and/or considerations can be recommended for future projects in this area* and *what barriers are there to undertaking further research in this area and how could these be overcome?)*

An important consideration is the confidentiality issue. For this reason, more detailed information regarding Vibrio remote sensing, oil spill management and molecular methodologies were not immediately available.

I would recommend encouraging this training for future projects as it gives the benefit of gaining knowledge of new methodologies from overseas that can then be more easily implemented in Australia.

FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION?

(e.g. IP protection, licensing, sales, revenues etc)

None applicable.

ACKNOWLEDGEMENTS

I would like to acknowledge my main PhD supervisor Mark Tamplin for his invaluable guidance and help with the organization of this activity. His critical review of this report is much appreciated. Big thanks to Angelo DePaola from the FDA, Rachel King from the oyster consortium, Anthony Zammit from the NSW food authority, Tony Troup from the oyster industry in NSW and Peter Kosmeyer from the oyster industry in Tasmania for their support. This activity would not have been possible without the financial support from the SeafoodCRC and the TIAR-UTAS. I am very grateful to all the people in USA who have collaborated directly and indirectly in this training: Jessica Jones, Jay Grimes, Nick Noriea, Marcia Pendelton, Adrianne Flowers, Kimberly Griffitt, John Bowers, Bill Walton and Charles Kaysner. Finally, I would like to express special thank to my family for their encouragement.

REFERENCES

DePaola, A, Nordstrom, JL, Bowers, JC, Wells, JG & Cook, DW 2003, 'Seasonal abundance of total and pathogenic *Vibrio parahaemolyticus* in Alabama oysters', Applied and Environmental Microbiology, vol. 69, no. 3, pp. 1521-1526.

Fernandez-Piquer, J, Ross, T, Bowman, JP & Tamplin, ML 2009, 'Viability of *Vibrio parahaemolyticus* in Australian Pacific Oysters ', Oral presentation at the 7th International Conference Molluscan Shellfish Society , 14-19 June 2009, Nantes, France [Conference Extract].

Fernandez-Piquer, J, Ross, T, Bowman, JP & Tamplin, ML 2009, 'Validation of a *Vibrio parahaemolyticus* predictive model in two different species of Australian oysters', Poster presentation at the Vibrios in the Environment International Conference, 7-12 November 2010, Biloxi, USA [Conference Extract]

Grimes et al., Vibrio remote sensing report, 2008, <http://www.eol.ucar.edu/projects/ohhi/vibrio/archive/>, (4/11/2010)

Johnson, C, Flowers, A, Noriea III, N, Zimmerman, AM, Bowers, JC, DePaola, A & Grimes, DJ 2010, 'Relationships between environmental factors and pathogenic vibrios in the northern Gulf of Mexico', In press. Applied and Environmental Microbiology.

Nordstrom, JL, Vickery, MCL, Blackstone, GM, Murray, SL & DePaola, A 2007, 'Development of a multiplex real-time PCR assay with an internal amplification control for the detection of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters', Applied and Environmental Microbiology, vol. 73, no. 18, pp. 5840-5847.

Parveen, S, Hettiarachchi, K, Bowers, J, Jones, J, Tamplin, ML, McKay, R, Beatty, W, Brohawn, K, DaSilva, L & DePaola, A 2008, 'Seasonal distribution of total and pathogenic *Vibrio parahaemolyticus* in Chesapeake Bay oysters and waters', International Journal of Food Microbiology, vol. 128, pp. 354-361.

Phillips, AMB, DePaola, A, Bowers, J, Ladner, S & Grimes, DJ 2007, 'An evaluation of the use of remotely sensed parameters for prediction of incidence and risk associated with *Vibrio parahaemolyticus* in Gulf Coast Oysters (Crassostrea virginica)', Journal of Food Protection, vol. 70, no. 4, pp. 879-884.

US FDA, Gulf of Mexico Oil Spill Update, 2010, <http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/Seafood/ucm210970.htm>, (02/12/2010)

Yoon, K, Min, K, Jung, Y, Kwon, K, Lee, J & Oh, S 2008, 'A model of the effect of temperature on the growth of pathogenic and nonpathogenic *Vibrio parahaemolyticus* isolated from oysters in Korea', Food Microbiology, vol. 25, pp. 635-641.