Spat Production of the Greenshell™ mussel

Perna canaliculus

in

New Zealand

Sam Buchanan

A thesis submitted in fulfilment of the requirements for the degree
Doctor of Philosophy in Zoology,
University of Auckland,
New Zealand,
September 1998

♦

ABSTRACT

The research presented in this thesis was undertaken in order to develop an understanding of the biology of *Perna canaliculus* sufficient to allow for commercial hatchery based production of Greenshell™ mussel spat. Hatchery production is an alternative to unreliable and inconsistent wild spat collection.

In a *Perna canaliculus* population followed for one year spawning occurred in early spring and late summer. Three quantitative histological measures of gonad maturity utilising image analysis technology and a qualitative classification system were compared. Measuring the relative surface area comprised of gametes on histological sections was found to be the most reliable method. A practical gonad visual index to determine the reproductive condition of adults for the selection of broodstock was developed and found to be highly effective as a means of predicting induced spawning success. Serotonin was not effective for inducing spawning of *Perna canaliculus*. Temperature shock and the use of stripped gametes was however found to be a reliable spawning induction method.

Relative gamete concentration, gamete age, temperature, sperm half life and gamete contact times were all found to have effects on fertilisation success for *Perna canaliculus*. Sperm concentration and the conditions of sperm aging were particularly important. Fertilisation kinetics of *Perna canaliculus* gametes modelled using the Vogel-Czihak-Chang-Wolf method suggested that 5% of sperm-egg contacts lead to successful fertilisation.

Broodstock management protocols that could be used to condition the adult of *Perna canaliculus* were investigated in order to enhance and prolong the natural reproductive season. Research suggested that for successful broodstock conditioning animals should already have begun gametogenesis at the time conditioning is commenced. Successful conditioning of *Perna canaliculus* was achieved at temperatures between 10 and 16°C over a period of about 50 days. A diet ration above 2-3% of the dry meat mass per day is suggested. A trial examining non-algal diet supplements suggest a mixture of yeast and lipid emulsion may have some potential value. Photoperiod manipulation did not effect the reproductive condition of *Perna canaliculus*.

The yield of veliger larvae was significantly enhanced if embryo culture water was treated with 1.0 mg/l EDTA. Veliger yield was not significantly affected at densities below 50 embryos/ml.

Perna canaliculus larvae grew most rapidly and survived well at the salinity of 35 ppt. Larvae grew most rapidly when cultured at low densities. Experiments suggest that early larvae can be cultured at 5-10/ml, however late stage larvae grew most rapidly when cultured at 1/ml. *Perna canaliculus* larvae displayed best growth and good survival if fed a mixed flagellate-diatom diet comprising *Isochrysis galbana (T-Iso)* and *Chaetoceros calcitrans*. The optimal diet ration, as a function of larval size, increased from about 20 cells/µl *Isochrysis galbana (T-Iso)* to around 150 cells/µl through the larval development period.

Thyroxine between the concentrations of $10^{-5}$and 10^{-8} M did not have an observable effect on larval developmental rate or eye spot development. Down welling settlement systems were found to be generally successful for *Perna canaliculus* larvae. L-DOPA was also demonstrated to enhance the settlement and metamorphosis of *Perna canaliculus* pediveligers.

ACKNOWLEDGMENTS

I would like to acknowledge the many people and various institutions that have contributed to this thesis and the Greenshell™ mussel spat project.

I am particularly indebted to my beautiful wife, Maria whose patience, understanding and love supported me throughout the sometimes difficult or frustrating times. Luis, my little boy, you have been the joy of my days that made any tedium or setback pale into insignificance. Thank you both for tolerating the long days and lost weekends.

I would also like to thank my father Roger and mother Liz, brother Tom and sister Clare for being such a great family. Particularly to my parents, thanks for giving me the confidence and the opportunity to undertake this thesis and putting me through the 10 years of tertiary education; I owe you a yatch!

I would like to thank my academic supervisors, Dr Russ Babcock of the University of Auckland for his advice and input and Dr Henry Kaspar from the Cawthron institutes for his constant support and guidance. To the many other people who have contributed their time and effort to reviewing various chapters and publications, thank you, your criticism and counsel are greatly appreciated. Particularly thank you Russ and Henry and also Bob Creese, Andrew Jeffs, Jonathan Gardner and Rodney Roberts.

I owe special gratitude to the excellent colleagues at Cawthron and the Glenhaven Aquaculture Centre whom I have had the pleasure to work with over the last few years and who have contributed their talents and insights, sweat and yes, even blood, to the mussel project and the hatchery. These include Steven Brown, Tim Mills, Bevan Fraser, Caroline Lapworth, James Holland, Anabel Thomas, Rodney Roberts, Chris Molloy, Sean Handley, Mike Taylor, Henry Kaspar, Graeme Robertson, Adrian Parlane, Peter and George Dixon, and to all the others - you know who you are. Thanks also to all those people from around the world that have contributed their ideas or time to the project, particularly thank you Ron Reitz for the lipid analyses and to Jose Fuentes and Jose Molares for your gracious hospitality and interest.

To those in the industry who have supported this project I owe a special thanks, for without your advice, guidance, financial and practical support this project would not have ever started. I hope

that the contribution of this thesis build towards a better, more secure Greenshell™ mussel industry for all those involved. In particular I would like to thank the Mussel Industry Council as a whole and individually Phil Driver, Bridget Steed, Paul Lupi, John Hannah, Don Mitchel, Rob Pooley, Bruce Cartwell and Tracey Osborne. I would like to specifically thank Tracey Osborne, John Hannah, Andrea Blackburne and Colin Stuart at Sealord Shellfish Ltd for their generous support and of course providing most of the thousands of mussels I have fed, spawned, executed, dissected, frozen and baked in the course of this project.

I would like to acknowledge the Foundation for Science Research and Technology for funding this project from 1994, the FRST 1997 spat review panel, and the various named and unnamed referees that kindly made the time to review our proposals. Particularly thanks to Bob Creese, Patricia Bergquist, Susan Utting, Ian Whyte, Philippe Douillet, Sandy Shumway. And to those involved in the most recent mussel application I have been honoured to work with you; Phil Mladenov, Sultan Mahmud, Jonathan Gardner and Smita Apte.

A special thanks to the people of New Zealand who have invested in this project with your hard earned taxes. I have worked hard to spend them well and hope the findings of this thesis bring dividends to those that deserve it.

And finally to my friend *Perna c.*, thanks for all the entertainment.

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

TABLE OF PHOTOGRAPHS