Development of tools for the sustainable management of genetics in polyploid Pacific oysters (*Crassostrea gigas*)

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Statement of co-authorship

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Located in chapter two.
Penny Miller was the primary author and with Anthony Koutoulis, Rene Vaillancourt, Nick Elliott and Peter Kube contributed to the idea, its formalisation and development, as well as assisted with refinement and presentation.

Paper 2, Genetic diversity and pedigree assignment in tetraploid Pacific oysters (*Crassostrea gigas*):
Located in chapter three.
Penny Miller was the primary author and with Anthony Koutoulis, Rene Vaillancourt, Nick Elliott and Peter Kube contributed to the idea, its formalisation and development, as well as assisted with refinement and presentation.

Paper 3, Assignment of parentage in triploid species using microsatellite markers with null alleles, an example from Pacific oysters (*Crassostrea gigas*):

Located in chapter four.

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Thesis Abstract

The commercial production of triploid Pacific oysters (*Crassostrea gigas*) has grown rapidly in recent years. There is now a push to move away from commonly used mass spawning techniques towards single pair cross selective breeding programs in an effort to improve growth and disease resistance within the triploid product. Before this can be achieved, there is a need to understand some of the fundamental genetics behind polyploid production and to develop molecular tools and techniques that can be used in establishing breeding programs. This dissertation developed and utilised suites of microsatellite markers to determine the baseline diversity of native, naturalised and cultured diploid oysters. It was found that the high diversity within naturalised oysters may provide a genetic reservoir for future breeding programs. The same microsatellite markers were used to determine diversity and pedigree assignment within a mass spawned tetraploid population across two successive generations. The first generation showed a high diversity, which significantly decreased in the second generation produced via mass spawning. This was most likely due to a low number of effective broodstock and skewed parental contributions and highlights the benefits of using single pair crosses over mass spawning to control inbreeding. A method for assigning pedigrees in triploids, produced by crossing diploids with tetraploids, was developed. This method will allow the pedigree of strongly performing triploids to be traced back so that the same or closely related broodstock can be used in single pair cross selection programs to produce future generations. To determine the long term stability of tetraploid oysters, the aneuploid frequency was analysed using flow cytometry across three generations. No difference was observed which suggests that either aneuploidy is occurring at a lower rate than previously predicted or that aneuploid oysters are being removed from the system

through hatchery grading or early mortality. Flow cytometry is not sensitive enough to detect small scale chromosome loss. Hence, fluorescent in situ hybridisation (FISH) using microsatellite markers was trialled. This was unsuccessful due to the inconsistency of the markers. The molecular tools, techniques and results described within this dissertation will aid in the development of single pair cross selective breeding programs for the improvement of triploid oysters.

Contents