

**Sperm Cryopreservation in
Australian Farmed Greenlip
(*Haliotis laevis*) and Blacklip
(*H. rubra*) Abalone**

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CONTENTS

LIST OF TABLES.....	V
LIST OF FIGURES.....	VII
DECLARATION	X
ABSTRACT	XI
ACKNOWLEDGEMENTS.....	XIV
CHAPTER 1 : GENERAL INTRODUCTION	1
CHAPTER 2 : SPERM CRYOPRESERVATION IN MARINE MOLLUSC: A REVIEW*	15
2.1 ABSTRACT	15
2.2 INTRODUCTION	17
2.3 CRYOPRESERVATION	19
2.3.1 Sperm collection method.....	19
2.3.2 Extender.....	21
2.3.3 Cryoprotectant agent	22
2.3.4 Sperm dilution/concentration before cryopreservation	24
2.3.5 Cooling rate	25
2.3.6 Thawing temperature.....	27
2.3.7 Sperm quality assessment.....	27
2.4 IMPROVEMENT OF MARINE MOLLUSCAN SPERM CRYOPRESERVATION TECHNIQUES	35
2.4.1 Living condition	35
2.4.2 Broodstock nutrition.....	36
2.4.3 Timing of sperm collection	37
2.4.4 Age of broodstock and sperm.....	38
2.4.5 Sperm quality assessment indicators	39
2.4.6 Sugars, amino acids and vitamins	40
2.5 CONCLUSION	41
CHAPTER 3 : CRYOPRESERVATION OF SPERM IN FARMED AUSTRALIAN GREENLIP ABALONE <i>HALIOTIS LAEVIGATA</i> *	43
3.1 ABSTRACT	43
3.2 INTRODUCTION	45
3.3 MATERIALS AND METHODS.....	47
3.3.1 Broodstock.....	47
3.3.2 Gamete collection.....	48

3.3.3 Chemical solution preparation.....	49
3.3.4 Equipment setup.....	50
3.3.5 Sperm quality evaluation methods.....	50
3.3.6 Experiments.....	51
3.3.7 Statistical analysis.....	54
3.4 RESULTS.....	55
3.4.1 Effects of CPA types and concentrations on sperm motility.....	55
3.4.2 Effects of equilibration times on sperm motility.....	55
3.4.3 Effects of different rack heights on sperm motility.....	57
3.4.4 Effects of thawing temperatures on sperm motility.....	57
3.4.5 Comparison of sperm to egg ratios on fertilization rate.....	58
3.4.6 Effects of sugar types and concentrations on sperm motility and fertilization rate.....	59
3.4.7 Comparison of PMI, MMP and AI between sperm cryopreserved with 6% DMSO and 6% DMSO + 1% glucose.....	61
3.5 DISCUSSION.....	62
CHAPTER 4 : EFFECTS OF BROODSTOCK AGE AND SPERM COLLECTION TIME OVER A NATURAL SPAWNING PERIOD ON SPERM CRYOPRESERVATION IN FARMED GREENLIP ABALONE*	69
4.1 ABSTRACT.....	69
4.2 INTRODUCTION.....	70
4.3 MATERIAL AND METHOD.....	71
4.3.1 Broodstock and gamete collection.....	71
4.3.2 Experimental procedure.....	72
4.3.3 Statistical analysis.....	73
4.4 RESULTS.....	74
4.5 DISCUSSION.....	77
CHAPTER 5 : GREENLIP ABALONE (<i>HALIOTIS LAEVIGATA</i> DONOVAN, 1808) SPERM CRYOPRESERVATION USING A PROGRAMMABLE FREEZING TECHNIQUE AND TESTING THE ADDITION OF AMINO ACID AND VITAMIN*	79
5.1 ABSTRACT.....	79
5.2 INTRODUCTION.....	81
5.3 MATERIAL AND METHODS.....	83
5.3.1 Animals and gamete preparation.....	83
5.3.2 Chemicals.....	84
5.3.3 Equipment.....	84

5.3.4 Sperm quality evaluation methods	85
5.3.5 Experiments	86
5.3.6 Statistical analysis	88
5.4 RESULTS	90
5.4.1 Effects of different permeable CPAs on post-thaw sperm motility	90
5.4.2 Effects of cooling rate and DMSO concentration on post-thaw sperm motility	90
5.4.3 Effects of thawing temperature on post-thaw sperm motility	91
5.4.4 Effects of endpoint temperature on post-thaw sperm motility	92
5.4.5 Assessment of types and concentrations of sugar, amino acid and vitamin on post-thaw sperm motility and fertilization rate	93
5.4.6 Assessment of post-thaw sperm plasma membrane integrity (PMI), mitochondrial membrane potential (MMP) and acrosome integrity (AI)	97
5.5 DISCUSSION	99
5.6 CONCLUSIONS	103
CHAPTER 6 : IMPROVEMENT IN NON-PROGRAMMABLE SPERM CRYOPRESERVATION TECHNIQUES IN FARMED GREENLIP ABALONE <i>HALIOTIS LAEVIGATA</i> *	104
6.1 ABSTRACT	104
6.2 INTRODUCTION	105
6.3 MATERIALS AND METHODS.....	106
6.3.1 Animals and gamete preparation	106
6.3.2 Chemicals	106
6.3.3 Sperm cryopreservation and quality evaluations.....	107
6.3.4 Experiments	108
6.3.5 Statistical analysis	109
6.4 RESULTS	110
6.4.1 Effects of different monosaccharides on post-thaw sperm quality in farmed greenlip abalone	110
6.4.2 Effects of the addition of amino acid and vitamin on post-thaw sperm quality in farmed greenlip abalone	112
6.5 DISCUSSION.....	116
CHAPTER 7 : CRYOPRESERVATION OF SPERM IN FARMED BLACKLIP ABALONE (<i>HALIOTIS RUBRA</i>) *	119
7.1 ABSTRACT	119
7.2 INTRODUCTION	120
7.3 MATERIALS AND METHODS.....	122

7.3.1 Animal sources and gamete collection	122
7.3.2 Chemical and equipment preparation.....	123
7.3.3 Sperm quality assessment.....	123
7.3.4 Experiments.....	124
7.3.5 Statistical analysis	126
7.4 RESULTS.....	127
7.4.1 Effects of CPA types and concentrations on sperm motility.....	127
7.4.2 Effects of equilibration times on sperm motility.....	127
7.4.3 Effects of rack heights on sperm motility	128
7.4.4 Effects of thawing temperatures on sperm motility	129
7.4.5 Effects of sugar types and concentrations on sperm motility.....	130
7.4.6 Comparisons of sperm to egg ratios on fertilization rate	131
7.5 DISCUSSION	133
CHAPTER 8 : GENERAL DISCUSSION, CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS	136
8.1 GENERAL DISCUSSION	136
8.2 CONCLUSIONS	141
8.3 FUTURE RESEARCH DIRECTIONS	142
REFERENCES	145
APPENDIX A: SPERM CRYOPRESERVATION PROTOCOL FOR FARMED ABALONE IN AUSTRALIA.....	167

LIST OF TABLES

Table 1.1 Abalone aquaculture production (tonnes) from major producing countries and regions.....	2
Table 1.2 Abalone species farmed in major countries and regions in the world.....	3
Table 1.3 Abalone aquaculture production (tonnes) and value (\$000) in Australia. The symbol “-” means missing values.....	5
Table 2.1 Sperm collection method, extender, cryoprotectant agent (CPA) or CPA/co-CPA combination and dilution/concentration used in marine mollusc sperm cryopreservation.	30
Table 2.2 Freezing methods and steps, thawing temperatures and outcomes in marine mollusc sperm cryopreservation.	32
Table 3.1 Sperm motilities (%) after exposure to CPAs at different concentrations for 10 min on ice, $n = 3$	55
Table 3.2 Comparison of plasma membrane integrity (PMI), mitochondrial membrane potential (MMP) and acrosome integrity (AI) between sperm cryopreserved in 6% DMSO and 6% DMSO + 1% glucose, $n = 3$	61
Table 4.1 Comparisons in post-thaw sperm motilities (%) and fertilization rates (%) between sperm collected at different times during a natural spawning period and between 2 and 3 years old greenlip abalone, $n = 3$	74
Table 4.2 Comparisons in plasma membrane integrity, mitochondrial membrane potential and acrosome integrity values in fresh sperm collected from 2 and 3 years old greenlip abalone at different time during the spawning season, $n = 3$	75
Table 4.3 Comparisons in plasma membrane integrity, mitochondrial membrane potential and acrosome integrity values in post-thaw sperm collected from 2 and 3 year old greenlip abalone at different time during the spawning season, $n = 3$	76
Table 5.1 Comparison of plasma membrane integrity (PMI), mitochondrial membrane potential (MMP) and acrosome integrity (AI) between sperm cryopreserved in 10% DMSO and 10% DMSO + 0.6% glycine, $n = 3$	98
Table 6.1 Comparison of plasma membrane integrity, mitochondrial membrane potential and acrosome integrity between sperm cryopreserved in 6% DMSO plus different monosaccharides in farmed greenlip abalone, $n = 3$	111
Table 6.2 Post-thaw sperm fertilization rates (%) between sperm cryopreserved in selected cryoprotective mediums in farmed greenlip abalone, $n = 3$	112
Table 6.3 Comparison of plasma membrane integrity, mitochondrial membrane potential and acrosome integrity between sperm cryopreserved in 6% DMSO + 0.6% glycine plus different monosaccharides in farmed greenlip abalone, $n = 3$	112
Table 6.4 Comparison of plasma membrane integrity, mitochondrial membrane	

potential and acrosome integrity between sperm cryopreserved in 6%
DMSO + 1% glucose and its combination with 0.6% glycine or 0.02% L-
ascorbic acid in farmed greenlip abalone, $n = 3$ 115

LIST OF FIGURES

Figure 1-1 Farmed major abalone type in Australia.....	6
Figure 3-1 Sperm motilities (%) after exposure to selected cryoprotectant solutions at equilibration time of 5, 10, 30 and 60 min, $n = 3$. Different letters within each cryoprotectant solution indicate significant difference between different equilibration times.	56
Figure 3-2 Sperm motilities (%) after exposure to 8% EG, PG or DMSO at equilibration time of 10 and 20 min, $n = 3$. Different letters within each cryoprotectant solution indicate significant difference between equilibration times.	56
Figure 3-3 Post-thaw sperm motilities (%) after being frozen at different rack heights in 8% DMSO, EG or PG for 10 min and stored in LN for at least 2 h, $n = 3$. Different letters within each cryoprotectant solution indicate significant difference between different rack heights.	57
Figure 3-4 Post-thaw sperm motilities (%) after cryopreservation in 6, 8 or 10% DMSO and thawed at different temperatures, $n = 3$. Different letters within each cryoprotectant solution indicate significant difference between different thawing temperatures.	58
Figure 3-5 Post-thaw sperm fertilization rates (%) at different sperm to egg ratios, $n = 3$. Different letters indicate significant difference.....	59
Figure 3-6 Post-thaw sperm motilities (%) after addition of different types and concentrations of sugar into 6% DMSO, $n = 3$. Different letters within each CPA combination indicate significant difference between different sugar concentrations.	60
Figure 3-7 Comparison of post-thaw sperm fertilization rates in 6% DMSO and its combination with 1% glucose or 2% sucrose, $n = 3$. Different letters indicate significant difference.	60
Figure 5-1 Post-thaw sperm motilities (%; relative to control) after being cryopreserved in 8% PG, DMSO or EG, $n = 3$. Bars with different letters differ significantly ($P < 0.05$).....	90
Figure 5-2 Post-thaw sperm motilities (%; relative to control) after being frozen at different cooling rates in 6, 8 or 10% DMSO in a programmable freeze controller, $n = 3$. Different capital letters within each cooling rate indicate significant differences between DMSO concentrations. Different lowercase letters within each DMSO concentration indicate significant differences between cooling rates ($P < 0.05$).....	91
Figure 5-3 Sperm motilities (%; relative to control) after being thawed at different temperatures in a programmable freeze controller, $n = 3$. Different letters indicate significant differences ($P < 0.05$).	92
Figure 5-4 Post-thaw sperm motilities (%; relative to control) after being frozen to different endpoint temperatures in a programmable freeze controller before being transferred into LN, $n = 3$. Bars with different letters differ	

significantly ($P < 0.05$).....	93
Figure 5-5 Comparison of post-thaw sperm motilities (%; relative to control) after being frozen in 10% DMSO or its combination with different types and concentrations of sugar, $n = 3$. Bars with different capital letters within each sugar concentration (+ 10% DMSO) differ significantly between different sugar types ($P < 0.05$). Bars with different lowercase letters within each sugar type (+ 10% DMSO) differ significantly between different sugar concentrations ($P < 0.05$).....	94
Figure 5-6 Comparisons of post-thaw sperm motilities (%; relative to control) between 10% DMSO (control) and its combination with different concentrations of glycine, $n = 3$. Bars with different letters differ significantly ($P < 0.05$).....	95
Figure 5-7 Comparisons of post-thaw sperm motilities (%; relative to control) between 10% DMSO (control) and its combination with different concentrations of taurine, $n = 3$. Bars with different letters differ significantly ($P < 0.05$).....	95
Figure 5-8 Comparisons of post-thaw sperm motilities (%) between 10% DMSO (control) and its combination with different concentrations of L-ascorbic acid, $n = 3$. Bars with different letters differ significantly ($P < 0.05$).....	96
Figure 5-9 Effects of 10% DMSO and its combination with 4% sucrose, 0.6% glycine, 0.02% L-ascorbic acid or 0.2% taurine on post-thaw sperm fertilization rate (%) at different sperm to egg ratios, $n = 3$. Bars with different capital letters within each cryoprotectant solution differ significantly between different sperm to egg ratios ($P < 0.05$). Bars with different lowercase letters within each sperm to egg ratio differ significantly between different cryoprotectant solutions ($P < 0.05$).....	97
Figure 6-1 Comparisons of post-thaw sperm fertilization rate (%) at different sperm to egg ratios in sperm cryopreserved in 6% DMSO plus different monosaccharides in farmed greenlip abalone, $n = 3$. Bars with different capital letters in each sperm to egg ratio indicate significant difference ($P < 0.05$) between cryoprotective mediums. Bars with different lowercase letters in each cryoprotective medium indicate significant difference ($P < 0.05$) between different sperm to egg ratios.	111
Figure 6-2 Comparisons of post-thaw sperm motilities (%) between sperm cryopreserved in 6% DMSO + 1% glucose (control) and combinations of 6% DSMO + 1% glucose with different concentrations of taurine in farmed greenlip abalone, $n = 3$. Bars with different letters indicate significant difference ($P < 0.05$).....	113
Figure 6-3 Comparisons of post-thaw sperm motilities (%) between sperm cryopreserved in 6% DMSO + 1% glucose (control) and combinations of 6% DSMO + 1% glucose with different concentrations of glycine in farmed greenlip abalone, $n = 3$. Bars with different letters indicate significant difference ($P < 0.05$).....	114
Figure 6-4 Comparisons of post-thaw sperm motilities (%) between sperm cryopreserved in 6% DMSO + 1% glucose (control) and combinations of 6% DSMO + 1% glucose with different concentrations of L-ascorbic acid	

in farmed greenlip abalone, $n = 3$. Bars with different letters indicate significant difference ($P < 0.05$).....	114
Figure 6-5 Comparisons of post-thaw sperm fertilization rate (%) at different sperm to egg ratios between sperm cryopreserved in 6% DMSO + 1% glucose (control) and combinations of 6% DMSO + 1% glucose with 0.6% glycine, 0.02% L-ascorbic acid or 0.4% taurine in farmed greenlip abalone, $n = 3$. Different capital letters in each sperm to egg ratio indicate significant difference ($P < 0.05$) between cryoprotective mediums. Bars with lowercase letters in each cryoprotective medium indicate significant difference ($P < 0.05$) between different sperm to egg ratios.....	115
Figure 7-1 Sperm motilities (%) after 10 min exposure to different CPAs at various concentrations on ice, $n = 3$. Different letters within each CPA concentration indicate significant difference between different CPAs.	127
Figure 7-2 Sperm motilities (%) after equilibration in 8% DMSO, EG or PG from 10, 20, 30 or 60 min, $n = 3$. Different letters within each CPA indicate significant difference between different equilibration times.....	128
Figure 7-3 Post-thaw sperm motilities (%) after exposure to LN vapour at different rack heights above LN surface in 8% DMSO, EG or PG, $n = 3$. Different letters within each CPA indicate significant difference between different rack heights.....	129
Figure 7-4 Post-thaw sperm motilities (%) after being thawed at different temperatures in 6, 8 or 10% DMSO, $n = 3$. Different letters within each CPA indicate significant difference between different thawing temperatures.	130
Figure 7-5 Post-thaw sperm motilities (%) after cryopreservation in different types and concentrates of sugars, $n = 3$. Different letters within each concentration indicate significant difference between different CPAs.	131
Figure 7-6 Post-thaw sperm fertilization rates (%) at different sperm to egg ratios after cryopreservation in 6% DMSO or its combination with 1% or 2% glucose, $n = 3$. Different letters within each sperm to egg ratio indicate significant difference between different CPAs.....	132

DECLARATION

I certify that this work contains no material which has been accepted for any other degree or diploma in any university or institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

In addition, I certify that no part of this work will be submitted for any other degree or diploma in any university or institution without the prior approval of the University of Flinders.

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ABSTRACT

Greenlip *Haliotis laevis* and blacklip *H. rubra* abalone as well as their hybrid are the major abalone farmed in Australia. To ensure the long-term sustainable and competitive development of the industry, genetic improvement programs have been applied. However, the efficiency of these programs has been compromised due to: (1) asynchronous spawning in males and females within and between species; (2) the short spawning window period in a breeding season; and (3) risks associated with keeping superior broodstock alive and healthy. Sperm cryopreservation is a proven technique to overcome similar issues and sperm collected from wild greenlip abalone has been investigated and 90% post-thaw sperm fertilization rates have been achieved. Nevertheless, when the developed protocol was applied to sperm from farmed stocks, low and highly variable results were observed, hindering its application. The Australian abalone aquaculture industry now relies entirely on domesticated farmed abalone as broodstock. Therefore, understanding factors causing this discrepancy, and development of sperm cryopreservation techniques suitable for Australian farmed abalone species, are the focus of this PhD project.

Factors affecting sperm cryopreservation and strategies to improve the sperm quality were evaluated using both programmable and/or non-programmable freezing techniques in farmed greenlip and blacklip abalone. In greenlip abalone, broodstock physiological conditions, such as broodstock age and sperm collection time over a natural spawning period, were also evaluated.

Among the single cryoprotectant agent [dimethyl sulfoxide (DMSO), propylene glycol, ethylene glycol, and glycerol] evaluated, DMSO produced the best post-thaw sperm motility and/or fertilization rates using both programmable and non-

programmable freezing techniques in farmed greenlip abalone. However, to achieve the highest post-thaw sperm fertilization rates of 60 to 70%, a higher DMSO concentration and a sperm to egg ratio were required in the former technique (10% and 40,000:1) than in the latter one (6% and 10,000:1). The addition of sugar (glucose, sucrose or trehalose) in DMSO to further improve the post-thaw sperm quality showed that the post-thaw sperm motility was significantly improved by the addition of 4% sucrose using programmable freezing technique, whereas the post-thaw sperm fertilization rates were not. In contrast, when a non-programmable freezing technique was used, the addition of 1% glucose in 6% DMSO significantly improved both post-thaw sperm motility and fertilization rates, with the latter reaching 80%. Fluorescent stain analyses revealed that the addition of glucose significantly improved the post-thaw sperm plasma membrane integrity (PMI) and mitochondrial membrane potential (MMP).

Data from the addition of amino acids (taurine and glycine) and vitamin (L-ascorbic acid) revealed that the addition of 0.6% glycine in 10% DMSO or 6% DMSO + 1% glucose significantly improved the post-thaw sperm fertilization rates to over 90% when using the programmable and non-programmable freezing techniques, respectively. The addition of glycine improved the post-thaw sperm MMP with application of the former technique, whereas PMI and acrosome integrity (AI) with application of the latter one. After the addition of 0.6% glycine, the optimal sperm to egg ratio remained the same in programmable freezing technique whereas this ratio reduced from 10,000:1 to 2,000:1 in the non-programmable freezing technique. Flow cytometry analyses showed higher post-thaw sperm PMI, MMP and AI values were achieved when using non-programmable versus programmable freezing techniques.

Evaluation of the effects of glucose, fructose and galactose using the non-

programmable freezing technique indicated that in farmed greenlip abalone, galactose and fructose had a similar ability to protect sperm from cryoinjury as glucose. The replacement of glucose with either of these monosaccharides resulted in a similar level of post-thaw sperm fertilization rate, PMI, MMP and AI.

Results from evaluation of broodstock physiological conditions in farmed greenlip abalone showed that sperm collected at the middle of a natural spawning period had a better ability to tolerate the cryopreservation processes than those collected at the beginning or at the end of a natural spawning period, resulting in significantly higher post-thaw sperm motility, fertilization rate, PMI, MMP and AI. In contrast, little difference in these parameters was found between 2 and 3 years old animals.

Among the single cryoprotectant agents assessed in farmed blacklip abalone using the non-programmable freezing technique, 6% DMSO achieved the highest post-thaw sperm motility, which was the same as found for farmed greenlip abalone.

Further addition of sugar (glucose, sucrose or trehalose) in 6% DMSO showed that the addition of 2% glucose significantly improved the post-thaw sperm quality, producing the highest post-thaw sperm fertilization rate of 70% at a sperm to egg ratio of 10,000:1, although the rate was significant lower than the control (83%).

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