# Enhancement of tetraploid and triploid production in the Australian Pacific Oyster industry

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AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE

## Project No. 2012/728

***This project was conducted by Dr. Standish K. Allen, Jr., Director of the Aquaculture Genetics and Breeding Technology Center, Virginia Institute of Marine Science, College of William and Mary***
***&***
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## NON-TECHNICAL SUMMARY

**PROJECT NO:** Enhancement of tetraploid and triploid production in the Australian Pacific Oyster industry

**PRINCIPAL INVESTIGATOR**: Dr. Standish K. Allen, Jr.

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

- Enhance R&D of tetraploid technology now based at SCL
- Assist with research of CRC investigator Penny Miller concerning genetic diversity and aneuploidy in Pacific oyster
- Develop cutting edge tools with CSIRO to assess advances in polyploidy technology

**NON TECHNICAL SUMMARY:**

- Spawned and set 12 triploid test lines comprised of putative POMS resistant ASI brood stock – for subsequent testing in POMS affected areas
- Spawns a 2012 generation of SCL tetraploids
    - 2 – commercial lines
    - 1 – select line
- Edited two Penny Miller manuscripts on pedigree assignment in tetraploids and genetic management of tetraploids
- Initiated the development of a new line of specialty oysters for SCL, destined to be a tetraploid breeding population
- Began development of new method of producing tetraploids (McTets) that, if successful, can reduce the generation time for incorporating important traits into tetraploid lines
- Ran season long investigation into the reproductive potential of tetraploid x tetraploid matings, called “the matrix”
- Enabled cytogenetics sampling for Penny Miller from test crosses produced for the above objective
- Began the process of incorporating putative POMS resistance into tetraploid lines
- Consulted, to SCL and CRC, on aspects of the reorganization of ASI, including the incorporation of tetraploid technology into the ASI program

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

**I. Spawned and set 12 triploid test lines comprised of putative POMS resistant ASI brood stock – for subsequent testing in POMS affected areas of NSW**

The following crosses were made using putative POMS-resistant (R) and POMS susceptible (S) families derived from ASI testing. About 200,000 larvae were removed ultimately for setting.

| | | | D-larvae | Hatch rate | |
|---|---|---|---|---|---|
| Type | Family | Culture ID | M | Culture | Family |
| R | 4 | 3YC1201 | 3.2 | 32% | |
| R | 4 | 3YC1202 | 3.3 | 33% | 32.5% |
| R | 26 | 3YC1203 | 7.0 | 70% | |
| R | 26 | 3YC1204 | 5.0 | 50% | 60.0% |
| R | 27 | 3YC1205 | 5.3 | 53% | |
| R | 27 | 3YC1206 | 3.9 | 39% | 45.8% |
| S | 3 | 3YC1207 | 4.2 | 42% | |
| S | 3 | 3YC1208 | 4.0 | 40% | 40.8% |
| S | 7 | 3YC1209 | 3.6 | 36% | |
| S | 7 | 3YC1210 | 5.3 | 53% | 44.4% |
| S | 22 | 3YC1211 | 5.3 | 53% | |
| S | 22 | 3YC1212 | 6.1 | 61% | 57.2% |

Table 1: Hatch rate of triploid ASI cultures after 24 hours. Type refers to Resistant or Susceptible families, with actual family listed next. Each family (and its replicate) had a different culture name from 3YC1201 to 3YC1212. Number of D-larvae is given in millions.

**II. Spawns a 2012 generation of SCL tetraploids**

During the spawning of the above ASI lines and during the execution of the early rounds of our "matrix" experiment, mass spawns were accomplished to produce the next generation of commercial tetraploids, TN12a and TN12b representing different sets of parents. The last such generation was TN11 but those are only juveniles now and not sufficient for commercial spawns. TN12 was produced from TN08 (produced in 2008). Now with the TN12 generation, there will be two co-existing lines at SCL – even and odd year, which not only provide assurance of sufficient numbers of tetraploids for commercial culture but also provides a second genetic resource for the company.

In addition to the TN12 spawns, a later spawn was accomplished in January consisting of individuals selected specifically for commercially important traits such as shape and depth, named TNS12. We are not certain whether selection of this type will be efficient in tetraploids as selection can be slower in tetraploids in general. However, with the back-up of sufficient unselected parents in TN12 and TN11, it is worth a shot. Later, there will have to be testing of this line to gauge if selection is working.

**III. Work with Penny Miller**

PI Allen spent some time editing Penny's manuscript, "Considerations for maintaining tetraploid Pacific oysters (*Crassostrea gigas*) as broodstock for triploids: a case study of diversity and pedigree assignment," which saw two rounds of editing. Also comments were provided to her for an early draft of "Development of a new R script, POLYPARENT, to

determine parental assignments in polyploids and its application in triploid Pacific Oysters (*Crassostrea gigas*)."

During the matrix experiments, Penny came to assist and to set up sampling for cytogenetic analysis of early embryos from the various crosses. She plans to use this material for at least part of her CRC supported PhD research, "Genetic diversity, pedigree assignment and aneuploidy in cultured Pacific Oyster (*Crassostrea gigas*) diploid, triploid and tetraploid population." Chromosome spreads will be made from the two – eight cell embryos and aspects of aneuploidy examined.

**IV. Initiated the development of a new line of specialty oysters for SCL, destined to be a tetraploid breeding population**

The creation of tetraploid is a multigenerational process, starting with inducing triploidy in the first generation and then tetraploidy in the second. At minimum, this takes 3-4 years. Anticipating the market for future triploids, then, is an important planning step in the tetraploid breeding process.

Four distinct triploid populations were produced during the PI's tenure. One is a color variant that has potential for market expansion, perhaps overseas. Three others are lines that were created in anticipation of the eventual need for disease resistant lines. All four of these "chemical" triploid spawns (named 3C1202, -04, -05, and -06) will be brought through the setting and juvenile stages at SCL and then planted to the field. If all goes well, they will become tetraploid populations in 2015.

**V. Began development of new method of producing tetraploids (McTets) that, if successful, can reduce the generation time for incorporating important traits into tetraploid lines**

The French research group IFREMER published a paper a number of years ago called "A complimentary method for production of tetraploid *Crassostrea gigas* using crosses between diploids and tetraploids with cytochalasin B treatments." It was reported to be a complimentary method to the patented method that 4Cs Breeding Technologies, Inc. has been marketing (called 4C tetraploids). In principal, it would cut the generation time for producing new lines of tetraploids, in half. Its utility in Australia would be to introgress desirable traits, particularly disease resistance, into existing tetraploid lines. Because this is potentially a faster breeding method, and because the author of the "Complimentary" paper is named McCombie, we have classified this method as McTets.

Despite a number of casual attempts by the PI and an ongoing research project with the eastern oyster, *C. virginica*, this method has not been successful. Then again, eastern oysters and Pacific oysters are quite different. We produced several spawns using this technique. It turns out that the difficult aspect of this method is keeping the tetraploids alive through the larval period. Considerable resources were spent on these activities and the body of data will likely be worthy of a manuscript describing the difficulties of this method, which, frankly, were glossed over in the original publication. In fact, it may be an

alternative method, *per se*, but it is not likely a complimentary method if heaven and earth have to be moved just to obtain a successful spawn.

**VI. Ran season long investigation into the reproductive potential of tetraploid x tetraploid matings, called "the matrix"**

Tetraploids are like a different species. That is, their condition of having twice as much genetic material and its evolutionarily selected diploid parent produces a much different physiology, especially surrounding reproduction. While quite fertile, there have been issues with the reproducibility of tetraploid x tetraploid crosses (called 4M crosses), particularly variation in hatching rate. Several hypotheses have been raised as to why this is true and we explored two in a series of four experiments that lasted all season. Either the variation is caused by the state of sexual maturity of the parents, or the variation is caused by specific combinations of parents, some of which work and some that do not.

These projects were designed with discussions and assistance from Peter Kube and Nick Elliot at CSIRO. Peter will be analysing the data from the highly successful experiments and we are planning to publish the material. Little has been published about tetraploid oyster breeding.

**VII. Consulted to SCL and CRC on aspects of the reorganization of ASI, including the incorporation of tetraploid technology into the ASI program**

Besides the expertise in oyster polyploids, the PI – in his day job – runs a large, successful breeding program at the Virginia Institute of Marine Science – the Aquaculture Genetics and Breeding Technology Center. Accordingly, the PI has a considerable amount of expertise in, and many opinions about, how to run a breeding program. At this juncture, the ASI program is on the verge of reorganizing. This has considerable ramifications for the industry as well as the main hatcheries that supply most of the seed in Australia. It also has profound implications for the future of tetraploid technology in Australia, especially the merging of the two IPs.

## ABOUT THE PROJECT/ACTIVITY

### BACKGROUND AND NEED

The overall goal for SCL is to make tetraploid technology work as effectively as anywhere in the world. In many ways, SCL is ahead of the game, with dedicated facilities and technicians to pursue this goal. The objective of this Visiting Expert project is to get SCL fully on this path and to continue the improvement of tetraploid technology in Australia, and by example, the world. There is currently only a hand full of commercial operations producing natural triploids and even fewer assessing breeding technologies required to enhance the breeding of tetraploid populations.

At SCL, commercial production of natural triploid oysters in Australia has been successful for a number of years. The regeneration of tetraploids through large mass populations of tetraploids is accomplished each year by SCL research staff. They also have the know-how

for the production of both natural mass spawning of tetraploids and chemical induction of tetraploids.

The opportunity exists to enhance our tetraploid lines through the infusion of genetics from the industry owned breeding program (Australian Seafood Industries). Pre-2010 the infusion of new germ plasm was intended to improve the morphological characteristics of the tetraploids but now the priority from industry is to produce high survival triploids. This request from industry is based on the following two, and likely very distinct, traits.

1. Specific resistance to OsHV-1 µVar
2. Increase fitness in general oyster populations to combat mortalities in South Australia or Tasmania probably due to the high metabolism of triploids in food poor waters

The breeding of specific tetraploid lines is a new challenge filled with a number of, as yet, answered questions about the biology and genetics of tetraploids. Long term breeding objectives will require answers to these questions and this Visiting Expert project aims to initiate some of this work and set a course for future work for SCL, CSIRO and VIMS, and train SCL staff.

**RESULTS** – see above

## INDUSTRY IMPACT

**PROJECT OUTCOMES (THAT INITIATED CHANGE IN INDUSTRY)**

The development of tetraploids is a multigenerational process that, in some ways, has only begun, starting with this project. As indicated above, the activity from this project has initiated about 10 potential new lines of tetraploids:

- 4 x 3C triploid spawns to become tetraploids
- 1 x McTet
- 2 x 4C spawns
- 3 x 4M crosses

The project has brought together CSIRO with tetraploid technology and may provide a catalyst to the resolution of marrying ASI and tetraploid IP.

**SUMMARY OF CHANGE IN INDUSTRY**
**WHAT FUTURE AND ONGOING CHANGES ARE EXPECTED?**

Australia's breeding program of the future is going to be a very different animal from that of today. It will be lighter on its feet, more collaborative, adaptive, efficient, and speak with one informed voice. It will embrace polyploidy as a major breeding tool of the 21st Century. It will provide life blood for the established growers seeking improvements in efficiency and "transfusion" in the form of new lines for those segments of the industry that are under pressure from Mother Nature, either in SA or NSW.

**WHAT BARRIERS ARE THERE FOR CHANGES TO OCCUR?**

ASI needs to reorganize into a sustainable and professional breeding program that will provide a continuous source of improved genetic material that can be introgressed in tetraploids. Presently the program faces a sunset with the pending loss of CRC funds in the near future, causing myopia. At the same time, the management of ASI does not have cohesive leadership that would allow it to marry its IP with tetraploid technology.

**IF NOT ALREADY HAPPENING, WHEN WILL THE CHANGES OCCUR?**

N/A

**WHAT IS THE LIKELIHOOD THAT THESE CHANGES WILL OCCUR?**

With an organized and focused breeding program, genetic change *will* be obtained.

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

Currently, the tetraploid technology is held exclusively at SCL. This project enabled direct talks with SCL and its Board about opening the technology industry wide in Australia.

## COMMUNICATION OF PROJECT/EXTENSION ACTIVITIES

**WHAT IS THE OUTPUT THAT NEEDS TO BE COMMUNICATED?**
**WHO IS/ARE THE TARGET AUDIENCE/S?**
**WHAT ARE THE KEY MESSAGES?**

Communication occurred with a number of industry members during a visit to NSW with GM Wells, including Rob Moxham, John Stubbs, and Jeff Diemar. We also paid a visit to Wayne O'Conner at NSW Fisheries, and visited Southern Cross Hatchery in Port Stephens. Also during this visit, the PI interacted with Drs. Ika Paul-Pont and Richard Whittington from Sydney University.

The PI had a meeting with Ben and Graeme Cameron in Tasmania, and with Phil Lamb and the Spring Bay Mussel group in Triabunna. The PI also interacted on two occasions with the SCL Board, once in a strategic meeting about the future of ASI and tetraploid breeding in January, where Len Stephens was also attending.

Information will be disseminated through SCL quarterly newsletter to over 200 customers, researchers, and government agencies throughout Australia and Internationally. Multiple interactions with CSIRO and Penny Miller, and her advisors Drs. Rene Vaillancourt and Anthony Koutoulis, were afforded.

PI Allen attended Sense-T Aquaculture Project workshop on the 19$^{th}$ of November and contributed to group discussion on the potential uses of this technology within the oyster industry.

## LESSONS LEARNED AND RECOMMENDED IMPROVEMENTS

### WHAT IS YOUR FEEDBACK?

This work was accomplished at a small R&D facility at SCL. What is stunning is that there is a complete lack of institutional facilities or even research direction in matters pertaining to the shellfish industry. Case in point, ASI hatchery activities have to occur in a short time span in rented facilities, apparently at relatively high cost. For an industry as mature and sophisticated as the Australian oyster industry not to have a resource for R&D, such as the experiments carried out here, is – as my feedback – inexcusable.

### FURTHER ACTION REQUIRED IN REGARDS TO COMMERCIALISATION?

n/a

### ACKNOWLEDGEMENTS

The PI extends his heartfelt gratitude to the management and employees of SCL, particularly research "buddy" Andy Day, Breeding Manager Scott Parkinson, and General Manager Kerry Wells. My stay was assured comfortable by the Staff of SCL, particularly the motherly care of Jacqui Singleton. The PI is especially grateful for the professional, and otherwise, conversations with fellow gene jockey Peter Kube.