Antiviral immune responses in abalone and influence of potential abiotic and biotic factors

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Abbreviations

AbHV	Abalone herpesvirus
AVG	Abalone viral ganglioneuritis
DMSO	Dimethyl sulfoxide
EMEM	Eagle's minimal essential medium
HSV-1	Herpes simplex virus type 1
NBT	Nitroblue tetrazolium
OD	Optical density
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFU	Plaque-forming unit
qPCR	Quantitative real time polymerase chain reaction
RT	Room temperature
SO	Superoxide anion
THC	Total haemocyte count

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

12/11/2012

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Abstract

Abalone (Haliotidae) are marine gastropod mollusks and important aquaculture species worldwide. Unfortunately, severe mortality of abalone caused by a herpesvirus (AbHV) has been reported in Australia. The manifestation of disease involves an interaction between virus, environment and abalone immunity (Chapter 1). Therefore, this research aimed to investigate the presence of antiviral activity in abalone *Haliotis laevigata* (greenlip), *H. rubra* (blacklip) and their hybrid. Due to the lack of molluscan cell lines for culturing AbHV, antiviral activity of abalone was assessed against a similar neurotropic herpesvirus, herpes simplex virus type 1 (HSV-1) using the plaque assay. Assessment of antiviral activity was complemented with other immune assays, measuring total haemocyte count (THC), phagocytosis, phenoloxidase activity, respiratory burst and antibacterial activity against *Vibrio* spp. to provide an overall view of immune status in abalone after exposure to various biotic and environmental factors.

A number of abalone organs were screened for anti-HSV-1 activity, but only the haemolymph (20%, v/v) and the lipophilic extract of digestive gland (3,000 μg ml^{-1}) were found to substantially decrease the number and size of virus plaques (Chapter 2). Haemolymph inhibits viral infection at an early stage (e.g. viral entry) whereas the antiviral effect of the lipophilic extract is greatest when added one hour after infection (e.g. the intracellular stage of viral infection).

There was considerable variation in the levels of antiviral and antibacterial activity in the haemolymph among abalone within the same aquaculture family lines and natural populations in different geographic locations (Chapter 3). Antiviral and antibacterial

activity increased slightly with an increase in shell length. However, there was no significant effect of gender or spawning status on antiviral or antibacterial status.

Concomitant with strong antiviral activity against HSV-1 in a lipophilic extract of *Ulva lactuca* and *Spyridia filamentosa*, higher antiviral activity was detected in the digestive gland lipid extract of abalone fed *Ulva lactuca* (64.2% at 650µg ml^{-1}) or *Spyridia filamentosa* (69.51%) compared to abalone fed pellets (47.42%) or pellets supplemented with *Arthrospira maxima* (46.3%) or *Dunaliella salina* (46%) (Chapter 4). There was no influence of diet on the humoral antiviral activity, indicating antiviral factors in the haemolymph are likely to be innately biosynthesized by the abalone.

Sampling of wild-caught *H. rubra* showed a significant correlation between temperature and antiviral or antibacterial activity, with higher activity in summer than in winter months (Chapter 5). However, antibacterial activity was compromised in favour of antiviral activity as the water temperatures peaked in summer. A controlled laboratory experiment with water temperature raised from 18 to 21 or 24 °C showed that THC and SO increased at day 1 and then dropped back to control levels by days 3 and 7. By comparison, the humoral immune parameters showed a delayed response with antibacterial and antiviral activity significantly increasing on days 3 and 7, respectively. Consistent with the field study, antibacterial activity became significantly depressed after prolonged exposure to elevated temperatures. Consequently, abalone may have more resilience to viruses than bacterial pathogens under conditions of elevated temperature.

Experimental transmission trials were undertaken using an immersion model to study the abalone immune response to infection with AbHV (Chapter 6). The infection

status of abalone was confirmed by real-time PCR. THC decreased by 38.8% in moribund abalone, but increased by 42.6 and 13.6% in apparently healthy abalone that were PCR-negative and PCR-positive for AbHV, respectively, in comparison to the non-infected group. The level of SO decreased in abalone confirmed as PCR-positive for AbHV, by 30.8% in moribund abalone and by 7.2% in apparently healthy abalone. However, for apparently healthy abalone that were PCR-negative after viral challenge, SO significantly increased, by 59.3%, in comparison to uninfected controls. These results suggest that THC and SO provide potential immune markers for AbHV infection status.

In conclusion, abalone have at least two antiviral compounds with different modes of action against viral infection. Humoral antiviral factors appear to be constitutively produced and are influenced by high temperature but not by diet or infection status. Further investigation is required to establish whether the individual variability in antimicrobial activity is heritable in breeding programs and whether higher activity confers greater resistance to disease.

Acknowledgement

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On a more personal note, I would like to thank my wife and my parents for their endless love and encouragement. They always stay beside me to mentally support me throughout my study. To my son Quang Dang and my daughter An Dang, you give me so much fun and energy.

Thesis Structure

This thesis encompasses five discrete experiments, each of which is presented in an independent manuscript format. Thus, some repetition of background and methods may be found between chapters. Each chapter has independent hypotheses and aims, and these complement the overall aims mentioned in the introductory chapter. For chapters that have been published or have been accepted for publication in peer-reviewed journals, the literature citation is given on the title page of that chapter. Within each manuscript, the "study" refers to the particular experiment associated with only that chapter. References to other experiments are made by formal citation of published or submitted work. Although the nominative form "we" is used in journal submitted manuscripts of Chapter 2-6, the work presented was undertaken by myself under supervision of the coauthors.

My supervisors, Peter Speck and Kirsten Benkendorff, are listed on all papers due to intellectual input in experimental design, interpretation and feedback on manuscript drafts. Contributions of other co-authors are listed for chapters below.

- Chapter 3: Prof. Mehdi Doroudi is a named investigator on the original Seafood CRC grant that funded this project and he had intellectual input into the idea of screening different geographic populations and family lines. Ben Smith is my industry mentor and he facilitated access to farmed family lines and helped set up the spawning experiment.

- Chapter 4: Yan Li facilitated bacterial culture for antibacterial assay and helped set up the phagocytosis assay.

- Chapter 6: Dr Mark Crane is a co-investigator on the Seafood CRC grant that funded this research and he facilitated the experiments at AAHL, arranged animal ethics approval for the infection experiment and had intellectual input into the experimental design and interpretation. Dr Serge Corbeil and Lynette Williams helped set up the infection experiments and facilitated the PCR and interpretation. John Hoad facilitated cell culture work at AAHL.

Chapter 1 is a general introduction to the concepts involved in this research, as well as a presentation of the aims and significance of this work. Chapter 2 contains a manuscript, published in *Journal of General Virology*, that describes the *in vitro* antiviral activity against herpes simplex virus in abalone *Haliotis laevigata*. Chapter 3 contains a paper published in *Aquaculture* that describes variation in the antiviral and antibacterial activity of abalone *H. laevigata, H. rubra* and their hybrid in South Australia. Chapter 4 contains a manuscript, published in *Aquaculture*, which identified the effects of micro- and macro-algal diet supplementations on growth and immunity of greenlip abalone, *H. laevigata*. Chapter 5 contains a manuscript published in *Fish and Shellfish Immunology* that documents the influence of elevated temperatures on immune responses of abalone, *H. rubra*. Chapter 6 contains a manuscript, submitted to *Fish and Shellfish Immunology* (3rd July 2012), which describes immunological changes in response to herpesvirus infection in abalone *Haliotis laevigata* and *Haliotis rubra* hybrids. Chapter 7 represents a general conclusion and summary of the entire study, drawing together the results and implication from all papers.