Seafood CRC Combined Visiting Expert and Research Travel Grant Application: Visit by Dr. Standish K. Allen Jr. from the Virginia Institute of Marine Science to Australia and fluorescent in situ hybridisation training by CRC PhD student Penny Miller prior to Dr Allen's visit

Penny Miller

AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

Project No. 2012/727

This project was conducted by Penny Miller, PhD student of the University of Tasmania in conjunction with Shellfish Culture Limited and the CSIRO

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NON-TECHNICAL SUMMARY

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PRINCIPAL INVESTIGATOR: Penny Miller

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(PROJECT) OBJECTIVES OF RESEARCH TRAVEL GRANT/ INDUSTRY BURSARY:

- Develop Reliable techniques for Fluorescent in situ hybridisation (FISH) of Pacific Oysters.
- Scientific write up of techniques for diploid, triploid and tetraploid Pacific Oysters using FISH.
- Capture high quality images that can later be published in peer reviewed journals, equipment not available in Tasmania

NON TECHNICAL SUMMARY:

Fluorescent in situ hybridisation (FISH) a genetic technique that involves fluorescently labelling chromosomes so that each can be identified individually under a high powered microscope. This technique has not been applied to Pacific Oysters in Australia and, to date, has not been used on any polyploid oysters. Despite being highly accurate and reliable, FISH analysis is not a commonly used technique. Penny travelled to Canberra to work with Tariq Ezaz of the University of Canberra on troubleshooting her FISH protocol to work on Pacific oysters. Eventually, the protocol worked, but not consistently or at a strong enough level for chromosomes to be individually identified. It was determined that, due to their small size and weak signals, fluorescently labelled microsatellites are not a reliable method for karyotyping oysters, particularly polyploids where chromosomes tend to overlap. A different probe (PNA) was also trialled. Again this was inconsistent, but the signals were stronger than the microsatellites. This probe is worth mapping and further investigation, however, time, money and sampling constraints prevented any additional study.

Given the limited success of the FISH work, Standish's visit was used to improve analysis and write up of two other studies: "Considerations for maintaining tetraploid Pacific Oysters (*Crassostrea gigas*) as broodstock for selectively bred triploids: a case study of diversity" and "Parental assignment in triploid Pacific oysters (*Crassostrea gigas*): development of a new R

script, POLYPARENT." These studies are currently undergoing final supervisory comments, and should be submitted for publishing this month.

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

- Using existing techniques FISH of Pacific oysters using microsatellites is difficult, inefficient and non-informative.
- Determination of the potential for the PNA probe to mapped in Pacific oysters
- Improvement of two scientific manuscripts to be submitted for publishing

ABOUT THE PROJECT/ACTIVITY

BACKGROUND AND NEED

FISH could be an important tool for detecting the aneuploid frequency in tetraploid oyster populations. This is important because a decrease in tetraploid genetic stability could potentially reduce the efficiency of breeding programs and may have carry over impacts on the triploid commercial product.

RESULTS

It was determined that using existing techniques microsatellite markers for FISH analysis in polyploid oysters is difficult, inconsistent and uninformative. Instead, future studies should focus on different markers such as the PNA probe or BACs.

INDUSTRY IMPACT

PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)

Due to the difficulty and inconsistency of using microsatellite makers for FISH analysis in Pacific oysters, the predicted industry impact was not achieved.

SUMMARY OF CHANGE IN INDUSTRY

(What immediate changes might be expected for business/industry?)

None so far.

WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?

Future research is needed into different fluorescent probes that can individually identify chromosomes. Additionally, more research is needed to obtain better metaphase spreads. Once the aneuploid frequency can be obtained using FISH, a better understanding of the stability of tetraploid oysters can be gained. This will effect breeding management decisions such as what age class is best to use as broodstock and how often new tetraploids should be spawned or induced.

WHAT BARRIERS ARE THERE FOR CHANGES TO OCCUR?

The major barrier for this to occur is obtaining probes that consistently and reliably identify individual chromosomes. As seen in this study, this can be a very difficult and time consuming process. Much more research is needed.

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)

I do not know of any other researcher currently investigating the cytogenetics of polyploid oysters. However, aneuploid frequency is an important question for industry and it is likely, now that the genome for Pacific oysters is mapped, that a protocol for using BACs in Pacific oysters to analyse aneuploidy will be developed in the near future.

WHAT IS THE LIKELIHOOD THAT THESE CHANGES WILL OCCUR?

(e.g. 50% chance that four businesses will adopt project findings)?

It is too soon to judge, as results of the rate of chromosome loss need to be determined before changes to industry can occur.

WHAT BARRIERS ARE THERE TO DOPTION 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.)

Again this will depend of the frequency of aneuploidy within a tetraploid population.

COMMUNICATION OF PROJECT/EXTENSION ACTIVITIES

WHAT IS THE OUTPUT THAT NEEDS TO BE COMMUNICATED?

That FISH using microsatellite markers is not practical. Other probes (e.g. PNA or BACs), however, could be more successful.

WHO IS/ARE THE TARGET AUDIENCE/S?

Researchers that want to determine the aneuploid frequency in Pacific oysters

WHAT ARE THE KEY MESSAGES?

Using existing techniques, FISH using microsatellite markers to identify tetraploid Pacific oyster chromosomes is inconsistent and uninformative.

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?)

Future research into other FISH probes, such as BACs or PNA, is needed

COMMUNICATION CHANNELS

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

Channel	*Who by*	*When*
Thesis chapter	***Penny Miller***	***December***

LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS

WHAT IS YOUR FEEDBACK?

Whilst no changes to industry were brought about by this study, the research undertaken was still highly important. The use of microsatellite markers to fluorescently label tetraploid oyster chromosomes was potentially more time and cost effective than other probes. If it had worked, it would have provided a valuable insight into the genetic stability of tetraploid oysters. A paper “Bouilly, K., Chaves, R., Leitao, A., Benabdelmouna, A., Guedes-Pinto, H.,

2008. Chromosomal organization of simple sequence repeats in the Pacific oyster (*Crassostrea gigas*): (GGAT)(4), (GT)(7) and (TA)(10) chromosome patterns. J. Genet. 87, 119-125." had detailed the success of this method on a small scale. Hence the difficulties and inconsistencies encountered were very unexpected.

FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION?

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

None as yet

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