

Final Report

Development of formulated diets for cultured abalone

**David A.J. Stone, Matthew S. Bansemer
and
James O. Harris**

Project No. 2010/736



**AUSTRALIAN
SEAFOOD
COOPERATIVE
RESEARCH CENTRE**

November 2014



This project was conducted by

Principal Investigator

Associate Professor David A.J. Stone
 SARDI Aquatic Sciences
 Phone: 08 8207 5482
 Email: David.Stone@sa.gov.au

Co-Investigators

Dr James O. Harris
 Flinders University
 Phone: 08 8201-5974
 Email: james.harris@flinders.edu.au

Dr Louise L. Adams
 University of Tasmania
 Phone: 03 6324 3818
 Email: Louise.Adams@utas.edu.au

This report may be cited as:

Stone, D.A.J., Bansemer, M.S. and Harris, J.O. (2014). Final Report. Development of formulated diets for cultured abalone. Prepared by the South Australian Research and Development Institute (Aquatic Sciences), Adelaide. CRC Project No. 2010/736. 131pp.

ISBN: 978-1-921563-68-3

Copyright, 2014: The Australian Seafood CRC Company Ltd, the Fisheries Research and Development Corporation and the South Australian Research and Development Institute.

This work is copyright. Except as permitted under the *Copyright Act* 1968 (Cth), no part of this publication may be reproduced by any process, electronic or otherwise, without the specific written permission of the copyright owners. Neither may information be stored electronically in any form whatsoever without such permission.

The Australian Seafood CRC is established and supported under the Australian Government's Cooperative Research Centres Program. Other investors in the CRC are the Fisheries Research and Development Corporation, Seafood CRC company members, and supporting participants.

Office Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042
Postal Box 26, Mark Oliphant Building, Laffer Drive, Bedford Park SA 5042
Tollfree 1300 732 213 Phone 08 8201 7650 Facsimile 08 8201 7659
Website www.seafoodcrc.com ABN 51 126 074 048

Important Notice

Although the Australian Seafood CRC has taken all reasonable care in preparing this report, neither the Seafood CRC nor its officers accept any liability from the interpretation or use of the information set out in this document. Information contained in this document is subject to change without notice.



Australian Government
Fisheries Research and
Development Corporation



An Australian Government Initiative



Non-Technical Summary

2010/736: Development of formulated diets for cultured abalone

PRINCIPAL INVESTIGATOR:

Associate Professor David A.J. Stone
Sub-Program Leader Aquaculture Nutrition and Feed Technology
SARDI Aquatic Sciences
Email:david.stone@sa.gov.au
Phone: 08 8207 5350
ADDRESS: 2 Hamra Avenue, West Beach, SA, 5022

PROJECT OBJECTIVES:

- 1. Determine optimum dietary protein requirements for juvenile (1-year-old) and sub-adult (2-year-old) greenlip abalone at 14, 18 and 22°C.**
- 2. Determine optimum dietary protein requirements for post-weaned sub-juvenile (6-month-old) greenlip abalone at 14, 17 and 20°C.**
- 3. Develop an abalone on-farm grow-out trial manual.**
- 4. Develop and test starter feeds and improved grow-out feeds for greenlip and hybrid abalone in commercial settings.**

ABSTRACT

The key research findings described in this project addressed the two highest research priorities identified by the Australian Abalone Growers' Association (AAGA) in 2009, prior to the commencement of this project:

1. Improve our understanding of the effects of seasonal water temperatures on the growth of abalone; and
2. Improve our understanding of the effects of dietary protein on the growth of abalone.

Members of the AAGA were interested in determining if multi-diet feeding strategies designed specifically to provide the optimum dietary protein level to the abalone in response to animal age and seasonal fluctuations in water temperature improve production. Additionally, the planned overall outcome from this project was to develop commercial diet formulations and feeding strategies that deliver a >10% improvement in productivity across an entire grow-out period for greenlip (*Haliotis laevis*) and hybrid abalone (*H. laevis* × *H. rubra*). To achieve this outcome, a series of laboratory-based experiments were designed to improve our understanding of the optimum dietary protein levels for greenlip abalone and also characterise the growth and feed utilisation of greenlip abalone of different age classes at a range of seasonal temperatures (14 - 22°C) representative of those experienced by abalone in land-based facilities in southern Australia (Chapters 2 and 3). This information was then used to design and run three commercial on-farms trials at Great Southern Waters, Coastal Seafarms and Kangaroo Island Abalone (Chapters 4 and 5). The on-farm trials comprised a series of three long-term (>18 months) studies, using commercial culture practices, to evaluate the growth, feed utilisation and survival of greenlip and hybrid abalone using two different feeding strategies:

1. Single-diet feeding strategy: the current production method of feeding one standard protein diet for the entire trial;

2. Multi-diet feeding strategy: fed a sequential combination of “high protein” / “low protein” grow-out diets for the entire trial.

Results from the laboratory-based experiments demonstrated that water temperature significantly affected the specific growth rate (SGR) of three age classes of greenlip abalone (6-month-old, 1-year-old and 2-year-old). The growth of the two younger classes of abalone was improved as water temperatures increased from 14 to 18 and 14 to 22°C; however, the growth rate of the larger 2-year-old abalone was only improved as temperatures increased from 14 to 18°C. Based on this information, it was deemed plausible to shorten the commercial production cycle of abalone by heating land-based nursery systems in order to gain accelerated growth before transfer to grow-out systems. This concept is currently being evaluated on-farm by several of AAGA companies, with promising results.

The optimal dietary crude protein (CP) levels were identified for all three age classes of abalone and were all influenced by water temperature. We recommended that the dietary protein levels for commercial greenlip and hybrid abalone diets were altered to ensure that higher protein diets are used during periods of rapid growth. Australian feed companies now produce commercial abalone grow-out diets containing ~35% CP. Australian abalone farmers use the new diets routinely to improve productivity.

An abalone on-farm grow-out trial manual was developed in consultation with AAGA and participating feed companies and used to standardise the evaluation of multi-diet versus single diet feeding strategies for abalone. The new high protein commercial diets were used in combination with the pre-existing standard protein diets to develop the multi-diet feeding strategies that were evaluated in three long-term (>18 months) on-farm trials run at Great Southern Waters, Coastal Seafarms and Kangaroo Island Abalone (Chapter 5).

Results were positive. Great Southern Waters reported a 9.3% improvement in biomass gain of hybrid abalone with the high protein diet feeding strategy. This was achieved with no differences in survival, minimal increases in feed input and a 7% improvement in feed conversion ratios (FCR). This then resulted in a 9.5% improvement in sales revenue after feed costs were included. There are numerous benefits of producing larger abalone quicker. Of most relevance, the overall duration of a typical three year production cycle for hybrid abalone may be shortened by 1.9 to 3.4 months by feeding high protein diets.

This response was not even across farm trials and was due to several factors, including insufficient replication, resulting in a reduction of statistical power and the ability to discern significant differences between treatments. In order to achieve adequate statistical power (Power of test, $P = 0.80$), we recommend eight replicate slab tanks per treatment in all future on-farm studies. Both farms, which produced inconclusive results, used thinning practices of harvesting and restocking with middle graded abalone to reduce stocking densities. This practice effectively diluted growth responses. Future trials should use controlled thinning by partially harvesting to reduce tank biomass to avoid over-crowding, as practiced during the trial at Great Southern Waters. In retrospect, in the early stages of the development of multi-diet feeding strategies for abalone, it may have been more beneficial to prove the concept of improved abalone growth on these farms using a high protein diet alone for the entirety of the trials.

Overall, the use of alternate feeding strategies investigated in this study, compared to the previous practice of feeding the standard protein level diet alone, were advantageous, and would result in savings for both fixed and variable costs during the commercial production of land-based abalone. Considerable savings could also be gained from reduced summer mortality of stock during shortened production cycles. By adopting the alternate feeding strategies and diets, farmers may harvest abalone sooner, and reduce exposure to one less summer. This factor alone could result in improved productivity, and when combined with

savings made with improvements in biomass and feed efficiency gains, we could expect a >10% improvement in sales revenue across the entire grow-out period for cultured abalone.

OUTCOMES ACHIEVED

The planned outcome of the project, to deliver a >10% improvement in productivity across an entire grow-out period for greenlip and hybrid abalone to industry has been achieved.

In order to achieve the planned outcome, four Objectives were established, in consultation with AAGA and the participating feed companies, and a series of laboratory based experiments (Chapters 2 and 3), and on-farm commercial trials (Chapters 4 and 5) were designed and carried out to completion. The four Objectives, along with the outcome for each, are provided below:

Objective 1: *Determine optimum dietary protein requirements for juvenile (1-year-old) and sub-adult (2-year-old) greenlip abalone at different temperatures*

Objective 2: *Determine optimum dietary protein requirements for sub-juvenile (6-month-old) greenlip abalone at different temperatures*

The outcomes for Objectives 1 and 2 were achieved.

- Prior to this project, Australian feed companies produced commercial diets containing crude protein levels of 25 - 30% for the production of greenlip and hybrid abalone in Australia; this has now changed to 35% (Chapters 2, Stone et al. 2013; Chapter 3, Bansemer et al. 2015).
- Australian abalone farmers use the new diets routinely to improve productivity.
- Optimum dietary protein levels for greenlip abalone also varied with age and water temperature (Chapters 2, Stone et al. 2013; Chapter 3, Bansemer et al. 2015).
- It was also deemed plausible to shorten the commercial production cycle of abalone by heating land-based nursery systems to gain accelerated growth (\geq 50%) before transfer to grow-out systems. This concept is currently being evaluated on-farm by several of the AAGA companies, with promising results.

We identified potential scope to go forward and design on-farm trials (Chapter 4) to evaluate the concept of multi-diet feeding strategies on-farm in the production of greenlip and hybrid abalone with the aim of improving growth and feed utilisation and to reduce the duration of the production cycle (Chapter 5).

Objective 3: *Develop an abalone on-farm grow-out trial manual*

The outcome from Objective 3 was achieved.

- Following extensive consultation with AAGA members, staff of participating abalone feed companies and research providers, a manual was produced (Chapter 4, Appendix 4), distributed and used successfully for the on-farm feeding strategy trials with greenlip and hybrid abalone (Chapter 5).

Objective 4. *Develop and test starter feeds and improved grow-out feeds for greenlip*

and hybrid abalone in commercial settings

The outcomes from Objective 4 were achieved.

- Based on results in Chapters 2, 3, 4 and 5, a recommendation was made to the project stakeholders to adopt multi-diet feeding strategies for abalone production.
- A food conversion ratio (FCR) improvement of 7% and a biomass gain of 9.3% combined to give us an annual 9.5% increase in basic sales revenue for hybrid abalone fed the high protein diet during the Great Southern Waters trial (Chapter 5).
- The overall duration of a typical three year production cycle of abalone may be shortened by 1.9 to 3.4 months. By shortening the production cycle AAGA farmers may reduce the mortality rate of larger, more valuable, abalone by reducing their exposure to a third summer.
- Australian feed companies now offer commercial abalone grow-out diets of varying protein levels.
- AAGA members have adopted the use of feeding new high protein diets, or multi-diet feeding strategies.

Overall, the outcome of the development of diet formulations and feeding strategies that deliver a >10% improvement in productivity across the entire grow-out period for abalone to the industry was achieved.

The Australian abalone producers now have the confidence to use newly formulated high protein diets, produced by Australian feed companies, in combination with previously existing diets to improve weight gain, feed utilisation and productivity. Additionally, AAGA members, Australian feed companies and research providers worked closely to achieve these goals. All groups have identified the importance of nutritional research in relation to growth and health and improved productivity for abalone farms. The confidence developed in gaining a significant return on their research and development investment during this project has resulted in AAGA members deciding to pursue this line of research into the future.

AAGA members, feed companies representatives and researchers have agreed on the need to increase our understanding of abalone nutrition, physiology and health. This has led to the development of a proposal for the establishment of an Abalone Research Centre for Excellence, based in South Australia.

LIST OF OUTPUTS PRODUCED

The majority of the research described in this project has been extended to the broader scientific community. Apart from the extension of results to project stakeholders, numerous presentations were given by project participants to extend the information arising from this project. Project information was extended to other members of the aquaculture and feed industry, government departments, the general public and members of the scientific community. Information was disseminated at domestic and international scientific conferences, ASCRC and industry workshops and directly to feed company representatives.

Some of the most notable research outputs include:

Reports and publications (Appendix 1)

- One final report;

- One on-farm feed trial manual;
- Seven peer-reviewed scientific publications;
- Three Honours theses;
- One Masters thesis;
- Eleven conference abstracts; and
- Numerous industry presentations.

Student training (Appendix 2)

There has been a considerable student training component to this project, resulting in the training of:

- Greater than forty undergraduate extra-mural work experience students;
- Five part-time undergraduate student projects;
- Four Honours students (Three students completed and one student to complete by May 2015);
- One Masters Student; and
- Seven PhD students (all current and due for completion in 2015 and 2016).

All of the Honours and postgraduate students presented their research at industry meetings, and domestic and international conferences. Several students have also published their research in internationally peer-reviewed journals (Appendix 1). A major benefit of the student training component is the output of new industry entrants, trained with relevant skills that will contribute to future industry development. Several students have already obtained work within the industry.

Other peripheral research associated with the project has provided great insight into the survival, energetics, digestive tract health, water quality and product quality of a range of age classes of greenlip and hybrid abalone in relation to diet manipulation at optimal and sub-optimal water temperatures. This information is essential in improving feeding practices on-farm, especially in relation to times of high water temperatures when oxygen levels are at their lowest (Appendices 1 and 2).

ACKNOWLEDGEMENTS

This research was supported by the Fisheries Research and Development Corporation (FRDC) and the Australian Seafood Cooperative Research Centre (ASCRC) and seafood industry partner the Australian Abalone Growers' Association.

The authors would also like to acknowledge the support of the South Australian Research and Development Institute (SARDI) and Marine Innovation Southern Australia (MISA) for their financial support of Dr David Stone and the provision of the SARDI Aquatic Sciences experimental facilities at West Beach, SA.

The authors would also like to thank the following people for their direct and valuable contributions:

Mr Tim Rudge, Miss Sarah Smith and Mr Liam Bryce of Coastal Seafarms, Portland, Victoria.

Mr Anton Krsinich and Miss Lucy Saunders of Great Southern Waters Pty Ltd, Indented Head, Victoria.

Mr David Connell of Kangaroo Island Abalone, South Australia.

Mr Joel Scanlon of Aquafeeds Australia (formerly Adam and Amos Abalone feeds) for his input into experimental design and diet formulations for the laboratory experiments.

Dr Tom Coote and Mr Kym Heidenreich of Eyre Peninsula Aquafeeds for their input into experimental design and diet formulations for the laboratory experiments.

Dr Rhys Hauler, Dr Matthew Bransden and Dr Nicole Ruff of Skretting Australia for their input into experimental design and diet formulations for the laboratory experiments.

Mr Raymond Cultura of SARDI Aquatic Sciences, Australasian Experimental Stockfeed Extrusion Centre, for the manufacture of the experimental diets.

Hanru Wang for her technical work during the laboratory-based experiments at SARDI Aquatic Sciences, West Beach.

Numerous Flinders University, University of Adelaide and University of Tasmania students, including Krishna-Lee Currie, Elise Schaefer, Duong Ngoc Duong, Thanh Hoang Hai, Brett Lange, Georgia Mercer and Kurniati Nur for their technical assistance during the laboratory-based experiments at SARDI Aquatic Sciences, West Beach.

Mr Bansemer holds an Australian Seafood Cooperative Research Centre scholarship (2011/251). The authors would also like to acknowledge this support.

Contents

Chapter 1. Introduction.....	6
1.1 Need	6
1.2 Objectives	7
Chapter 2. The determination of the optimum protein requirements for juvenile and sub-adult greenlip abalone at water temperatures of 14, 18 and 22°C	8
Abstract.....	8
Introduction	9
Aim.....	10
Materials and Methods	10
Results	14
Discussion.....	21
Conclusions and Recommendations	24
Chapter 3. The determination of the optimum protein requirements for post-weaned sub-juvenile greenlip abalone at 14, 17 and 20°C	25
Abstract.....	25
Introduction	26
Aim.....	26
Materials and Method.....	27
Results	29
Discussion.....	33
Conclusions and Recommendations	37
Chapter 4. The development of an on-farm abalone grow-out trial manual.....	38
Abstract.....	38
Introduction	39
Materials and Methods	39
Conclusions and Recommendations	39
Chapter 5. Commercial validation of improved feeding strategies for abalone.....	40
Abstract.....	40
Introduction	41
Aim.....	41
Materials and Methods	42
Results	48
Discussion.....	61
Conclusions and Recommendations	66
Chapter 6. General Discussion.....	67
Chapter 7. Benefits and Adoption.....	73
Chapter 8. Further Development	74
Chapter 9. Planned Outcomes	75
CRC Output	76

Public Benefit Outcomes	76
Private Benefit Outcomes.....	77
Linkages with CRC Milestone Outcomes.....	77
Chapter 10. Conclusion.....	77
Chapter 11. References	79
Chapter 12. Appendices.....	85
Appendix 1. Publications, reports and presentations arising from this project.....	I
A1.1 Manuscripts for Peer-Reviewed Journals.....	I
A1.2 Reports	II
A1.3 Abstracts and Presentations at Scientific Conferences	II
A1.4 Presentations at other Meetings.....	III
Appendix 2. Student project details	VI
A2.1 PhD Student Projects.....	VI
A2.2 Masters Student Project.....	VI
A2.3 Honours Student Projects	VI
Appendix 3. Basic increase in sales revenue calculations for hybrid abalone during the Great Southern Waters on-farm trial.....	VIII
A3.1 Background.....	VIII
A3.2 Methods	VIII
A3.3 Results.....	VIII
A3.4 Conclusions and Recommendations	IX
Appendix 4. Abalone on-farm grow-out trial manual.	XI
A4.1 Introduction.....	XV
A4.1.1 Potential of multi-diet feeding strategies.....	XV
A4.1.2. Purpose of the on-farm manual.....	XV
A4.2 Aim	XVII
A4.3 Materials and Methods.....	XVII
A4.3.1 Overview of on-farm experimental design	XVII
A4.3.2 Dedicated farm staff for on-farm trial	XIX
A4.3.3 Experimental animals.....	XIX
A4.3.4 Experimental system.....	XIX
A4.3.4.1 <i>Allocation of treatments to culture tanks</i>	XIX
A4.3.4.2 <i>Preparation of culture tanks</i>	XXI
A4.3.5 Stocking of trials.....	XXI
A4.3.5.1 <i>Example of systematic interspersation for stocking of trials</i>	XXII
A4.3.5.2 <i>Tagging procedures (optional)</i>	XXIII
A4.3.6 Feeds, feed storage and feeding.....	XXIII
A4.3.6.1 <i>Feeds</i>	XXIII
A4.3.6.2 <i>Feed storage</i>	XXIII
A4.3.6.3 <i>Feeding</i>	XXIV
A4.3.7 Sampling procedures for weight checks and harvesting	XXIV
A4.3.7.1 <i>Labelling of bags for sample collections</i>	XXIV

A4.3.7.2 <i>Monthly weight checks</i>	XXV
A4.3.7.2.1 Method to sample abalone at weight checks	XXV
A4.3.7.3 <i>Three monthly weight checks</i>	XXVI
A4.3.7.4 <i>Harvesting at 9 months to reduce stocking density</i>	XXVI
A4.3.7.5 <i>Re-stocking at 9 months with reduced stocking densities</i>	XXVII
A4.3.7.5.1 Method to randomly select abalone from middle 1/3 of culture tank for restocking.....	XXVIII
A4.3.7.6 <i>Harvesting at 18 months at the completion of the trial</i>	XXVIII
A4.3.8 Summary of data collection and staff allocation for the trial	XXIX
A4.3.8.1 <i>Summary for stocking</i>	XXIX
A4.3.8.2 <i>Summary for monthly weight checks</i>	XXX
A4.3.8.3 <i>Summary for three monthly weight checks</i>	XXX
A4.3.8.4 <i>Summary for intermediate stocking density reduction harvest</i>	XXX
A4.3.8.5 <i>Summary for intermediate re-stocking</i>	XXXI
A4.3.8.6 <i>Summary for final harvest</i>	XXXI
A4.3.9 Calculation of growth performance and feed efficiency indices.....	XXXII
A4.3.10 Environmental monitoring of experimental systems.....	XXXII
A4.3.11 Tank cleaning and mortality monitoring and reporting	XXXIII
A4.3.12 Statistical analyses.....	XXXIV
A4.4 Acknowledgements	XXXV
A4.5 References	XXXVI
A4.6 Appendix A4.1	XXXVII
A4.6.1 Tagging method	XXXVII

List of figures

Figure 2.1. a & b. Dry matter leaching loss of experimental abalone diets at three water temperatures (Fig. 2.1a; 14, 18 & 22°C) at 1, 2, 4, 8 & 16 h post submersion (Fig. 2.1b).	14
Figure 2.2. a & b. Specific growth rate of 1-year-old (Fig. 2.2a) and 2-year-old (Fig. 2.2b) abalone fed a range of dietary protein levels (1-year-old: 27, 30, 33 & 36%; 2-year-old: 24, 27, 30 & 33%) at three different water temperature for 84 d.	15
Figure 2.3. a & b. Apparent economic feed conversion ratio of 1-year-old (Fig. 2.3a) and 2-year-old (Fig. 2.3b) abalone fed a range of dietary protein levels (1-year-old *: 27, 30, 33 & 36%; 2-year-old: 24, 27, 30 & 33%) at three different water temperature for 84 d.	19
Figure 2.4. a & b. Protein deposition for 1-year-old (Fig 2.4a) and 2-year-old (Fig. 2.4b) abalone fed a range of dietary protein levels (1-year-old: 27, 30, 33 & 36%; 2-year-old: 24, 27, 30 & 33%) at three different water temperature for 84 d.	21
Figure 3.1. Specific growth rate of greenlip abalone at three water temperature fed four dietary protein levels for 91 d.	31
Figure 3.2. Feed consumption rate for greenlip abalone fed four dietary protein levels at three water temperature for 91 d.	32
Figure 3.3. Apparent economic feed conversion ratio (FCR) for greenlip abalone fed four dietary protein levels at three water temperature for 91 d.	32
Figure 5.1. Flow diagram of the timing of events used for the 18 month on-farm trials.	46
Figure 5.2. Water temperatures in slab tanks for each feeding strategy treatment over the entire on-farm trial at Great Southern Waters (15/11/12 - 30/5/14; 561 d).	49
Figure 5.3. Dissolved oxygen levels in slab tanks for each feeding strategy treatment over the entire on-farm trial at Great Southern Waters (15/11/12 - 30/5/14; 561 d).	49
Figure 5.4. Tank salinity levels in slab tanks for each feeding strategy treatment over the entire on-farm trial at Great Southern Waters (15/11/12 - 30/5/14; 561 d).	49
Figure 5.5. Tank pH levels in slab tanks for each feeding strategy treatment over the entire on-farm trial at Great Southern Waters (15/11/12 - 30/5/14; 561 d).	50
Figure 5.6. Individual weights of hybrid abalone fed the standard or high protein diet feeding strategies at Great Southern Waters over the duration of the entire trial (15/11/12 - 30/5/14; 561 d, 18.3 months).	51
Figure 5.7. Water temperatures profile in slab tanks over the entire on-farm trial at Coastal Seafarms (11/9/12 - 26/6/14; 653 d).	53
Figure 5.8. Individual weights of greenlip abalone fed the single or multi-diet feeding strategies during Phase 1 (11/9/12 to 13/9/13, 12 months) of the on-farm trial at Coastal Seafarms.	54
Figure 5.9. Individual weights of greenlip abalone fed the single or multi-diet feeding strategies during Phase 2 (13/9/13 to 26/6/14, ~21 months) of the on-farm trial at Coastal Seafarms.	56
Figure 5.10. Water temperature profile over the entire duration of the on-farm trial at Kangaroo Island Abalone (11/5/12 - 28/3/14; 686 d; ~23 months).	58
Figure A4.1. Flow diagram of the timing of stocking, sampling and harvest events used for the 18 month on-farm trials.	XVIII
Figure A4.2. An example of random or systematic allocation of the different feeding strategy treatments to the culture tanks.	XX
Figure A4.3. Description of the location for sample collection at monthly and 3 monthly weight checks.	XXVI
Figure A4.4. Diagram of the area used (middle third of culture tank) to randomly collect abalone for re-stocking for second phase of experiment.	XXVIII

List of tables

Table 2.1. Ingredient and nutrient composition of experimental diets.	12
Table 2.2. Summary of water quality for each temperature system.	13
Table 2.3. Growth performance and soft tissue composition of 1-year-old greenlip abalone.	16
Table 2.4. Growth performance and soft tissue composition of 2-year-old greenlip abalone.	17
Table 3.1. Summary of water quality for each water temperature system.....	29
Table 3.2. Growth performance, feed efficiency and nutrient retention of greenlip abalone at three water temperature fed four dietary protein levels.....	30
Table 5.1. The biochemical composition of the commercial diets used in the on-farm trials.	43
Table A4.1. Water quality variables and measurement frequencies for on-farm trial tanks.	XXXIII

Chapter 1. Introduction

The value of abalone cultured in Australia has developed from <\$1 million in 1998 - 1999 to more than \$17 million (includes scallops, abalone and giant clams) in 2007 - 2008 (ABARE 2010) and approximately 960 tonnes of cultured abalone were produced in 2013 valued at approximately \$34 million (Australian Abalone Growers' Association (AAGA) personal communication). Abalone aquaculture in Australia began by examining work done in other countries (Cropp 1989), and recommending that kelp be used, as it is in other countries, as a food. It was rapidly identified that this was an inappropriate and uneconomical food, which led to research into food for abalone. McShane et al. (1994) identified that the toughness of kelp was a major deterrent for the use of this alga for Australian temperate abalone, and Hone (1992) led a workshop on artificial diets for abalone, which identified many areas requiring research in order to develop artificial diets for our species. Much of the development of this industry was underpinned by work done through the Fisheries Research and Development Corporation's (FRDC) Abalone Aquaculture Subprogram (AAS), which funded research into production constraints identified at the time of this subprogram (1994 - 2006). Examples of this previous work include research into protein and lysine (Coote 1998), environmental (Burke et al. 2001), and lipid requirements (Dunstan et al. 2001).

During the development of any new species for culture, there are several stages that require investigation at the laboratory level, and confirmation at farm-scale operations. This covers areas such as environmental requirements, stocking densities, reproductive biology, and nutrition and growth of larvae, juveniles and adults. Much of this work was done within the AAS, but since this time, farmers have had the opportunity to evaluate if the completed work addressed their concerns, and to fine-tune the next round of questions that need addressing.

It has become apparent that although substantial research was conducted into the development of appropriate diets for Australian abalone, more is required. Fleming et al. (1996) reviewed the research conducted on artificial diets for abalone, including Australian species. Research by Van Barneveld et al. (1998) into oil type and level and their interaction with other nutrients, Coote (1998) into protein, lipid and amino acid nutrition for juvenile greenlip abalone at 20°C, and Dunstan et al. (2001) into the level and type of oil, provided information needed for diet formulation in terms of oils. Research within the AAS resulted in protein levels being set at 35% on a dry weight basis, carbohydrates at 60% and vitamins and minerals at 4.5% (Fleming et al. 1996). The crude protein values were then re-evaluated for juvenile green lip abalone by Coote (1998) and Coote et al. (2000) and reported to be ~27%. Although several studies contributed to this formulation, all used juvenile abalone at single temperatures, therefore, no effort was put in to distinguish if nutritional requirements differ once the abalone begin to mature. In 2009, the AAGA met and came up with a set of priorities, which ranked nutritional research highly. Therefore, in spite of the background research done on temperate Australian abalone, perceptions exist that the diets could be improved and formulated to meet the protein requirements of abalone at different life stages and water temperatures.

1.1 Need

It was estimated that 1000 tonnes of formulated feeds were used to achieve the current level of production of cultured Australian abalone in 2010. Feed is considered as the major variable cost (up to 30%) associated with abalone production (Mr Justin Fromm, personal communication), so minor improvements in feeds or feed efficiency may result in large improvements in productivity. There are currently three major feed manufacturers supplying

the abalone grow-out sector in Australia and, prior to this project, each feed company typically offered one formulation of feed for the entire 2.5 to 3 year grow-out stage of the production cycle of greenlip or hybrid abalone. It is a common practice in other sectors of the livestock industry to use a range of different diet formulations throughout the production cycle to satisfy the requirements of animals of different life stages and during different seasons. It is also well established that wild abalone have at least two distinct feeding strategies as they develop with young cryptic abalone grazing on benthic organisms whilst older animals feed on macroalgae.

It was considered that improvements in commercial feeds formulated for abalone, specifically for different life stages and/or water temperatures (seasonal/inter-annual/climate change), were likely to deliver improvements in productivity across an entire grow-out period. As a result of a research and development planning meeting held by AAGA, other industry participants, the Australian Seafood Cooperative Research Centre (ASCRC) and research providers in 2009, the AAGA perceived that the current commercial abalone feeds did not contain the required nutrient combinations to meet the genetic potential for growth.

Australian abalone feed producers had based dietary formulations on information from previous FRDC funded projects. The information from the FRDC projects, including a range of ingredient nutrient availability and requirement data, resulted in the standard of Australian abalone feeds surpassing Japanese abalone feeds (considered as the benchmark at the time). AAGA had identified research in this area to be their highest priority within the ASCRC.

The need to optimise artificial diets for abalone fits within the Seafood CRC's research Program 1 - Production Innovation. It is expected that improvements in the efficiency of abalone culture, either in terms of a higher biomass at the end of the standard culture period, or by shortening the culture period, will lead to increases in the production and profitability of cultured abalone. Consultation with AAGA members resulted in the following research objectives for this project.

1.2 Objectives

There were four objectives in this project:

1. Determine optimum dietary protein requirements for juvenile (1-year-old) and sub-adult (2-year-old) greenlip abalone at 14, 18 and 22°C (Chapter 2);
2. Determine optimum dietary protein requirements for post-weaned sub-juvenile (6-month-old) greenlip abalone at 14, 17 and 20°C (Chapter 3);
3. Develop an abalone on-farm grow-out trial manual (Chapter 4); and
4. Develop and test starter feeds and improved grow-out feeds for greenlip and hybrid abalone in commercial settings (Chapter 5).

Chapter 2. The determination of the optimum protein requirements for juvenile and sub-adult greenlip abalone at water temperatures of 14, 18 and 22°C

David A.J. Stone^{1,2,3,4*}, James O. Harris^{2,4}, Hanru Wang³, Georgia J. Mercer², Elise N. Schaefer², Matthew S. Bansemer².

^{1*} South Australian Research and Development Institute, Aquatic Sciences Centre, 2 Hamra Ave, West Beach, 5024, SA, Australia

² Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide, 5001, SA, Australia

³ University of Adelaide, School of Animal and Veterinary Sciences, Faculty of Sciences, Roseworthy, 5371, SA, Australia

⁴ Marine Innovation Southern Australia

*Corresponding author: Phone: +61-8-8207 5350, Fax: +61-8-8207 5481, E-mail: david.stone@sa.gov.au

Abstract

Land based grow-out of greenlip abalone (*Haliotis laevis*) in Australia is predominantly practiced using a single diet feeding strategy despite geographical and seasonal differences in water temperature which influence feed intake and growth. This 12 week study investigated the interactions between two abalone year classes (1-year-old, 1.8 g; 2-year-old, 22.9 g), three water temperatures (14, 18, 22°C) and four dietary protein levels (1-year-old, 27, 30, 33, 36% crude protein (CP); 2-year-old, 24, 27, 30, 33% CP) to evaluate the potential to use diets to suit specific water temperatures and aged animals. Diets were formulated to be iso-energetic (~12.5 MJ kg⁻¹ digestible energy), contain fat levels of ~3.6% and digestible protein levels ranging from 17.99 to 28.57%. Feed was fed to excess daily and uneaten food was collected. Temperature significantly affected the specific growth rate (SGR) of both year classes of abalone. There was no significant effect of dietary protein level on SGR; however, abalone compensated for a reduction of dietary protein by consuming more feed. This was evident with significant increases in feed conversion ratios (FCR) as dietary protein levels decreased for both year classes. With the exception of abalone grown at 14°C, where protein deposition decreased with increasing dietary protein level, protein deposition increased with an increase in dietary protein for both year classes at higher water temperatures. For 1-year-old abalone, as temperature increased from 14 to 22°C the optimum crude dietary protein levels increased from ~29 to ~35%. For 2-year-old abalone, the optimum crude protein level appeared to be lower, and increased from 24% at 14°C to 34% at 22°C. There is scope to use multi-diet feeding strategies in the production of greenlip abalone (Chapter 5). We recommend that the dietary protein levels for commercial greenlip abalone are altered to ensure that higher protein diets are used during periods of rapid growth which may be attained, at, and above, the previously reported optimal water temperature of 18°C (~35% CP).

The work described in this Chapter addressed Objective 1 of this project and has been published in the international peer reviewed journal, Journal of Shellfish Research: Stone D.A.J., Harris J.O., Wang H., Mercer G.J., Schaefer E.N., Bansemer M.S. 2013. Dietary protein level and water temperature interactions for greenlip abalone *Haliotis laevis*. Journal of Shellfish Research 32, 119-130.

Introduction

The aquaculture production of Australian greenlip abalone (*Haliotis laevis*) occurs predominantly in land-based systems, which experience large seasonal fluctuations in water temperatures, and are reliant on formulated feeds. Typically, once juvenile greenlip abalone are weaned off microalgae they are on-grown using a single diet feeding strategy with crude protein levels in the range of 27 - 30%. This range is based on previous research conducted by Coote et al. (2000) on one size class of juvenile greenlip abalone (0.5 - 2.5 g) at 20°C. Wild abalone have a distinct shift in dietary preference over their life cycle, initially feeding on microalgae, then as sub-adults shifting to a predominately macroalgae diet (Won et al. 2010). The reported protein levels (dry matter basis) of microalgae and macroalgae used for cultured abalone range from 12 - 35% (Brown et al. 1997) and 11 - 19% (Mai et al. 1994), respectively. This shift suggests that their nutritional requirements may change over time, with respect to protein. There is significant scope to capitalise on this shift in feeding behaviour, and in combination with investigations into protein requirements at fluctuating seasonal water temperatures, develop and implement multi-diet feeding strategies aimed at improving the productivity of the greenlip abalone culture industry.

Temperature is an important environmental factor and significantly impacts on the growth of organisms (Hochachka and Somero 2002). Water temperatures experienced during the grow-out of greenlip abalone seasonally oscillate between 10 to 25°C. The optimum temperature for growth, and the 50% critical thermal maxima, of greenlip abalone (82 mm shell length [SL]) were reported to be 18.3 and 27.5°C, respectively (Gilroy and Edwards 1998). Steinarsson and Imsland (2003) reported size dependent variation in the optimum growth temperature of red abalone (*H. rufescens*) with temperature optimum for growth having a symmetrical, concave relationship with shell length from 16 mm to maturity, reaching a peak of 17.8°C at 44 mm SL. Cho and Kim (2012) investigated the effects temperature (20 - 26°C) on the growth performance of juvenile (4.7 g) *H. discus hannai* and reported an optimum of 20°C for their culture. Green et al. (2011) reported a significant reduction in the growth rate of *H. midae* as temperature increased from 18 to 24°C.

The reported optimal dietary protein levels for a range of abalone species are variable (Fleming et al. 1996). Uki et al. (1986) and Taylor (1992), using casein as the main protein source, reported optimal crude protein levels of 38% for *H. discus hannai*, and at least 30% for *H. kamtschatkana*, respectively. Sales et al. (2003), also using casein as the main dietary protein source, reported an optimum protein level of 36% (dry matter basis) for 5 g *H. midae* cultured at 16°C, and reported depressed growth at a dietary protein level of 28%. These findings differ from the lower optimum crude protein level of 27% reported for juvenile greenlip abalone (0.6 - 2.5 g) cultured at 20°C (Coote et al. 2000). Similarly, Bautista-Teruel and Millamena (1999) reported an optimum crude protein level of 27% for juvenile *H. asinina* (0.6 - 3.0 g) at 27 to 31°C. Some initial research has been carried out to investigate the interactions of animal size and dietary protein level with a view to develop multi-diet feeding strategies in related abalone species. Britz and Hecht (1997), reported maximum growth of *H. midae* at 18°C occurred in large (7.8 ± 0.25 g) abalone fed a crude dietary protein level of 44%, while the maximum growth occurred in small (0.2 ± 0.01 g) abalone at 34%. Shipton and Britz (2001a) also demonstrated differences in the protein requirements of juvenile (10 - 20 mm initial SL; 0.5 g) and young adult (40 - 50 mm initial SL; 20 g) *H. midae*.

Upon reviewing the results from the above studies it becomes apparent that variables such as species, animal size, water temperature and nutrient digestibility may play a pivotal role in the differences observed. All of the above studies used “high quality” protein sources, and while some studies gave consideration to the “ideal protein ratio” concept, no studies formulated on a digestible protein basis. Protein is an expensive, yet essential dietary

component for abalone and it is crucial that it is palatable, digestible and has an adequate amino acid profile to support maximum protein deposition and growth (Britz 2006a, Britz and Hecht 1997; Bautista-Teruel et al. 2003).

Aim

This study aims to investigate the interactions of dietary protein level and water temperature on the growth performance and feed utilisation of 1- and 2-year-old age classes of greenlip abalone. In this study, test diets were formulated to ensure a gradation of digestible protein levels to cover the range considered to be commercially applicable to land-based production of abalone in south-eastern Australia. We selected a range of highly palatable and digestible ingredients, formulated at realistic inclusion levels, using protein and energy digestibility data reported for greenlip abalone by Fleming et al. (1998) and Vandeppeer (2005). With respect to amino acid composition, diets were formulated using the “ideal protein ratio” concept using soft tissue amino acid values for similar sized greenlip abalone reported by Coote et al. (2000). All diets contained ~3.5% lipid, as recommended for greenlip abalone by Van Barneveld et al. (1998) and Dunstan et al. (2000), and contained crude and digestible energy levels of ~17.4 and ~12.5 MJ kg⁻¹, respectively. The diets were tested at a range of seasonal temperatures (14, 18, 22°C) representative of those experienced by growing abalone in land-based facilities in south-eastern Australia.

Materials and Methods

Experimental animals

Greenlip abalone of two distinct year class cohorts (1- and 2-year-olds were spawned in September 2010 and 2009, respectively) were purchased from South Australian Mariculture at Boston Point, Port Lincoln, South Australia in September 2011. Abalone of each year class were fed a commercial diet (Eyre Peninsula Aquafeed Pty Ltd, Lonsdale, South Australia, Australia) prior to the experiment and had not been previously used for any other experiments. Upon arrival at the SARDI Aquatic Science Centre, West Beach, Adelaide the abalone were placed in a flow through seawater system overnight and then stocked into the experimental system at ambient water temperature (15°C) over the following two days.

Experimental system

The temperature (20 ± 1°C) and photoperiod (12 h low intensity fluorescent lighting at 3.4 Lux; equates to dark limit of civil twilight under a clear sky: 12 h dark) controlled experimental facility consisted of three identical temperature controlled (14, 18, 22°C) salt water systems supplied with flow-through UV treated seawater (Model 025120-2, 120 w, Emperor Aquatics, Pottstown, PA, USA). Each system was comprised of a 780 L sump, 780 L intermediate tank, 780 L header tank (Solid Nally MegaBins, MS7800; Viscount Plastics Pty Ltd., Hawthorn East, Vic, Australia) and thirty six 12.5 L blue plastic culture units (Nally IH305, Viscount Plastics Pty Ltd.; length, 39.2 cm; width, 28.8 cm; 11.0 cm depth; bottom surface area of 1,129 cm²) with a water depth of 2.5 cm. Water temperature control was achieved by using either chillers (3 hp 240 v 50 hz; Daeil Cooler Co., Ltd., Busan, Korea) or 3kw immersion heaters (240 V, 3 kw, JQ20; Austin and Cridland, Carlton, NSW, Australia). The culture units were each provided with temperature controlled flow-through water from the reservoir by gravity feed at a rate of 300 mL min⁻¹. Water level was set at 2.5 cm in each culture unit using a standpipe with a mesh screen (0.8 mm nominal mesh size) on the outlet to retain uneaten food.

Stocking

Abalone were anaesthetised using 1 mL (1-year-old, n = 960) or 2 mL (2-year-old, n = 480) of Ethyl p-aminobenzoate (Sigma Chemicals, Balcatta, WA, Australia) L⁻¹ of seawater in a 40 L plastic container with 30 L of oxygenated ambient temperature (15°C) seawater. Twenty 1-

year-old and ten 2-year-old animals were screened from larger populations, weighed, measured and stocked, using systematic interspersions, into four replicate culture units treatment⁻¹ combination. A sample of abalone from each year class were also collected, shucked and stored frozen at -20°C for proximate analysis. Due to the low ambient water temperature (15°C) at stocking, a one week acclimation period was used to slowly raise or lower the water temperature (~1°C d⁻¹) to the desired temperatures of 14, 18 or 22°C. The water temperature was then maintained at the desired treatment temperatures (± 1.0°C) throughout the remainder of the 84 d experiment. Dead abalone were recorded, measured, weighed and replaced with abalone of a similar weight and size that had been held at the same treatment water temperature and fed a commercial diet.

Diets and feeding

The proximate composition of the ingredients was analysed. The diets were then formulated, mixed and extruded into flat pellets using a commercial pasta-machine (La Prestigiosa medium, IPA, Vicenza, Italy) and cold pressing technology, and contained nominal protein levels of 27, 30, 33, 36% crude protein for 1-year-old abalone and 24, 27, 30 and 33% crude protein for 2-year-old abalone. The ingredient composition and chemical composition of the diets are displayed in Table 2.1. All diets were formulated to be iso-energetic on a digestible basis. The diets were also formulated, using book values, so the ratio of each essential amino acid to lysine was equal to, or greater than, that analysed in the soft body tissue of *H. laevigata* reported by Coote et al. (2000). The different diet series were chosen according to the general understanding of the specific requirements of proteins for the small and large animals. Feed rates were maintained in the range of 4.00 - 5.25% biomass⁻¹ d⁻¹ (1-year-old) and 1.00 - 1.75% biomass⁻¹ d⁻¹ (2-year-old) total biomass of abalone tank⁻¹ d⁻¹. These rations were in excess of the animals daily requirements. The rations were adjusted based on the biomass at stocking and from monthly weight checks for all treatments. Feeding was carried out at 4:00 pm daily. Cleaning and collection of food waste took place daily at 8:30 am and was done by sieving the entire tank contents through a fine mesh. The wet uneaten feed was collected and stored frozen at -20°C. The wet uneaten feed was dried in an oven at 105°C for 16 h. The amount of dry feed consumed was calculated by subtracting the amount of dry uneaten feed from the amount of dry feed offered. We then determined the proportion of uneaten feed that was lost through the collection net without animals in the tank; and then used this correction factor to calculate the corrected apparent feed intake tank⁻¹.

At the end of the experiment feeding was stopped at 24 h prior to harvest to ensure digestive tracts were empty before harvesting. Eight 1-year-old abalone and four 2-year-old abalone from each tank were collected, shucked and stored frozen at -20°C and later pooled for each tank to analyse whole tissue proximate composition.

The dry matter leaching loss of each diet was determined in triplicate after a period of 1, 2, 4, 8 and 16 h immersion at 14, 18 and 22°C. This was achieved by placing 1 g of each diet into 25 ml of salt-water at the required temperature and then extracting the supernatant, by syringe, and then oven drying the remaining pellet at 105°C for 16 h.

Water quality

Throughout the study water quality was maintained at levels appropriate for good growth of greenlip abalone (Table 2.2). Water temperature and dissolved oxygen (mg L⁻¹ and % saturation) were measured daily using an OxyGuard™ Handygamma dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). The pH was measured daily using a meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA). Salinity (g L⁻¹) was determined weekly using a portable salinity refractometer, model RF20 (Extech Instruments, Nashua, NH, USA). Light intensity was measured using a LI-COR 1400 Quantum light meter (LI-COR Environmental, Lincoln, NE, USA).

Table 2.1. Ingredient and nutrient composition of experimental diets.

<i>Ingredient composition (% diet as fed)</i>	<i>Nominal crude protein level (%)</i>				
	24	27	30	33	36
Salmon fish meal	4.00	4.00	4.00	4.00	4.00
Solvent extracted soybean meal	16.40	18.90	21.40	23.90	26.47
Lupins (de-hulled)	17.95	20.80	23.60	26.40	29.14
Waxy maize starch	30.40	30.67	29.07	27.59	19.96
Pregelatinised waxy maize starch	15.40	10.00	5.62	1.15	0.00
Wheat gluten meal	5.00	5.00	5.00	5.00	5.00
Casein	4.43	5.48	6.53	7.59	8.63
Diatomaceous earth	2.61	1.76	1.79	1.77	4.60
Fish oil	1.61	1.22	0.84	0.46	0.10
EPA Vitamin/mineral premix ¹	0.20	0.20	0.20	0.20	0.20
Sodium alginate	0.30	0.30	0.30	0.30	0.30
Vitamin E	0.01	0.01	0.01	0.01	0.01
Calcium sulphate	0.50	0.43	0.36	0.30	0.22
Monosodium phosphate	0.75	0.72	0.68	0.65	0.61
Arginine	0.27	0.31	0.37	0.41	0.46
Threonine	0.17	0.20	0.23	0.27	0.30
<i>Biochemical composition (% diet as fed) Analysed and calculated</i>					
Moisture	10.10	10.35	10.48	10.61	10.30
Crude protein	24.20	27.00	31.10	34.30	37.30
Digestible protein (calculated)	17.99	20.27	23.54	26.13	28.57
Lipid	3.60	3.60	3.60	3.70	3.50
Gross energy (MJ kg ⁻¹)	17.5	17.0	17.3	17.6	17.3
Digestible energy (MJ kg ⁻¹) (calculated)	12.7	12.3	12.4	12.6	12.5
Ash	5.68	5.02	5.31	5.24	8.17
NFE (calculated) ²	66.52	64.38	59.99	56.76	51.03
Digestible CP:GE (g MJ ⁻¹)	14.14	16.57	19.06	20.79	22.80
<i>Calculated amino acids (% diet as fed)</i>					
Arginine	1.95	2.22	2.50	2.77	3.04
Histidine	0.64	0.72	0.80	0.89	0.97
Isoleucine	1.13	1.28	1.44	1.59	1.74
Leucine	1.86	2.10	2.35	2.59	2.83
Lysine	1.34	1.52	1.71	1.90	2.09
Methionine	0.41	0.46	0.51	0.57	0.62
Phenylalanine	1.16	1.31	1.46	1.61	1.76
Threonine	1.07	1.22	1.37	1.53	1.67
Tryptophan	0.27	0.30	0.34	0.37	0.41
Valine	1.26	1.42	1.59	1.75	1.92

¹ The vitamin mineral premix was provided by Eyre Peninsula Aquafeed (Lonsdale, SA, Australia).² Digestible protein and energy values based on data reported by Fleming et al. (1998) and Vandeppeer (2005).³ NFE = Nitrogen free extract = 100 % - (protein % + lipid % + ash %).

Table 2.2. Summary of water quality for each temperature system.

Temperature system	Temperature (°C) ^{1,3}	Dissolved oxygen (mg L ⁻¹) ^{1,2,3}	Dissolved oxygen (% saturation) ^{1,2,3}	pH ^{1,2,3}	Salinity (mg L ⁻¹) ^{1,2,4}
14°C	14.1 ± 0.20 (13.2 - 14.7)	8.0 ± 0.19 (6.8 - 8.7)	99.1 ± 1.64 (88.4 - 104.2)	8.3 ± 0.05 (8.1 - 8.4)	35.0 ± 0.62 (34.0 - 36.0)
18°C	18.1 ± 0.22 (17.6 - 19.0)	7.3 ± 0.26 (6.2 - 8.1)	95.7 ± 2.96 (79.2 - 102.7)	8.3 ± 0.04 (8.2 - 8.4)	35.0 ± 0.62 (34.0 - 36.0)
22°C	22.0 ± 0.41 (21.0 - 23.0)	6.7 ± 0.41 (5.3 - 8.1)	92.5 ± 4.45 (73.6 - 101.6)	8.3 ± 0.05 (8.2 - 8.4)	35.0 ± 0.62 (34.0 - 36.0)

¹ Values means ± standard deviation, values in parentheses represent the range of values.

² Data for DO, pH and salinity for entire experiment, while the data for water temperature is from end of temperature acclimation period.

³ n = 83.

⁴ n = 12.

Performance indices

All data reported for individual animal performance was based on the individual data recorded from each tank, whereas biomass gain and FCR have taken the weight of mortality into account. Performance indices for weight gain, specific growth rate (SGR) and apparent nutrient efficiency and deposition ratios were calculated as described by Stone et al. (2003). The condition factor (CF) was calculated using the equation derived from *H. midae* by Britz and Hecht (1997): $CF = 5575 \times (\text{weight [g]} / \text{length [mm]})^{2.99}$. The optimum protein levels for each year class at each water temperature were estimated using the average of the X_{\max} values of the SGR and protein deposition. The X_{\max} and Y_{\max} values for 1-year-old animals were determined directly from the equation for the second order polynomial relationship, whereas, the X_{\max} and Y_{\max} values for 2-year-old animals were determined directly off the graph.

Biochemical analyses

The proximate composition analyses of ingredients, diets and whole body tissue were conducted according to methods in the British Pharmacopoeia Commission (2004) or DIN Standards (2000). Abalone collected (5 tank⁻¹) for whole body proximate analyses were pooled, freeze dried then ground. Tissue samples were freeze-dried to a constant weight at -50°C to determine moisture content. Diet samples were oven dried to a constant weight at 105°C for 16 h to determine moisture content. Crude protein (N × 6.25) was determined by the Kjeldahl method (BP A219 H Determination of Nitrogen). Crude lipid was analysed using a Soxtherm rapid extraction system (Gerhardt GmbH and Co. KG, Königswinter, Germany) with petroleum liquid (BP 100°C) as the extracting solvent. Ash was determined by a muffle furnace at 550°C for 16 h. Gross energy content was determined using a bomb calorimeter (DIN Standards, 2000), calibrated with benzoic acid).

Statistical analyses

Homogeneity of variances among mean values was assessed using Levene's test for equality of variance errors. Data from each year class were analysed separately using two-factor Analysis of Variance (ANOVA) with water temperature as the first factor and dietary protein level as the second factor. Where significant interactions were observed the data from all treatment combinations for the given variable were analysed using one-factor ANOVA. Student Newman-Keuls (SNK) test, was used to identify significant differences among multiple treatment means. Regressions were also applied to SGR, FCR and protein deposition data in an attempt to determine the optimum protein level for growth of each year

class. A significance level of $P < 0.05$ was used for all statistical tests. All statistical analyses were done using IBM SPSS, Version 19 for Windows (IBM SPSS Inc., Chicago, IL, USA). All values are presented as means \pm standard error of the mean (SE).

Results

General observations

There were no significant differences in the initial weight, tank biomass and shell length between treatments for 1-year-old, or 2-year-old abalone at the commencement of the experiment ($P > 0.05$). The overall mean value for the abalone of each year class were as follows: initial weight (1.75 ± 0.01 and 22.93 ± 0.09 g); initial tank biomass (34.96 ± 0.09 and 229.29 ± 0.88 g tank⁻¹); initial shell length (23.31 ± 0.03 and 56.64 ± 0.08 mm) for 1- and 2-year-old abalone, respectively. Animals exhibited normal signs of feeding behaviour throughout the study and no gross signs of disease were observed. Overall, the mortality rate for the experiment was 2.92% and the majority of the mortalities occurred within the first two to three weeks following stocking. Interestingly, there was a significant effect of water temperature on mortality rate of 1-year-old abalone (two-factor ANOVA; $P < 0.001$). A higher mortality rate was observed at 14°C ($6.60 \pm 1.09\%$) compared to either 18°C ($1.25 \pm 0.56\%$) or 22°C ($1.25 \pm 0.72\%$). There was no significant effect of protein level on mortality rate ($P = 0.421$), and there was no significant interaction between water temperature and protein level ($P = 0.054$). The mortality rate ($2.71 \pm 0.83\%$) of 2-year-old abalone was not significantly affected by water temperature ($P = 0.676$), protein level ($P = 0.421$) or the interaction between these two factors ($P = 0.877$).

The analysed protein contents of the diets were slightly higher than the formulated nominal values (Table 2.1). Diet dry matter leaching loss was significantly affected ($P < 0.001$) by diet type, water temperature, and immersion time in water. There were no significant interactions between these factors ($P > 0.05$). The differences in percent leaching losses between diet types were significant, but relatively small (Diet 1, 5.83 > Diet 2, 3.73 = Diet 3, 3.68 = Diet 4, 4.05 = Diet 5, 3.86%; three-factor ANOVA, pooled SE, 0.316%, SNK). Leaching loss increased with increasing water temperature (Figure 2.1a) and with increasing submersion time until 8h (Figure 2.1b).

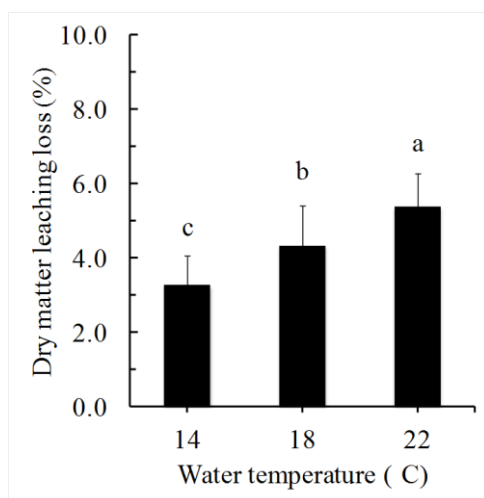


Figure 2.1a.

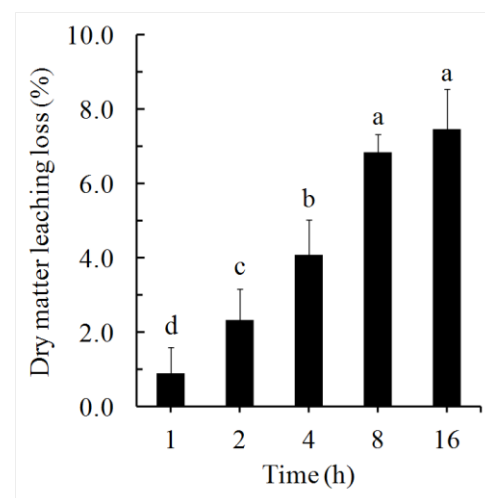


Figure 2.1b.

Figure 2.1. a and b. Dry matter leaching loss of experimental abalone diets at three water temperatures (Fig. 2.1a; 14, 18, 22°C) at 1, 2, 4, 8 and 16 h post submersion (Fig. 2.1b). Diet type NS; Water temperature Sig.; Time Sig.; Interaction NS; Values that share the same superscript are not significantly different, $P > 0.05$, three-factor ANOVA, SNK, mean \pm SE, $n = 15$ for temperature and 9 for time.

Growth performance

There were significant effects ($P < 0.001$) of water temperature on final individual weight (Table 2.3) and specific growth rate (SGR) for 1-year-old abalone (Figure 2.2a). There were significant increases in final individual weight and SGR observed as the temperature increased from 14 to 22°C. There was no significant effect of protein level on final individual weight ($P = 0.449$) or SGR ($P = 0.200$). There were no significant interactions between water temperature and protein level for final individual weight or SGR, $P = 0.439$ and 0.237 , respectively. However, there were trends for an increase in final weight and SGR of 1-year-old abalone at 22°C as protein level increased from 27% to 33% (Table 2.3, Figure 2.2a). Whereas, in comparison a relatively neutral and negative response was observed to increasing protein level for weight gain and SGR of 1-year-old abalone cultured at 18 and 14°C, respectively. There were also significant effects ($P < 0.001$) of water temperature on final individual weight and SGR for 2-year-old abalone. There was a significant increase in final individual weight (Table 2.4) and SGR (Figure 2.2b) observed for 2-year-old abalone as the temperature increased from 14 to 18 and 22°C. There was no significant difference between 18 and 22°C for final individual weight ($P = 0.424$) and SGR ($P = 0.796$). There was no significant effect of protein level on final individual weight ($P = 0.692$) or SGR (Figure 2.2b; $P = 0.465$). There were no significant interactions between water temperature and protein level for final individual weight or SGR, $P = 0.811$ and 0.936 , respectively.

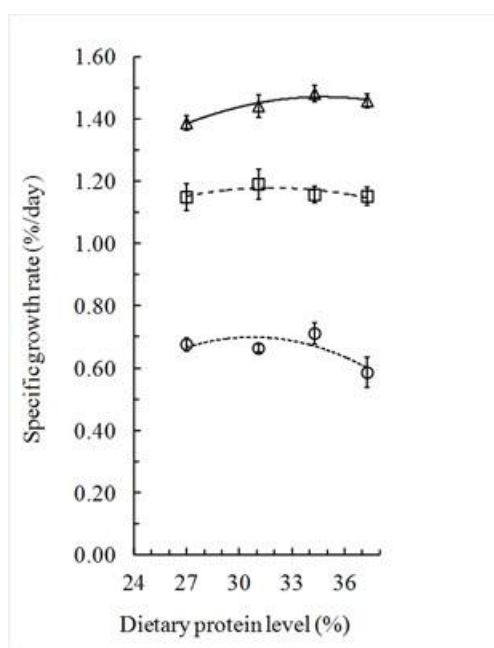


Figure 2.2a

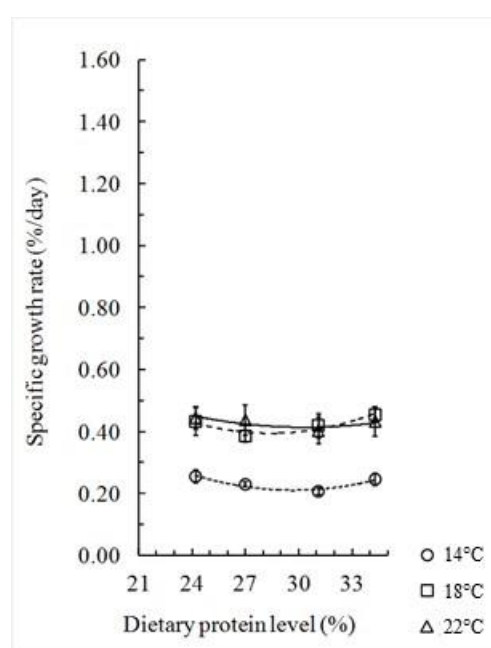


Figure 2.2b

Figure 2.2. a and b. Specific growth rate of 1-year-old (Fig. 2.2a) and 2-year-old (Fig. 2.2b) abalone fed a range of dietary protein levels (1-year-old: 27, 30, 33, 36%; 2-year-old: 24, 27, 30, 33%) at three different water temperature for 84 d.

$n = 4$ tanks, mean \pm SE.

1-year-old* 14°C: $y = -0.0023x^2 + 0.14x - 1.48$, $R^2 = 0.62$, $X_{max} = 30.8$, $Y_{max} = 0.70$; 18°C: $y = -0.0011x^2 + 0.070x + 0.069$, $R^2 = 0.60$, $X_{max} = 31.6$, $Y_{max} = 1.17$; 22°C: $y = -0.0014x^2 + 0.099x - 0.25$, $R^2 = 0.95$, $X_{max} = 35.3$, $Y_{max} = 1.50$).

2-year-old* 14°C: $y = 0.0016x^2 - 0.093x + 1.59$, $R^2 = 0.9172$, $X_{max} = 24.2$, $Y_{max} = 0.26$; 18°C: $y = 0.0018x^2 - 0.11x + 1.88$, $R^2 = 0.86$, $X_{max} = 24.2$, $Y_{max} = 0.43$; 22°C: $y = 0.0009x^2 - 0.057x + 1.28$, $R^2 = 0.66$; $X_{max} = 24.2$, $Y_{max} = 0.44$).

* X_{max} and Y_{max} values for 1-year-old abalone are determined from the second order polynomial equation, whereas, the X_{max} and Y_{max} values for 2-year-old abalone determined directly off the graph.

Table 2.3. Growth performance and soft tissue composition of 1-year-old greenlip abalone.

Temperature (°C)	14 ¹				18 ¹				22 ¹				SE ¹	ANOVA							
	27	30	33	36	27	30	33	36	27	30	33	36		Temp (°C) (A)			Protein level (%) (B)				AxB
														14	18	22	27	30	33	36	
<i>Growth performance</i>																					
Final weight (g fish ⁻¹)	3.09	3.05	3.16	2.84	4.57	4.75	4.65	4.66	5.58	5.90	6.00	6.03	0.15	Z	Y	X	ns	ns	ns	ns	ns
Biomass gain (g tank ⁻¹) ²	23.50	26.61	27.67	25.11	56.01	59.12	60.17	57.62	80.32	83.42	84.48	81.92	2.08	Z	Y	X	ns	ns	ns	ns	ns
Final shell length (mm)	28.91	28.67	29.05	28.04	34.76	34.93	34.73	34.89	37.54	38.10	38.36	38.18	0.36	Z	Y	X	ns	ns	ns	ns	ns
Shell growth rate (µm d ⁻¹)	66.53	63.63	68.93	57.73	136.73	138.63	135.43	137.63	167.73	176.48	180.00	175.73	4.31	Z	Y	X	ns	ns	ns	ns	ns
Condition factor	0.74	0.75	0.74	0.74	0.63	0.65	0.64	0.63	0.61	0.62	0.61	0.63	0.01	X	Y	Z	ns	ns	ns	ns	ns
Feed consumption rate (mg individual ⁻¹ d ⁻¹)	62.17 ^f	62.48 ^f	55.80 ^f	59.24 ^f	136.73 ^c	138.27 ^c	109.16 ^e	118.24 ^{de}	168.30 ^a	158.38 ^b	123.25 ^d	123.05 ^d	3.31	*	*	*	*	*	*	*	*
<i>Soft tissue composition</i>																					
Moisture (%)	81.75	82.24	82.55	82.35	79.49	80.11	79.89	79.66	77.29	78.37	78.38	79.81	0.52	X	Y	Z	ns	ns	ns	ns	ns
Protein (% dry)	66.45 ^{ab}	66.30 ^{ab}	63.86 ^{abc}	67.47 ^a	60.70 ^{cd}	62.70 ^{bc}	64.74 ^{abc}	65.50 ^{abc}	58.57 ^d	62.06 ^{bc}	64.26 ^{abc}	63.85 ^{abc}	1.01	*	*	*	*	*	*	*	*
Fat (% dry)	6.59	7.28	6.18	6.37	6.61	6.27	5.87	5.83	6.91	7.09	6.23	5.98	0.30	ns	ns	ns	x	x	y	y	ns
Ash (% dry)	11.36	10.15	11.49	11.13	10.73	10.49	10.75	10.75	9.71	9.65	9.92	10.86	0.60	ns	ns	ns	ns	ns	ns	ns	ns
NFE (% dry)	16.58	16.27	18.48	15.04	21.96	20.55	18.64	17.93	24.81	21.20	19.60	19.32	1.32	Y	X	X	x	xy	xy	y	ns
Energy (MJ kg ⁻¹ dry)	20.18	20.00	19.85	21.00	20.30	19.68	19.98	20.15	20.18	20.30	20.10	19.98	0.23	ns	ns	ns	ns	ns	ns	ns	ns

¹ Mean ± pooled SE; n = 4.

² Biomass gain = (weight of final animals + weight of mortalities) - (weight of stocked animals + weight of replacement animals); ns: denotes non-significant ($P > 0.05$); A x B = two-factor ANOVA interactions; X, Y, Z: For variables with a significant effect of temperature and no interaction, values without a common upper case letter are different (X indicates the highest value; $P < 0.05$); w, x, y, z: For variables with a significant effect of protein level and no interaction, values without a common lower case letter are significantly different (w indicates the highest value; $P < 0.05$); * denotes significant interaction ($P < 0.05$); ^{a, b, c, d, e}: For variables with a significant interaction, differences in all protein levels are compared across all temperatures (one-factor ANOVA, SNK test), values without a common superscript are significantly different (^a indicates the highest value; $P < 0.05$). NFE = Nitrogen free extract = 100 % - (protein % + lipid % + ash %).

1-year-old initial soft tissue content of protein (66.41% dry), lipid (6.01% dry), ash (12.08% dry), NFE (15.50% dry) and energy (19.95 MJ kg⁻¹ dry).

Table 2.4. Growth performance and soft tissue composition of 2-year-old greenlip abalone.

Temperature (°C)	14 ¹				18 ¹				22 ¹				SE ¹	ANOVA									
	Nominal protein (%)		24	27	30	33	24	27	30	33	24	27		30	33	Temp (°C) (A)			Protein level (%) (B)				AxB
	14	18														22	24	27	30	33			
<i>Growth performance</i>																							
Final weight (g fish ⁻¹)	28.72	27.78	27.58	28.22	32.25	31.49	32.28	33.84	33.09	33.72	32.35	33.02	1.03	Y	X	X	ns	ns	ns	ns	ns		
Biomass gain (g tank ⁻¹) ²	54.45	48.60	46.50	53.45	98.63	87.11	97.44	110.07	103.35	104.94	97.16	102.49	9.38	Y	X	X	ns	ns	ns	ns	ns		
Final shell length (mm)	60.19	59.38	59.65	59.48	62.79	62.25	62.33	62.62	63.42	64.54	64.37	64.47	0.60	Z	Y	X	ns	ns	ns	ns	ns		
Shell growth rate (µm d ⁻¹)	40.23	35.33	36.15	29.60	75.40	65.93	67.88	73.78	82.65	89.83	88.85	97.45	5.83	Z	Y	X	ns	ns	ns	ns	ns		
Condition factor	0.77	0.77	0.76	0.78	0.76	0.76	0.78	0.80	0.76	0.73	0.71	0.72	0.02	X	X	Y	ns	ns	ns	ns	ns		
Feed consumption rate (mg fish ⁻¹ day ⁻¹)	136.20 ^e	155.78 ^d	168.71 ^{cd}	131.37 ^e	199.06 ^b	209.55 ^b	208.45 ^b	169.43 ^{cd}	228.58 ^a	228.03 ^a	208.68 ^b	174.38 ^c	4.74	*	*	*	*	*	*	*	*		
<i>Soft tissue composition</i>																							
Moisture (%)	80.66	80.35	80.68	82.43	80.76	80.71	81.08	82.92	80.18	82.81	80.18	80.42	0.79	ns	ns	ns	ns	ns	ns	ns	ns		
Protein (% dry)	60.90	63.30	65.19	65.78	59.03	60.44	60.47	64.36	57.72	58.68	62.18	64.00	1.32	X	Y	Y	y	xy	wx	w	ns		
Fat (% dry)	6.96	6.17	7.27	6.52	6.52	5.74	6.20	6.41	5.59	5.43	6.23	5.44	0.36	X	X	Y	ns	ns	ns	ns	ns		
Ash (% dry)	9.32	8.67	9.08	9.60	8.90	8.90	9.62	8.94	8.39	9.26	8.70	9.23	0.42	ns	ns	ns	ns	ns	ns	ns	ns		
NFE (% dry)	22.82	21.87	18.46	18.10	25.56	24.94	23.71	20.29	28.31	26.63	22.83	21.33	1.49	Y	X	X	x	x	y	y	ns		
Energy (MJ kg ⁻¹ dry)	20.18	20.08	20.00	20.08	19.85	20.25	19.83	20.23	19.85	19.48	19.85	20.20	0.20	ns	ns	ns	ns	ns	ns	ns	ns		

¹ Mean ± pooled SE; n = 4.

² Biomass gain = (weight of final animals + weight of mortalities) - (weight of stocked animals + weight of replacement animals); ns: denotes non-significant ($P > 0.05$); A x B = two-factor ANOVA interactions; X, Y, Z: For variables with a significant effect of temperature and no interaction, values without a common upper case letter are different (X indicates the highest value; $P < 0.05$); w, x, y, z: For variables with a significant effect of protein level and no interaction, values without a common lower case letter are significantly different (w indicates the highest value; $P < 0.05$); * denotes significant interaction ($P < 0.05$); ^{a, b, c, d, e}: For variables with a significant interaction, differences in all protein levels are compared across all temperatures (one-factor ANOVA, SNK test), values without a common superscript are significantly different (^a indicates the highest value; $P < 0.05$). NFE = 100% - (protein% + fat% + ash%).

2-year-old initial soft tissue content of protein (64.54% dry), lipid (5.68% dry), ash (10.67% dry), NFE (19.11% dry) and energy (20.22 MJ kg⁻¹ dry).

There was a significant effect of water temperature on biomass gain for 1-year-old abalone (Table 2.3; $P < 0.001$). A progressive significant increase in biomass gain was recorded as the temperature increased from 14 to 22°C. There was also a significant effect of water temperature on biomass gain for 2-year-old abalone (Table 2.4; $P < 0.001$). The biomass gain was significantly lower at 14°C compared to either 18 or 22°C. There was no significant effect of protein level on biomass gain for 1-year-old ($P = 0.342$) or 2-year-old abalone ($P = 0.631$), and there was no significant interaction between the two factors for either 1-year-old ($P = 0.414$) or 2-year-old ($P = 0.900$) abalone.

There were significant effects of water temperature on final shell length, shell growth rate and condition factor for 1-year-old abalone (Table 2.3; $P < 0.001$). Significant increases in final shell length and shell growth rate were observed as the temperature increased from 14 to 22°C. In contrast, there was a significant decrease in condition factor observed as the temperature increased condition factor from 14 to 22°C. There was no significant effect of protein level on final shell length ($P = 0.623$) or shell growth rate ($P = 0.527$) or condition factor ($P = 0.572$). However, for shell growth rate similar trends were observed in response to increasing protein level at each temperature when compared to weight gain (Table 2.3) and SGR (Figure 2.2a) of abalone of the same year class. There were no significant interactions between water temperature and protein level for final shell length ($P = 0.424$), shell growth rate ($P = 0.427$) or condition factor ($P = 0.924$) for 1-year-old abalone. For 2-year-old abalone there were significant increases in final shell length and shell growth rate observed as the temperature increased from 14 to 22°C (Table 2.4; $P < 0.001$). In contrast, the condition factor of 2-year-old abalone was significantly lower at 22°C (Table 2.4; $P = 0.005$) compared to 14 and 18°C. There was no significant effect of protein level on final shell length ($P = 0.995$), shell growth rate ($P = 0.893$) or condition factor ($P = 0.697$). However, there was a trend for shell growth rate to increase in response to increasing protein level at 22°C for 2-year-old abalone, but this did not equate to an increase in SGR (Figure 2.2b). There were no significant interactions between water temperature and protein level for final shell length ($P = 0.692$), shell growth rate ($P = 0.424$) or condition factor ($P = 0.496$) for 2-year-old abalone.

Feed utilisation

There were significant effects of water temperature ($P < 0.001$) and protein level $P < 0.001$) on feed consumption rate (Table 2.3) and apparent economic feed conversion ratio (FCR; Figure 2.3a) for 1-year-old abalone. There were also significant interactions observed between these variables for feed utilisation ($P < 0.001$). The interaction for feed consumption rate may be explained by the neutral response of feed intake to additional protein at 14°C compared to the significant reductions in feed intake which began at the 33% protein level at 18°C, and at the 30% protein level at 22°C (Table 2.3; one-factor ANOVA, SNK). The interaction for apparent FCR may be explained by the significant positive relationship between FCR and protein level at 14°C, compared to the significant negative relationships observed between FCR and protein level at both 18 and 22°C (Figure 2.3a; one-factor ANOVA, SNK). There were significant effects of water temperature on feed consumption rate for 2-year-old abalone (Table 2.4; $P < 0.001$). There were also significant effects of protein level on feed consumption rate ($P < 0.001$). There was also a significant interaction between water temperature and protein level for feed consumption rate ($P < 0.001$). The interaction for feed consumption rate may be explained by the significantly negative relationship between this variable and protein level at 22°C, compared to the positive relationships at both 14 and 18°C up to the 30% protein level (Table 2.4; one-factor ANOVA, SNK).

There were significant effects of water temperature on FCR for 2-year-old abalone (Figure 2.3b; $P < 0.001$). The FCR at 14°C was significantly higher than either 18 or 22°C. There were also significant effects of protein level on FCR. The FCRs of 27 and 30% protein being significantly higher than 33% protein, whereas 24 and 33% protein were not significantly

different ($P = 0.082$). There was no significant interaction between water temperature and protein level for FCR ($P = 0.338$).

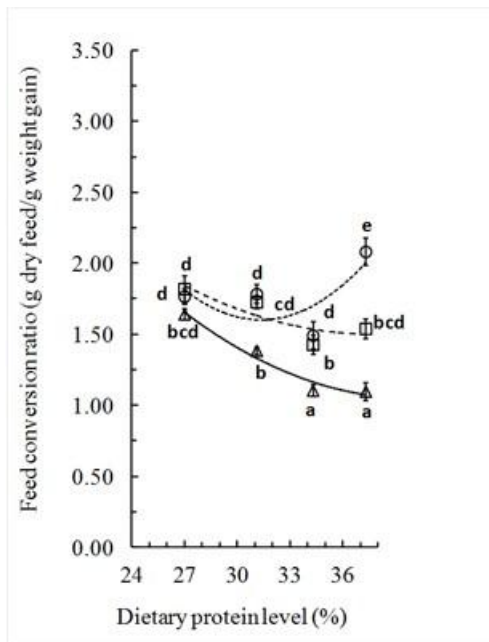


Figure 2.3a

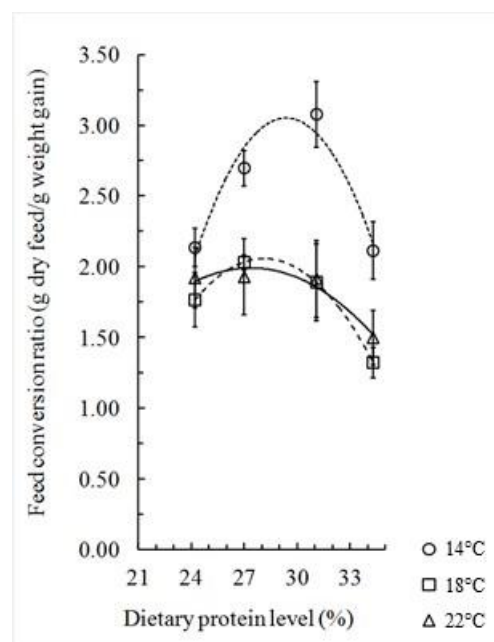


Figure 2.3b

Figure 2.3. a and b. Apparent economic feed conversion ratio of 1-year-old (Fig. 2.3a) and 2-year-old (Fig. 2.3b) abalone fed a range of dietary protein levels (1-year-old *: 27, 30, 33, 36%; 2-year-old: 24, 27, 30, 33%) at three different water temperature for 84 d.

$n = 4$ tanks, mean \pm SE.

1-year-old, 14°C: $y = 0.011x^2 - 0.71x + 12.69$, $R^2 = 0.51$, $X_{max} = 31.3$, $Y_{max} = 1.61$; 18°C: $y = 0.0029x^2 - 0.22x + 5.68$, $R^2 = 0.76$, $X_{max} = 37.3$, $Y_{max} = 1.48$; 22°C: $y = 0.0036x^2 - 0.29x + 6.82$, $R^2 = 0.97$, $X_{max} = 37.3$, $Y_{max} = 1.05$).

2-year-old * 14°C: $y = -0.037x^2 + 2.15x - 28.47$, $R^2 = 0.93$, $X_{max} = 24.2$, $Y_{max} = 2.14$; 18°C: $y = -0.019x^2 + 1.079x - 13.11$, $R^2 = 1.00$, $X_{max} = 34.3$, $Y_{max} = 1.32$; 22°C: $y = -0.0098x^2 + 0.54x - 5.36$, $R^2 = 0.94$, $X_{max} = 34.3$, $Y_{max} = 1.50$).

* X_{max} and Y_{max} values for 1-year-old are determined from the second order polynomial equation, whereas, the X_{max} and Y_{max} values for 2-year-old are determined directly off the graph.

Soft tissue composition

For 1-year-old greenlip abalone there was a significant effect of water temperature on soft tissue moisture composition ($P < 0.001$). Moisture content was inversely related to increasing water temperature (Table 2.3). There was no significant effect of protein level ($P = 0.091$) and the interaction between the two factors ($P = 0.373$). There was no significant effect of water temperature ($P = 0.686$), protein level ($P = 0.129$) or interaction between the two factors ($P = 0.104$) on soft tissue moisture composition of 2-year-old greenlip abalone (Table 2.4).

Final dry tissue protein content of 1-year-old abalone was significantly affected by water temperature ($P < 0.001$) and protein content ($P < 0.001$; Table 2.3). There was also a significant interaction for tissue protein content ($P = 0.034$). The interaction is explained by the significant reduction in tissue protein for the 1-year-old abalone fed the 27% crude protein diet at 22°C, while tissue protein was not significantly affected within the two other water temperature groups (Table 2.3, one-factor ANOVA, SNK). There were significant effects of water temperature on final dry tissue protein content for 2-year-old abalone (Table 2.4; $P = 0.003$). Tissue protein was significantly higher at 14°C. Tissue protein levels were significantly ($P < 0.001$) positively related to increasing dietary protein levels. There was no

significant interaction between water temperature and protein level for final tissue protein content ($P = 0.758$).

There were no significant effects of water temperature on soft tissue fat content for 1-year-old abalone ($P = 0.075$; Table 2.3). However, there was a significant effect of dietary protein level ($P = 0.003$), with abalone fed diets containing 27 and 30% crude protein having slightly, though significantly, higher fat levels compared to those fed 33 or 36% crude protein. There were no significant interactions between the two factors ($P = 0.699$). For 2-year-old abalone the fat content at 14°C was significantly higher than either 18 or 22°C (Table 2.4; $P = 0.001$). There was no significant effects of protein level on fat content ($P = 0.065$), and there was no significant interaction between water temperature and protein level ($P = 0.733$).

There was no significant effect of water temperature ($P = 0.073$; 0.638) or dietary protein level on the dry soft tissue ash content of 1-year-old ($P = 0.404$) or 2-year-old abalone (0.654) and there were no significant interactions between the two factors ($P = 0.830$; 0.404) (Tables 2.3 and 2.4), respectively. There was no significant effect of water temperature ($P = 0.372$; 0.217) or dietary protein level ($P = 0.118$; 0.352) on the dry soft tissue energy content of 1 or 2-year-old abalone and there were no significant interactions ($P = 0.054$; 0.298) (Tables 2.3 and 2.4), respectively.

There was a significant effect of water temperature on soft tissue NFE content for 1- and 2-year-old abalone (Tables 2.3 and 2.4; $P < 0.001$). For 1- and 2-year-old abalone the NFE contents were significantly lower at 14°C compared to either 18 or 22°C. There was a significant effect of protein level on NFE content on 1-year-old ($P = 0.015$) and 2-year-old abalone ($P < 0.001$). For 1-year-old abalone there was a significant progressive reduction in NFE content as the dietary protein content increased from 27 to 36% (Table 2.3). While the NFE content of 2-year-old abalone fed the 30 and 33% crude protein diets were significantly lower than those of the abalone fed diets containing 24 and 27% crude protein (Table 2.4). There was no significant interaction between the two factors for either year class ($P = 0.245$; $P = 0.894$).

Nutrient utilisation

There was a significant effect of water temperature (Figure 2.4a; $P < 0.001$) and protein level on protein deposition ($P = 0.015$) for 1-year-old abalone. There was also a significant interaction between water temperature and protein level for protein deposition ($P = 0.018$). The interaction for protein deposition for 1-year-old abalone may be explained by the significant positive response to increasing protein level occurring at 33% protein level at 22°C compared to the non-significant neutral and significant negative responses observed at 18 and 14°C, respectively (Figure 2.4a; one-factor ANOVA, SNK). There was a significant effect of water temperature ($P = 0.041$) and protein level on protein deposition for 2-year-old abalone (Figure 2.4b; $P = 0.048$) and there was a significant interaction between water temperature and protein level for protein deposition ($P = 0.008$). The interaction may be explained by the significant increase in protein deposition observed at the 33% protein level at 22°C compared to the non-significant responses to increasing protein level recorded at 18 and 14°C (Figure 2.4a; one-factor ANOVA, SNK).

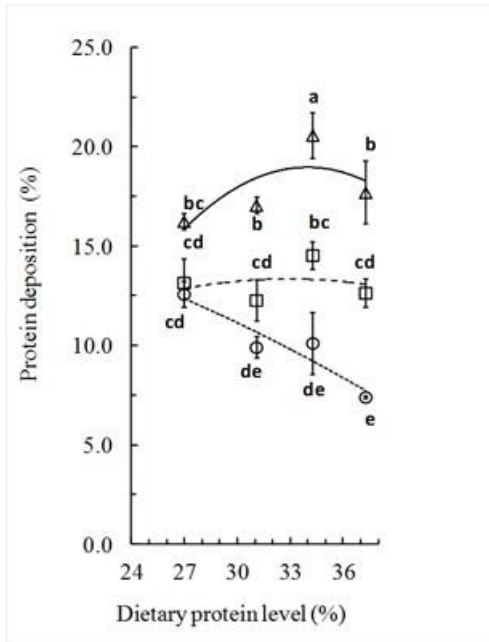


Figure 2.4a

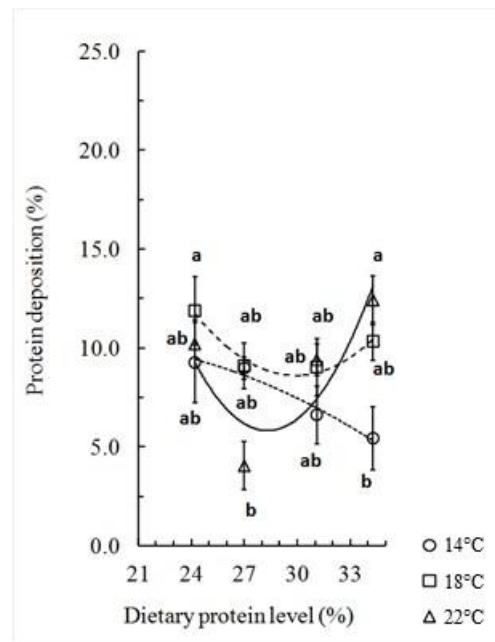


Figure 2.4b

Figure 2.4. a and b. Protein deposition for 1-year-old (Fig 2.4a) and 2-year-old (Fig. 2.4b) abalone fed a range of dietary protein levels (1-year-old: 27, 30, 33, 36%; 2-year-old: 24, 27, 30, 33%) at three different water temperature for 84 d.

n = 4 tanks, mean \pm SE.

1-year-old * 14°C: $y = -0.0062x^2 - 0.051x + 18.27$, $R^2 = 0.88$, $X_{max} = 27.1$, $Y_{max} = 12.33$; 18°C: $y = -0.014x^2 + 0.92x - 1.75$, $R^2 = 0.047$, $X_{max} = 32.8$, $Y_{max} = 13.31$; 22°C: $y = -0.063x^2 + 4.30x - 54.13$, $R^2 = 0.53$, $X_{max} = 34.0$, $Y_{max} = 19.00$)

2-year-old * 14°C: $y = -0.016x^2 + 0.53x + 5.93$, $R^2 = 0.97$, $X_{max} = 24.2$, $Y_{max} = 9.26$; 18°C: $y = 0.096x^2 - 5.74x + 94.47$, $R^2 = 0.96$, $X_{max} = 34.3$, $Y_{max} = 10.33$; 22°C: $y = 0.2049x^2 - 11.599x + 170.01$, $R^2 = 0.7386$, $X_{max} = 34.3$, $Y_{max} = 12.41$).

* X_{max} and Y_{max} values for 1-year-old are determined from the second order polynomial equation, whereas, the X_{max} and Y_{max} values for 2-year-old are determined directly off the graph.

Discussion

Our overarching goal was to optimise abalone growth, by formulating specific levels of dietary protein for seasonal water temperatures throughout the production cycle. The information obtained from this study was essential to achieving this goal, and was used to formulate diets in the on-farm trials (Chapter 5) which evaluated optimum dietary protein levels and feeding strategies in commercial situations.

Animals fed actively on the experimental diets and displayed no apparent gross signs of disease. The mortality rate observed in this study was low and compared favourably with mortality rates observed at commercial facilities during routine tank harvesting/stocking procedures. The growth rates recorded in this study were favourable when compared to other laboratory based studies by Coote et al. (2000) and Vandeppeer (2005), and also comparable to those observed in commercial facilities. Coote et al. (2000) reported an SGR of $1.03\% d^{-1}$ for juvenile greenlip abalone stocked at ~ 1 g (19 mm shell length) and grown at $20^\circ C$ for 85 d. Vandeppeer (2005) also reported an SGR of $1.05\% d^{-1}$ for greenlip abalone (2.25 g; 25 mm shell length) grown for a period of 50 d at $18^\circ C$.

In this study, animals were fed to excess and feed was not a limiting factor. When feed is not limiting, temperature is the most important environmental factor influencing the growth rate

of an animal (Britz et al. 1997). The optimal water temperature for greenlip abalone is 18.3°C (Gilroy and Edwards 1998); below this, a reduction in growth may occur, as the metabolic rate of a poikilothermic organism is temperature dependent (Quartararo et al. 1998; Freeman 2001). In this study, the SGR of 1-year-old abalone were more responsive to increases in water temperatures than 2-year-old abalone. Size dependent optimal water temperature for growth has also been observed in red abalone (*H. rufescens*) with a symmetrical, concave relationship peaking at a shell length of 44 mm at 17.8°C (Steinarsson and Imsland 2003). The optimal water temperature for juvenile *H. discus hannai* (4.7 g) growth was at 20°C (Cho and Kim 2012). An optimal water temperature of 20°C was similarly observed in *H. midae* (Britz et al. 1997). Britz et al. (1997) fed a commercial diet to *H. midae* (1 g, 18 mm SL) over three months and observed a significant increase in growth rate as temperature increased from 12 to 20°C with mortality rates between 1% to 4.4%. However, at water temperatures above 20°C the growth rate was significantly reduced and mortalities increased drastically from 12% at 22°C to 43% at 24°C (Britz et al. 1997). Green et al. (2011) also reported significant reductions in the growth rate of *H. midae* as temperature increased from 18 to 22 to 24°C. In contrast, in the present study as water temperatures increased from 14 to 22°C, 1-year-old greenlip abalone were observed to have significant increases in SGR and shell growth rate with relatively low mortalities. While 2-year-old greenlip abalone differed in their response and maximum growth was attained at 18°C and remained constant at 22°C with no apparent increase in mortality. Greenlip abalone have previously been observed to have a critical thermal maxima, defined as the point at which abalone can no longer hold on to the substrate, and is considered equivalent to death, at a water temperature of 27.5°C (Gilroy and Edwards 1998). Abalone in this study clearly had not reached their thermal tolerance level.

The growth parameters of 1-year-old abalone were more responsive to both temperature and protein level than 2-year-old abalone. Although care must be exercised when comparing results from different studies, differences in the growth rate between juvenile and sub-adult greenlip abalone were similarly observed between other studies by Coote et al. (2000) and Vandeppeer (2005). Similarly, Britz and Hecht (1997) observed higher growth rates in *H. midae* ranging from 1.5-2.2% body weight d⁻¹ for 0.2-1 g abalone compared to 0.1-0.5% body weight d⁻¹ for 7-14 g abalone. Basal metabolic rate and maintenance requirements are proportional to body weight, and are greater in larger animals, as such, SGR has a tendency to be higher in smaller animals. In addition, subsequent histological examination showed that eggs were present in 2-year-old greenlip abalone prior to the commencement of the experiment, and as such, abalone might have partitioned some energy for reproductive maturation instead of for somatic growth.

Growth rates alone do not appear to be a sensitive measure of the effects of dietary protein inclusion for abalone, when fed to excess, in short term experiments such as this one. No significant effect of protein level on final individual weight gain, SGR, or shell growth rate were observed for either year class. For 1-year-old greenlip abalone held at either 18 or 22°C, there was a general trend for growth parameters to increase as protein level increased, and reached an optimum between 32.2% and 34.7% crude protein, respectively. In comparison, the growth rates of 2-year-old greenlip abalone were lower. As previously mentioned, the animals in the present study were fed to excess. The protein availability initially limits protein accumulation by an animal. If protein is not limiting, protein accumulation is limited by the dietary energy content (Coote et al. 2000). Animals eat to satisfy their energy requirements. Digestible dietary energy (~12.5 MJ kg⁻¹) was constant between diets in the present study, while the protein to energy ratio increased from 14.14 to 22.80 with increasing protein level. Bautista-Teruel and Millamena (1999) determined, by regression analysis, the optimal dietary protein level for *H. asinina* (0.6 g) at 27-31°C to be 27% with an estimated metabolisable energy level of 12.98 MJ kg⁻¹. In the present study we observed that greenlip abalone fed low-protein diets increased their food consumption rate,

as an offset to increase protein intake, resulting in no significant difference in growth rates between different protein levels in the diet.

2-year-old animals in our study exhibited slow and highly variable growth rates. Highly variable growth rates have been observed in protein requirement studies in a number of other abalone species (Britz 1996b; Coote et al. 2000). Britz (1996b) and Coote et al. (2000) concluded that variable growth rates reduced statistical power, and resulted in an inability to detect significant treatment effects, other than between the most extreme dietary treatments. Britz (1996b) concluded that an increase in the duration of the experiment might have led to significant dietary effects of economic importance. Alternately, it may be desirable to use a restrictive feeding method to halt compensatory nutrient intake in future nutrient requirement studies for greenlip abalone.

Considering the inability to estimate optimal dietary protein levels by significant differences in growth rates alone; an estimate was attained by investigating the combined responses of SGR, feed intake, FCR and protein deposition. Significant differences were observed in feed intake, FCR and protein deposition in relation to increasing dietary protein level for both 1- and 2-year-old greenlip abalone. In this study, feed intake was significantly reduced as dietary protein levels increased and as a result, 1-year-old abalone at 18°C and 22°C had significant improvements in FCR as dietary protein increased. 1-year-old abalone at 22°C had significantly higher protein deposition at 34.3% protein, while a non-significant increase was observed at 18°C as dietary protein increased. The improved FCR of 1-year-old abalone, based on lower feed intakes, coupled with a numerically higher SGR and improved protein deposition at higher dietary protein levels suggests that the optimal crude protein level at 12.5 MJ kg⁻¹ digestible energy at 18 and 22°C is 32.2% (24.4% DP) and 34.7% (26.7% DP), respectively.

Significant reductions in feed intake with increasing dietary protein level were also observed at 18 and 22°C for 2-year-old greenlip abalone. There were also strong negative second order polynomial relationships between protein level and FCR for 2-year-old abalone at these temperatures (Figure 2.3b) indicating that FCR improved at the 34.3% CP level. In contrast to 1-year-old abalone, 2-year-old abalone did not show an improved SGR or protein deposition at higher dietary protein levels. These results suggest that 2-year-old animals should be fed with 34% CP (25.9% DP) at 18 or 22°C. However, more research is needed to determine the optimal level of dietary protein for these 2-year-old abalone which are larger and have a slower growth rate.

Regarding the results of SGR, FCR and protein deposition at 14°C we recommend an optimal level of 29.0% CP (21.9% DP) and 24% CP (17.8% DP) for 1- and 2-year-old greenlip abalone, respectively.

The optimal dietary protein levels for greenlip abalone recommended from this study are higher than previously reported by Coote et al. (2000). They are similar to those reported by Taylor (1992) for *H. kamtschatkana* ($\geq 30\%$ crude protein). However, they are considerably lower than the level of 38% crude protein recommended by Uki et al. (1986) for *H. discus hannai*, or by Britz (1996b) who recommended a much higher value of 47% crude protein for *H. midae*. Assuming that abalone in the aforementioned studies were unable to increase their feed intake when fed lower protein diets, Coote et al. (2000) hypothesised that the difference in their study, to his, occurred due to sub-optimal essential amino acid profiles in the experimental diets. In addition to Coote et al. (2000) hypothesis, we also hypothesise that the higher differences may have also been due to the unavailability of nutrients due to the diets not being formulated on a digestible basis, using reliable apparent digestibility coefficients, developed specifically for the species tested. The higher protein level recommended by Uki et al. (1986) and Britz (1996b) may have also occurred due to species-specific protein requirements.

Interestingly, and although inconclusive, the results suggest similar or slightly lower optimum protein levels for larger 2-year-old versus 1-year-old greenlip abalone. In contrast, Britz and Hecht (1997), using fish meal as the major protein source, reported maximum growth of *H. midae* at 18°C occurred in large (20 months; 36 mm initial SL; 7.8 g) abalone fed a crude dietary protein level of 44%, while the maximum growth occurred in small (6 months; 11 mm initial SL; 0.2 g) abalone at 34%. Higher protein deposition occurred in larger abalone fed a protein level of 34 or 44%. In contrast, small abalone had higher protein deposition when fed a protein level of 24 or 34% (Britz and Hecht 1997). This may indicate species differences, so further research is needed.

In our study, significantly higher condition factors for 1- and 2-year-old greenlip abalone were observed as water temperature decreased. Britz et al. (1997) also observed this response in *H. midae* and hypothesised the higher condition factor occurred due to a decrease in energy requirements and a partitioning of energy into glycogen reserves at lower water temperatures. However, the results in our study conflict with this hypothesis, as NFE and fat levels were observed to increase with increasing water temperature, and increasing dietary protein levels, in both age classes (Tables 2.3 and 2.4). This indicates species differences in relation to energy utilisation and suggests further research is required. It is also possible that the reduced condition factor observed in our study was due to an increased proportion of new shell being put forward by the faster growing abalone at the higher water temperatures. Meat and shell growth has been reported to be independent in related mollusc species (Palmer 1981).

Conclusions and Recommendations

There were marked differences between the growth performance, feed utilisation and nutrient deposition of 1- and 2-year-old greenlip abalone in relation to increasing water temperatures and dietary protein levels. In south-eastern Australia, commercial diets for greenlip abalone currently contain crude protein levels in the range of 25 - 30%, and are used over the entire grow-out period of the production cycle. We recommend that the dietary protein levels for commercial greenlip abalone are altered to ensure that higher protein diets are used during periods of rapid growth which may be attained, at, and above, the previously reported optimal water temperature of 18°C (~35% CP). Lower protein diets may be used for larger, slower growing 2-year-old abalone during periods of cold water temperatures. Further research will be needed to clarify the change, over time, for optimum dietary protein levels between 1- and 2-year-old greenlip abalone. Additionally, the optimum dietary protein levels for larger 3-year-old+ greenlip abalone will also need to be determined. With regards to improved growth at increasing water temperatures, it may be a viable option to heat nursery systems to improve growth and productivity, and ultimately reduce the length of the production cycle for greenlip abalone. Based on the information obtained from the present study we would recommend there is great potential to introduce multi-diet feeding strategies that provide optimum protein levels for different life stages, and at different seasonal water temperatures, experienced throughout the production cycle of greenlip abalone.

Chapter 3. The determination of the optimum protein requirements for post-weaned sub-juvenile greenlip abalone at 14, 17 and 20°C

Matthew S. Bansemer¹, James O. Harris^{1,2}, Duong N. Duong¹, Jian G. Qin^{1,2}, David A.J. Stone^{1,2,3,4*}.

¹ Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide, 5001, SA, Australia

² Marine Innovation Southern Australia

^{3*} South Australian Research and Development Institute, Aquatic Sciences Centre, 2 Hamra Ave, West Beach, 5024, SA, Australia

⁴ University of Adelaide, School of Animal and Veterinary Sciences, Faculty of Sciences, Roseworthy, 5371, SA, Australia

*Corresponding author: Phone: +61-8-8207 5350, Fax: +61-8-8207 5481, E-mail: david.stone@sa.gov.au

Abstract

In this 91 d study, the interaction between four dietary crude protein (CP) levels (27, 30, 33 and 36% CP) and three water temperatures (14, 17 and 20°C) on the growth and feed utilisation of post-weaned, sub-juvenile 6-month-old greenlip abalone (*Haliotis laevis*; 0.91 g) were investigated. Diets were formulated to be iso-energetic (12.5 MJ kg⁻¹ digestible energy), containing fat levels of ~3.6% and digestible protein from 17.99% to 28.57%. Abalone were fed to excess at 16:00 h daily, and uneaten feed was collected the following day. The specific growth rate (SGR) of abalone improved significantly as water temperatures increased from 14 to 17 to 20°C. In addition, apparent protein deposition was significantly higher in abalone at 17 and 20°C compared to abalone at 14°C. There was no significant effect of dietary protein level on SGR, but faster growing abalone at 20°C compensated by consuming more feed when fed low dietary protein levels. In contrast, a significant positive relationship was observed between dietary protein level and feed consumption rate in slower growing abalone at 14 and 17°C. A non-significant tendency for the apparent feed conversion ratio (FCR) to improve was observed in abalone fed high protein diets at 20°C, while at 14°C, abalone had a significantly poorer FCR, especially when fed high dietary protein levels. Based on results from the current study, it is plausible to heat land-based nursery systems in order to gain accelerated growth of juveniles before transfer to grow-out systems. Additionally, no benefits were apparent by feeding abalone high protein diets at 14 or 17°C. Therefore, we recommend a dietary protein level of 29% CP at 14 and 17°C, the minimum recommended for 1-year old greenlip abalone (Stone et al. 2013, Chapter 2). While the SGR of abalone at 20°C was not influenced by dietary protein, the feed consumption rate decreased and there was a tendency for FCR to improve as protein level increased. Therefore, we recommend that it may be beneficial for abalone to be switched to a diet containing ~35% CP at temperatures > 20°C to improve growth and productivity.

Please note: Initially, this study was designed to compare the growth performance of juvenile greenlip (*Haliotis laevis*) and hybrid abalone (*H. laevis* × *H. rubra*) in response to increasing temperatures and protein levels. As a result of high mortality levels of hybrid abalone due to “walk outs” from the experimental system, and after discussions with the Chief Executive of AAGA, it was decided to excluded hybrid abalone from the study and analyse greenlip abalone alone.

This Chapter addresses Objective 2 of this project and is published in Aquaculture: Bansemer M.S., Harris J.O., Qin J.G., Duong D.N., Stone D.A.J. (2015). Growth and feed utilisation of juvenile greenlip abalone (*Haliotis laevis*) in response to water temperatures and increasing dietary protein levels. Aquaculture 436, 13-20.

Introduction

Greenlip abalone (*Haliotis laevisgata*) are primarily grown in land-based systems throughout southern Australia. The water temperature during grow-out affects almost every aspect of on-farm production (Britz et al. 1997), and can range from below 10°C in Tasmania during winter to above 24°C in South Australia during summer. The optimal water temperature for growth for a Tasmanian greenlip abalone strain (82 mm shell length [SL]) was 18.3°C (Gilroy and Edwards, 1998), while the optimal water temperature for growth for a South Australian greenlip abalone strain (23 mm SL) was 22°C (Stone et al. 2013, Chapter 2, Figure 2.2a). The temperature dependent response in abalone growth may be attributed to genetics or animal size differences, the latter of which has also previously been reported in red abalone (*Haliotis rufescens*) (Steinarsson and Imsland, 2003).

Once juvenile greenlip abalone are weaned off a microalgae diet, they are fed formulated diet for approximately three years until they reach market size. Dietary protein plays a major role in the nutritional value of formulated diets, as optimal growth is dependent on maximising protein deposition, which is limited by dietary protein availability (Fleming and Hone, 1996; Britz and Hecht, 1997; Shipton and Britz, 2001b). The optimal dietary protein level is dependent on a number of factors including the abalone species, abalone size, water temperature, ingredient digestibility and dietary energy level (Bautista-Teruel and Millamena, 1999; Stone et al. 2013, Chapter 2; Bansemer et al. 2014). The abalone industry is relatively new compared to other aquaculture sectors such as fin fish, which have successfully developed pre-starter, starter, grower and finisher diets for different grow-out stages (Ng and Romano, 2013; Sarker et al. 2013). There is an increased demand to introduce multi-diet feeding strategies for greenlip abalone by optimising the dietary protein level for each age class and water temperature throughout the production cycle. However, the optimal dietary protein level for greenlip abalone throughout their whole production cycle is not clear. Prior to 2013, Australian abalone diets were formulated to contain ~27% crude protein (CP) based on a growth experiment for juvenile greenlip abalone (0.55-0.94 g) at 20°C (Coote et al. 2000). Currently however, Australian abalone feed contains 30 to 35% CP as suggested by recent research for greenlip abalone (1.75 g) at 22°C (Stone et al. 2013, Chapter 2), but the authors also reported the optimal dietary protein level is dependent on both age (1- and 2-year-old abalone) and water temperature (14, 18 and 22°C). Further research focused on the nutritional requirements of greenlip abalone soon after weaning (~ 6-month-old abalone) is required to improve our understanding on feed formulation for juvenile abalone.

Aim

In this study, our aim was to identify the optimal dietary CP level for post-weaned sub-juvenile greenlip abalone (6-month-old) at 14, 17 and 20°C. On-farm, the water temperature fluctuates throughout the grow-out period in land-based facilities in southern Australia; the water temperatures selected in the current study represent the temperature range typically occurring from autumn, through winter, to early summer experienced by post-weaned sub-juvenile greenlip abalone. The nominal dietary CP levels used in this study were 27, 30, 33 and 36%. These levels are considered to be commercially applicable to land-based abalone production in southern Australia. Diets used in this study were formulated on a digestible protein basis and contained highly palatable and digestible ingredients at realistic inclusion levels, using protein and energy digestibility data reported for greenlip abalone (Fleming et al. 1998; Vandeppeer, 2005). Diets were formulated using the “ideal protein ratio” concept such that the ratio of each essential amino acid to lysine was equal to, or greater than, the soft tissue amino acid values for greenlip abalone (Coote et al. 2000). Diets contained ~3.5% lipid (Van Barneveld et al. 1998; Dunstan et al. 2000), and ~17.4 MJ kg⁻¹ crude and ~12.5 MJ kg⁻¹ digestible energy levels. The results of this study will contribute towards the development of diets suitable for post-weaned abalone at different water temperatures.

Materials and Method

Experimental animals and system

Greenlip abalone (weight 0.91 ± 0.01 g; shell length 19.46 ± 0.02 mm; $n = 864$) were purchased from South Australian Mariculture (Port Lincoln, South Australia, Australia) in April 2013. Prior to stocking, abalone were held in a flow through seawater system at South Australia Research and Development Institute Aquatic Science Centre (SARDI ASC) (West Beach, South Australia, Australia) for two weeks and fed a commercial diet (~30% CP; Eyre Peninsula Aquafeed Pty Ltd, Lonsdale, SA, Australia).

The experiment was conducted in a photoperiod and temperature controlled laboratory described in Stone et al. (2013, Chapter 2). The photoperiod was 12 h low intensity fluorescent lighting at 3.4 lux: 12 h dark. The air temperature was adjusted based on the incoming water temperature and ranged from 16.0 - 19.3°C. Three identical culture systems (14, 17 or 20°C) were supplied with 30 µm sand-filtered, UV treated seawater (Model 025120-2, 120w, Emperor Aquatics, Pottstown, PA, USA). Sixteen 12.5-L blue plastic culture units (Nally IH305, Viscount Plastics Pty Ltd.; 39.2 × 28.8 × 11.0 cm) per system were each supplied with flow-through seawater (300 mL min⁻¹). Water depth was held at 2.5 cm using a standpipe with a mesh screen (0.8 mm) on the outlet to retain uneaten feed. Water temperature was held at 14, 17 or 20°C ($\pm 1^\circ\text{C}$) throughout the experiment through the use of either immersion heaters (240 V, 3 kw, JQ20; Austin and Cridland, Carlton, NSW, Australia) or chillers (3 hp, 240 V, 50 Hz: Daeil Cooler Co., Ltd., Busan, Korea).

Stocking

Abalone were gently pried from the substrate using a spatula. Eighteen animals were weighed, measured and stocked into one of four replicate culture units per treatment combination. Animals were acclimated to the system for 16 d and fed their respective diets. After seven d the water temperature was either lowered or raised slowly (1°C d⁻¹) to the desired water temperatures (14, 17 or 20°C) and was maintained at these levels ($\pm 1^\circ\text{C}$) throughout the remainder of the 75 d experiment. Dead abalone during the experiment were measured, weighed, recorded, and replaced with abalone of a similar weight and size that had been held at each respective water temperature and fed the commercial formulated diet.

Diets and feeding

At each temperature, animals were fed with one of four dietary protein levels (27, 30, 33 and 36% CP; Stone et al. 2013, Chapter 2, Table 2.1). The proximate composition of the ingredients was analysed prior to diet formulation. Diets were formulated on a digestible protein and isoenergetic basis, based on data reported for greenlip abalone (Fleming et al., 1998; Vandeppeer, 2005). Solvent-extracted soybean meal, de-hulled lupins, casein and fish meal were used as the main dietary protein source, while fish oil, fish oil and de-hulled lupins were used as the main dietary lipids source. Diets were also formulated, using book values, so that the ratio of each essential amino acid to lysine was equal to, or greater than, that analysed to the soft body tissue of greenlip abalone (Coote et al. 2000). Due to the difficulty to determine the amino acid requirement of abalone (Shipton et al. 2002), applying the "ideal protein concept" was concluded to be an acceptable alternative (Fleming et al., 1996).

Diets were cold-pressed into flat pellets (4 × 3 × 2 mm thick) using a commercial pasta machine (La Prestigiosa medium; IPA. Vicenza. Italy). The dry matter leaching loss for each diet was determined in triplicate by submerging the diet (1 g) in seawater (25 mL) at 14, 17 and 20°C for 16 h. After 16 h, the supernatant was removed, by syringe, and the remaining pellets were dried at 105°C for 16 h. The dry matter leaching loss for all diets was highest at 20°C, but was less than 8% dry weight. Abalone were fed to excess of their daily requirements (4% of the abalone biomass d⁻¹) at 16:00 h. Feed rates were maintained at these levels throughout the study based on monthly weight checks. Tanks were cleaned and

uneaten feed was collected by sieving the entire tank contents through a fine mesh at 08:30 h and stored at -20°C, and was later dried at 105 °C for 16 h. Daily feed consumption was estimated by the difference between feed offered and uneaten feed in dry weight. The proportion of uneaten feed lost between 08:30 to 16:00 h, from leaching and by sieving the entire tank contents through a fine mesh without animals in the tank, at the respective water temperatures, was used as a correction factor to calculate the apparent feed consumption rate.

Biochemical analysis and water quality analysis

At the commencement of the experiment, the soft tissue of 50 animals (n = 4 replicates) were collected, shucked and stored at -20°C to analyse the initial soft tissue proximate composition. At the conclusion of the experiment, ten abalone from each tank were collected, shucked and stored at -20°C. The abalone were later pooled for each tank for the analysis of soft tissue proximate composition. The proximate composition analyses of ingredients, diets, and whole body tissue were conducted according to methods in the British Pharmacopoeia Commission (2004) or German Institute for Standardization (2000).

All data reported for animal performance were based on the pooled data from each tank. All calculations using abalone weight were based on wet values, while feed use values were based on dry values:

- Biomass gain (g tank^{-1}) = (final weight + Σ mortality weight) - (initial weight + Σ replacement weight)
- Specific growth rate (SGR, $\% \text{ d}^{-1}$) = (ln final weight - ln initial weight) / time (d) \times 100
- Shell growth rate ($\mu\text{m d}^{-1}$) = (final shell length - initial shell length) / time (d)
- Condition factor = $5575 \times (\text{weight [g]} / \text{length [mm]}^{2.99})$ (Britz and Hecht, 1997)
- Apparent feed consumption = feed offered – uneaten feed collected – ([total feed offered \times % leaching loss without animals] + [uneaten feed collected / % retained without animals \times % leaching loss without animals]) / 2 (Stone et al. 2013, Chapter 2)
- Apparent feed conversion ratio (FCR) = feed consumed / abalone weight gain
- Apparent protein efficiency ratio (PER) = abalone weight gain / protein consumed
- Apparent energy efficiency ratio (EER) = abalone weight gain / energy consumed
- Apparent protein deposition = (final soft body protein - initial soft body protein) / protein intake \times 100
- Apparent energy deposition = (initial soft body energy - initial soft body energy) / energy intake \times 100

Water quality parameters were measured daily and were maintained throughout the study at appropriate levels for the growth of abalone (Table 3.1). Water temperature was measured using a thermometer. Dissolved oxygen (mg L^{-1} and % saturation) was measured using a dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark). The pH was measured using a meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA). Salinity (g L^{-1}) was measured using a portable salinity refractometer (model RF20, Exttech Instruments, Nashua, NH, USA). Light intensity was measured using a LI-COR 1400 Quantum light meter (LI-COR Environmental, Lincoln, NE, USA).

Statistical analyses

IBM SPSS, Version 20 for Windows (IBM SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Homogeneity of variances and normality among mean values were assessed using Levene's test for equality of variance errors and the standardized residuals against the predicted mean plot, respectively. All percentage data was arcsine transformed

before analyses. All variables were analysed using two-factor ANOVA, with water temperature as the first factor and dietary protein level as the second factor. Student Newman-Keuls post-hoc tests were used to detect significant differences between treatments. Linear and second order polynomial regression analyses were also applied to SGR, feed consumption rate ($\text{mg individual}^{-1} \text{d}^{-1}$) and FCR. A significance level of $P < 0.05$ was used for all statistical tests. All values are presented as means \pm standard error of the mean (SE) unless otherwise stated. If SE was < 0.01 it is reported as “0.01”.

Table 3.1. Summary of water quality for each water temperature system.

Nominal water temperature	Actual water temperature ($^{\circ}\text{C}$) ^{1,2,3}	Dissolved oxygen (mg L^{-1}) ^{1,2,4}	Dissolved oxygen (% sat) ^{1,2,4}	pH ^{1,2,4}	Salinity (ppt) ^{1,2,4}
14 $^{\circ}\text{C}$	14.0 \pm 0.1 (13.8 - 14.1)	8.0 \pm 0.3 (7.1 - 8.5)	99.3 \pm 1.4 (95.8 - 103.0)	8.14 \pm 0.05 (7.95 - 8.26)	35.7 \pm 0.57 (34.0 - 38.0)
17 $^{\circ}\text{C}$	17.0 \pm 0.3 (16.1 - 17.9)	7.6 \pm 0.2 (7.0 - 8.0)	98.2 \pm 1.2 (94.5 - 101.8)	8.15 \pm 0.04 (7.99 - 8.26)	35.7 \pm 0.57 (34.0 - 38.0)
20 $^{\circ}\text{C}$	19.9 \pm 0.3 (19.0 - 20.9)	7.3 \pm 0.2 (6.9 - 7.7)	97.2 \pm 1.6 (91.3 - 102.0)	8.15 \pm 0.04 (8.02 - 8.26)	35.7 \pm 0.57 (34.0 - 38.0)

¹ Values means \pm standard deviation, values in parentheses represent the range of values.

² Data for DO, pH and salinity for entire experiment, while the data for water temperature is from end of temperature acclimation period.

³ n = 75.

⁴ n = 91.

Results

General observations

The analysed protein content of the diets was slightly higher than the formulated nominal values (Stone et al. 2013, Chapter 2, Table 2.1). There were no significant differences in the initial weight and shell length between treatments ($P > 0.05$). The overall mortality for the study was 4.05%, but was significantly higher at 14 $^{\circ}\text{C}$ (7.64%) compared to 17 $^{\circ}\text{C}$ (2.09%) and 20 $^{\circ}\text{C}$ (2.43%) ($P = 0.006$; Table 2.1). Mortalities were not significantly influenced by dietary protein level ($P = 0.592$) or interaction between water temperature and dietary protein level ($P = 0.309$).

Growth performance

Water temperature had a significant effect on the final weight and shell length of greenlip abalone ($P < 0.001$; 14 < 17 < 20 $^{\circ}\text{C}$; Table 3.2). Final individual weight and shell length were not significantly affected by dietary protein level ($P = 0.801$ and $P = 0.965$, respectively) or by the interaction of these two factors ($P = 0.924$ and $P = 0.965$, respectively).

Biomass gain, SGR and shell growth rate were also significantly affected by water temperature ($P < 0.001$; 14 < 17 < 20 $^{\circ}\text{C}$; Table 3.2). Dietary protein level had no significant effect on SGR ($P = 0.772$), biomass gain ($P = 0.799$) or shell growth rate ($P = 0.840$) and there were no significant interactive effects between water temperature and dietary protein level on biomass gain ($P = 0.958$), SGR ($P = 0.927$) or shell growth rate ($P = 0.989$). In addition, there was no significant linear or second order polynomial relationship between dietary protein level and SGR for abalone at 14, 17 or 20 $^{\circ}\text{C}$ ($P > 0.05$; Figure 3.1). Condition factor was significantly affected by water temperature ($P < 0.001$; 14 > 17 > 20 $^{\circ}\text{C}$; Table 3.2), but not significantly affected by dietary protein level ($P = 0.472$), or the interactive effects between these two factors ($P = 0.732$).

Table 3.2. Growth performance, feed efficiency and nutrient retention of greenlip abalone at three water temperature fed four dietary protein levels.

Temperature (°C)	14 ¹				17 ¹				20 ¹				SE	ANOVA							
	27	30	33	36	27	30	33	36	27	30	33	36		Temp (°C) (A) ³			Protein level (%) (B) ⁴				AxB ⁵
														14	17	20	27	30	33	36	
<i>Growth performance</i>																					
Mortality (%)	5.56	2.78	9.72	12.50	2.78	1.39	2.78	1.39	4.17	2.78	1.39	1.39	0.82	Z	Y	Y	NS		NS		
Initial weight (g)	0.91	0.91	0.92	0.92	0.91	0.91	0.92	0.91	0.91	0.91	0.92	0.91	0.01	NS			NS		NS		
Final weight (g)	1.59	1.47	1.53	1.49	1.92	1.97	1.95	1.96	2.58	2.50	2.57	2.47	0.06	X	Y	Z	NS		NS		
Biomass gain (g tank ⁻¹)	11.74	10.19	10.49	10.20	18.18	18.97	19.02	18.80	29.97	28.40	29.81	28.03	1.16	X	Y	Z	NS		NS		
SGR (% d ⁻¹)	0.62	0.53	0.56	0.54	0.82	0.86	0.82	0.84	1.15	1.11	1.14	1.10	0.04	X	Y	Z	NS		NS		
<i>Somatic growth parameters</i>																					
Initial shell length (mm)	19.40	19.46	19.55	19.53	19.36	19.51	19.48	19.48	19.37	19.41	19.51	19.45	0.02	NS			NS		NS		
Final shell length (mm)	22.11	21.71	22.04	21.96	24.14	24.34	24.23	24.17	26.67	26.45	27.02	26.61	0.30	X	Y	Z	NS		NS		
Shell growth rate (µm d ⁻¹)	29.98	24.96	27.57	26.60	52.65	53.17	52.61	55.89	80.27	78.21	83.41	79.12	3.34	X	Y	Z	NS		NS		
Condition factor	0.85	0.83	0.82	0.81	0.78	0.79	0.79	0.80	0.78	0.78	0.75	0.76	0.01	Z	Y	X	NS		NS		
<i>Feed utilisation</i>																					
Feed consumption rate (mg individual ⁻¹ d ⁻¹)	15.66 ^{de}	14.60 ^e	16.16 ^{de}	18.44 ^{cd}	16.96 ^{de}	18.31 ^{cd}	18.60 ^{cd}	20.46 ^c	28.56 ^a	25.91 ^{ab}	25.84 ^{ab}	24.61 ^b	0.69	*	*	*	*	*	*	*	
Feed consumption rate (g kg abalone ⁻¹ d ⁻¹)	10.94	10.39	10.53	12.95	10.89	13.03	14.08	14.50	20.92	21.89	18.90	19.01	0.67	X	Y	Z	NS		NS		
Apparent FCR	2.17	2.35	2.55	2.95	1.55	1.58	1.65	1.79	1.55	1.51	1.42	1.43	0.08	Z	Y	Y	NS		NS		
<i>Nutrient retention</i>																					
Apparent PER	1.55	1.24	1.06	0.83	2.21	1.83	1.63	1.35	2.15	1.94	1.87	1.69	0.07	Y	Z	Z	z	y	y	x	NS
Apparent PD	17.90	14.55	12.40	10.25	24.56	16.41	16.77	12.41	23.90	18.58	20.97	15.29	0.84	Y	Z	Z	z	y	y	y	NS
Apparent EER	2.47	2.23	2.06	1.79	3.51	3.31	3.17	2.91	3.42	3.49	3.63	3.65	0.11	X	Y	Z	NS				NS
Apparent ED	10.69	8.52	9.08	7.33	15.02	12.79	11.40	10.55	13.71	13.63	14.22	13.03	0.43	Y	Z	Z	z	zy	zy	y	NS
<i>Proximate composition²</i>																					
Moisture (%)	75.15	75.80	75.12	75.60	74.18	74.90	75.44	76.27	74.62	74.70	74.81	75.72	0.14	NS			y	zy	zy	z	NS
Protein (% dry)	50.95	54.77	51.51	54.51	52.03	46.77	53.44	49.58	53.35	48.65	55.20	49.12	0.92	NS			NS				NS
Fat (% dry)	5.49	5.06	5.17	5.40	5.29	5.08	5.18	4.91	4.69	4.95	4.51	4.20	0.07	Z	Z	Y	NS				NS
Ash (% dry)	10.27	10.57	9.92	10.34	9.84	10.28	10.50	10.76	10.35	10.29	9.85	11.05	0.10	NS			NS				NS
Energy (MJ kg ⁻¹ dry)	19.92	20.02	19.94	20.11	20.02	19.56	19.97	19.62	19.88	19.65	20.04	19.38	0.07	NS			NS				NS

SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; PD, protein deposition; EER, energy efficiency ratio; ED, energy deposition.

¹ Mean ± pooled SE (n = 4).

² Initial soft tissue content of greenlip abalone (dry): protein (46.79%), lipid (4.07%), ash (13.92%), and energy (18.71 MJ kg⁻¹ dry).

³ X, Y, Z: For variables with a significant effect of temperature and no interaction (Z indicates the highest value; $P < 0.05$).

⁴ w, x, y, z: For variables with a significant effect of protein level and no interaction (z indicates the highest value; $P < 0.05$).

* Denotes parameters with a significant interaction (A × B; $P < 0.05$) and difference in protein level are compared across all water temperatures (one-factor ANOVA, SNK test; ^a indicates the highest value; $P < 0.05$).

NS: denotes no significant differences ($P > 0.05$).

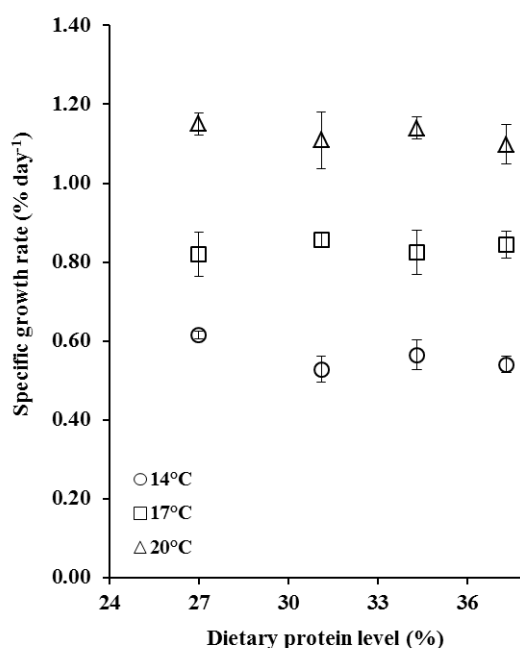


Figure 3.1. Specific growth rate of greenlip abalone at three water temperature fed four dietary protein levels for 91 d. n = 4 tanks, mean \pm SE. There were no significant relationships between dietary protein level and specific growth rate for abalone at 14, 17 or 20°C ($P > 0.05$).

Feed use

Water temperature had a significant effect on feed consumption rate ($\text{mg abalone}^{-1} \text{d}^{-1}$) ($P < 0.001$), while dietary protein level did not ($P = 0.184$). However, feed consumption rate was significantly affected by the interaction between water temperature and dietary protein level ($P = 0.002$; Table 3.2). When compared to abalone fed other dietary protein levels at their respective water temperatures, the feed consumption rate by abalone fed 36% dietary CP level at 17°C was significantly higher, while the feed consumption rate by abalone fed 36% dietary CP level at 20°C was significantly lower. The feed consumption rates of abalone at 14 and 17°C were similar when fed the same dietary protein level. The feed consumption rate of abalone at 20°C was significantly higher than abalone at 14 and 17°C (Table 3.2). In addition, regression analyses indicated that there were significant moderate positive second order polynomial and linear relationships between dietary protein level and feed consumption rate for abalone at 14°C ($R^2 = 0.536$, $P = 0.007$) and 17°C ($R^2 = 0.501$, $P = 0.002$), respectively (Figure 3.2). In contrast, regression analyses indicated that there was a significant moderate negative linear relationship between dietary protein level and feed consumption rate for abalone at 20°C ($R^2 = 0.501$, $P = 0.002$; Figure 3.2). The feed consumption rate ($\text{g kg abalone}^{-1} \text{d}^{-1}$) was significantly affected by water temperature ($P < 0.001$; $14 < 17 < 20^\circ\text{C}$) while dietary protein level have no significant effect on feed consumption rate ($P = 0.632$) and there was no significant interaction between water temperature and dietary protein level ($P = 0.133$).

The apparent FCR was significantly affected by water temperature ($P < 0.001$; $14 > 17 = 20^\circ\text{C}$; Table 3.2), while dietary protein level had no significant influence on FCR ($P = 0.111$) and there was no significant interaction between water temperature and dietary protein level ($P = 0.137$). However, regression analyses indicated that there was a significant moderate positive second order polynomial relationship between dietary protein level and FCR for abalone at 14°C ($R^2 = 0.406$, $P = 0.034$; Figure 3.3). While there were no significant relationships between dietary protein level and FCR for abalone at 17 or 20°C ($P > 0.05$), there were positive and negative tendencies, respectively (Figure 3.3).

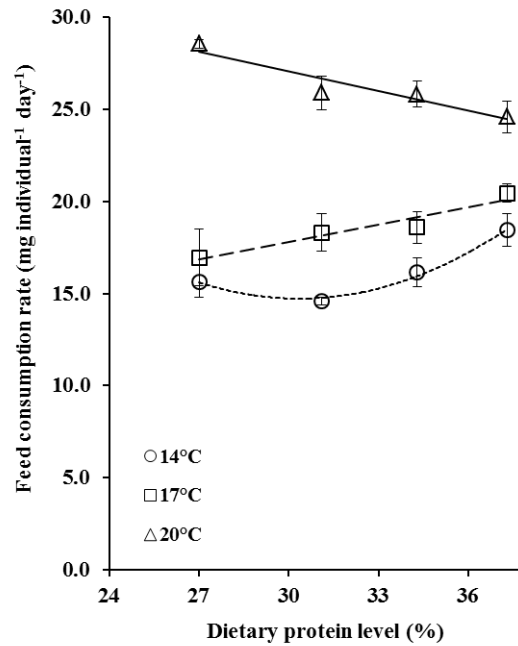


Figure 3.2. Feed consumption rate for greenlip abalone fed four dietary protein levels at three water temperature for 91 d.

n = 4 tanks, mean ± SE.

Second order polynomial relationships: 14°C, $y = 0.78x^2 - 4.753x + 86.871$, $R^2 = 0.536$, $P = 0.007$.

Linear relationships: 17°C: $y = 0.314x + 8.392$, $R^2 = 0.296$, $P = 0.029$; and 20°C: $y = -0.357x + 37.778$, $R^2 = 0.501$, $P = 0.002$.

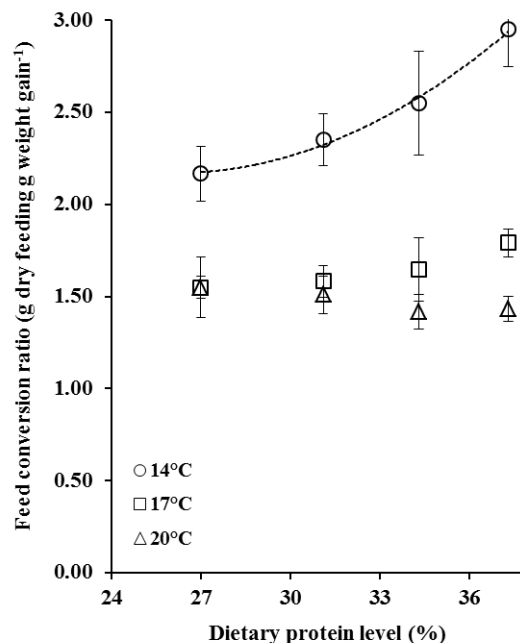


Figure 3.3. Apparent economic feed conversion ratio (FCR) for greenlip abalone fed four dietary protein levels at three water temperature for 91 d.

n = 4 tanks, mean ± SE.

Second order polynomial relationships: 14°C, $y = 0.006x^2 - 0.317x + 6.307$, $R^2 = 0.406$, $P = 0.034$.

There were no significant relationships between dietary protein level and FCR for abalone at 17 or 20°C ($P > 0.05$).

Soft tissue composition

Dietary protein level had a significant effect on the soft tissue moisture content ($P = 0.018$). The soft tissue moisture content was significantly higher in abalone fed 36% CP compared to abalone fed 27% CP (Table 3.2). There were no significant differences between abalone fed other diets. Water temperature had no significant effect on soft tissue moisture content ($P = 0.364$), and there were no significant interactions between water temperature and dietary protein level ($P = 0.441$). Soft tissue protein content in greenlip abalone was not significantly affected by water temperature ($P = 0.556$) or dietary protein level ($P = 0.637$), and there were no significant interactions between these two factors ($P = 0.606$). Soft tissue fat content was significantly influenced by water temperature ($P < 0.001$; $14 = 17 > 20^{\circ}\text{C}$), but was not significantly affected by dietary protein level ($P = 0.073$), and there were no significant interactions between water temperature and dietary protein level ($P = 0.595$). Soft tissue ash content was not significantly affected by water temperature ($P = 0.962$) and dietary protein level ($P = 0.252$), and there were no significant interactions between these two factors ($P = 0.211$). Soft tissue energy was not significantly affected by water temperature ($P = 0.231$) and dietary protein level ($P = 0.317$), and there were no significant interactions between these two factors ($P = 0.519$; Table 3.2).

Nutrient use

The apparent PER was significantly affected by water temperature ($P < 0.001$; $14 < 17 = 20^{\circ}\text{C}$) and dietary protein level ($P < 0.001$). The PER of abalone fed a diet containing 27% CP compared to all other diets was significantly superior to that in all other treatments, while the PER of abalone fed a diet containing 36% CP was significantly inferior to that in all other treatments. The PER of abalone fed diets containing 30 and 33% CP was not significantly different ($P > 0.05$). There was no significant interaction between water temperature and dietary protein level for PER ($P = 0.771$).

Water temperature had a significant effect on apparent protein deposition ($P < 0.001$; $14 < 17 = 20^{\circ}\text{C}$). Protein deposition was significantly influenced by dietary protein level ($P = 0.002$), and was significantly superior in abalone fed a diet containing 27% CP compared to abalone fed other CP levels. The protein deposition of abalone fed 30, 33 or 36% CP was not significantly different ($P > 0.05$). There was no significant interaction between water temperature and dietary protein level ($P = 0.567$).

The apparent EER was significantly influenced by water temperature ($P < 0.001$; $14 < 17 < 20^{\circ}\text{C}$). The EER was not significantly affected by dietary protein level ($P = 0.268$) or the interaction between water temperature and dietary protein level ($P = 0.429$). The apparent energy deposition was significantly affected by water temperature ($P < 0.001$; $14 < 17 = 20^{\circ}\text{C}$; Table 3.2) and dietary protein level ($P = 0.013$). Abalone fed 27% CP had a significant superior energy deposition compared to abalone fed 36% CP ($27 = 30 = 33\%$ CP; $30 = 33 = 36\%$ CP). There was no significant interaction between these two factors ($P = 0.451$).

Discussion

The experimental animals fed actively on diets throughout the study and growth rates were comparable to those observed in commercial facilities and other laboratory-based studies (Coote et al. 2000; Vandeppeer, 2005; Stone et al. 2013, Chapter 2, Figure 2.2a). For example, Coote et al. (2000) reported a SGR for greenlip abalone (~ 1 g) of $1.03\% \text{ d}^{-1}$ at 20°C for 85 d (Coote et al. 2000), Vandeppeer (2005) reported a SGR for greenlip abalone (2.3 g) of $1.05\% \text{ d}^{-1}$ at 18°C for 50 d, while Stone et al. (2013, Chapter 2, Figure 2.2a) reported a calculated peak SGR of $1.48\% \text{ d}^{-1}$ for greenlip abalone (1.8 g) over 84 d at their optimal water temperature of 22°C .

Water temperature is a key environmental variable that affects the survival, growth, feed consumption, nutritional requirements and digestive physiology of abalone (Britz et al. 1997; Edwards and Condon, 2001; Vandeppeer, 2006; Schaefer et al. 2013; Stone et al. 2013, Chapter 2; Stone et al. 2014). In the current study, the SGR, biomass gain, apparent protein deposition, PER, shell growth and feed consumption rate of greenlip abalone all significantly increased with water temperature from 14 to 20°C. Significantly improved SGR with increasing water temperature was previously reported for 1-year-old greenlip abalone up to 22°C (Stone et al. 2013, Chapter 2, Figure 2.2a), and similarly in South African abalone (*Haliotis midae*) up to 20°C (Britz et al. 1997). In the current study, improved growth and protein deposition as water temperature increased may have occurred due to an increased efficiency at utilising dietary components, particularly protein, due to temperature dependent feed intake and digestive enzyme activity (Britz et al. 1997; Edwards and Condon, 2001; Hochachka and Somero, 2002). Edwards and Condon (2001) reported significantly higher (75%) protease activity as temperature increased from 9 to 24°C in blacklip abalone (*Haliotis rubra*) and suggested that this would contribute to improved growth rates up to this species' optimal water temperature of 17°C (Gilroy and Edwards, 1998).

The optimal water temperature for the growth of greenlip abalone is controversial. Gilroy and Edwards (1998) reported a calculated optimal of 18.3°C for Tasmania stock, while more recently Stone et al. (2013, Chapter 2, Figure 2.2a) reported an optimal of 22°C for South Australian stock. Although 22°C was not used in the current study, the SGR of abalone was significantly superior at 20°C compared to 17 or 14°C, providing further support for Stone et al. (2013, Chapter 2). The possible discrepancy may have been due to genetic differences as Gilroy and Edwards (1998) worked with a Tasmanian strain, while in the current study and Stone et al. (2013, Chapter 2) used a more heat tolerant South Australian strain. Additionally, Gilroy and Edwards (1998) used larger greenlip abalone (82 mm SL) compared to smaller abalone (19 mm SL) in the current study. In addition, water temperature optima may also decline with size, where previous research showed age-dependent differences when water temperature was raised from 18 to 22°C; the growth rates of 1-year-old greenlip abalone (23 mm SL) significantly increased, whereas the growth rate of 2-year-old abalone (57 mm SL) did not (Stone et al. 2013, Chapter 2, Figure 2.2a and 2.2b). Steinarsson and Imsland (2003) reported similar size dependent optimal water temperature for red abalone (*Haliotis rufescens*), which peaked at 17.8°C for 44 mm SL abalone, and declined to 14.5°C for 98 mm SL abalone. Lastly, the optimal water temperature reported by Gilroy and Edwards (1998) were not determined from growth studies, but were estimated from behavioural studies on temperature preference. These discrepancies highlight the importance of species, strain and size-specific data from experiments concerning the variable of interest, and to not rely on models generated from other variables. The superior growth rate at high water temperatures observed in the current study further support the benefits of heating nursery systems during periods of low temperatures to improve growth. A cost-benefit analysis of implementing temperature controlled nursery systems is currently being undertaken on abalone farms throughout southern Australia. Further research investigating the optimal water temperature for different sized greenlip abalone, similar to Steinarsson and Imsland (2003), may be beneficial to increase on-farm production, especially with regards to implementing temperature controlled systems for other year classes of abalone.

To improve production without additional infrastructure, diet manipulation, particularly dietary protein levels, also leads to improve growth (Britz, 1996; Britz and Hecht, 1997; Coote et al., 2000; Stone et al., 2013, Chapter 2). Protein is an expensive dietary component and plays a major role in abalone nutrition. The optimal dietary protein level has been the focus of numerous studies for a range of abalone species, including *H. midae* (Britz, 1996; Britz and Hecht, 1997), *H. rubra* (Dunstan, 2010), Green Ormer (*Haliotis tuberculata*) (Mai et al. 1995), Pacific abalone (*Haliotis discus hannai*) (Mai et al. 1995) and greenlip abalone (Coote et al. 2000; Stone et al. 2013, Chapter 2). The aims of these studies have been to reduce on-farm

expense and increase production (Britz, 1996; Fleming and Hone, 1996; Britz and Hecht, 1997; Shipton and Britz, 2001b). The protein requirements of greenlip abalone have been the focus of two previous studies, both studies formulated diets using the “ideal protein concept” (Coote et al. 2000; Stone et al. 2013, Chapter 2). Coote et al. (2000) used highly digestible protein sources (casein and semolina) and reported an optimal dietary CP level of 27% for juvenile greenlip abalone (0.55 - 0.94 g) at 20°C. A more recent investigation by Stone et al. (2013, Chapter 2) used highly digestible protein sources (solvent-extracted soybean meal, de-hulled lupins, casein and fish meal) and reported water temperature- and size-dependent optimal dietary protein level for greenlip abalone. The optimal dietary CP level increased from ~29.0 to 32.2 to 34.7% CP for 1-year-old abalone and from 24 to 34% CP for 2-year-old abalone as water temperature increased from 14 to 18 and 22°C, respectively (Stone et al. 2013, Chapter 2). Due to space limitations in Stone et al. (2013, Chapter 2), the protein requirements of the younger age class of greenlip abalone (~ 6 month old), investigated in the current study, could not be determined at the same time as 1- and 2-year old abalone. Although caution should be exercised when comparing between studies, the aim of the current study was to provide further information on the optimal dietary protein level of this age class.

In the current study, the SGR of abalone was not significantly affected by dietary protein level. However, the benefit of high protein diets to SGR is masked by differences in feed consumption that affected the FCR of abalone. As abalone were fed to satiation throughout the experiment, the significant negative relationship between dietary protein level and feed consumption rate for abalone at 20°C indicates that these faster growing abalone up-regulated feed intake when fed low protein diets to increase protein intake and achieve near-maximum growth potential. In contrast, a significant positive relationship between feed consumption rate and dietary protein levels occurred in slow growing abalone at 14 and 17°C. The positive relationship between dietary protein and feed consumption rate resulted in a significant positive relationship between dietary protein level and FCR for abalone at 14°C. There were no significant relationships between dietary protein level and FCR for abalone at 17°C and 20°C, but a slight positive and negative tendency between dietary protein level and FCR, respectively. These results suggest that the interactive effects of water temperature and dietary protein on feed consumption rate and FCR may be influenced by increased digestive enzyme activity at warmer water temperatures (Edwards and Condon, 2001), differences in the energetics of abalone at different water temperatures (Duong et al. 2014), reduced gastrointestinal transit time (Currie, 2013) or alterations to the gastrointestinal tract morphology (Schaefer et al. 2013).

Feeding abalone high levels of dietary protein, up to 36%, did not necessarily translate to increased soft tissue protein deposition. In contrast, superior protein deposition was previously reported in 1-year-old greenlip abalone fed increasing dietary protein levels at 22°C (Stone et al. 2013, Chapter 2, Figure 2.4a). At sub-optimal water temperatures, below 22°C, abalone may deaminate excess protein to supply energy for metabolism rather than protein deposition and tissue growth, subsequently resulting in increased feed costs and ammonia excretion (Chaitanawisuti et al. 2011). A recent finding by Duong et al. (2014), from samples collected from the same animals used in the current study, indicated that ammonia excretion was significantly higher when abalone were fed diets containing 36% CP compared to abalone fed 27% CP, further supporting this hypothesis. While dietary ingredients used in the current study were selected due to their relatively high protein and energy digestible coefficients (Fleming et al. 1998; Vandeppeer, 2005), to reduce feed costs the energy requirements of abalone should ideally be satisfied by dietary carbohydrates (Dunstan, 2010). Increasing the dietary digestible energy supplied by carbohydrates as dietary protein level increased may have reduced the use protein for energy and in turn, increases the utilisation of dietary protein for protein deposition and growth. However, in practical diet formulations, as we utilised in the present study, there is limited room to economically manipulate the formulation to increase digestible carbohydrate levels. Lipid

inclusion levels used in the current study are optimal for greenlip abalone (Dunstan et al. 2000). As such, providing energy from this source is also limited. It would be beneficial in future studies to investigate different novel dietary ingredients, such as dried macroalgae, with particular consideration to the carbohydrate content, composition and carbohydrate digestibility, to achieve a protein sparing effect (Dunstan, 2010). This may also lead to further improvements to growth and reduction to feed cost.

Test diets were formulated with input from all of the Australian commercial abalone feed producers, and were designed to be isoenergetic, while maintaining the optimal crude lipid level of ~3.5% for greenlip abalone (Dunstan et al. 2000). Due to the inherent lipid content of the other dietary protein sources, fish oil levels decreased as dietary protein level increased. Fish oil, and the inherent fish oil in fish meal, contain essential long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA): eicosapentaenoic acid (20:5n-3, EPA), docosapentaenoic acid (22:5n-3, DPA) and docosahexaenoic acid (22:6n-3, DHA) (Bautista-Teruel et al. 2011). LC n-3 PUFA are important for cellular membrane structure and function, controlling and regulating cellular metabolism, and many other aspects of animal physiology (Bautista-Teruel et al. 2011). The LC n-3 PUFA fatty acid profile of the salmon fish meal and salmon oil used in the current study were not analysed. However, using the analysed crude lipid level of salmon fish meal and salmon fish oil (16 and 100%, respectively; Table 2), and the fatty acid levels of Atlantic salmon by-products (Nichols et al. 2002), the EPA, DPA, DHA and Σ LC n-3 PUFA levels were calculated to be lowest in the 36% CP diet, 0.071, 0.023, 0.108 and 0.202%, respectively. There are conflicting reports in the literature pertaining to the LC n-3 PUFA requirement of abalone. Dunstan et al. (2000) suggested the EPA and DHA requirement of greenlip abalone was 0.3% of the diet, especially at cooler water temperatures, which would suggest that the 36% CP diet in the current study may have been LC n-3 PUFA deficient. However, Dunstan et al. (2000) also concluded that maximum growth would be achieved, with no addition of fish oil, when the dietary fish meal inclusion was greater than 8% (fish meal lipid level of 8%; EPA= 0.014; DPA= 0.002; DHA = 0.011; Σ LC n-3 PUFA = 0.026%), which was far exceeded in all diets used in the current study. In addition, the gene expression of Δ -6 desaturase and elongase 2 and the bioaccumulation of LC n-3 PUFA demonstrated in hybrid abalone (*H. laevigata* \times *H. rubra*) suggest that this closely related hybrid are able to desaturate and chain elongate the precursor α -linolenic acid (18:3n-3, ALA) to EPA, and DHA to a lesser extent (Mateos et al. 2011). In the current study, the lipid fractions of de-hulled lupin meal, and to a lesser extent solvent extracted soybean meal, contained moderate levels of the precursor ALA (Chiofalo et al. 2012; Monteiro et al. 2012), and likely supplemented dietary LC n-3 PUFA. Moreover, Hernández et al. (2013) reported that if ALA is present in the diet, growth of abalone (*H. tuberculata*) is not compromised after 200 days compared to control animals fed a fish oil diet.

Throughout the study, no apparent gross disease symptoms, but a low number of mortalities were observed, which compared favourably with commercial facilities during routine tank harvesting of stocking procedures. However, significantly higher mortalities at 14°C (7.64%) compared to 17°C (2.09%) and 20°C (2.43%) were observed. Stone et al. (2013, Chapter 2) similarly observed significantly higher mortalities at 14°C (6.60%), than 18°C (1.25%) or 22°C (1.25%). In contrast, summer mortality is the major concern to the abalone farmers in southern Australia during periods of high summer water temperatures (>22°C) where mortality rates can be up to 50% (Vandepeer, 2006). The moderate, yet significantly higher mortalities at low water temperatures observed in the current study might be easily overlooked on-farm. A higher amount of “walk-outs” during the dark period, particularly after monthly weight checks, occurred at 14°C compared to 17 and 20°C. These animals were typically found alive, and returned to their respective tank immediately. “Walk-outs” were also observed in *H. midae* when held at 12 - 20°C, but they do not seem to be water temperature related (Britz et al. 1997). The reason for “walk-outs” and the higher mortalities at 14°C deserves further investigation as it may be currently overlooked on-farm.

Conclusions and Recommendations

In conclusion, this study adds to the knowledge of the nutritional requirement of greenlip abalone throughout their production cycle, by investigating the optimal dietary protein level for post-weaned, 6-month-old sub-adult greenlip abalone at a more precise water temperature range. When considering SGR, feed consumption rate, FCR and protein deposition, there were no apparent benefits to feed 6-month-old greenlip abalone high protein diets at 14 or 17°C. Therefore, we recommend a dietary protein level of 29% CP (21.9% digestible protein) with a digestible energy level of 12.5 MJ kg⁻¹ at 14 and 17°C, which was the minimum recommendation for 1-year-old greenlip abalone by Stone et al. (2013, Chapter 2). Although care must be taken when comparing between studies, as different sized animals and water temperatures were used, Stone et al. (2013; Chapter 2) previously recommended a dietary protein level of 34.7% CP (26.7% digestible protein) for slightly larger 1-year old greenlip abalone at 22°C. In the current study, although dietary protein had no significant effect on SGR of abalone at 20°C, abalone consumed less feed and there was a tendency for an improvement of the FCR, but also a significantly lower PER, as animals were fed increased dietary protein level. Therefore, we suggest that it may be beneficial for greenlip abalone to be switched to a diet containing 34.7% CP (26.7% digestible protein) once the water temperature reaches 20°C. These dietary protein recommendations were derived using greenlip abalone that have been selected for growth and survival at higher summer water temperatures, such as those experienced in South Australian waters and Port Phillip Bay, Victoria. It may be beneficial to switch to higher protein diets at lower temperatures in areas where abalone have been selected to grow at lower water temperatures, such as Tasmania and coastal Victoria. Further research to determine the appropriate water temperature to switch to higher protein diets in Tasmania and coastal Victoria may be required to fine tune on-farm feeding practices. The current study and Stone et al. (2013, Chapter 2) provide a much greater of the protein requirements for 6-month, 1-year and 2-year old greenlip abalone at a range of relevant water temperatures.

Chapter 4. The development of an on-farm abalone grow-out trial manual

David A.J. Stone^{1,2,3*}, Matthew S. Bansemer, James O. Harris^{2,3}.

^{1*} South Australian Research and Development Institute, Aquatic Sciences Centre, 2 Hamra Ave, West Beach, 5024, SA, Australia

² Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide, 5001, SA, Australia

³ Marine Innovation Southern Australia

*Corresponding author: Phone: +61-8-8207 5350, Fax: +61-8-8207 5481, E-mail: david.stone@sa.gov.au

Abstract

An abalone on-farm grow-out manual was developed in consultation with members of the Australian Abalone Growers' Association (AAGA) and staff of the participating feed companies. The manual described relevant methods to run on-farm grow-out trials with abalone. The manual was distributed to participating AAGA farms and commercial feed companies and was accepted and used to standardise the methods between farms to evaluate multi-diet versus single diet feeding strategies for abalone in commercial settings reported in Chapter 5.

The work described in this Chapter addressed Objective 3 of this project and appears in Appendix 4 of this report: Stone, D.A.J. Bansemer, M.S. and Harris, J.O. (2014). Abalone on-farm grow-out trial manual. Prepared by the South Australian Research and Development Institute (Aquatic Sciences), Adelaide. Part of CRC Project No. 2010/736. 27pp.

Introduction

To ensure the trials described in Chapter 5 of this report were conducted in a controlled manner between farms, an abalone on-farm grow-out trial manual was developed. The manual was developed in close consultation with participating AAGA farmers and members and the participating feed companies. The development of the manual involved several farm visits and phone interviews with AAGA members and feed company personal. An overview of the manual was presented, for further comment, at the AAGA meeting at SARDI West Beach in September 2010. The manual was then finalised and distributed for use on-farm.

Materials and Methods

The manual addressed a range of culture variables which included, but was not limited to, the following:

- Anaesthetic methods;
- Grading and management interventions;
- Measuring methods;
- Synchronising water quality measurements;
- Stocking densities;
- Feed rates;
- Feeding times and frequencies;
- Type of culture units;
- Replication;
- Water flow;
- Performance indices to be measured; and
- The use of designated farm persons to run studies.

Conclusions and Recommendations

Once the manual was completed, a hard copy was distributed to participating industry members and used successfully in the on-farm trials (Chapter 5). A PDF of the manual was also sent to the CEO of the AAGA for distribution to other association members. The manual appears in Appendix 4 of this report.

Chapter 5. Commercial validation of improved feeding strategies for abalone

David A.J. Stone^{1,2,3*}, Matthew. S Bansemer², Anton Krsinich⁴, Lucy Saunders⁴, Tim Rudge⁵, Liam Bryce⁵, David Connell⁶, James O. Harris^{2,3}.

^{1*} South Australian Research and Development Institute, Aquatic Sciences Centre, 2 Hamra Ave, West Beach, 5024, SA, Australia

² Flinders University, School of Biological Sciences, GPO Box 2100, Adelaide, 5001, SA

³ Marine Innovation Southern Australia

⁴ Great Southern Waters Pty. Ltd., 366 The Esplanade, Indented Head, 3223, VIC

⁵ Coastal Seafarms, 9b Market Ct, Portland, 3305, VIC

⁶ Kangaroo Island Abalone, North Coast Road, Kangaroo Island, 5223, SA

*Corresponding author: Phone: +61-8-8207 5350, Fax: +61-8-8207 5481, E-mail: david.stone@sa.gov.au

Abstract

Three >18 month on-farm trials evaluated growth and feed utilisation of greenlip and hybrid abalone using commercially formulated diets and two different feeding strategies. The trials at Coastal Seafarms and Kangaroo Island Abalone tested diets formulated and produced by Skretting and EP Aquafeed, respectively, and used the following feeding strategies: 1. single-diet feeding strategy, the current production method of feeding one standard protein diet for the entire trial; and 2. multi-diet feeding strategy, fed a sequential combination of “high protein” / “low protein” grow-out diets for the entire trial. The trial at Great Southern Waters used diets produced by Skretting, but used a different feeding strategy that consisted of a standard protein diet for the entire trial versus a high protein diet for the entire trial. Great Southern Waters reported a 9% improvement in biomass gain of hybrid abalone with the high protein diet feeding strategy. This was achieved with no differences in survival and minimal difference in feed input between dietary treatments. At the completion of the on-farm trials at Coastal Seafarms and Kangaroo Island Abalone, no significant differences in growth and feed utilisation were detected when using the multi-diet feeding strategy. Numerous factors were identified that limited the ability to draw conclusions from these two trials and recommendations are provided to improve future on-farm trials. In retrospect, in the early stages of the development of multi-diet feeding strategies for abalone, it may have been more beneficial to prove the concept of improved abalone growth on these farms using a high protein diet alone for the entirety of the trials. This is consistent with the results reported for 1-year-old greenlip abalone fed high protein diets during periods of high growth (Stone et al. 2012, Chapter 2). Based on an abalone farm gate value of \$25 kg⁻¹ (AAGA personal communication), for an additional feed input cost of \$2, an annual increase (9.5%) in sales revenue of \$31 m⁻² of tank area was achieved for hybrid abalone produced using the high protein diet feeding strategy. Additionally, the overall duration of a typical three year production cycle for hybrid abalone may be shortened by 1.9 to 3.4 months by feeding high protein diets. Considerable savings could also be gained from reduced summer mortality of stock during shortened production cycles. By adopting these high protein diets and feeding strategies, farmers may harvest abalone sooner, and reduce exposure to one less summer. This factor alone could result in improved productivity, and when combined with savings made with 6 - 9% improvements in weight, biomass and feed efficiency gains, a >10% improvement in productivity across the entire grow-out period for greenlip and hybrid abalone may be achieved.

The work described in this Chapter addressed Objective 4 of this project and is to be prepared for publication in the internationally peer reviewed journal, *Aquaculture*.

Introduction

Multi-diet feeding strategies, aimed at meeting differences in protein requirements of an animal at different seasonal temperatures and ontogenic stages of development, are commonly used throughout the grow-out cycle to improve productivity for a range of farmed terrestrial and aquatic organisms (National Research Council 1993; Hardy 2002). Multi-diet feeding strategies may also provide other critically important outcomes such as minimised nitrogenous effluent outputs and associated culture unit and environmental pollution impacts (National Research Council 1993). Multi-diet strategies may have the potential to improve abalone growth and farm productivity. There is evidence that demonstrates marked difference in the growth performance and feed utilisation of related abalone species of different size classes in relation to changes in dietary protein level (Britz and Hecht 1997; Bautista-Teruel and Millamena 1999). Temperature has also been demonstrated to have a significant effect on growth rate of abalone (Steinarsson and Imsland 2003; Stone et al. 2013, Chapter 2; Bansemer et al. 2015, Chapter 3). These differences suggest the use of multi-diets feeding strategies for abalone could improve growth and farm productivity.

Prior to this project, apart from using specialised nursery diets, or altering the feeding rate in response to seasonal fluctuations in water temperatures, Australian abalone producers did not routinely alter the protein content of the commercial grow-out diets in response to changing animal sizes or water temperature (Stone et al. 2013, Chapter 2). Several feed companies supplied grow-out diets to the AAGA farmers. The ingredient composition of the grow-out feeds differed between manufacturers, but typically contained similar crude protein levels ranging from 27 - 30%. This range of dietary protein was based on research by Coote (1998) and Coote et al. (2000), that used juvenile greenlip abalone of one size class (25 mm SL) and one water temperature (20°C).

Results from the laboratory experiments described in Chapters 2 and 3 demonstrated differences in the growth performance and feed efficiency of post-weaned sub-juvenile (6-month-old), juvenile (1-year-old) and sub-adult (2-year-old) greenlip abalone (*Haliotis laevis*) in relation to changes in dietary protein level and water temperature (Stone et al. 2013, Chapter 2; Bansemer et al. 2015, Chapter 3). In summary, very little growth occurred for each size class of abalone at the lower water temperature of 14°C, regardless of the dietary protein level. However, the growth performance and feed utilisation of 1-year-old greenlip abalone at warmer water temperatures was improved by using 31 to 35% dietary protein. In contrast, there was little to no gain by increasing the protein level above 27% for the larger 2-year-old abalone, regardless of water temperature. The results suggested that there was considerable scope to incorporate a multi-diet feeding strategy, specifically targeting dietary protein level manipulation at seasonal water temperatures, into the production cycle of abalone in land-based culture in Australia, or for that matter, elsewhere. Hence, a series of long term (>18 month) on-farm trials were run at three AAGA farms; Great Southern Waters, Coastal Seafarms and Kangaroo Island Abalone.

Aim

The aim of the study was to evaluate the potential of high protein or multi-diet feeding strategies versus a single diet feeding strategy utilising a pre-existing standard dietary protein level to improve the growth performance and feed utilisation of greenlip (*Haliotis laevis*) and hybrid abalone (*H. laevis* × *H. rubra*) under commercial conditions across the normal production cycle.

Materials and Methods

The detailed description of the methods used for the on-farm trials are provided in Appendix 4 (Abalone on-farm grow-out trial manual). A brief summary description of the methods specifically used on each farm is presented below.

Participating farms and feed companies

Initially, six land-based commercial abalone farms (three greenlip and three hybrid producers) and three commercial abalone feed producing companies were to be directly involved in the on-farm trials. However, due to industry constraints, only three abalone farms (2 greenlip and 1 hybrid producers) and two feed companies were able to participate.

The three abalone companies were:

1. Coastal Seafarms. Contact: Mr Tim Rudge, General Manager, 9b Market Ct, Portland, Victoria, 3305 (Greenlip abalone);
2. Great Southern Waters Pty. Ltd. Contact: Anton Krsinich, CEO, 366, The Esplanade, Indented Head, Victoria, 3223 (Hybrid abalone); and
3. Kangaroo Island Abalone. Contact: David Connell, General Manager, North Coast Road, Kangaroo Island, South Australia, 5223 (Greenlip abalone).

The two participating feed companies were:

1. Eyre Peninsula Aquafeeds. Contacts: Dr Thomas Coote, 44 Donegal Rd, Lonsdale, South Australia, 5160 (serviced KIA); and
2. Skretting Australia. Contact: Dr Matthew Bransden, 26 Maxwells Road, Cambridge, Tasmania, 7170 (serviced CSF and GSW).

A fourth farm, Southern Ocean Mariculture (Port Fairy, Victoria), was designated to use hybrid abalone fed the Aquafeeds Australia diets, but due to high summer water temperatures and limitations with stock movements, the farm had to opt out of the trial. As a result, Aquafeeds Australia was not able to test their diets in this component of the project.

Diet formulations, feeding strategies and feed rates

The farm-based trials were designed to evaluate commercial diet formulations combined with different feeding strategies for the production of juvenile and sub-adult greenlip and hybrid abalone grown across normal commercial production cycles, at varying life cycle stages, whilst exposed to seasonal fluctuations in ambient water temperatures. The biochemical composition of the diets and a description of the diets and feeding strategies evaluated in each on-farm trial are provided in Tables 5.1 and 5.2, respectively.

At each farm, the growth performance of abalone fed the newly formulated starter and grow-out diets were evaluated against the growth performance of abalone fed the pre-existing grow-out diet, alone. This resulted in each farm using up to three different diet formulations within the two feeding strategies (Table 5.2). The feed companies used the information developed in Chapter 2 to formulate practical diets of appropriate protein and energy levels for juvenile and sub-adult abalone (Table 5.1).

Table 5.1. The biochemical composition of the commercial diets used in the on-farm trials.

Item (as fed)	Eyre Peninsula Aquafeeds diets ¹			Skretting Australia diets ²	
	Low protein (LP)	Standard protein (SP)	High protein (HP)	Standard protein (SP)	High protein (HP)
Moisture (%)	9.00	9.00	9.00	9.00	9.00
Crude protein (%)	27.10	29.60	33.80	30.00	35.00
Lipid (%)	3.80	3.66	4.20	4.00	4.00
Ash (%)	5.60	6.50	6.50	6.00	4.00
Carbohydrate (%)	49.50	48.90	41.60	49.00	48.00
Nitrogen free extract (%) ³	63.50	60.24	55.44	60.00	57.00
Gross energy (MJ kg ⁻¹) ⁴	18.80	18.80	19.20	19.00	19.60

¹ Diet composition data from Eyre Peninsula Aquafeeds.

² Diet composition data from Skretting Australia.

³ Nitrogen free extract (%) = 100% - [protein (%) + lipid (%) + ash (%)].

⁴ Dietary gross energy content (MJ kg⁻¹) calculated using the dietary values (g kg⁻¹) for crude protein, lipid and nitrogen free extract multiplied by the following gross energy values for protein, 23.6 MJ kg⁻¹; lipid, 39.5 MJ kg⁻¹; and carbohydrate, 17.2 MJ kg⁻¹ (NRC, 1995).

On-farm culture systems

The culture systems at each farm used for each trial all represented the typical culture systems and conditions utilised in the commercial production of abalone.

Great Southern Waters committed 16 concrete slab tanks to the trial (eight replicate slab tanks feeding strategy¹). The slab tanks were 16.1 m long and 2.5 m wide, with a laminar flow water depth of 4.6 cm at the inlet end and 1.5 cm at the outlet end. Tanks were supplied with ambient temperature seawater at a varying flow rates throughout the year according to the season (1/11/12 - 31/1/13, 180 L min⁻¹; 1/2/13 – 31/10/13, 170 L min⁻¹; 1/11/13 - end, 180 L min⁻¹). Slab tanks were cleaned regularly using tipper flushers.

Coastal Seafarms committed eight concrete slab tanks to the trial (four replicate slab tanks feeding strategy¹). The slab tanks were 16 m long and 2.5 m wide, with a laminar flow water depth of 3.0 cm. Tanks were supplied with ambient temperature seawater at a flow rate of 170 L min⁻¹. Tanks were cleaned regularly using tipper flushers.

Kangaroo Island Abalone committed ten (five replicate systems feeding strategy¹) PVC pipe systems to the trial between 18/5/12 to 20/11/12. Each system was comprised of 75 m of 100 mm diameter PVC pipe (effective surface area = 16 m²). The PVC systems were provided with ambient temperature seawater at a flow rate of 15 to 30 L min⁻¹. Pipes were cleaned regularly by flushing with seawater at 150 L min⁻¹ for 2 min. After the first thinning harvest (20/11/12) abalone were transferred to eight plastic slab tanks (area, 26.4 m²; 11 m long and 2.4 m wide, with a laminar flow water depth of 1.5 cm). This resulted in four replicate slab tanks per feeding strategy. Slab tanks were used until the completion of the trial. The slab tanks were provided with ambient temperature seawater at a flow rate of 100 to 150 L min⁻¹, and were situated in a shed covered with two layers of black shade cloth. Tanks were cleaned regularly using tipper flushers.

Table 5.2. A description of the diets and feeding strategies evaluated in each on-farm trial.

Abalone farm	Feed company	Abalone type	Feeding strategy	Crude protein (% CP, as fed)	Feed rate (% biomass d ⁻¹)	When fed	Comments
Great Southern Waters	Skretting Australia	Hybrid	1. Single diet, standard protein level	Standard protein (SP) ¹ : 30.0	1 - 3, daily for both feeding strategies ²	Entire trial	Abalone for both feeding strategies were weaned onto the standard protein Skretting Halo diet prior to the experiment
			2. Single diet, high protein level	High protein (HP): 35.0			
Coastal Seafarms	Skretting Australia	Greenlip	1. Single diet, standard protein level	Standard protein (SP) ¹ : 30.0	1 - 3, six d week ⁻¹ for both feeding strategies ²	Entire trial	Abalone for both feeding strategies were weaned onto the standard protein Skretting Halo diet prior to the experiment
			2. Multi-diet, mix of protein levels	Standard protein (SP) ¹ : 30.0 High protein (HP): 35.0			
Kangaroo Island Abalone	Eyre Peninsula Aquafeeds	Greenlip	1. Single diet, standard protein level	Standard protein (SP): 30.0	Phase 1: 1 - 3 ² , daily Phase 2 and 3: fed visually to demand daily	Entire trial	At the start of the trial Abalone were weaned directly onto the diets used for each of their respective feeding strategies. Initially, abalone were fed with 3 mm pellets and then at 3 and 7 months they were switched to 5 and 7mm pellets, respectively.
			2. Multi-diet, mix of protein levels	Low protein (LP): 27.1 Standard protein (SP): 29.6 High protein (HP): 33.8			

¹ Skretting Australia standard protein diet was Halo diet.

² The actual feed rates varied over the course of the experiment depending on water temperature and abalone size.

History of abalone prior to the commencement of the on-farm trials

Prior to the commencement of each trial, abalone used at each farm were cultured using normal commercial methods. A brief summary of the culture conditions used at each respective farm are provided below.

Great Southern Waters bred hybrid abalone on-site. Prior to the commencement of the trial juvenile hybrid abalone were cultured indoors, under low light conditions, in slab tanks provided with flow-through seawater at ambient temperatures, and fed the Skretting Halo diet (4 mm).

In May 2012, greenlip abalone bred at SAM Abalone Pty. Ltd. (Boston Point, Port Lincoln, South Australia) were transferred to Coastal Seafarms at 7 months of age and cultured in deep water plastic lined nursery tanks that contained stone 'hides' as refuges for the abalone. The tanks were supplied with filtered ambient temperature seawater, and situated under a single layer of shade mesh. Abalone were fed the Skretting Halo diet (4 mm).

At Kangaroo Island Abalone, greenlip abalone were bred on-site and cultured indoors in nursery tank systems. The nursery systems were provided with flow-through seawater at ambient temperatures. During this period abalone fed on the algal growth on the sheets. The abalone were not weaned onto commercial diets prior to the commencement of the trial.

Experimental procedures

The experimental procedures used in the on-farm trials were based on those reported in Appendix 4 and outlined in Figure 5.1. Variations to the sampling methods outlined in this figure occurred as a result of water temperature restrictions on handling and stock movements. Alterations were appropriate and are presented with the results in this Chapter. Mortalities on each farm were also counted and recorded on a regular basis.

Stocking and running of on-farm trials

The on-farm trials were planned to start in October/November 2010, run for 18 months and cover two summer growing periods. However, due to a delay in obtaining data from the laboratory experiments, a late start to all of the on-farm trials occurred. Following consultation with AAGA members, the trials started when possible and were extended to cover an additional summer. The trials ran for 561 d (18.3 months; 15/11/12 - 30/5/14), 653 d (~21 months; 11/9/12 - 26/6/14) and 686 d (~23 months; 11/5/12 - 28/5/13) at Great Southern Waters, Coastal Seafarms and Kangaroo Island Abalone, respectively.

A description of the sampling carried out at stocking is provided in Figure 5.1. Each trial was stocked, sampled and harvested using the commercial methods practiced at each farm.

At Great Southern Waters, the on-farm trial ran in one phase. The trial was stocked by the 15/11/12, with medium graded advanced post-weaned juvenile hybrid abalone (initial weight 3.2 g; initial shell length (SL) 29 mm) at 88 kg abalone slab per tank (~25,000 abalone slab tank⁻¹) (Table 5.3). As abalone grew, to ensure that excessive stocking densities did not interfere with the growth of abalone, all slab tanks were partially harvested on four separate occasions throughout the course of the trial. The trial concluded on the 30/5/2014 (total duration 18.3 months, 561 d).

Great Southern Waters also weighed, measured and tagged 120 abalone per tank on the 16/1/13 using plastic FPN glue-on shellfish tag (Hallprint Pty. Ltd. Hindmarsh Valley, South Australia, Australia) and Supa Glue Gel (Selleys® Quick Fix™, Selleys®, Padstow, New South Wales, Australia). During the course of the trial the tagged abalone were carried forward through each thinning harvest and re-stocking event and were re-weighed, measured and recorded ~15 months (tagged duration 453 d) later, just prior to final harvest (14/4/2014).

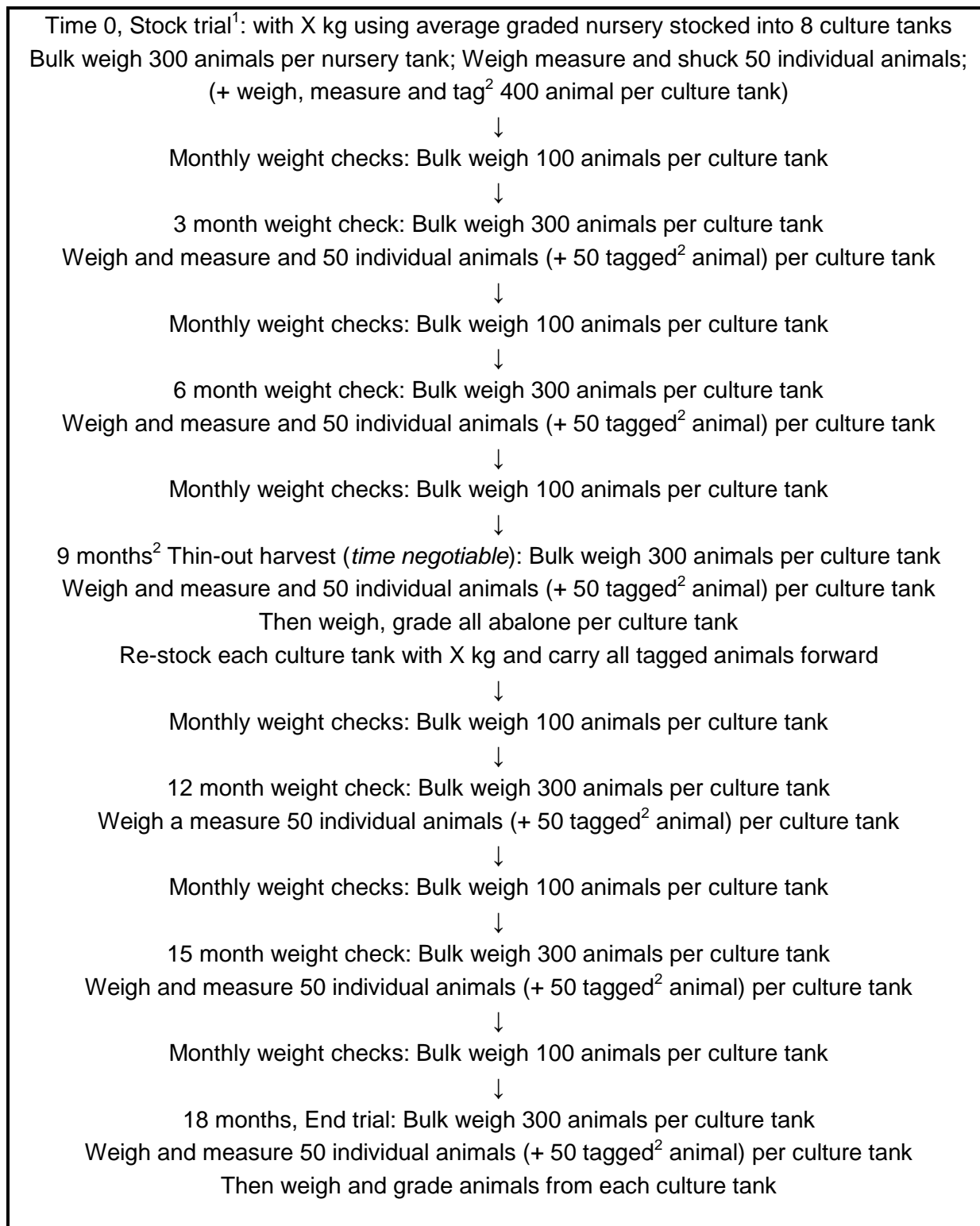


Figure 5.1. Flow diagram of the timing of events used for the 18 month on-farm trials.
¹ The stocking and thinning harvest dates were negotiable and are described in the stocking and running of trials sections in this Chapter of the report;
² Tagging was optional and was only carried out at GSW.

Over the course of the trial at Great Southern Waters, abalone were sub-sampled on a regular basis, generally according to the procedure described in Figure 5.1. Water quality was also measured in the culture tanks on a regular basis. The variables measured were dissolved oxygen (mg L^{-1}), pH, salinity (g L^{-1}) and temperature ($^{\circ}\text{C}$).

During the stocking of the Great Southern Waters trial, one tank (E22) received too many abalone. The data from this tank was excluded and resulted in the high protein diet feeding strategy having seven replicate slab tanks compared to the eight tanks for the single-diet feeding strategy.

At Coastal Seafarms, the on-farm trial ran in two distinct phases for a total of 653 d (~21 months). For Phase 1 (11/9/12 - 13/9/13; 367 d; ~12 months), the trial was stocked on the 11/9/12 with advanced post-weaned juvenile greenlip abalone (average initial weight 2.2 g; average initial SL 22 mm) at 99 kg abalone per slab tank. Over the course of this phase, abalone were sub-sampled on a regular basis according to the procedure described in Figure 5.1. During the course of Phase 1 a further four thinning harvest events took place on the 22/1/13, 27/2/13, 27/3/13 and the 9/4/13, with 100, 75, 37.5 and 25 kg of abalone removed from each slab tank, respectively. At the conclusion of Phase 1, a thinning harvest, grading and re-stocking event was completed on the 13/9/13.

For Phase 2 (13/9/13 - 26/6/14; 286 d; ~9.5 months) of the trial at Coastal Seafarms, 200 kg of medium grade abalone (single-diet strategy, $22.05 \text{ g abalone}^{-1}$; multi-diet strategy, $21.45 \text{ g abalone}^{-1}$) were stocked back into each respective slab tank. Over the course of the first 3 months of this phase abalone were sub-sampled on a regular basis according to the procedure described in Figure 5.1, then they were bulk weighed. This phase concluded on the 26/6/14, at which point all abalone were harvested and bulk weighed.

At Kangaroo Island Abalone, pre-weaned greenlip abalone underwent two thinning harvest, grading and re-stocking events on the 20/11/12 and 28/5/13, which resulted in three separate phases for the trial. For Phase 1, abalone (initial weight 0.6 g; initial SL 15.0 mm) were stocked into each PVC pipe system on the 11/5/12. On the 20/11/12, after 6.4 months (193 d), abalone were harvested from the PVC pipe system, weighed and measured. This process completed Phase 1 of the trial.

Phase 2 of the Kangaroo Island Abalone trial commenced on the 20/11/12. Sixty kg of previously weighed and medium graded greenlip abalone (3.3 g; 29 mm SL) from each PVC pipe system, for the respective treatment, were systematically transferred forward to eight slab tanks (four tanks feeding strategy⁻¹) and cultured for 6.3 months (189 d). On the 28/5/13, abalone were harvested from the slab tanks, weighed and measured. This process completed Phase 2.

Phase 3 of the Kangaroo Island Abalone trial commenced on the 28/5/13. Seventy four kg of medium graded abalone (17 g) were brought forward from their respective treatments and transferred back into their respective slab tanks and on-grown for a further ~10 months (304 d). There was also an additional thinning event during this phase of the trial (28/12/13), where 45 kg was removed from each slab tank to minimise overcrowding. Tanks were harvested and the trial concluded on the 28/3/14. Overall, the entire trial at Kangaroo Island Abalone ran for a total of 686 d (~23 months). Sampling procedures at this farm deviated from those described in Figure 5.1. Over the course of the trial abalone were only sub-sampled at each thinning harvest, re-stocking event and were bulk weighed at the commencement and completion of each phase of the trial.

Variables measured

All calculations using abalone weight and feed weights were based on wet values:

- Apparent biomass gain (kg tank^{-1}) = (final weight + thinning harvest weight) - (initial weight)
- Specific growth rate (SGR, $\% \text{ d}^{-1}$) = $(\ln \text{ final weight} - \ln \text{ initial weight}) / \text{time (d)} \times 100$
- Apparent feed conversion ratio (FCR) = feed consumed / biomass gain
- Apparent protein efficiency ratio (PER) = abalone weight gain / protein consumed
- Apparent energy efficiency ratio (EER) = abalone weight gain / energy consumed
- Shell growth rate ($\mu\text{m d}^{-1}$) = (final shell length - initial shell length) / time (d)
- Condition factor = $5575 \times \text{weight g} / \text{length cm}^{2.99}$ (Britz and Hecht, 1997)
- Meat:shell ratio = final meat weight / final shell weight
- Meat:whole weight ratio = final meat weight / final whole weight

A basic economic analysis of the growth performance of abalone from the Southern Waters on-farm trial was calculated to determine the increase in sales revenue, after feed costs (including freight), on a tank per experiment (561 d) basis and also on a tank y^{-1} basis (Appendix 3). The economic analysis was based on an abalone farm gate value of $\$25 \text{ kg}^{-1}$ (AAGA personal communication), and the differences in feed input costs and yields for hybrid abalone grown using the standard protein diet versus the high protein diet feeding strategy from the Great Southern Waters on-farm trial.

Statistics

Homogeneity of variances among mean values was assessed using Levene's test for equality of variance errors. Data for each farm was analysed separately. Data for each variable was analysed separately using Analysis of Variance (ANOVA). Where interactions were observed, the differences among means were assessed using one-factor ANOVA. A significance level of $P < 0.05$ was used for all statistical tests. All statistical analyses were done using IBM SPSS, Version 21 for Windows (IBM SPSS Inc., Chicago, IL, USA). All values are presented as means \pm standard error of the mean (SE).

Results

Great Southern Waters on-farm trial

The water temperature in the tanks at Great Southern Waters experienced normal seasonal fluctuations throughout the trial, which ranged from $10.1 - 24.7^\circ\text{C}$ with a mean of 17.5°C , which was similar between treatments (Figure 5.2). The mean dissolved oxygen levels were 6.12 and 6.31 mg L^{-1} for the high protein diet and standard protein diet feeding strategies, respectively, and ranged from $4.21 - 9.14 \text{ mg L}^{-1}$ (Figure 5.3). Dissolved oxygen levels were lowest in all slab tanks during the period of mid-summer to early autumn. Salinity levels were similar between treatments (standard protein diet, 34.5 vs. high protein diet, 34.7 g L^{-1} ; Figure 5.4) and ranged from $34.5 - 41.1 \text{ g L}^{-1}$. Initially, salinity levels were in the range of $37 - 38 \text{ g L}^{-1}$ up until late June 2013 when levels spiked to above 40 g L^{-1} for a period approximately one month, where they sharply dropped to levels of $\sim 36 \text{ g L}^{-1}$ for the remainder of the trial. The pH level of the tanks was similar between feeding strategies (mean, 8.37 ; minimum, 8.37 ; maximum, 8.74) and tended to increase as the trial progressed. The tendency for the progressive increase was linked directly to the pH level of the incoming seawater supply (mean, 8.57 ; minimum, 8.09 ; maximum, 8.82) (Figure 5.5) and may have also been linked to the rapid drop in salinity that was also observed over the same period (Figure 5.4).

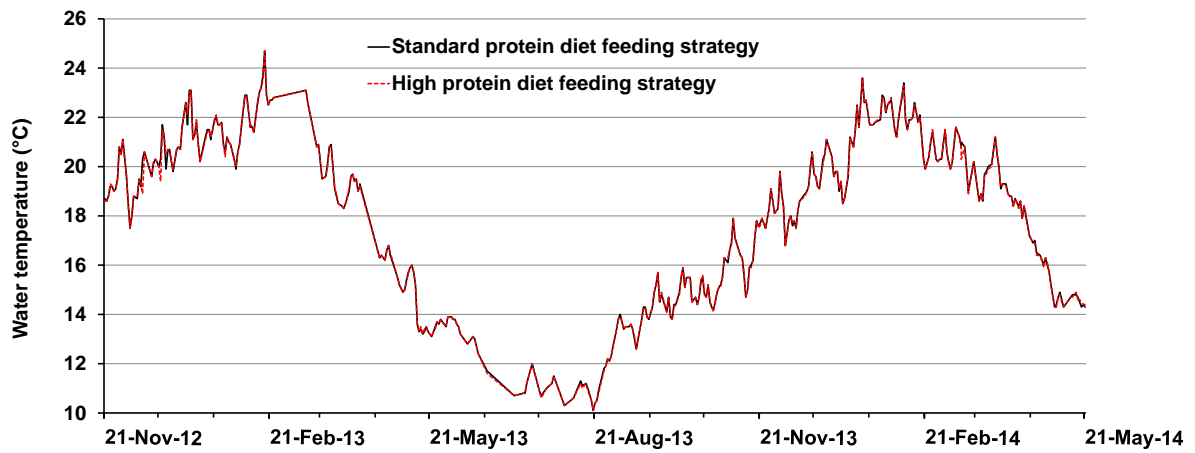


Figure 5.2. Water temperatures in slab tanks for each feeding strategy treatment over the entire on-farm trial at Great Southern Waters (15/11/12 - 30/5/14; 561 d).

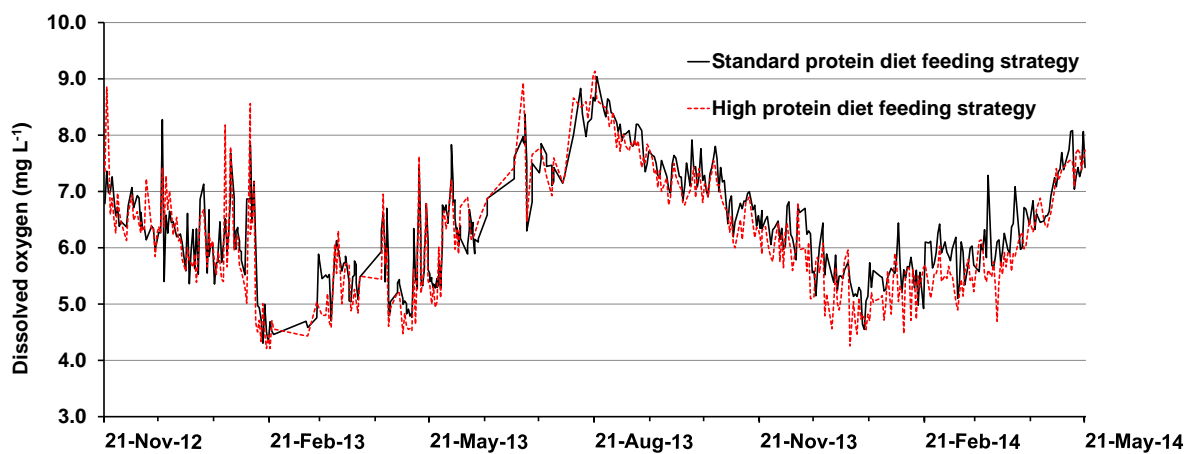


Figure 5.3. Dissolved oxygen levels in slab tanks for each feeding strategy treatment over the entire on-farm trial at Great Southern Waters (15/11/12 - 30/5/14; 561 d).

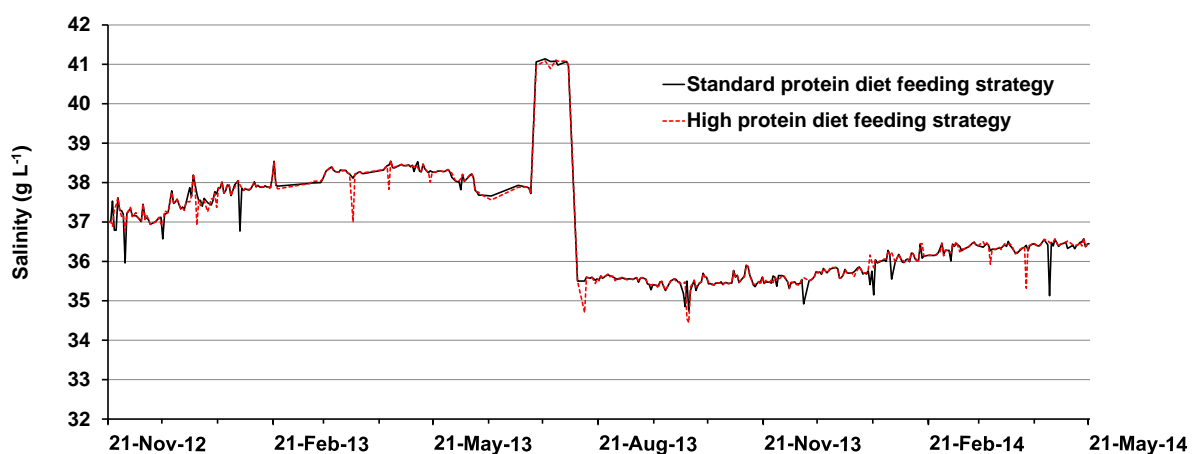


Figure 5.4. Tank salinity levels in slab tanks for each feeding strategy treatment over the entire on-farm trial at Great Southern Waters (15/11/12 - 30/5/14; 561 d).

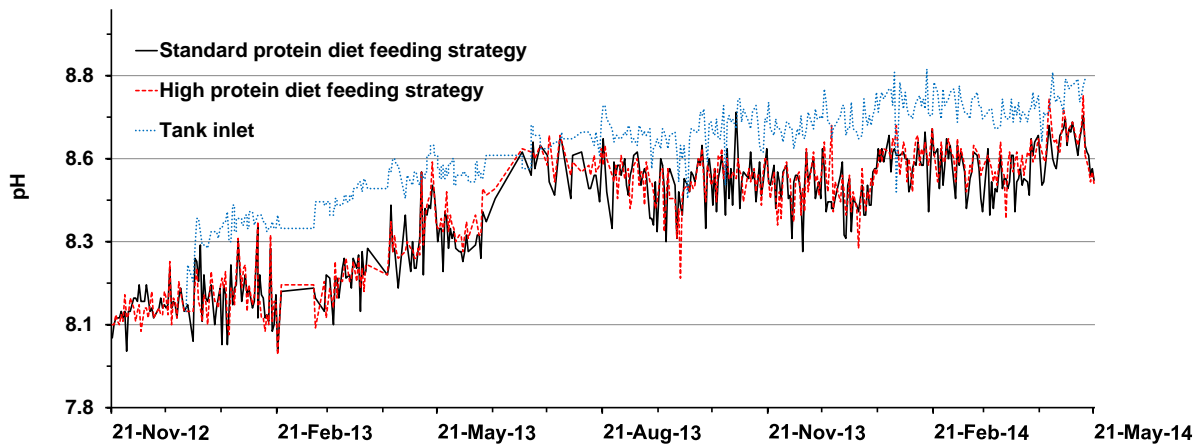


Figure 5.5. Tank pH levels in slab tanks for each feeding strategy treatment over the entire on-farm trial at Great Southern Waters (15/11/12 - 30/5/14; 561 d).

There was a significant effect of diet and time on the mean individual weight of hybrid abalone over the course of the on-farm trial at Great Southern Waters ($P < 0.001$; two-factor ANOVA; Figure 5.6), and there was no significant interaction between the two factors ($P = 0.055$). There was a significant 6% increase in the mean individual weight of hybrid abalone fed the high protein compared to the standard protein diet strategy. The individual weight of hybrid abalone significantly progressively increased at each sampling time over the duration of the trial (Figure 5.6).

The high protein feeding strategy significantly improved the growth performance of hybrid abalone at the end of the trial at Great Southern Waters (Table 5.3). The final individual weight ($P = 0.029$), final weight gains ($P = 0.030$) and SGR ($P = 0.020$) of hybrid abalone fed the high protein diet feeding strategy were significantly higher (5.2, 5.4 and 1.7%, respectively) than abalone fed the standard protein feeding strategy (Table 5.3; one-factor ANOVA). The condition factor of the hybrid abalone fed the high protein feeding strategy was also significantly higher (5.9%) than for the abalone fed the standard protein diet strategy ($P = 0.032$; one-factor ANOVA). There were no significant differences for any of the other variable measured between the standard protein versus the high protein diet feeding strategy for hybrid abalone at the end of the trial ($P > 0.05$; Table 5.3). Nevertheless, compared to the standard protein diet feeding strategy, the corresponding percent increase for final biomass (8.6%) and biomass gain (9.3%), apparent FCR (7.1%) for abalone fed the high protein diet feeding strategy were all numerically superior, with probabilities (P) below 0.100 and approaching the 0.05 level of significance (Table 5.3).

Based on an abalone farm gate value of \$25 kg⁻¹ (AAGA personal communication) and a biomass gain of 9.3%, a substantial 9.5% increase in sales revenue of \$31 m⁻² of slab tank area y⁻¹ was attained, after taking into account an extra \$2 of feed costs (including freight), for hybrid abalone produced using the high protein diet feeding strategy (Appendix 3, Table A3.1).

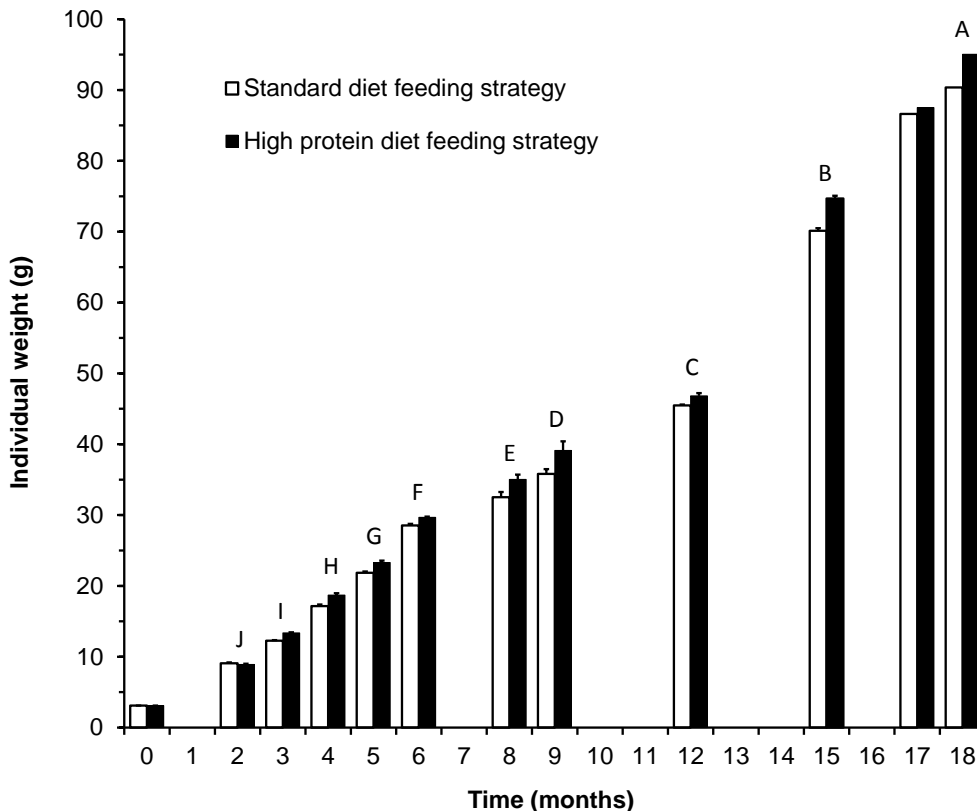


Figure 5.6. Individual weights of hybrid abalone fed the standard or high protein diet feeding strategies at Great Southern Waters over the duration of the entire trial (15/11/12 - 30/5/14; 561 d, 18.3 months).

The standard protein diet strategy (n = 8) used the standard protein (30.0% CP) Skretting Halo diet for the entire trial.

The high protein diet strategy (n = 7) used the high protein (35.0% CP) Skretting diet.

Each mean value is derived from 500 individually weighed abalone per tank.

Apart from initial values (n = 3), all other values are means \pm SE.

Initial and tagged abalone data at 17 months were excluded from statistical analysis.

Tagged abalone had been handled previously and initial values were derived from abalone from the holding tanks prior to stocking.

There were significant effects ($P < 0.001$) of feeding strategy (high protein diet > standard protein diet) and time on the individual weight of abalone over the course of the trial (progressively significantly increased between each sampling time). No significant interactions occurred between the two factors ($P = 0.055$). Different letters denote significant differences between times ($P < 0.001$; two-factor ANOVA, SNK test).

For tagged hybrid abalone, there were no significant differences between any of the variables for either feeding strategy at the completion of the on-farm trial (Table 5.4.; $P > 0.05$; one-factor ANOVA). However, apart from final shell length and shell growth rate, there was a tendency for all variables to be numerically greater for tagged abalone from the high protein compared to the standard protein diet feeding strategy.

There was a high rate of tag shedding recorded for hybrid abalone in the trial at Great Southern Waters. On the 16/1/13, 120 abalone from each tank were tagged. After 453 d, only $38.3 \pm 9.9\%$ of the tagged abalone were recovered.

Table 5.3. Performance of juvenile hybrid abalone at the completion of the Great Southern Waters trial (15/11/12 - 30/5/14; 561 d).

Item ¹	Standard protein diet strategy ⁴	High protein diet strategy ⁵	P value
Survival (%)	98.73 ± 0.27	98.68 ± 0.27	0.897
Initial biomass (kg tank ⁻¹) (15/11/12)	77.78 ± 0.59	77.56 ± 0.63	0.800
Final biomass (kg tank ⁻¹) (30/5/14)	1159.5 ± 37.8	1259.5 ± 28.5	0.060
Biomass gain (kg tank ⁻¹)	1081.7 ± 38.2	1181.9 ± 28.6	0.062
Total feed offered (kg tank ⁻¹)	1065.3 ± 10.5	1088.8 ± 7.21	0.096
Apparent Feed conversion ratio (FCR)	0.99 ± 0.08	0.92 ± 0.05	0.082
Apparent protein efficiency ratio (PER)	3.38 ± 0.11	3.11 ± 0.07	0.051
Apparent energy efficiency ratio (EER)	5.34 ± 0.17	5.54 ± 0.12	0.379
Initial weight (g abalone ⁻¹)	3.11 ± 0.02	3.10 ± 0.03	0.800
Final weight (g abalone ⁻¹) ²	90.36 ± 1.37 ^b	95.05 ± 1.30 ^a	0.029
Weight gain (g abalone ⁻¹)	87.25 ± 1.38 ^b	91.94 ± 1.31 ^a	0.030
Specific growth rate (SGR % d ⁻¹)	0.600 ± 0.004 ^b	0.610 ± 0.003 ^a	0.021
Initial shell length (mm) ³	29.49 ± 0.19	28.81 ± 0.63	
Final shell length (mm) ²	80.51 ± 0.58	80.42 ± 0.51	0.916
Shell growth rate (µm d ⁻¹)	80.45 ± 0.58	80.37 ± 0.52	0.917
Condition factor (CF)	1.01 ± 0.02 ^b	1.07 ± 0.01 ^a	0.032

¹ Means (± SE) for each variable in each row that do not share the same superscript are significantly different ($P < 0.05$; one-factor ANOVA, SNK test).

² Final weight and shell lengths determined from 500 abalone tank⁻¹.

³ The initial shell length was determined from batches of 50 abalone from each of the 11 slab tanks that were used to stock the experimental tanks.

⁴ Standard protein diet (Skretting, 30.0% CP) strategy (n = 8) fed for the entire trial.

⁵ High protein diet (Skretting, 35.0% CP) strategy (n = 7) fed for the entire trial.

Table 5.4. Growth performance of tagged juvenile hybrid abalone at the completion of the tagging section of the Great Southern Waters trial (16/1/13 - 14/4/14; 453 d).

Item ¹	Standard protein diet strategy ²	High protein diet strategy ³	P value ¹
Initial weight (g abalone ⁻¹)	9.07 ± 0.22	8.92 ± 0.14	0.560
Final weight (g abalone ⁻¹)	86.62 ± 1.73	87.53 ± 1.11	0.674
Weight gain (g abalone ⁻¹)	77.55 ± 1.62	78.62 ± 1.13	0.607
Weight gain (%)	857 ± 20.00	883 ± 19.38	0.365
Specific growth rate (SGR % d ⁻¹)	0.697 ± 0.006	0.705 ± 0.006	0.351
Final shell length (mm)	80.51 ± 0.58	80.42 ± 0.52	0.916
Condition factor (CF)	0.967 ± 0.010	0.981 ± 0.012	0.243

¹ No significant differences between feeding strategies (one-factor ANOVA; means ± SE).

² Standard protein diet (Skretting, 30.0% CP) strategy (n = 8) fed for the entire trial.

³ High protein diet (Skretting, 35.0% CP) strategy (n = 7) fed for the entire trial.

Coastal Seafarms on-farm trial

This trial was run in two distinct Phases. During Phase 1, the biomass of several of the tanks was potentially reduced by water rats. A large number of shells were found adjacent to the tanks. The rats may have had a preference to predate on the abalone from the slab tanks nearest to the wall of the shed, but this could not be confirmed. The actual weight of abalone eaten by the rats was not determined, therefore, the data reported for this Phase of the trial needs to be evaluated with caution.

Coastal Seafarms did not report any unusual weather events during the course of the on-farm trial and the tanks experienced seasonal fluctuations in water temperature that ranged from 11.5 - 23.2°C, with a mean value of 16.7°C (Figure 5.7).



Figure 5.7. Water temperatures profile in slab tanks over the entire on-farm trial at Coastal Seafarms (11/9/12 - 26/6/14; 653 d).

Over the course of Phase 1 of the Coastal Seafarms trial, greenlip abalone were sampled and measured at regular intervals. Feeding strategy ($P = 0.001$) and time ($P < 0.01$) had significant effects on the individual weight of greenlip abalone (two-factor ANOVA; Figure 5.8). However, there was also a significant interaction between these two factors ($P = 0.017$). The interaction may be explained by the greater growth response of the greenlip abalone in the single-diet feeding strategy during the early stages of the trial (months 1 - 5) compared to the reduced growth response of abalone fed the same treatment in the latter stages of the trial (months 6 - 12). In contrast, greenlip abalone fed the multi-diet feeding strategy continued to grow and were significantly superior after 10 and 11 months (Figure 5.8; $P < 0.01$; one-factor ANOVA; SNK test).

At the completion of Phase 1 of the trial at Coastal Seafarms, there were no significant differences in survival, final biomass, biomass gain, individual and final weights, individual weight gain, FCR, energy efficiency ratio, final shell length, shell growth rate or condition factor of greenlip abalone between feeding strategies (Table 5.5; $P > 0.05$; one-factor ANOVA). There was a significant effect of feeding strategy on protein efficiency ratio (Table 5.5; $P = 0.006$; Single-diet strategy > multi-diet strategy; one-factor ANOVA).

There was no significant effect of feeding strategy on the total biomass of greenlip abalone in each grade class at the end of Phase 1 of the Coastal Seafarms trial ($P = 0.653$; two-factor ANOVA; Table 5.5). There was a significant effect of grade class on the total biomass ($P < 0.001$). There was also a significant interaction between the two factors ($P = 0.008$). The interaction may be explained by the lower total biomass of abalone in the medium grade class from the multi-diet strategy compared to the single-diet feeding strategy, in conjunction

with the greater total biomass of abalone from the large grade class in the multi-diet strategy compared to the single-diet feeding strategy (Table 5.5; one-factor ANOVA; SNK).

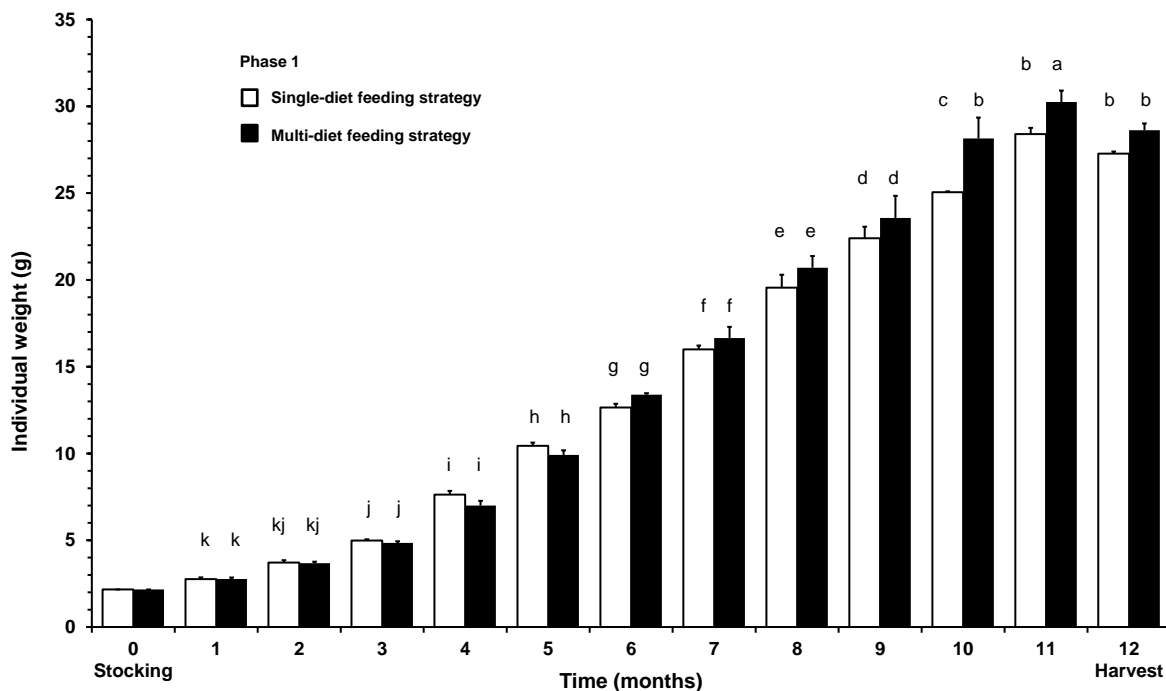


Figure 5.8. Individual weights of greenlip abalone fed the single or multi-diet feeding strategies during Phase 1 (11/9/12 to 13/9/13, 12 months) of the on-farm trial at Coastal Seafarms.

All values are means \pm SE, $n = 4$ and are determined from sub-samples of abalone from each tank. Initial individual stocking weights excluded from statistical analysis.

Mean values amongst feeding strategies and time that have different letters are significantly different ($P < 0.05$; one-factor ANOVA, SNK test).

There was no significant effect of feeding strategy on the proportion of final biomass of greenlip abalone in each grade class at the end of Phase 1 ($P = 1.000$; two-factor ANOVA; Table 5.5). There was a significant effect of grade class on the proportion of final biomass in each grade class ($P < 0.001$) and there was a significant interaction between the two factors ($P = 0.002$). The interaction is explained by the smaller proportion of abalone in the medium grade class from the multi-diet strategy compared to the single-diet feeding strategy, in conjunction with the greater proportion of abalone in the large grade class from the multi-diet strategy compared to the single-diet feeding strategy (Table 5.5; one-factor ANOVA; SNK).

Feeding strategy had no significant effect on the final individual weight of greenlip abalone at the end of Phase 1 of the Coastal Seafarms trial ($P = 0.805$; two-factor ANOVA; Table 5.5). There was a significant effect of grade class on the final individual weight of abalone ($P < 0.001$; large > medium > small; Table 5.5). There was no significant interaction between the two factors ($P = 0.511$). Between 11 and 12 months, there were also significant and numerical decreases in the individual weights of greenlip abalone fed the multi- and single-diet feeding strategies, respectively (Figure 5.8). The decrease in weights may have been a result of more pronounced dehydration due to the extended use of anaesthesia at the 12 month harvest sampling time. At 12 months, the entire population of abalone from each slab tank were exposed to anaesthesia over a prolonged period at harvested prior to weighing; whereas, abalone sub-sampled at 11 months were chipped individually and weighed rapidly.

Table 5.5. Survival, growth performance, feed utilisation and grade distribution of greenlip abalone (11/9/12 - 13/9/13; 367 d) at the end of Phase 1 of the Coastal Seafarms trial.

Item ^{1,2,3}	Single-diet strategy	Multi-diet strategy	P value
Survival (%)	98.83 ± 0.45	98.58 ± 0.48	0.718
Initial biomass (kg) (11/9/12)	99.0 ± 0.00	99.0 ± 0.00	
Final biomass (kg) (13/9/13)	772.6 ± 23.17	757.1 ± 15.47	0.599
Biomass gain (kg)	673.6 ± 23.17	658.1 ± 15.47	0.599
Initial individual weight (g abalone ⁻¹)	2.2 ± 0.00	2.2 ± 0.00	
Final individual weight (g abalone ⁻¹)	22.64 ± 0.95	22.08 ± 0.76	0.664
Individual weight gain (g abalone ⁻¹)	20.44 ± 0.95	19.88 ± 0.76	0.664
Specific growth rate (µm d ⁻¹)	0.64 ± 0.01	0.63 ± 0.01	0.681
Feed consumed (kg tank ⁻¹)	837.0 ± 0.00	837.0 ± 0.00	
Apparent feed conversion ratio (FCR)	1.25 ± 0.04	1.28 ± 0.03	0.595
Apparent protein efficiency ratio (PER) ⁴	2.69 ± 0.09 ^a	2.23 ± 0.05 ^b	0.006
Apparent energy efficiency ratio (EER)	4.24 ± 0.15	4.03 ± 0.10	0.286
Initial shell length (mm)	22.0 ± 0.00	22.0 ± 0.00	
Final shell length (mm)	54.99 ± 0.48	54.26 ± 0.83	0.478
Shell growth rate (µm d ⁻¹)	89.90 ± 1.35	87.90 ± 2.26	0.478
Condition factor (CF)	0.78 ± 0.12	0.80 ± 0.14	0.487
<i>Total biomass per grade class (kg)⁴</i>			
Small grade class	29.05 ± 2.55 ^c	20.93 ± 1.53 ^c	
Medium grade class	254.55 ± 2.88 ^a	201.36 ± 2.87 ^b	< 0.001
Large grade class	251.48 ± 8.16 ^a	297.31 ± 15.22 ^a	
<i>Proportion of biomass per grade class (%)⁴</i>			
Small grade class	5.46 ± 0.51 ^d	4.06 ± 0.42 ^d	
Medium grade class	47.13 ± 3.53 ^b	38.83 ± 0.90 ^c	< 0.001
Large grade class	47.41 ± 3.35 ^b	57.11 ± 1.29 ^a	
<i>Individual weight of abalone per grade class (g)⁵</i>			
Small grade class	12.65 ± 0.126 ^C	12.65 ± 0.263 ^C	
Medium grade class	22.05 ± 0.299 ^B	21.45 ± 0.412 ^B	< 0.001
Large grade class	34.25 ± 0.472 ^A	34.60 ± 0.653 ^A	

¹ Means ± SE (n = 4), except for overall mean individual weights (n = 8).

² Means for individual variables were determined from 50 individual abalone tank⁻¹.

³ Means for individual grade classes determined from bulk weights of 100 abalone tank⁻¹.

⁴ Means for apparent protein efficiency ratio, total biomass or proportion of biomass per grade class for either feeding strategy that do not share the same superscript are significantly different ($P < 0.05$; one-factor ANOVA, SNK test).

⁵ Individual weights per grade class for either feeding strategy that do not share the same upper-case superscript are significantly different ($P < 0.05$; two-factor ANOVA, SNK test).

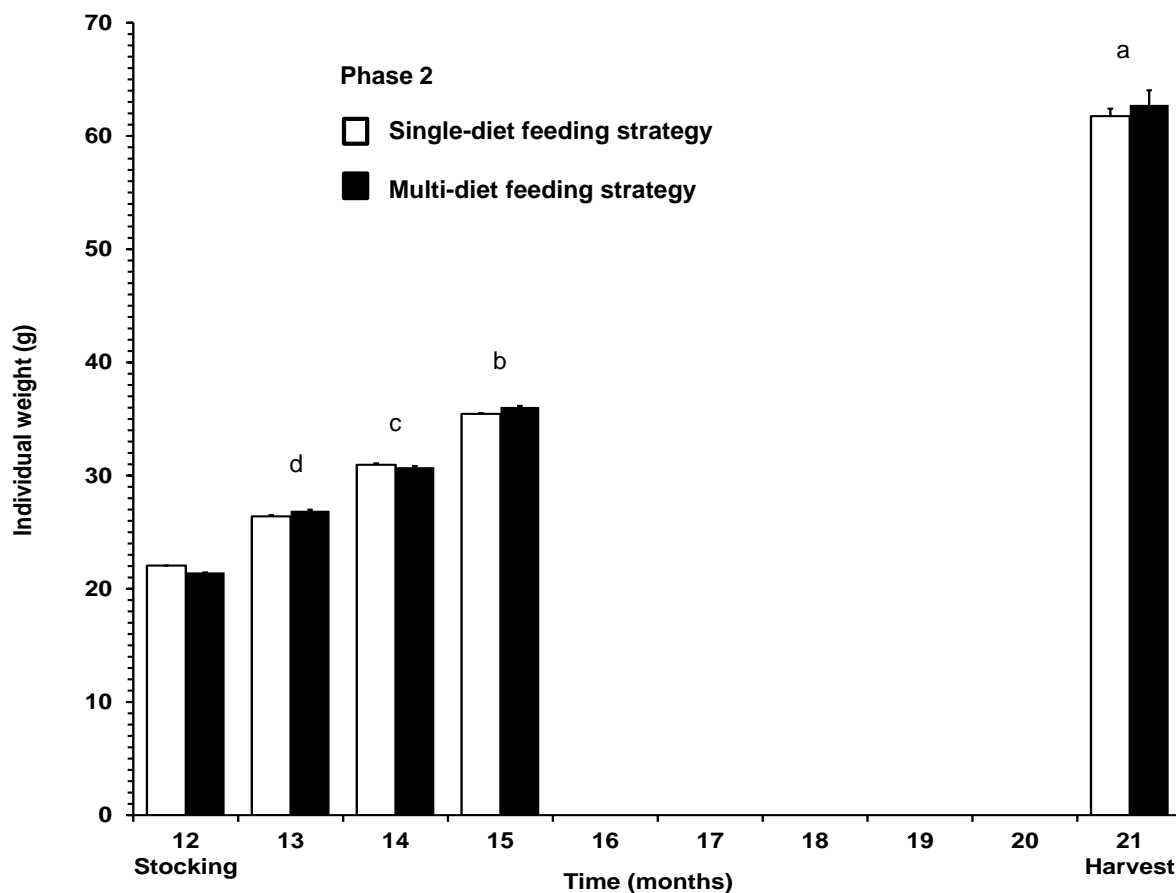


Figure 5.9. Individual weights of greenlip abalone fed the single or multi-diet feeding strategies during Phase 2 (13/9/13 to 26/6/14, ~21 months) of the on-farm trial at Coastal Seafarms.

All values are means (\pm SE, $n = 4$) determined from sub-samples of abalone from each tank. Twelve month stocking data excluded from the statistical analysis.

Mean values for each sampling time with different letters are significantly different over time ($P < 0.05$; two-factor ANOVA, SNK test).

Over the course of Phase 2 of the Coastal Seafarms trial, abalone were also sampled regularly for individual weight determinations. There was no significant effect of feeding strategy on the individual weight of greenlip abalone ($P = 0.403$), but there was a significant effect of time ($P < 0.001$; Figure 5.9; $21 > 15 > 14 > 13$ months) and there was no significant interaction between the two factors ($P = 0.897$, two-factor ANOVA; SNK test).

At the end of Phase 2, all abalone were removed from the slab tanks and weighed. There were significant differences in the protein efficiency ratio (single-diet $>$ multi-diet, $P = 0.002$), final shell length (single-diet $>$ multi-diet, $P = 0.006$) and shell growth rate (single-diet $>$ multi-diet, $P = 0.022$) of greenlip abalone between feeding strategies (Table 5.6; one-factor ANOVA). There was no significant difference for any of the other variables measured for greenlip abalone between feeding strategies at the end of this Phase of the trial (Table 5.6; $P > 0.05$; one-factor ANOVA).

Table 5.6. Growth and feed utilisation of greenlip abalone at the end of Phase 2 of the Coastal Seafarms trial (13/9/13 - 26/6/14; 286 d).

Item ^{1,2,3}	Single-diet strategy	Multi-diet strategy	P value
Initial biomass (kg) (13/9/13)	200.0 ± 0.00	200.0 ± 0.00	
Final biomass (kg) (26/6/14)	520.8 ± 9.04	513.3 ± 4.77	0.491
Biomass gain (kg)	320.8 ± 9.04	313.3 ± 4.77	0.491
Initial individual weight (g)	22.05 ± 0.00	21.45 ± 0.00	
Final individual weight (g)	65.06 ± 0.95	60.84 ± 1.69	0.072
Individual weight gain (g)	43.01 ± 0.95	39.39 ± 1.69	0.111
SGR (% d ⁻¹)	0.37 ± 0.005	0.36 ± 0.010	0.246
Feed consumed (kg tank ⁻¹)	558.0 ± 0.00	558.0 ± 0.00	
Apparent feed conversion ratio (FCR)	1.07 ± 0.02	1.08 ± 0.01	0.499
Apparent protein efficiency ratio (PER)	1.92 ± 0.05 ^a	1.60 ± 0.02 ^b	0.002
Apparent energy efficiency ratio (EER)	3.03 ± 0.09	2.88 ± 0.04	0.177
Initial shell length (mm)	54.99 ± 0.50	54.26 ± 0.83	
Final shell length (mm)	77.35 ± 0.46 ^a	74.67 ± 0.44 ^b	0.006
Shell growth rate (µm d ⁻¹)	78.16 ± 1.60 ^a	71.34 ± 1.55 ^b	0.022
Condition factor (CF)	0.806 ± 0.009	0.850 ± 0.017	0.069

¹ Values means ± SE (n = 4).

² Mean individual values were determined from the mean of 50 abalone from each tank.

³ Means values within each row that do not share the same superscript are significantly different ($P < 0.05$; one-factor ANOVA, SNK test).

Kangaroo Island Abalone trial

Kangaroo Island Abalone farm experienced seasonal fluctuations in water temperature over the course of the trial, which averaged 17.8°C and ranged from 13.4 - 23.2°C. During both summer/autumn seasons water temperatures were high for extended periods from late January to mid-March (Figure 5.10).

Greenlip abalone were initially fed the lower protein diet as part of the multi-diet feeding strategy for the majority of Phase 1 (11/5/12 - 30/9/12). This did not result in a reduction of growth performance when compared to that of the abalone fed the single diet feeding strategy that used the normal protein level diet over the same period (Table 5.7).

The condition factor, meat to whole weight ratio and the meat to shell ratio of the greenlip abalone fed the multi-diet feeding strategy were significantly higher than those of the abalone fed the single-diet strategy ($P < 0.010$; one-factor ANOVA; Table 5.7). There were no significant differences for any of the other variables measured from greenlip abalone fed the single-diet versus the multi-diet feeding strategy during Phase 1 of the trial ($P > 0.05$; Table 5.7).



Figure 5.10. Water temperature profile over the entire duration of the on-farm trial at Kangaroo Island Abalone (11/5/12 - 28/3/14; 686 d; ~23 months).

Table 5.7. Growth performance and feed utilisation of pre-weaned greenlip abalone at the end of Phase 1 of the Kangaroo Island Abalone trial (11/5/12 - 20/11/12; 193 d).

Item ^{1,2,3}	Single-diet strategy ⁴	Multi-diet strategy ⁵	<i>P</i> value
Initial weight (g abalone ⁻¹)	0.6	0.6	
Final weight (g abalone ⁻¹)	3.31 ± 0.18	3.30 ± 0.11	0.964
Weight gain (g abalone ⁻¹)	2.71 ± 0.18	2.70 ± 0.11	0.964
Weight gain (%)	452.3 ± 30.7	450.3 ± 18.0	0.959
Specific growth rate (SGR % d ⁻¹)	0.84 ± 0.03	0.84 ± 0.01	0.977
Initial shell length (mm)	15.0	15.0	
Final shell length (mm)	29.36 ± 0.61	28.97 ± 0.31	0.587
Shell growth rate (µm d ⁻¹)	74.39 ± 3.15	72.41 ± 1.6	0.591
Condition factor (CF)	0.72 ± 0.01 ^b	0.75 ± 0.01 ^a	0.008
Meat weight (g)	2.01 ± 0.12	2.13 ± 0.08	0.850
Shell weight (g)	1.22 ± 0.07	1.18 ± 0.04	0.557
Meat:whole weight ratio (%)	62.65 ± 0.15 ^b	63.82 ± 0.23 ^a	0.003
Meat:shell weight ratio (%)	169.40 ± 1.22 ^b	178.75 ± 1.74 ^a	0.003

¹ Means ± SE, n = 5 PVC pipe systems, except for initial weight and length (n = 1).

² Individual weights, lengths, shell and meat weights for each tank were determined from 30 abalone from each PVC pipe system.

³ Means for each variable in each row that do not share the same superscript are significantly different (*P* < 0.05; one-factor ANOVA, SNK test).

⁴ The single-diet strategy used the standard protein (29.6% CP) Eyre Peninsula Aquafeed diet for the duration of Phase 1 of the trial.

⁵ The multi-diet strategy used the low protein (27.1% CP) Eyre Peninsula Aquafeed diet for five months and then, when the water temperature reached 16°C (1/10/12), switched to the high protein diet (33.8% CP) for the remainder of Phase 1 of the trial.

During late January to mid-March of 2013 during Phase 2 of the on-farm trial at Kangaroo Island Abalone, water temperatures were high (>22°C) for extended periods and high mortalities were observed in greenlip abalone across both feeding strategy treatments (Table 5.8). The survival of greenlip abalone was significantly higher when fed the multi-diet feeding strategy with the high protein diet (33.8% CP; 55.43% survival) compared to the

single-diet feeding strategy using the standard protein diet (29.6% CP; 49.63% survival; $P = 0.003$; one-factor ANOVA; Table 5.8). The final biomass and biomass gain of abalone, at the end of Phase 2 of the trial, were numerically lower (~30%) for the single-diet feeding strategy compared to the multi-diet feeding strategy (Table 5.8). There were no significant differences for any of the other response variable measured from greenlip abalone between feeding strategy treatments at the conclusion of Phase 2 of the trial ($P > 0.05$; Table 5.8).

When comparing different sampling methods, there were no significant differences in the individual weight of abalone determined using the average of 50 individuals or the average of the bulk weight of 300 abalone from each slab tank at the conclusion of Phase 1 of the trial ($P = 0.241$; one-factor ANOVA Table 5.8).

Table 5.8. Survival and growth performance of greenlip abalone at the end of Phase 2 of the Kangaroo Island Abalone trial (20/11/12 - 28/5/13; 189 d).

Item ¹	Single-diet strategy ⁵	Multi-diet strategy ⁶	<i>P</i> value
Survival (%)	49.63 ± 0.59 ^b	55.43 ± 1.06 ^a	0.003
Initial biomass (kg) (20/11/12)	60.0	60.0	
Final biomass (kg) (28/5/13)	143.85 ± 4.70	174.35 ± 16.37	0.124
Biomass gain (kg)	83.85 ± 4.70	114.35 ± 16.37	0.124
Initial weight (g abalone ⁻¹)	3.31 ± 0.18	3.30 ± 0.11	0.964
Final weight (g abalone ⁻¹) ²	19.72 ± 0.79	18.10 ± 0.72	0.179
Final weight (g abalone ⁻¹) ³	19.60 ± 0.96	18.01 ± 0.31	0.167
Weight gain (g abalone ⁻¹) ³	16.42 ± 0.79	14.80 ± 0.72	0.181
Weight gain (%)	495.9 ± 23.90	448.4 ± 21.83	0.192
Specific growth rate (SGR % d ⁻¹)	0.92 ± 0.019	0.87 ± 0.02	0.171
Initial shell length (mm)	29.36 ± 0.61	28.97 ± 0.31	0.587
Final shell length (mm)	54.25 ± 0.81	52.45 ± 0.58	0.121
Shell growth rate (µm d ⁻¹)	131.67 ± 4.28	124.23 ± 3.05	0.206
Condition factor (CF)	0.69 ± 0.01	0.71 ± 0.01	0.176
Meat weight (g) ⁴	11.65 ± 0.53	10.78 ± 0.42	0.244
Shell weight (g) ⁴	8.01 ± 0.56	6.96 ± 0.19	0.120
Meat:whole weight ratio (%)	58.95 ± 0.63	60.30 ± 0.33	0.109
Meat:shell weight ratio (%)	145.93 ± 4.35	150.04 ± 2.26	0.197

¹ Means ± SE (n = 4 slab tanks) for each variable in each row that do not share the same superscript are significantly different ($P < 0.05$; one-factor ANOVA, SNK test).

² Individual weights and shell lengths were determined from 50 abalone tank⁻¹.

³ Individual weights were determined from average bulk weight of 300 abalone tank⁻¹.

⁴ Individual shell and meat wet weights were determined from 30 shucked abalone tank⁻¹.

⁵ The single-diet strategy used the standard protein (29.6% CP) Eyre Peninsula Aquafeed diet for the duration of Phase 2 of the trial.

⁶ The multi-diet strategy used the high protein (33.8% CP) Eyre Peninsula Aquafeed diet for five months and switched to the standard protein (29.6% CP) diet once the water temperature dropped below 16°C (1/6/13) for the remainder of Phase 2 of the trial.

At the end of Phase 3 of the trial at Kangaroo Island Abalone, there was no significant difference in the survival, final biomass ($P = 0.738$) or biomass gain ($P = 0.738$) of abalone for either feeding strategy ($P > 0.05$; one-factor ANOVA; Table 5.9). There were no major numerical differences for any of the other variable measured from greenlip abalone fed the

single-diet versus the multi-diet feeding strategy at the end of Phase 3 of this trial (Table 5.9).

Table 5.9. Growth performance of greenlip abalone after ~10 months (304 d) at the end of Phase 3 of the Kangaroo Island Abalone trial (28/5/13 - 28/3/14).

Item ^{1,2,3,4}	Single-diet strategy ⁵	Multi-diet strategy ⁶	<i>P</i> value
Survival (%)	91.21 ± 0.93	92.64 ± 0.45	0.214
Final biomass (kg) (28/5/13)	260.5 ± 6.61	257.0 ± 7.47	0.738
Biomass gain (kg)	186.5 ± 6.61	183.0 ± 7.47	0.738
Initial weight (g abalone ⁻¹) ⁷	17.0	17.0	
Final weight (g abalone ⁻¹) ⁷	77.14	75.4	
Weight gain (g abalone ⁻¹) ⁷	60.14	58.4	
Weight gain (%) ⁷	407.35	376.77	
Specific growth rate (SGR % d ⁻¹) ⁷	0.498	0.490	

¹ There was no significant difference for mean values for variables (means ± SE, n = 4 slab tanks) between feeding strategies (*P* > 0.05; one-factor ANOVA).

² Initial biomass weights were determined from the total bulk weight of the abalone tank⁻¹.

³ Final biomass was determined from the total bulk weight of abalone from each tank.

⁴ Initial and final weights were determined from the mean of 100 abalone treatment⁻¹.

⁵ The single-diet strategy used the standard protein (29.6% CP) Eyre Peninsula Aquafeed diet for the duration of Phase 3 of the trial.

⁶ The multi-diet strategy used the low protein (27.1% CP) Eyre Peninsula Aquafeed diet for five months and then, when the water temperature rose above 16°C (1/10/13), switched to the high protein diet (33.8% CP) for the remainder of Phase 3 of the trial.

⁷ One value based on the average of a bulks sample was provided, therefore, no statistical analyses for these variables were possible.

Discussion

The aim of the three on-farms trials at Great Southern Waters, Coastal Seafarms and Kangaroo Island Abalone was to evaluate the potential of high protein diet or multi-diet feeding strategies to improve the growth performance and feed utilisation of greenlip (*Haliotis laevis*) and hybrid abalone (*H. laevis* × *H. rubra*) under commercial conditions.

The protein and temperature studies carried out in Chapters 2 (Stone et al. 2013) and 3 (Bansemer et al. 2015) provided us with a foundation to better understand the response of different aged (size) abalone to differing dietary protein levels during seasonal fluctuations in water temperature. These results provided invaluable information for participating Australian abalone feed companies, and research providers, to better formulate the diets and feeding strategies for greenlip and hybrid abalone that were tested in the on-farm trials. The actual ingredient formulations of the commercial test diets remained confidential and were retained by each company as proprietary information. Throughout the project, participating Australian abalone feed companies and AAGA members worked closely together and were proactive with input during the design phase of the on-farm trials. Feed companies were also flexible and prompt with the production and delivery of the commercial diets to the participating AAGA farms. AAGA farm managers and staff designated to oversee and run the on-farm trials were also flexible, conscientious and prompt with the delivery of results. There was no negative feedback from farmers regarding the water stability or palatability of the diets to abalone. The trials successfully tested a combination of newly formulated commercial starter and grow-out diets versus fed pre-existing grow-out diets on the growth performance of abalone across the normal production cycle.

During the trial at Great Southern Waters, hybrid abalone displayed a significant, 6% increase in individual weight when cultured using the high protein diet feeding strategy compared to the standard protein diet feeding strategy (Figure 5.6). At the completion of the Great Southern Waters trial, a significant 5.4% improvement in SGR resulted in an extra 100 kg of abalone per slab tank, which equated to a 9.3% increase in biomass by using the high protein diet compared to the abalone fed the standard protein diet feeding strategy (Table 5.3). It is important to note that this gain in biomass was obtained with a 2.2% increase in feed input weight, as feed was utilised 7.1% more efficiently (Table 5.3). The FCR for abalone fed the high protein diet feeding strategy was superior, with a probability value (*P*) below 0.100 and approaching the 0.05 level of significance (Table 5.3). However, the high protein diet has a 5% cost premium compared to the standard protein diet (Appendix 3). Despite this, based on an abalone farm gate value of \$25 kg⁻¹ (AAGA personal communication), there was a 9.5% increase in sales revenue of \$31 m⁻² of tank area y⁻¹ for hybrid abalone produced using the high protein diet compared to the standard protein diet feeding strategy (Appendix 3, Table A3.1). This return was achieved with the additional feed cost of \$2 m⁻² of tank area year⁻¹.

Additionally, over the course of the Coastal Seafarms trial, similar statistically significant and numerical trends were observed for individual weight of sub-sampled greenlip abalone during the later stages of Phase 1 (Figure 5.8) and 2 (Figure 5.9), respectively. If greenlip abalone in these tanks were not interrupted by grading and restocked with medium grade abalone from each treatment between trial Phases, the trends for individual weight increases observed in Figures 5.8 and 5.9 suggests that the abalone fed the multi-diet feeding strategy may have significantly exceeded the weight of those fed the single diet feeding strategy at the completion of the trial. Bearing this in mind, and as previously suggested, future on-farm growth trials should avoid using this procedure of thinning, grading and restocking. We

recommend that in order to reduce tank biomass, the practice of controlled partial thinning of the tanks, as used at Great Southern Waters, would be the preferred method.

Data reported by Stone et al. (2013; Chapter 2) indicates that superior protein deposition may be achieved in greenlip abalone fed to apparent satiation with high protein diets (>33% crude protein). In each on-farm trial, abalone from each feeding strategy treatment were fed to excess at the same feed rate (% of biomass). This suggests that abalone fed the high protein diet or multi-diet feeding strategy in all farm trials may have been over-fed, which may have resulted in the reduced apparent protein efficiency ratios observed over the course of the Great Southern Waters (Table 5.3) and Coastal Seafarms trials (Table 5.5 and 5.6). Providing the same feed rate to both treatments in the trials may have resulted in a higher proportion of wasted feed for the high protein diet or the multi-diet feeding strategy. For example, in the Great Southern Waters trial, given that hybrid abalone fed the high protein diet feeding strategy returned a 7.1% superior FCR. In addition, considering the results from the laboratory experiment in Chapter 2 (Stone et al. 2013, Figure 2.2a), where 1-year-old greenlip abalone were 17% and 20% more efficient at utilising diets containing 33% crude protein compared to 30% protein at 18 and 22°C, respectively, it may be possible to reduce feed rates and still end up with the same improvements to growth rates. Assuming this is the case, we suggest that refining the feed rates used for multi-diet feeding strategies would also result in additional improvements in FCR, apparent protein efficiency ratios and savings in costs related to freight, feed storage, handling and use. Reducing the feed input, particularly in the early stages of production, would also lead to improvements in water quality, which in turn, would lead to improvements in growth, survival and ultimately farm productivity. However, a reduction in feeding rate will result in feeding fewer diet chips at each event. This could be problematic, as farmers like to provide a sufficient spread of feed chips to ensure abalone have equal opportunity to feed at high stocking densities. To overcome this problem, the simplest solution would be to reduce the feed chip size to still deliver the same number of pellets to a given area of tank space.

The improvements in growth and biomass gain, during the Great Southern Waters trial, were accompanied by a significant (~6%) improvement in the condition factor of abalone fed the high protein diet feeding strategy (Table 5.3). The improved condition factor was obtained without any additional shell growth compared to the abalone produced using the standard protein diet feeding strategy. Interestingly, significantly or numerically improved condition factors were also observed in greenlip abalone fed the multi-diet feeding strategies during Phase 1 (Table 5.8) and 2 (Table 5.9) of the Kangaroo Island Abalone trial, respectively. Similar trends were also observed for improved condition factor in greenlip abalone fed the multi-diet feeding strategy during both phases of the Coastal Seafarm trial (Tables 5.5 and 5.6). Improvements in condition factor suggest that abalone are depositing protein as new tissue rather than shell growth and relates to an increased shell volume and meat weight, which in turn, may equate to an improvement in the meat:shell ratio (Cho 2010). A significant improvement in the meat:shell ratio of greenlip abalone was also evident in the multi-diet feeding strategy during Phase 1 of the Kangaroo Island Abalone trial (Table 5.8). This is an important consideration when producing abalone to be shucked prior to canning and other markets.

After 12 months, greenlip abalone from Phase 1 of the Coastal Seafarms trial were harvested and graded. Interestingly, at this point there were no significant differences in mean individual weight of abalone, biomass or biomass gain from each tank between feeding strategies. However, although they had not reached harvest size, distinct statistical differences in the proportion of greenlip abalone in each grade class were observed between feeding strategies (Table 5.5). Compared to the single-diet feeding strategy, the multi-diet feeding strategy had a significantly greater proportion of animals in the large grade class (~19%), a significantly smaller proportion in the medium grade class (~19%), and a similar proportion in the small grade class (Table 5.5). This suggests that although the apparent

mean weight of a tank full of abalone may be the same, in response to feeding strategy, over time a larger proportion of abalone cultured using the multi-diet feeding strategy may reach market size earlier.

With regard to the performance of different age (size) of abalone fed the multi-diet and single diet feeding strategies, results suggest that weight gains using the high protein diet appeared to be made early with the younger (smaller) greenlip abalone at Coastal Seafarms during Phase 1 of the trial (Figure 5.8) compared to older (larger) greenlip abalone during Phase 2 (Figure 5.9). This growth phenomena, although less pronounced, was also evident with hybrid abalone during the trial at Great Southern Waters (Figure 5.6). This finding is consistent with results from Stone et al. (2013, Chapter 2, Table 2.3, Figure 2.2a). However, even though the difference in growth appears to be maintained, relative gains were reduced as greenlip abalone grew larger in their second year (Figure 5.8 and 5.9). This is also consistent with results reported for *H. midae* by Britz and Hecht (1997), who reported younger (smaller) abalone grew significantly faster, on a percentage basis, compared to older (larger) abalone, respectively. Speed at which abalone attain harvest size is of commercial importance. Any advancement in growth captured early in the production cycle, assuming that all things are equal during the latter stages of the production cycle, should result in an earlier arrival at harvest (Stone et al. 2013, Chapter 2; Britz and Hecht, 1997). This was also apparent in the Phase 3 of the Kangaroo Island Abalone trial, where larger 17 g greenlip abalone were used, and after 10 months of growth there was no significant or appreciable difference in biomass gain or final individual weights between either feeding strategy (Table 5.9). However, as greenlip abalone were fed in a qualitative, rather than quantitative, manner during this phase of the trial, it is difficult to draw a solid conclusion on growth performance as the animals may have been over, or underfed. Nevertheless, when results with greenlip abalone are combined across the two farms it appears that larger abalone may be fed a reduced protein level during later stages and at cooler stages of the production cycle, with no apparent compromise to growth (Stone et al. 2013, Chapter 2, Figure 2.2b). This would result in a further savings. Although inconclusive, Stone et al. (2013, Chapter 2) suggested 2-year-old greenlip abalone require a similar or slightly lower dietary protein level for optimal growth compared to 1-y-old greenlip abalone. Clearly, further refinement of the multi-diet feeding strategy is warranted for older abalone during seasonal fluctuations in water temperatures.

During the Great Southern Water trial, a sub-sample of hybrid abalone were weighed, measured and tagged two months after stocking. The delay in the establishment of this process, in relation to the initial stocking date, was due to the small size of abalone. Tagged animals were weighed and measured again one month before the final harvest and completion of the trial. At the completion of the tagging component of the trial, there were no significant differences for the variables measured between either feeding strategy (Table 5.4; $P > 0.05$). However, apart from final shell length and shell growth rate, there was a tendency for all variables to be numerically greater for tagged abalone from the high protein diet compared to the standard protein diet feeding strategy. These results support the significant results observed in the non-tagged hybrid abalone over the entirety of the trial (Table 5.3), and confirms the improved growth of abalone when fed a high protein diet. In on-farm trials, tagged abalone may be used as a preliminary indicator of treatment effects, but we would not recommend this method as a sole indicator of growth performance. One potential cause for the lack of significant responses for the tagged hybrid abalone (Table 5.4) may have been due to the lost opportunity to capture growth during the first two months of favourable summer water temperatures between November 2011 and mid-January 2012. Stone et al (2013, Chapter 2) demonstrated that smaller 1-year-old greenlip abalone are more efficient at utilising higher protein diets for growth at their optimal water temperature of 22°C. With regards to tagging and tag recovery, there was a high rate of tag shedding (~38%) observed in Great Southern Waters trial. This suggests that future trials that utilise this practice may have to tag extra abalone in anticipation of this shedding rate.

During the project, there were difficulties in managing the on-farm research trials in a commercial context. Despite best efforts, that included the development and application of the on-farm manual to standardise methods, variation between farms occurred. Despite this, results from the on-farm trials, support the use of high protein diet or multi-diet feeding strategies to improve the production of cultured abalone. Significant improvements in growth performance and feed efficiencies were observed for hybrid abalone cultured using the high protein diet feeding strategy during the on-farm trial at Great Southern Waters (Tables 5.3 and 5.4; Figure 5.6), while the Coastal Seafarms trial detected minor improvement in individual weight gains and weight class distribution when greenlip abalone were fed the multi-diet feeding strategy (Table 5.5). However, Kangaroo Island Abalone on-farm trials did not detect any significant differences in growth or feed efficiencies for greenlip abalone cultured using the multi-diet feeding strategy. Several possible factors may have contributed to these outcomes:

- Insufficient tank replication.
- The practice of harvest, grading and restocking of tanks during the Coastal Seafarms and Kangaroo Island Abalone trials.
- Differences in feeding practices between farm trials.
- High mortality (>50%) during the summer of 2012/13 in the Kangaroo Island Abalone trial.
- Predation of abalone from the experimental tanks by water rats during the Coastal Seafarms trial.
- In retrospect, in the early stages of the development of multi-diet feeding strategies for abalone, it may have more beneficial to prove the concept of improved abalone growth on these farms using a high protein diet alone for the entirety of the trials. This is consistent with the results reported for 1-year-old greenlip abalone fed high protein diets during periods of high growth (Stone et al. 2012, Chapter 2).

Due to high data variability, there were difficulties to discern differences between treatments for growth and feed utilisation of abalone in the Kangaroo Island Abalone and Coastal Seafarms trials. This problem is typical of on-farm trials with low replication (Festing and Altman 2002). Insufficient tank replication may have led to a reduction of statistical power and the ability to discern significant differences. Therefore, a large number of replicates are required to achieve the power to detect statistically significant differences between treatment means. For statistical power analysis, the closer the power is to $P = 1.000$, the more powerful the experimental design (Festing and Altman 2002). In the Great Southern Waters trial, seven (high protein diet strategy) and eight replicates (standard protein diet strategy) tanks treatment⁻¹ provided sufficient power ($P = 0.624$) to discern a statistical significant difference of 5.2% between feeding strategies for weight gain (Table 5.3). Whereas, for biomass gain, the power ($P = 0.474$) was insufficient to detect a statistical difference between treatment means of 8.9%. Additionally, in the Kangaroo Island Abalone trial it was not possible to detect a 30% difference in tank biomass gain between feeding strategy treatments when using four replicate tanks (Table 5.8). In retrospect, it would have been desirable to increase replication for the Kangaroo Island Abalone and Coastal Seafarms trials to increase statistical power. Statistical power analysis was done to determine the number of replicate slab tanks required to observe a significant difference ($P < 0.05$) of 10% in biomass gain between treatments, for recommendations for future on-farm studies. This analysis was based on the results obtained from hybrid abalone fed the single protein feeding strategy treatment from the Great Southern Waters on-farm trial (mean, 1081.7 kg slab tank⁻¹; standard deviation, 108.05; two-tailed test, power of the test, $P = 0.80$; Statistical Solutions LLC 2014). Based on the results from this analysis, at least eight slab tanks per dietary treatment are the minimum requirement to observe a 10% significant difference in

biomass gain. However, this number will depend on the variability of the response being measured, which will also vary between farms and may be difficult to achieve given the restrictions of staffing, combined with production demands, placed on commercial operators. Investment in research and development is a serious proposition, where often gains may only be realised in small percentages, if at all. If trials are run with insufficient statistical power they may be doomed from the start and would not only result in a waste of time, resources and money in the present, they may also cost the industry well into the future, as cost savings procedures may be missed and are often not revisited until the turn-over of management occurs.

The practice of harvesting and restocking with middle graded abalone was practiced in both Kangaroo Island Abalone and Coastal Seafarms trials. At each harvest, grading and restocking event, abalone of the middle grade range from each feeding strategy treatment were used to restock the next phase of the trials. This practice essentially re-started the trial again, with common sized greenlip abalone, and inadvertently resulted in a dilution of the treatment response between each phase, and reduced the ability to discern statistical differences as larger animals have a lower growth potential (Stone et al. 2013, Chapter 2; Britz and Hecht, 1997). In retrospect, in future trials this practice should be avoided and replaced with controlled thinning of stocking densities throughout the trial by partial harvesting, as practiced during the trial at Great Southern Waters.

With regards to differences in feeding strategies between farms, Great Southern Waters and Coastal Seafarms fed the abalone of each feeding strategy with the same feed rate over the duration of the trials. During Phase 1 of the Kangaroo Island Abalone trial, abalone in both feeding strategies were also fed at the same rate. However, during Phases 2 and 3 of the Kangaroo Island Abalone trial, both feeding strategies were fed using commercial methods practiced at this farm, where feed was offered based on visual observations of demand. This feeding strategy may have inadvertently led to a bias and confounded results. Underfeeding abalone is the first limiting factor to the growth of abalone (Britz et al. 1997; Cho et al. 2009). For example, Cho et al. (2009) reported significantly lower weight gain after one week of starvation compared to a control group and concluded that starved juvenile *H. discus hannai* are unable to achieve full compensatory growth when supplied with excess feed after feed deprivation.

In addition, during late January to mid-March of 2013, during Phase 2 of the Kangaroo Island Abalone trial, water temperatures exceeded 22°C for extended periods and high mortalities were observed in greenlip abalone across tanks for both feeding strategy treatments (Table 5.8). The mortality event was not restricted to the trial tanks; it was also evident in larger abalone across the entire farm. As a result, the data was compromised during this Phase of the trial. However, an insight into the effect of high protein versus low protein diets on mortality was gained. The average survival of greenlip abalone was significantly higher when fed the multi-diet feeding strategy with the high protein diet (55.43%) compared to the single-diet feeding strategy (49.63%) using the standard protein diet (Table 5.8). It is worth noting that the final biomass and biomass gain of abalone, at the end of Phase 2 of the trial, were numerically lower (~30%) for the single-diet feeding strategy compared to the multi-diet feeding strategy (Table 5.8). The slightly higher, though not statistically significant, final weight for the abalone fed the single-diet feeding strategy during this phase of the trial may have been the result of the reduced stocking density due to the increased mortality experienced in this treatment. The effect of stocking density on the growth performance of hybrid abalone has previously been reported in a separate on-farm trial (Wassnig et al. 2010). The authors reported significantly lower weight gain in slab tanks when abalone were held at 20% above commercial stocking densities, compared to 20% below commercial stocking densities (Wassnig et al. 2010).

Finally, for Coastal Seafarms, ongoing predation on greenlip abalone by water rats during Phase 1 of the trial may have indiscriminately reduced tank biomass levels in some of the raceways, and in turn, affected (improved) growth performance and resulting feed efficiencies of greenlip abalone in an uncontrolled manner. As previously mentioned, Wassnig et al. (2010) measured improved growth performance of hybrid abalone associated with decreased stocking densities. The implementation of vermin control programs is recommended to reduce the predation of abalone in future on-farm trials.

Conclusions and Recommendations

The considerable investment in research and development by AAGA and all project participants during this project has been substantiated by the successful outcomes achieved in the laboratory and on-farm trials. The overarching outcome of this project, in collaboration with AAGA farmers and Australian abalone feed producers, was to develop diet formulations and feeding strategies that delivered a >10% improvement in productivity across the entire grow-out period for greenlip and hybrid abalone.

The results presented in this Chapter indicate that the outcome of the project has been achieved. Based on the results achieved during the course of this project, Australian abalone feed producers have altered the formulations of their commercial diets. The extremely positive results for abalone growth and feed utilisation from on-farm trials were achieved by using commercially formulated diets, developed in this project, which are now readily available for abalone producers. The high protein diet and multi-diet feeding strategies were successful in increasing weight and biomass gain, and when combined with improved feed efficiency, resulted in a 9.5% increase in the basic sales revenue with minimal 7.1% increase in feed input cost. Additionally, gains were made without an impact on mortality due to high protein diets. There are numerous benefits of producing heavier abalone quicker.

At the conclusion of the Great Southern Waters trial, hybrid abalone fed the high protein diet feeding strategy produced an extra 100 kg of biomass per slab tank. This equated to a 9.3% increase in biomass gain when fed the high protein diet feeding strategy over the abalone fed the standard protein diet feeding strategy. This weight gain was achieved in conjunction with a 7.1% improvement in FCR. Despite the 4.8% cost premium of the high protein diet, compared to the standard protein diet, there was a substantial return on additional feed input costs (Appendix 3, Table A3.1). The gains in growth and FCR resulted in a \$31 m⁻² tank area y⁻¹ increase in sales revenue for hybrid abalone produced using the high protein diet feeding strategy (Appendix 3, Table A3.1), which was achieved with an extra \$2 of feed input costs m⁻² tank area y⁻¹. Additionally, based on the results for individual weight gain (5.4%) and biomass gain (9.3%), from the Great Southern Waters trial, the overall duration of a typical three year production cycle may be shortened by 1.9 to 3.4 months by feeding high protein diets. This would result in a significant saving in both fixed and variable costs. There would also be considerable advantages to be gained from reduced mortality of stock during the shortened production cycle. Larger abalone are more prone to summer mortality (Lange et al. 2014; Stone et al. 2014). Using a high protein diet or multi-diet feeding strategy may allow farmers to harvest larger abalone sooner, which may reduce the exposure of valuable abalone to one less summer. This factor alone could result in improved productivity and cost savings, and when combined with savings made with increased growth rates and biomass gain, a >10% improvement in productivity across the entire grow-out period for greenlip and hybrid abalone may be achieved.

Chapter 6. General Discussion

The planned overall outcome from this project was the development of commercial diet formulations and feeding strategies that deliver a >10% improvement in productivity across an entire grow-out period for greenlip and hybrid abalone available to industry.

To achieve this outcome a series of laboratory-based experiments and commercial on-farm trials were designed with the following three aims:

1. Characterise the growth and feed utilisation of greenlip abalone of different age classes at a range of seasonal temperatures (14 - 22°C) representative of those experienced by abalone in land-based facilities in southern Australia in laboratory-based experiments (Chapters 2 and 3).
2. Improve our understanding of the optimum dietary protein levels for greenlip abalone growth in relation to age and seasonal fluctuations in water temperature in laboratory-based experiments (Chapters 2 and 3).
3. Use the information provided in Chapters 2 and 3 to design and run three on-farms trials at Great Southern Waters, Coastal Seafarms and Kangaroo Island Abalone to evaluate the potential of high protein diets or multi-diet feeding strategies to improve the growth performance and feed utilisation of greenlip (*Haliotis laevis*) and hybrid abalone (*H. laevis* × *H. rubra*) under commercial conditions (Chapters 4 and 5).

The rationale behind this approach was to use results from the laboratory-based experiments, which would lead to the recognition of the need for separate starter and grow-out feeds for greenlip and hybrid abalone. Results from the laboratory-based experiments would then be provided to the participating feed companies in order to formulate a series of commercial diets with varying protein levels for evaluation in the on-farm trials. Concurrently, the protocol for the on-farm trials would be developed collaboratively by the research providers, participating AAGA members and feed companies. In combination, the newly developed commercial feeds would be incorporated into multi-feeding strategies designed to provide optimal dietary protein for greenlip and hybrid abalone in relation to variations in animal age and seasonal variations in water temperature throughout the production cycle. The on-farm trials comprised a series of three long-term (>18 months) studies, using commercial culture practices, to evaluate the growth, feed utilisation and survival of greenlip and hybrid abalone using different feeding strategies.

Coastal Seafarms and Kangaroo Island Abalone used:

1. A single-diet feeding strategy that consisted of the current production method of feeding one standard protein diet for the entire trial; versus
2. A multi-diet feeding strategy that consisted of abalone being fed a sequential combination of “high protein” / “low protein” grow-out diets for the entire trial.

While Great Southern Waters working with Skretting chose to use:

1. A single diet feeding strategy that consisted of a standard protein diet throughout the entirety of the on-farm trial; versus.
2. A single diet feeding strategy that consisted of a high protein diet throughout the entirety of the on-farm trial.

Water temperature influences almost every aspect of abalone production, including the survival, growth, nutritional requirements, metabolic rate, digestive enzymatic activity and gastrointestinal morphology (Britz et al. 1997; Edwards and Condon 2001; Schaefer et al.

2013; Stone et al. 2013, Chapter 2; Stone et al. 2014; Bansemer et al. 2015, Chapter 3). Based on results from Stone et al. (2013, Chapter 2) and Bansemer et al. (2015, Chapter 3) the greatest improvement in production would occur by supplying smaller <1-year-old greenlip abalone with heated water (up to 22°C) in nursery systems during periods of low water temperatures. Stone et al. (2013, Chapter 2, Figure 2.2a) reported a ~74% and ~107% increase in SGR, respectively, for 1-year-old greenlip abalone cultured at 18 and 22°C compared to those cultured at 14°C. Heating nursery systems would ultimately lead to a reduction in the production time and cost. Implementing temperature controlled systems for the grow-out of other year-classes would also result in improved production. It is important to note that improvements in the growth response with increasing water temperature in larger abalone will not be as pronounced compared to smaller <1-year-old abalone. Stone et al. (2013, Chapter 2) reported the SGR of 1-year-old greenlip abalone (1.8 g) was 300% greater (Figure 2.2a) compared to 2-year-old (22.9 g) abalone at 18°C (Figure 2.2b). It is also important to consider that there were no significant differences in the SGR between 2-year-old greenlip abalone cultured at 18°C and 22°C (Stone et al. 2013, Chapter 2, Figure 2.2b). Therefore, water temperature may be heated to a lower temperature and still promote optimal growth in 2-year-old abalone. However, this practice may be cost prohibitive for the large volumes of water required for slab tank grow-out systems and may be better suited to deeper tank-based culture methods. Therefore, we recommend a cost-benefit analysis be undertaken to implement the heating of nursery systems, and possibly grow-out systems, to take advantage of the temperature dependent growth rate of greenlip abalone.

Protein is an expensive dietary component and plays a major role in abalone nutrition. The availability of dietary protein limits protein deposition and the optimal growth of abalone (Fleming and Hone 1996). The optimal dietary crude protein level has been the focus of numerous studies for a range of abalone species, including *H. midae* (Britz 1996; Britz and Hecht 1997), *H. rubra* (Dunstan 2010), *H. tuberculata* (Mai et al. 1995), *H. discus hannai* (Mai et al. 1995) and *H. laevigata* (Coote et al. 2000; Stone et al. 2013, Chapter 2; Bansemer et al. 2015, Chapter 3). These studies investigated the optimal protein level for one age class of abalone and one water temperature and only a handful of studies have previously explored the effect of biotic and abiotic factors on the protein requirements of abalone (Britz and Hecht 1997; Stone et al. 2013, Chapter 2; Bansemer et al. 2015, Chapter 3). Wild abalone have a distinct dietary shift during their life cycle. Abalone initially feed on benthic microalgae, then as sub-adults they shift to a predominately macroalgae diet (Shepherd and Turner 1985). The reported protein levels (dry matter basis) of microalgae and macroalgae used for cultured abalone range from 12% - 35% (Brown et al. 1997) and 11% - 19% (Mai et al. 1994), respectively. This dietary shift suggests that the dietary protein requirement of cultured abalone may also change over time. However, currently cultured abalone are fed a diet containing one protein level over the entire grow-out cycle. The implementation of diets containing different dietary protein levels suitable for animals of different life stages and different environmental conditions is common practice in a range of livestock species (Sawyer et al. 2004) and aquaculture fish species (NRC 1993). Further improvements to diet formulations for abalone, concentrating on optimising dietary protein level for environmental change and life stages, would ultimately lead to increased growth rates and feed utilisation and a reduction in production expenses.

The optimal dietary protein level for growth was investigated for post-weaned 6-month-old (Bansemer et al. 2015, Chapter 3), and 1- and 2-year-old greenlip abalone (Stone et al. 2013, Chapter 2). A previous study by Coote et al. (2000) reported an optimal dietary crude protein level of 27% for juvenile greenlip abalone (0.55 - 0.94 g) at 20°C. However, when considering the SGR, feed consumption, FCR and protein deposition for different age classes and water temperatures, the optimal dietary crude protein level increased from ~29.0 - 32.2 - 34.7% crude protein for 1-year-old greenlip abalone and from 24 to 34 and 34% crude protein for 2-year-old abalone as water temperature increased from 14 to 18 to

22°C, respectively (Stone et al. 2013, Chapter 2, Table 2.3, Figure 2.2a, 2.3a and 2.4a). In contrast, there were no apparent benefits to feed post-weaned, 6-month-old greenlip abalone high protein diets at 14 or 17°C (Bansemmer et al. 2015, Chapter 3, Table 3.2, Figure 3.1, 3.2 and 3.3). Therefore, we recommend post-weaned, 6-month-old sub-juvenile greenlip abalone be fed a diet containing 29% crude protein, the minimum recommendation for 1-year-old juvenile greenlip abalone by Stone et al. (2013, Chapter 2) at 14 and 17°C. For sub-juvenile abalone at 20°C, although dietary protein had no significant effect on SGR, abalone consumed less feed and there was a tendency for an improvement of the FCR when fed high protein diets. These results indicate it may be beneficial for post-weaned, sub-adult greenlip abalone to be switched to a diet containing 35% crude protein at 20°C (Bansemmer et al. 2015, Chapter 3), which was the dietary crude protein level recommended at 22°C for 1-year-old abalone by Stone et al. (2013, Chapter 2). These recommendations were rapidly adopted by the industry. Prior to this project Australian feed companies produced commercial diets containing crude protein levels of 25 - 30% for the production of greenlip and hybrid abalone in Australia. This has now changed, based on information developed in Chapter. 2, Australian feed companies now formulate commercial abalone diets containing 34 to 35% crude protein and are now used routinely by Australian abalone farmers to improve productivity.

An unexpected result in the laboratory-based experiments reported by both Stone et al. (2013, Chapter 2) and Bansemmer et al. (2015, Chapter 3) was the significantly higher mortality rates at 14°C compared to other water temperatures investigated. For example, the mortality rate of juvenile 1-year-old greenlip abalone at 14°C was 6.60%, while at 18°C and 22°C the mortality rate was 1.25% (Stone et al. 2013, Chapter 2) and was 7.64% at 14°C compared to 2.09% at 17°C and 2.43% at 20°C for post-weaned, sub-juvenile 6-month-old greenlip abalone (Bansemmer et al. 2015, Chapter 3). Summer mortality is the major concern to the abalone farmers in southern Australia during periods of high summer water temperatures (> 22°C) where mortality rates can be up to 50% in some culture units (Stone et al. 2014). However, the moderate, yet significantly higher mortality rates at low water temperatures observed in the smaller greenlip abalone in the laboratory-based experiments in the current study might be easily overlooked on-farm and deserves further investigation.

The recommendations provided for optimum protein levels, and growth in relation to manipulation of water temperature in this project are for greenlip abalone that have been bred using broodstock that have been sourced from areas that are exposed to higher summer water temperatures in South Australian gulf waters. Difference in optimum dietary protein levels may occur in areas where abalone have been selected to grow at lower water temperatures, such as Tasmania and coastal Victoria. Further research to determine the appropriate water temperature to heat nursery systems and to switch to higher protein diets in Tasmania and coastal Victoria may be required to fine tune on-farm feeding practices.

It is important to note that results achieved in laboratory-based experiments may differ from those achieved commercially. This is due to differences in a number of factors, including feeding practices, environmental conditions and stocking densities. Therefore, it is essential that concepts developed in laboratory-based experiments, which may lead to potential improvements in commercial production of abalone, are validated in on-farm trials. This is an essential step in the process of successful research, development and extension to commercial production. Validation of experimental results in this fashion improves the transition of results from the laboratory to the farm, as it gives producers confidence to incorporate successful trial results into routine commercial practices to improve productivity.

The information obtained from the laboratory-based studies (Stone et al. 2013, Chapter 2; Bansemmer et al. 2015, Chapter 3) were used, in consultation with members of the AAGA and staff of the participating feed companies, to develop the feeding strategies to be evaluated in three long-term (>18 months) on-farm trials at Great Southern Water, Coastal Seafarms and

Kangaroo Island Abalone. Prior to the commencement of the on-farm trials, an abalone on-farm grow-out trial manual, was developed, in collaboration with project participants (Chapter 4, Appendix 4). The manual was distributed to the participating farms, and used to standardise trial procedures to minimise the variability in results achieved between farms. The on-farm trials were run concurrently, and designed to evaluate commercial diet formulations for the production of juvenile and sub-adult greenlip and hybrid abalone throughout normal commercial production cycles, whilst exposed to seasonal fluctuations in ambient water temperatures.

Results from the on-farm trials were positive and indicated that there was value in using a high protein diet or multi-diet feeding strategies to improve production of hybrid abalone. Biomass gain and FCR improvements resulted in a 9.5% increase in sales revenue of \$31 m⁻² tank area y⁻¹, after taking into account an extra \$2 of feed costs (Appendix 3, Table A3.1).

Throughout the Coastal Seafarms trial, improvements in weight by feeding high protein diets associated with the multi-diet feeding strategies, compared to the standard protein diet of the single diet feeding strategy, primarily occurred during Phase 1, with smaller greenlip abalone (Chapter 5, Figure 5.8). During Phase 2 of the same trial, growth between feeding strategies reduced proportionally with size as greenlip abalone grew older (Figure 5.9). A similar, less pronounced response was observed with hybrid abalone during the Great Southern Waters trial (Figure 5.6). This variation in growth response in relation to dietary protein level and age may be used to further refine multi-diet feeding strategies for cultured abalone. Larger abalone may be fed diets containing lower dietary protein levels with no apparent compromise to growth. This approach would result in a further saving and be beneficial to water quality and may result in improved production. However, further research is required to refine this concept before implementation into commercial feeding practices.

The Coastal Seafarms and Kangaroo Island Abalone farm trials were unable to detect any significant improvement in greenlip abalone growth at the completion of each phase when using the multi-diet feeding strategies. However, in Phase 1 of the Coastal Seafarms trial before harvesting and restocking with middle graded abalone, the individual weight of abalone in the multi-diet feed strategy was significantly higher after 10 and 11 months (Chapter 5, Figure 5.8). Significant improvements in weight class distribution were also observed at the completion of this phase of the trial (Table 5.5). However, it appeared that these growth benefits were diluted once the trial was harvested and restocked using middle grade size abalone. If tank biomass was reduced through controlled partial harvesting, which was practiced in the trial at Great Southern Waters, significant improvements to growth performance may have been observed at the conclusion of the trial. In contrast, the trial at Kangaroo Island Abalone showed no improvement to growth, but a high summer mortality event (~50%) occurred during Phase 2 of the trial, which negatively influenced the results (Table 5.7, 5.8 and 5.9). Cultured abalone are prone to summer mortality in southern Australian regions, and as all farms experience periods of high summer water temperatures, it is difficult to eliminate this problem interfering with on-farm trials. However, it may be possible to minimise the likelihood of such mortality events occurring, or minimise their impacts, by careful advanced planning, to ensure that culture units are set at appropriate stocking densities during summer and early autumn periods, to minimise the effects of high water on the survival of abalone. Additionally, the use of dietary intervention with therapeutic compounds, such as antioxidants, may be incorporated into diets to reduce the impacts of these mortality events during periods of high, summer water temperatures (Lange et al. 2014; Stone et al. 2014).

During the project, there were difficulties in managing the on-farm trials in a commercial context. Each farm had set commercial production targets that had to be met, and often this resulted in alterations to trial maintenance and sampling procedures. However, given these

conflicting demands, overall each farm carried out the on-farm trials in a rigorous manner. Despite best efforts, that included the development and application of the on-farm growth trial manual (Chapter 4, Appendix 4) to standardise methods, and regular communication with the managers of each on-farm trial, variation in results between farms occurred. Despite this, results from the on-farm trials, support the use of high protein diets or multi-diet feeding strategies to improve the production of cultured abalone in land-based facilities throughout southern Australia.

It should be noted, a number of key factors have been identified to improve future trial designs and methodologies:

- Abalone should be fed on a biomass basis rather than by eye, as feeding by eye may limit feed intake, which is the first limiting factor in abalone growth (Britz et al. 1997). It may have also resulted in overfeeding and increased FCRs, reduced apparent protein efficiency ratios, reduced water quality and reduced growth.
- When reducing tank stocking densities to minimise the effects of overcrowding on abalone growth rates in tanks:
 - Harvesting and restocking with middle graded abalone, as practiced in the trials at Kangaroo Island Abalone and Coastal Seafarms, should be avoided. This practice resulted in a dilution of treatment responses and may have impacted the ability to discern statistical differences.
 - Thinning of stocking densities by controlled partial harvesting, as practiced during the Great Southern Waters trial, should be used instead.
- With regard to replication and statistical power, in order to achieve a 10% statistical difference ($P < 0.05$) in biomass gain we recommend that future on-farm trials commit eight culture units per treatment for adequate statistical power (Power of test, $P = 0.80$). A greater level of replication may be difficult to achieve on some farms given the restrictions of staffing, combined with production demands, placed on commercial operators. However, this number will vary and be dependent on the variability of the response measured. The variability for a given response will also differ between farms due to differences in abalone strains, on-farm culture environments and practices.
- Investment in research and development is a serious proposition, where often gains may only be realised in small percentages, if at all. If trials are run with insufficient statistical power they may be doomed from the start and would not only result in a waste of time, resources and money in the present, they may also cost the industry well into the future, as cost savings procedures may be missed, and are often not revisited until the turn-over of management occurs.
- Due to difference in tank design, the use of different strains of abalone (South Australian greenlip vs. Victorian greenlip abalone) or species (greenlip vs. hybrid abalone) may not allow for successful comparison between farms.
- Farms should have appropriate vermin control systems in place to minimise stock losses due to predation.

The overarching outcome of this project, in collaboration with AAGA farmers and Australian abalone feed producers, was to develop diet formulations and feeding strategies that delivered a >10% improvement in productivity across the entire grow-out period for greenlip and hybrid abalone. The results presented in this report indicate that the outcome of the project has been achieved. In conclusion, the investment to research and development by AAGA and all project participants during this project have been substantiated by the successful outcomes achieved in the laboratory and on-farm trials describe in this report.

Based on the results achieved during the course of this project, Australian abalone feed producers have altered the formulations of their commercial diets based on the results of the laboratory experiments in Chapters 2 and 3. Both laboratory-based studies identified significantly higher growth rates in abalone held at warmer water temperatures, up to 22°C (Stone et al. 2013, Chapter 2, Figure 2.2a; Bansemer et al. 2015, Chapter 3, Table 3.3). This needs further consideration as improved production, would occur from supplying smaller <1-year-old greenlip abalone with heated water (up to 22°C) in nursery systems during periods of low temperatures, which may reduce production costs. However, additional infrastructure, such as temperature controlled buildings, would be required to capitalise on improving production.

To capitalise on improved production without additional infrastructure, diet manipulation during periods of high growth (younger animals or at warmer water temperatures) would lead to improved production on-farm. The positive results for abalone growth and feed utilisation from on-farm trials were achieved by using commercially formulated diets, developed in this project, which are now readily available for abalone producers. In fact, as a result of this project, Skretting Australia, which have the largest market share of any of the Australian abalone feed companies, have switched over exclusively to the production of high protein diets for abalone grow-out in Australia. Adam and Amos abalone feeds and EP Aquafeeds now also offer a range of high protein grow-out diets. Additionally, based on the results for individual weight gain (5.4%) and biomass gain (9.3%), from the Great Southern Waters trial, the overall duration of a typical three year production cycle for hybrid abalone may be shortened by 1.9 to 3.4 months by feeding high protein diets. A reduced production cycle may also enable farmers to harvest larger abalone sooner, which may reduce the exposure of valuable stock to one less summer. This factor alone would result in significantly improved productivity and cost savings, and when combined with savings made with increased growth rates, biomass gain and feed efficiency, a >10% improvement in productivity across the entire grow-out period for abalone may be achieved.

Chapter 7. Benefits and Adoption

The key research findings described in this project have addressed the two highest research priorities of AAGA in 2009, prior to the commencement of this project:

1. Improve our understanding of the effects of season water temperatures on the growth of abalone; and
2. Improve our understanding of the effects of dietary protein on the growth of abalone.

The key research findings have been directly implemented by Australian commercial feed companies and AAGA members as they have come to hand. For example, prior to this project Australian feed companies produced commercial diets containing crude protein levels of 25 - 30% for the production of greenlip and hybrid abalone in Australia. This has now changed. Based on information developed in Chapter 2, Australian feed companies now formulate a range of commercial abalone grow-out diets containing crude protein levels that range up to 35%. Australian abalone farmers are now using these diets routinely to gain improved productivity. These findings have also helped the Australian feed companies to refine dietary formulations for inclusion into high protein diets and multi-diet feeding strategies to improve the commercial production of abalone (Chapters 2, 3, 4 and 5).

These findings have also helped the AAGA members by highlighting the potential of heating nursery systems (Chapters 2 and 3). We have improved our understanding and identified potential dietary interventions to reduce the impact of summer mortality on abalone production (Lange et al. 2014, Stone et al. 2014; Appendix 1). Following the ASCRC/AAGA research priority planning meeting held in Geelong, Victoria in May 2009, summer mortality was ranked as AAGA's third highest research priority after the two addressed within this project.

There has been a considerably large student training component to this project. This component has resulted in the training of at least 40 undergraduate work experience student, 5 part-time undergraduate student projects, three Honours students, one Masters student, and seven PhD students. All of the Honours and post-graduate students have had the opportunity to present their research results at industry meetings, domestic and international conferences and published their work in internationally peer-reviewed journals (Appendices 1 and 2). The main benefit of the large student training component is the output of new industry entrants trained with relevant skills that will contribute to future development of the industry. Several students have already obtained work in the industry.

Chapter 8. Further Development

This research has improved our understanding of the optimum dietary protein levels for juvenile greenlip abalone at seasonal water temperatures (Stone et al. 2013, Chapter 2; Bansemer et al. 2015, Chapter 3). It has also led to the development and validation of feeding strategies, utilising different dietary protein levels during different stages of production, to improve the productivity of commercial abalone culture. Results generated from the current research have been extended to industry and incorporated into commercial production and may form the basis for follow-on research in future projects. In the current research, there were several limitations and unsolved issues, or areas that were not covered in the experiments and trials, which require further investigation. Recommended areas for future research and development are outlined below:

- Validation of heated nursery systems to efficiently improve the early growth of abalone in order to reduce the length of the overall production cycle. Coastal Seafarms and Great Southern Waters are currently in the preliminary stages of evaluation.
- Refinement of the dietary nutrient requirements for earlier, and more successful, weaning of abalone. This area of research would evaluate the effects of the timing of weaning, and different dietary protein and other nutrient levels on the growth, health and survival of abalone over the subsequent stages of production.
- Refinement of multi-diet feeding strategies for larger abalone. In Chapters 2 and 3, where protein and temperature interactions were investigated a limited range of abalone age classes presented limitations to the design of the multi-diet feeding strategies for later stages of abalone (> 2-year-old). We suggest that further studies are undertaken with > 2-year-old abalone to refine the design of future multi-diet feeding strategies for on-farm validation and commercial use.
- The main focus of the research presented in this report was on the growth performance and feed utilisation. However, preliminary histological examination of the digestive tract morphology of abalone fed diets containing higher protein levels at different water temperatures was undertaken. A more comprehensive analysis of potential alterations to digestive tract morphology due to dietary and temperature effects is recommended, especially with regards to manipulating protein source, rather than level.
- Additional research is required to understand the effects of other bulk ingredients, commonly used in commercial diets, on digestive tract histology and health of abalone, particularly at high summer water temperatures.
- The examination of the microbial community in the digestive tract when abalone are fed diets containing higher protein levels at different water temperatures.
- Refinement of diets for high water temperatures and low dissolved oxygen levels for larger abalone in relation to productivity and summer mortality. Further research is required to understand the effects of the bulk ingredients commonly used in commercial diets on histology and microbial community in the digestive tract, and health, survival and growth of abalone at high summer water temperatures.

Combined, new information provided from these areas of research may contribute to increased productivity and expansion of the Australian abalone aquaculture industry.

Chapter 9. Planned Outcomes

The planned outcome of the project, to deliver a >10% improvement in productivity across an entire grow-out period for greenlip and hybrid abalone to industry, has been achieved. In order to achieve the planned outcome four Objectives were established, in consultation with AAGA and the participating feed companies, and a series of laboratory based experiments (Chapters 2 and 3), and on-farm commercial trials (Chapters 4 and 5) were designed and carried out to completion. The four Objectives, along with the outcome for each, are provided below.

Objective 1. *Determine optimum dietary protein requirements for juvenile (1-year-old) and sub-adult (2-year-old) greenlip abalone at different temperatures*

Objective 2. *Determine optimum dietary protein requirements for sub-juvenile (6-month-old) greenlip abalone at different temperatures*

The outcomes for Objectives 1 and 2 were achieved.

- Prior to this project, Australian feed companies produced commercial diets containing crude protein levels of 25 - 30% for the production of greenlip and hybrid abalone in Australia; this has now changed to 35% (Chapters 2, Stone et al. 2013; Chapter 3, Bansemer et al. 2015).
- Australian abalone farmer use the new diets routinely to improve productivity.
- Optimum dietary protein levels for greenlip abalone also varied with age and water temperature (Chapters 2, Stone et al. 2013; Chapter 3, Bansemer et al. 2015).
- It was also deemed plausible to shorten the commercial production cycle of abalone by heating land-based nursery systems to gain accelerated growth before transfer to grow-out systems. This concept is currently being evaluated on-farm by several of the AAGA companies, with promising results.

We identified potential scope to go forward and design on-farm trials (Chapter 4) to evaluate the concept of multi-diet feeding strategies on-farm in the production of greenlip and hybrid abalone with the aim of improving growth and feed utilisation and to reduce the duration of the production cycle (Chapter 5).

Objective 3. *Develop an abalone on-farm grow-out trial manual.*

The outcome from Objective 3 were achieved.

- Following extensive consultation with AAGA members, staff of participating abalone feed companies and research providers, a manual was produced (Chapter 4, Appendix 4), distributed and used successfully for the on-farm feeding strategy trials with greenlip and hybrid abalone (Chapter 5).

Objective 4. *Develop and test starter feeds and improved grow-out feeds for greenlip and hybrid abalone in commercial settings*

The outcomes from Objective 4 were achieved.

- Based on results in Chapters 2, 3, 4 and 5, a recommendation was made to the project stakeholders to adopt multi-diet feeding strategies for abalone production.

- A food conversion ratio (FCR) improvement of 7% and a biomass gain of 9.3% combined to give an annual 9.5% increase in basic sales revenue for hybrid abalone fed the high protein diet during the Great Southern Waters trial (Chapter 5).
- The overall duration of a typical three year production cycle of abalone may be shortened by 1.9 to 3.4 months. This may enable farmers to reduce the mortality rate of larger, more valuable, abalone by reducing their exposure to a third summer.
- Australian feed companies now offer commercial abalone grow-out diets of varying protein levels of up to 35%.
- AAGA members have adopted the use of feeding new high protein diets, or multi-diet feeding strategies.

Overall, the outcome of the development of diet formulations and feeding strategies that deliver a >10% improvement in productivity across the entire grow-out period for abalone to the industry was achieved.

The Australian abalone producers now have the confidence to use newly formulated high protein diets, produced by Australian feed companies, in combination with previously existing diets to improve the weight gain, feed utilisation and productivity. Additionally, AAGA members, Australian feed companies and research providers worked closely to achieve these goals. All groups have identified the importance of nutritional research in relation to growth and health and improved productivity for abalone farms. The confidence developed in gaining a significant return on their research and development investment during this project has resulted in AAGA members deciding to pursue this line of research well into the future.

AAGA members, feed companies representatives and researchers have agreed on the need to increase our understanding of abalone nutrition, physiology and health. This has led to the development of a proposal for the establishment of an Abalone Research Centre for Excellence, based in South Australia.

CRC Output

Public Benefit Outcomes

The majority of the research described in this project has been extended to the broader scientific community. Apart from the extension of results to AAGA and the Australian abalone feed companies, numerous presentations were given by project participants to extend the information arising from this project. Project information was extended to other members of the aquaculture and feed industry, government departments, the general public and members of the scientific community. Information was disseminated at domestic and international scientific conferences, ASCRC and industry workshops and directly to feed company representatives.

To date, one final report, an on-farm feed trial manual, seven peer-reviewed scientific publications, three Honours theses, one Masters thesis, eleven conference abstracts, and numerous presentations containing information specifically targeting ways to enhance the production of abalone, arose from this project (Appendix 1).

There has been a considerably large student training component in this project. This component has resulted in the training of greater than forty undergraduate extra-mural work experience students, five part-time undergraduate student projects, Four Honours students, one Masters Student and seven PhD students (Appendix 2). All of the Honours and Postgraduate students have had the opportunity to present their research results at industry

meetings, domestic and international conferences. Several students have also published their research in internationally peer-reviewed journals (Appendix 1). A major benefit of the student training component is the output of new industry entrants, trained with relevant skills that will contribute to future industry development. Several students have already obtained work within the industry.

Private Benefit Outcomes

The major private outcome of this project is the formulation and manufacture of improved diets for abalone production. AAGA members, in collaborations with commercial feed companies, have acted on this information and recommendations provided within this report, to provide significant improvements in the seasonal production of abalone in land-based operations. The commercial production diets and feeding strategies were designed to contain protein levels that meet the nutritional requirements of abalone at specific ages and for seasonal variations in temperatures to improve growth (6 - 9%) and feed utilisation (7%) of commercially produced abalone.

All information from this project has been extended to AAGA and staff at Australian feed companies, as it has come to hand, and they have acted on it to improve the sustainable production of abalone. Additionally, all information from Chapters 2 and 3 have been published, or are in press for publication, in internationally peer-reviewed scientific journals. This information has also been very well received when presented, both domestically and internationally, to the broader scientific community.

A large amount of information was also generated with regards to abalone growth, feeding behaviour and survival at optimal and sub-optimal temperatures (Schaefer et al. 2013; Stone et al. 2013, Chapter 2; Bansemer et al. 2015, Chapter 3; Lange et al. 2014; Stone et al. 2014). This information may form the basis to design and evaluate strategies to improve the early growth rate of juvenile abalone in heated nursery systems with an aim to reduce the overall production cycle and improve productivity.

Peripheral research associated with the project has also provided great insight into the feeding behaviour, growth, feed efficiency, water quality, energetic, product quality, digestive tract health and survival of a range of age classes of greenlip and hybrid abalone in relation to diet manipulation at optimal and sub-optimal water temperatures. This information is essential in developing feeding practices on-farm, especially in relation to times of high water temperatures when oxygen levels are lowest.

Linkages with CRC Milestone Outcomes

The outcomes from this project have addressed the following ASCRC Milestone outcomes:

- 1.3. Removal or reduction of key production constraints in selected aquaculture systems;
- 1.3.4. New low-cost aquaculture diets targeting improved feed conversion developed and evaluated; and
- 3.5. Production efficiency gains from genetic, health management and nutritional interventions quantified to inform long-term strategies and estimate commercial benefits.

Chapter 10. Conclusion

The overarching outcome of this project, in collaboration with AAGA farmers and Australian abalone feed producers, was to develop diet formulations and feeding strategies that delivered a >10% improvement in productivity across the entire grow-out period for greenlip and hybrid abalone. The results presented in this report indicate that the outcome of the project has been achieved. In conclusion, the investment in research and development by AAGA and all project participants during this project have been substantiated by the successful outcomes achieved in the laboratory and on-farm trials described in this report.

Based on project results, we recommend that the dietary protein levels for commercial greenlip abalone are altered to ensure that higher protein diets are used during periods of rapid growth which may be attained, at, and above, the previously reported optimal water temperature of 18°C (~35.0% CP). Australian abalone feed producers have now altered the formulations of their commercial diets based on the results of the laboratory experiments in Chapters 2 and 3 (Stone et al. 2013; Bansemer et al. 2015).

Both laboratory-based studies identified significantly higher growth rates in abalone held at warmer water temperatures, up to 22°C (Stone et al. 2013, Chapter 2, Figure 2.2a; Bansemer et al. 2015, Chapter 3, Table 3.3). This needs further consideration as improved production would occur from supplying smaller <1-year-old greenlip abalone with heated water (up to 22°C) in nursery systems during periods of low temperatures, which may reduce production costs. However, additional infrastructure, such as temperature controlled buildings, would be required to capitalise on improving production.

To capitalise on improved production without additional infrastructure, diet manipulation during periods of high growth (younger animals or at warmer water temperatures) would lead to improved production on-farm. The positive results for abalone growth and feed utilisation from on-farm trials were achieved by using commercially formulated diets, developed in this project, which are now readily available for abalone producers. The high protein diet and multi-diet feeding strategies were successful in increasing biomass gain, while improving FCR for hybrid abalone at Great Southern Waters. At the conclusion of the trial, an increase in basic sales revenue of \$31 m⁻² tank area y⁻¹; was achieved for the additional feed input cost of \$2 (Appendix 3). Additionally, these gains were made without an impact on mortality due to high protein diets.

Based on the results from the Great Southern Waters on-farm trial, the overall duration of a typical three year production cycle for hybrid abalone may be shortened by 1.9 to 3.4 months by feeding high protein diets. There are numerous benefits of producing heavier abalone quicker. Apart from savings for both fixed and variable costs, considerable savings may also be made from reduced mortality of stock during the shortened production cycle. For example, larger and older abalone are more prone to summer mortality. A shortened production cycle may allow farmers to harvest abalone sooner, which may reduce exposure of valuable stock to one less summer.

Overall, this project has provided the industry with new information to achieve a >10% improvement in productivity for abalone across the entire grow-out period.

Chapter 11. References

- ABARE, 2010. Australian Fisheries Statistics. Australian Bureau of Agricultural and Resource Economics.
- Bansemer, M.S., Qin, J.G., Harris, J.O., Howarth, G.S., Stone, D.A.J., 2014. Nutritional requirements and use of macroalgae as ingredients in abalone feed. *Reviews in Aquaculture*, doi: 10.1111/raq.12085.
- Bansemer, M.S., Harris, J.O., Qin, J.G., Duong, D.N., Stone, D.A.J., 2015. Growth and feed utilisation of juvenile greenlip abalone (*Haliotis laevis*) in response to water temperatures and increasing dietary protein levels. *Aquaculture* 436, 13-20.
- Bautista-Teruel, M.N., Koshio, S.S., Ishikawa, M., 2011. Diet development and evaluation for juvenile abalone, *Haliotis asinina* Linne: Lipid and essential fatty acid levels. *Aquaculture* 312, 172-179.
- Bautista-Teruel, M., Fermin, A., Koshio, S., 2003. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: animal and plant protein sources. *Aquaculture* 219, 645-653.
- Bautista-Teruel, M., Millamena, O., 1999. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: protein and energy levels. *Aquaculture* 178, 117-126.
- British Pharmacopoeia Commission, 2004. British pharmacopoeia. The Stationary Office, London, England.
- Britz, P.J., 1996a. The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae*. *Aquaculture* 140, 63-73.
- Britz, P.J., 1996b. Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae*, fed fishmeal-based semi-purified diets. *Aquaculture* 140, 55-61.
- Britz, P.J., Hecht, T., 1997. Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*. *Aquaculture* 156, 195-210.
- Britz, P.J., Hecht, T., Mangold, S., 1997. Effect of temperature on growth, feed consumption and nutritional indices of *Haliotis midae* fed a formulated diet. *Aquaculture* 152, 191-203.
- Brown, M.R., Jeffrey, S.W., Volkman, J.K., Dunstan, G.A., 1997. Nutritional properties of microalgae for mariculture. *Aquaculture* 151, 315-331.
- Burke, C.M., Harris, J.O., Hindrum, S.M., Edwards, S.J., Maguire, G.B., 2001. Environmental requirements of abalone. Final Report, FRDC Project No. 97/323, Tasmanian Aquaculture and Fisheries Institute, 154 pp.
- Chaitanawisuti, N., Kritsanapuntu, S., Santaweek, W., 2011. Effects of dietary protein and lipid levels and protein to energy ratios on growth performance and feed utilization of hatchery-reared juvenile spotted babylon (*Babylonia areolata*). *Aquaculture International* 19, 13-21.
- Chiofalo, B., Presti, V.L., Chiofalo, V., Gresta, F., 2012. The productive traits, fatty acid profile and nutritional indices of three lupin (*Lupinus* spp.) species cultivated in a

Mediterranean environment for the livestock. *Animal Feed Science Technology* 171, 230-239.

Cho, S.H., 2010. Effect of fishmeal substitution with various animal and/or plant protein sources in the diet of the abalone *Haliotis discus hannai* Ino. *Aquaculture Research* 41, 587-593.

Cho, S.H., Kim, D.S., 2012. Effects of Feed Type and Temperature on Growth of Juvenile Abalone, *Haliotis discus hannai* Ino. *Journal of the World Aquaculture Society* 43, 114-119.

Cho, S.H., Cho, Y.J., Yoo, J.H., 2009. Compensatory growth of juvenile abalone *Haliotis discus hannai* with different feeding regime. *Aquaculture Research* 40, 984-987

Coote, T.A., 1998. Optimising the nutrient specifications of manufactured feeds for farmed juvenile greenlip abalone (*Haliotis laevis* Donovan). PhD thesis, Department of Aquaculture, University of Tasmania, Australia. 143 pp.

Coote, T.A., Hone, P.W., Van Barneveld, R.J., Maguire, G.B., 2000. Optimal protein level in a semipurified diet for juvenile greenlip abalone *Haliotis laevis*. *Aquaculture Nutrition* 6, 213-220.

Cropp, R.A., 1989. Abalone culture in Tasmania. Dpt. Sea Fisheries, Tasmania, Technical Report 37.

Currie, K.L., 2013. Effect of water temperature and age on the gastrointestinal evacuation time and nutrient digestibility of greenlip abalone *Haliotis laevis*. School of Biological Sciences Honours thesis, Flinders University, Adelaide, Australia.

Daume, S., 2003. Abalone Aquaculture Subprogram: early life history of abalone (*Haliotis rubra*, *H. laevis*): settlement, survival and early growth. FRDC Report 1998/306.

DIN 51900-1, 2000. Testing of solid and liquid fuels - Determination of gross calorific value by the bomb calorimeter and calculation of net calorific value - Part 1. Deutsches Institut für Normung e.V. Beuth Verlag GmbH, Berlin.

Dunstan, G.A., 2010. A simple model for the determination of the relative utilization efficiency of protein by blacklip abalone (*Haliotis rubra* Leach). *Aquaculture Nutrition* 16, 1-12.

Dunstan, G. A., Volkman, J. K., Maguire, G. B., 2000. Optimisation of essential lipids in artificial feeds for Australian abalone. pp. 68. FRDC Project 94/85. CSIRO Marine Research, Hobart Tasmania.

Dunstan, G.A., Volkman, J.K., Maguire, G.B., 2001. Abalone Aquaculture Subprogram: optimisation of Essential Lipids in Artificial Feeds for Australian Abalone. FRDC Report 1994/85. CSIRO Marine Research, Hobart, Tasmania.

Duong, D.N., Harris, J.O., Qin, J.G., Stone, D.A.J., (2014) Bioenergetics of greenlip abalone (*Haliotis laevis*) and hybrid abalone (*H. laevis* × *H. rubra*) fed different protein levels at different water temperatures. World Aquaculture Conference, Adelaide, Australia. (Abstract) <https://www.was.org/meetingabstracts/ShowAbstract.aspx?Id=33149>

Edwards, S., Condon. C., 2001. Digestive protease characterization, localization and adaptation in blacklip abalone (*Haliotis rubra* Leach). *Aquaculture Research* 32, 95-102.

- Festing, M.F., Altman, D.G., 2002. Guidelines for the design and statistical analysis of experiments. *Institute of Laboratory Animal Research Journal* 43, 244-250.
- Fleming, A.E., Hone, P.W., 1996. Abalone aquaculture. *Aquaculture* 140, 1-4.
- Fleming, A.E., Van Barneveld, R.J., Hone, P.W., 1996. The development of artificial diets for abalone: A review and future directions. *Aquaculture* 140, 5-53.
- Fleming, A.E., Van Barneveld, R.J., Hone, P.W., Vandeppeer, M.E., Kruk, J.A., 1998. Complementary additivity of the digestibility coefficients of feed ingredients fed to juvenile greenlip abalone (*Haliotis laevis*). *Journal of Shellfish Research* 17, 641-647.
- Freeman, K.A., 2001. Aquaculture and Related Biological Attributes of Abalone Species in Australia - a Review. Fisheries Research Report No. 128, Fisheries Western Australia, Perth, 48 pp.
- German Institute for Standardization (DIN), 2000. Testing of solid and liquid fuels: determination of gross caloric value by the bomb calorimeter and calculation of net caloric value. Part 1 (51900-1). Berlin, Deutsches Institut für Normung e.V., Beuth Verlag GmbH.
- Gilroy, A., Edwards, S.J., 1998. Optimum temperature for growth of Australian abalone: preferred temperature and critical thermal maximum for blacklip abalone, *Haliotis rubra* (Leach), and greenlip abalone, *Haliotis laevis* (Leach). *Aquaculture Research* 29, 481-485.
- Green, A.J., Jones, C.L.W., Britz, P.J., 2011. The protein and energy requirements of farmed South African abalone *Haliotis midae* L. cultured at optimal and elevated water temperatures. *Aquaculture Research* 42, 1653-1663.
- Handlinger, J., Carson J., Harris J., Taylor D., 2006. Abalone Aquaculture Subprogram: potential for antibiotic use in abalone for disease control. FRDC Report 2000/205.
- Hardy, R.W., 2002. Rainbow trout, *Oncorhynchus mykiss*. In C.D. Webster and C.E. Lim, eds. Nutrient requirements and feeding of finfish for aquaculture, pp. 184-202. New York, CABI Publishing.
- Hernández, J., de la Para, A.M., Lastra, M., Viana, M.T., 2013. Effect of lipid composition of diets and environmental temperature on the performance and fatty acid composition of juvenile European abalone (*Haliotis tuberculata* L. 1758). *Aquaculture*, 412-413, 34-40.
- Hochachka, P.W., Somero, G.N., 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press, New York, USA.
- Hone, P.W., 1992. Biotechnology Aquaculture Workshop - Abalone Artificial Diets. Department of Primary Industries (Fisheries), Adelaide.
- Lange, B., Currie, K-L., Howarth, G.S., Stone, D.A.J., 2014. Dietary inclusion of grape seed extract and dried macroalgae improves the survival of greenlip abalone (*Haliotis laevis*) at high water temperatures. *Aquaculture* 433, 348-360.
- Mai, K., Mercer, J.P., Donlon, J., 1994. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino II: amino acid composition of abalone and six species of macroalgae with an assessment of their nutritional value. *Aquaculture* 128, 15-30.

Mai, K. Mercer, J.P., Donlon, J., 1995. Comparative studies on the nutrition of two species of abalone, *Haliotis tuberculata* L. and *Haliotis discus hannai* Ino. IV. Optimum dietary protein level for growth. *Aquaculture* 136, 165-180.

Mateos, H.T., Lewandowski, P.A., Su, X.Q., 2011. Dietary fish oil supplements increase tissue n-3 fatty acid composition and expression of delta-6 desaturase and elongase-2 in Jade Tiger Hybrid Abalone. *Lipids* 46, 741-751.

McShane, P.E., Gorfine H.K., Knuckey I.A., 1994. Factors influencing food selection in the abalone *Haliotis rubra* (Mollusca: Gastropoda). *Journal of Experiment Marine Biology and Ecology* 176, 27-37.

Monteiro, J., Li, F-J., MacLennan, M., Rabalski, A., Moghadasian, M.H., Nakamura, M.T., Ma, D.W.L., 2012. Menhaden oil, but not safflower or soybean oil, aids in restoring the polyunsaturated fatty acid profile in the novel delta-6-desaturase null mouse. *Lipids in Health and Disease* 11, 60.

National Research Council (NRC), 1993. Nutrient requirements of fish. Washington, D.C., National Academy Press, 114 pp.

Ng, W-K., Romano, N., 2013. A review of the nutrition and feeding management of farmed tilapia throughout the culture cycle. *Reviews in Aquaculture* 5, 220-254.

Nichols, P.D., Mooney, B.D., Elliot, N.G., 2002. Nutritional Value of Australian Seafood II. Factors Affecting Oil Composition of Edible Species. Commonwealth Science and Industrial Research Organisation (CSIRO) Marine Research and Fisheries Research and Development Corporation (FRDC), Australia.

Palmer, A.R., 1981. Do carbonate skeletons limit the rate of body growth? *Nature* 292, 150-152.

Quartararo, N., Allan, G.L., Bell, J.D., 1998. Replacement of fish meal in diets for Australian snapper, *Pagrus auratus*. *Aquaculture* 166, 279-295.

Taylor, B., 1992. Optimum protein levels in artificial diets for *Haliotis kamtschatkana*. *Journal of Shellfish Research* 11, 556.

Sales, J., Truter, P.J., Britz, P.J., 2003a. Optimum dietary protein level for growth in South African abalone (*Haliotis midae* L.). *Aquaculture Nutrition* 9, 85-89.

Sarker, P.K., Bureau, D.P., Hua, K., Drew, M.D., Forster, I., Were, K., Hicks, B., Vandenberg, G.W., 2013. Sustainability issues related to feeding salmonids: a Canadian perspective. *Reviews in Aquaculture* 5, 199-219.

Sawyer, J.E., Mathis, C.P., Davis, B., 2004. Effects of feeding strategy and age on live animal performance, carcass characteristics, and economics of short-term feeding programs for culled beef cows. *Journal of Animal Science* 82, 3646-3653.

Schaefer, E.N., Harris, J.O., Howarth, G.S., Bansemer, M.S., Stone, D.A.J., 2013. Comparative histological changes in the greenlip abalone *Haliotis laevigata* gastrointestinal tract in response to water temperature, different dietary protein levels, and animal age. *Journal of Shellfish Research* 32, 131-141.

- Shepherd, S., Turner, J., 1985. Studies of southern Australian abalone (genus *Haliotis*) VI: habitat preference, abundance, and predators of juveniles. *Journal of Experimental Marine Biology and Ecology* 93, 285-298.
- Shipton, T.A., Britz, P.J., 2001a. The effect of animal size on the ability of *Haliotis midae* L. to utilize selected dietary protein sources. *Aquaculture Research* 32, 393-403.
- Shipton, T.A., Britz, P.J., 2001b. The partial and total replacement of fishmeal with selected plant protein sources in diets for the South African abalone, *Haliotis midae* L. *Journal of Shellfish Research* 20, 637-645.
- Shipton, T.A., Britz, P.J., Walker, R.B., 2002. An assessment of the efficacy of two lysine microencapsulation techniques to determine the quantitative lysine requirement of the South African abalone, *Haliotis midae* L. *Aquaculture Nutrition* 8, 221-227.
- Statistical Solutions LLC., 2014. Accessed on the 20/10/14, <http://www.statisticalsolutions.net/pss_calc.php>.
- Steinarsson, A., Imsland, A.K., 2003. Size dependent variation in optimum growth temperature of red abalone (*Haliotis rufescens*). *Aquaculture* 22, 353-362.
- Stone, D.A.J., Allan, G.L., Anderson, A.J., 2003. Carbohydrate utilisation by juvenile silver perch *Bidyanus bidyanus* (Mitchell): III. The protein sparing effect of wheat starch based carbohydrates. *Aquaculture Research* 34, 123-134.
- Stone, D.A.J., Bansemmer, M.S., Lange, B., Schaefer, E.N., Howarth, G.S., Harris, J.O., 2014. Dietary intervention improves the survival of cultured greenlip abalone (*Haliotis laevigata* Donovan) at high water temperature. *Aquaculture* 430, 230-240.
- Stone, D.A.J., Harris, J.O., Wang, H., Mercer, G.J., Schaefer, E.N., Bansemmer, M.S., 2013. Dietary protein level and water temperature interactions for greenlip abalone, *Haliotis laevigata*. *Journal of Shellfish Research* 32, 119-130.
- Uki, N., Kemuyama, A., Watanabe, T., 1986. Optimum protein level in diets for abalone. *Bull. Japanese Society for Scientific Fisheries* 52.1005-1012.
- Van Barneveld, R.J., Fleming, A.E., Vandeppeer, M.E., Kruk, J.A., Hone, P.W., 1998. Influence of dietary oil and oil inclusion level in manufactured feeds on the digestibility of nutrients by juvenile greenlip abalone (*Haliotis laevigata*). *Journal of Shellfish Research* 17, 649-655.
- Vandeppeer, M., 2005. Abalone aquaculture subprogram: manufactured diet development. FRDC project No. 96/385. Fisheries Research and Development Corporation and the Abalone Aquaculture Subprogram 2005, Alice Springs, N.T, Australia.
- Vandeppeer, M., 2006. SARDI Publications, Abalone Aquaculture Subprogram: Preventing summer mortality of abalone in aquaculture systems by understanding interactions between nutrition and water temperature. South Australian Research and Development Institute (Aquatic Sciences), Adelaide, 85pp. SARDI Publication Number RD02/0035-2.
- Wassnig, M., Roberts, R.D., Krsinich, A., Day, R.W., 2010. Effects of water flow rate on growth rate, mortality and biomass return of abalone in slab tanks. *Aquaculture Research* 41, 839-846.

Won, N., Kawamura, T., Takami, H., Noro, T., Musashi, T., Watanabe, Y., 2010. Ontogenetic changes in the feeding habits of the abalone *Haliotis discus hannai*: field verification by stable isotope analyses. *Canadian Journal of Fisheries and Aquatic Sciences* 67, 347-356.

Chapter 12. Appendices

Appendix 1. Publications, reports and presentations arising from this project

A1.1 Manuscripts for Peer-Reviewed Journals

Bansemer, M.S., Harris, J.O., Qin, J.G., Duong, D.N., Stone, D.A.J., 2015. Growth and feed utilisation of juvenile greenlip abalone (*Haliotis laevis*) in response to water temperatures and increasing dietary protein levels. *Aquaculture* 436, 13-20.

Bansemer, M.S., Stone, D.A.J., in preparation. Temperature and diet type affects the feed intake of greenlip (*Haliotis laevis*) and hybrid (*H. laevis* x *Haliotis rubra*) throughout the night. Target, *Aquaculture*.

Bansemer, M.S., Qin J.G., Harris J.O., Howarth G.S., Stone D.A.J., 2014. Nutritional requirements and use of macroalgae as ingredients in abalone feed. *Reviews in Aquaculture*. doi: 10.1111/raq.12085

Buss, J., Jones, D., Harris, J.O., Lumsden, A., Bansemer, M.S., Stone, D.A.J., in press. The effect of feed density and diet type on the feeding behaviour of greenlip abalone (*Haliotis laevis*). *Marine and Freshwater Physiology*.

Currie, K-L., Davidson, H., Harris, J.O., Bansemer, M.S., Stone, D.A.J., in press. The effect of diet type and water temperature on the feeding behaviour of greenlip (*Haliotis laevis*) and hybrid abalone (*H. laevis* x *H. rubra*). Target, *Marine and Freshwater Physiology*.

Currie, K-L., Lange, B., Herbert, E.W., Harris, J.O., Stone, D.A.J., in preparation. Effect of water temperature and age on the gastrointestinal transit and nutrient digestibility of greenlip abalone (*Haliotis laevis*). Target, *Aquaculture*.

Lange, B., Currie, K-L., Howarth, G.S., Stone, D.A.J., 2014. Dietary inclusion of grape seed extract and dried macroalgae improves the survival of greenlip abalone (*Haliotis laevis*) at high water temperatures. *Aquaculture* 433, 348-360.

Schaefer, E.N, Harris, J.O., Howarth, G.S., Bansemer, M.S., Stone, D.A.J., 2013. Comparative histological changes in the greenlip abalone *Haliotis laevis* gastrointestinal tract in response to water temperature, different dietary protein levels, and animal age. *Journal of Shellfish Research* 32, 131-141.

Stone, D.A.J., Bansemer, M.S., Lange, B., Schaefer, E.N., Howarth, G.S., Harris, J.O., 2014. Dietary intervention improves the survival of cultured greenlip abalone (*Haliotis laevis* Donovan) at high water temperatures. *Aquaculture* 430, 230-240.

Stone, D.A.J., Harris, J.O., Wang, H., Mercer, G.J., Schaefer, E.N., Bansemer, M.S., 2013. Dietary protein level and water temperature interactions for greenlip abalone, *Haliotis laevis*. *Journal of Shellfish Research* 32, 119-130.

A1.2 Reports

Stone, D.A.J. Bansemer, M.S. and Harris, J.O. (2014). Abalone on-farm grow-out trial manual. Prepared by the South Australian Research and Development Institute (Aquatic Sciences), Adelaide. Part of CRC Project No. 2010/736. 27pp.

Stone, D.A.J., Bansemer, M.S. and Harris, J.O. (2014). Final Report. Development of formulated diets for cultured abalone. Prepared by the South Australian Research and Development Institute (Aquatic Sciences), Adelaide. CRC Project No. 2010/736. 131pp.

A1.3 Theses

Krishna-Lee Currie (2013). Gut transit time in cultured Australian greenlip abalone. Honours Thesis, Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia.

Brett Lange (2013). The effects of *Ulva* spp. and grape seed extract on the survival, digestive tract histology, immunology, and anti-oxidant enzyme activity of greenlip abalone (*Haliotis laevis*) at high water temperature. Honours Thesis, University of Adelaide, School of Animal and Veterinary Sciences, University of Adelaide, South Australia, Australia.

Kurniati Nur (2014). Protein digestibility assessment in hybrid and greenlip abalone under different commercial and experimental conditions. Masters Thesis, National Centre for Marine Conservation and Resource Sustainability, Australian Maritime College, University of Tasmania, Australia.

Elise Schaefer (2013). The effects of protein and temperature on the digestive tract structure of greenlip abalone (*Haliotis laevis*). Honours Thesis, Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia.

A1.4 Abstracts and Presentations at Scientific Conferences

Abstracts presented at the World Aquaculture Conference, Adelaide, South Australia, June 7th - 11th, 2014:

Bansemer, M.S., Qin, J., Harris, J.O., Howarth, G.S., Stone, D.A.J. Nutrient utilisation and growth of greenlip abalone (*Haliotis laevis*) fed nitrogen enriched macroalgae. <https://www.was.org/meetingabstracts/ShowAbstract.aspx?Id=33129>

Buss, J.J., Jones, D.A. Lumsden, A., Harris, J.O., Stone, D.A.J. Restricting feed rate has more effect than diet type on the feeding behaviour of greenlip abalone *Haliotis laevis* (Poster). <https://www.was.org/meetingabstracts/ShowAbstract.aspx?Id=33144>

Currie, K-L., Harris, J.O., Stone, D.A.J. Lower water temperature and increasing age on extend the gastrointestinal evacuation time of greenlip abalone *Haliotis laevis*. <https://www.was.org/meetingabstracts/ShowAbstract.aspx?Id=33150>

Duong, D.N., Stone, D.A.J., Qin, J., Harris, J. Bioenergetics of greenlip abalone (*Haliotis laevis*) and hybrid abalone (*H. laevis* × *H. rubra*) fed different protein levels at different water temperatures. <https://www.was.org/meetingabstracts/ShowAbstract.aspx?Id=33149>

Harris, J.O., Stone, D.A.J. The emergence and status of the Australian abalone aquaculture industry. <https://www.was.org/meetingabstracts/ShowAbstract.aspx?Id=33173>

Hoang Hai, T., Stone, D.A.J., Harris, J., Qin, J. Effects of dietary macroalgae and commercial diets on shell and flesh colour of greenlip abalone (*Haliotis laevis*). <https://www.was.org/meetingabstracts/ShowAbstract.aspx?Id=33143>

Lange, B.D., Stone, D.A.J., Howarth, G.S. Grape seed extract and dried macroalgae *Ulva lactuca* improve survival of greenlip abalone *Haliotis laevis* at high water temperature. <https://www.was.org/meetingabstracts/ShowAbstract.aspx?Id=33145>

Stone, D.A.J., Bansemer, M.S., Lange, B.D., Schaefer, E.N., Howarth, G.S., Harris, J.O. A temperature challenge model to investigate summer mortality in greenlip abalone (*Haliotis laevis*). <https://www.was.org/meetingabstracts/ShowAbstract.aspx?Id=33132>

Stone, D.A.J., Bansemer, M.S., Harris, J.O., Mercer, G.J., Wang, H. Dietary protein level and water temperature interactions for juvenile greenlip (*Haliotis laevis*) and hybrid abalone (*H. laevis* × *H. rubra*). <https://www.was.org/meetingabstracts/ShowAbstract.aspx?Id=33131>

Abstracts presented (oral presentations) at the 8th International Abalone Symposium Hobart, Tasmania, Australia, 6th - 11th May, 2012:

Schaefer, E.N.*, Harris, J.O., Stone, D.A.J. Howarth, G.S. Histological changes in *Haliotis laevis* in response to different dietary protein levels and water temperatures.

Stone, D.A.J. *, Harris, J., Mercer, G, Wang, H., Schaefer, E., Bansemer, M. Dietary protein level and water temperature interactions for greenlip abalone, *Haliotis laevis*.

A1.5 Presentations at other Meetings

Presentations at the SARDI Aquatic Sciences Seminar series, SARDI Aquatic Sciences, West Beach, SA, 2nd June, 2014:

Stone, D.A.J., Bansemer, M.S.*, Harris, J.O., Mercer, G.J., Wang, H. Dietary protein level and water temperature interactions for juvenile greenlip (*Haliotis laevis*) and hybrid abalone (*H. laevis* × *H. rubra*).

Stone, D.A.J., Bansemer, M.S., Lange, B.D., Schaefer, E.N., Howarth, G.S., Harris, J.O. A temperature challenge model to investigate summer mortality in greenlip abalone (*Haliotis laevis*).

Presentation at the Australian Abalone Growers' Association "Surviving Summer" Workshop, SARDI Aquatic Science Centre, West Beach, South Australia, 6th September 2013:

Stone, D.A.J. Surviving summer, Nutrition and how it works with an emphasis on antioxidants.

Presentations for the Annual Australian Abalone Growers' Association meeting, SARDI Aquatic Sciences, 5th September 2013:

Bansemer, M., Qin, J., Stone, D., Harris, J., Howarth, G. 2013. The effects of dietary macroalgae and water temperature on the digestive enzyme activity of abalone.

Buss, J., Jones, D., Currie, K.-L., Davidson, H., Lumsden, A., Stone, D., Harris, J., 2013. The effect of temperature, feed density and diet type on the feeding behaviour of greenlip and hybrid abalone.

Currie, K.-L. Stone, D., Harris, J., 2013. The effects of water temperature and animal size on the gut transit time of greenlip abalone.

Duong, D.N., Qin, J., Stone, D., Harris, J., 2013. The effects of water temperature on the bioenergetics of abalone fed macroalgae supplements.

Hoang Hai, T. Qin, J., Stone, D., Harris, J., 2013. The effects of water temperature on the bioenergetics of abalone fed macroalgae supplements.

Lange, B., Howarth, G. Stone, D.A.J. Antioxidant and dried *Ulva* spp. to improve survival of large greenlip abalone at high water temperatures.

Schaefer, E.N., Stone, D., Harris, J., Howarth, G. 2013. Nutrition of Australian abalone: Investigation into summer mortality.

Stone, D.A.J, Harris, O. and Ward, L. Development of diets for cultured abalone: Progress of lab-based and on-farm trials.

Presentations at the Annual Postgraduate Conference School of Biological Sciences, Flinders University, Adelaide, South Australia, 3rd - 5th July, 2013:

Bansemer, M., Qin, J., Stone, D.A.J., Harris, J., Howarth, G. A comparison of the growth performance of greenlip abalone fed live macroalgae or commercial formulated diets.

Duong, D.N., James Harris, David Stone, Jian Qin. Bioenergetics of greenlip and hybrid abalone fed different diets at different water temperatures.

Hoang, T.H., Qin, J., Harris, J., Stone, D. Effects of dietary macroalgae and pigment supplementation on abalone shell and flesh colour.

Schaefer, E.N., Harris, J., Stone, D., Howarth, G. Nutritional health in Australian abalone: an investigation into "summer mortality".

Presentation at the Australian Abalone Growers' Association AGM, SARDI Aquatic Science Centre, West Beach, South Australia, 22nd August 2012:

Stone, D.A.J, Harris, J., Ward, L. Development of formulated diets for cultured abalone"; Progress of lab-based nutritional trials and on-farm trials.

Presentations at the Annual School of Biological Sciences Annual Postgraduate Conference, Flinders University, June 2012:

Bansemer, M., Qin, J., Stone, D., Harris, J., Howarth, G. Improvement of abalone nutrition with macroalgae addition.

Hoang Hai, T., Qin, J., Harris, J., Stone, D. Effects of dietary macroalgae and pigment supplementation on abalone shell and flesh colour.

Presentation at the Australian Abalone Growers' Association Annual Seminar and General Meeting 2011. Queenscliff, Victoria July 29th:

Stone, D., Harris, J. and Ward, L. Nutritional research and workshop on the 2012 farm protocols.

Presentation at the Australian Abalone Growers' Association meeting at the Australasian Aquaculture Conference, Hobart, Australia, May 24th, 2010:

Stone D.A.J. Development of diets for cultured abalone (Australian Seafood CRC project development).

Presentation at the Australian Abalone Growers' Association meeting and CRC workshop at the Australasian Aquaculture Conference, Geelong, Victoria, May 7th, 2009:

Stone, D.A.J. South Australian Research and Development Institute/Marine Innovation South Australia (MISA): Aquaculture research and development capability.

Appendix 2. Student project details

A2.1 PhD Student Projects

Krishna-Lee Currie, PhD student. Project entitled “Optimisation of formulated diets to maximise growth and survival of cultured greenlip abalone, *Haliotis laevis*, at optimal and suboptimal temperatures”. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Dr James Harris, Assoc. Prof. David Stone and Prof. Gordon Howarth (University of Adelaide, School of Animal and Veterinary Sciences). Commenced 17th March 2014.

Mathew Bansemer, Premiers Science Research Fund 8 Scholarship and ASCRC Scholarship PhD program entitled “Improvement of abalone nutrition with macroalgae addition”. Faculty of Science and Engineering, School of Biology Sciences, Flinders University, South Australia, Australia. February 2012 - current. Supervisor: Assoc. Prof. Jian Qin; Co Supervisors: Assoc. Prof. David Stone, Dr James Harris, Prof. Gordon Howarth. Expected completion date March 2015.

Duong, Ngoc Duong PhD student. Project entitled “Bioenergetics of abalone fed different diets with macroalgae supplements”. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. August 15 2012 - current. Supervised by Dr James Harris, Prof. Jian Qin and Assoc. Prof. David Stone; Industry participants: Mr Joel Adam Scanlon (Aquafeeds Australia). Expected completion date August 2015.

Elise Schaefer, PhD program entitled “Nutrition of Australian abalone: Investigation into summer mortality”. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. November 2012 (FT.) - current. Supervisor: Dr James Harris; Co Supervisors: Assoc. Prof. David Stone, Prof. Jian Qin, Prof. Gordon Howarth. Expected completion date November 2015.

Hoang Hai, Thanh, PhD student. Project entitled “Impact of macroalgae and diet pigment supplementation on abalone shell and flesh colour”. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. August 15, 2012 - current. Supervised by Prof. Jian Qin, Dr James Harris and Assoc. Prof. David Stone. Expected completion date August 2015.

A2.2 Masters Student Project

Kurniati Nur, Master of Applied Science (Marine Environment) with Honours, Aquaculture program entitled “Protein digestibility assessment in hybrid and greenlip abalone under different commercial and experimental conditions”. National Centre for Marine Conservation and Resource Sustainability, Australian Maritime College, University of Tasmania, Australia. 2012 (FT) -2013. Supervisor: Dr Louise Adams; Co-supervisors: Dr Mark Adams, Dr Nural Amin (Abtas Ltd, Tasmania), Assoc. Prof. David Stone.

A2.3 Honours Student Projects

Jessica Buss, Honours student. Project entitled “The use of dietary additives to improve the survival of cultured greenlip abalone, *Haliotis laevis*”. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by

Dr James Harris, Assoc. Prof. David Stone. Awarded the Playford Scholarship 2014. Commenced July 2014.

Krishna-Lee Currie, Honours student. Project entitled “Gut transit time in cultured Australian greenlip abalone”. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. Supervised by Dr James Harris and Dr David Stone. Commenced February 15 2013; Graduated with 1st Class Honours 17/4/14. Awarded the Playford Scholarship 2013. Chancellors letter of Commendation 2013 and 2014. Awarded the University Medal 2014.

Brett Lange, Honours student. Project entitled “The effects of *Ulva* spp. and grape seed extract on the survival, digestive tract histology, immunology, and anti-oxidant enzyme activity of greenlip abalone (*Haliotis laevis*) at high water temperature”. University of Adelaide, School of Animal and Veterinary Sciences, University of Adelaide, South Australia, Australia. Supervised by Prof. Gordon Howarth and Assoc. Prof. David Stone. Commenced February 15 2013; Graduated with 1st Class Honours November 2013. Awarded the Playford Scholarship 2013.

Elise Schaefer: Honours student. Project entitled “The effects of protein and temperature on the digestive tract structure of greenlip abalone (*Haliotis laevis*)”. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. July 2011 - May 2012. (Mid-year intake). Supervised by Dr James Harris, Assoc. Prof. David Stone. Graduated with 1st Class Honours on the 24/5/2012

A2.4 Undergraduate Student Projects

Alyssa Lumsden, SARDI Summer Scholarship. Project entitled: Video monitoring of the feeding behaviour of greenlip abalone. Department of Earth and Environmental Science, Marine Biology, University of Adelaide. December-February 2012/13.

Krishna-Lee Currie and Hannah Davidson, Flinders 3rd year student project entitled: Video monitoring of the feeding behaviour of greenlip and hybrid abalone in relation to temperature and diet type. Faculty of Science and Engineering, School of Biological Sciences, Flinders University, South Australia, Australia. August-December 2012.

Appendix 3. Basic increase in sales revenue calculations for hybrid abalone during the Great Southern Waters on-farm trial

A3.1 Background

An economic analysis of the performance of abalone sales gain revenue after feed costs for the Great Southern Waters on-farm trial was carried out. The analyses were calculated on a \$ slab tank⁻¹ trial⁻¹ (561 d) basis and also on a \$ slab tank⁻¹ y⁻¹ (365 d) basis, based on the differences in biomass gain of hybrid abalone grown using the standard protein diet feeding strategy versus the high protein diet feeding strategy (Table A3.1). The on-farm trial used 50 m² slab tanks. The calculations in this table are simplistic and provide an estimation of the financial gain achieved from the biomass gain of hybrid abalone and do not include other costs including labour etc. used over the duration of the on-farm trial (561 d).

A3.2 Methods

Data was calculated on a \$ slab tank⁻¹ 561 d⁻¹ to represent the gain in sale revenue (after feed costs including freight) achieved from an individual slab tank at the end of the trial. Data was also calculated to standardise the gain in sale revenue on a \$ slab tank⁻¹ y⁻¹ basis and on a \$ m² y⁻¹ basis using straight line interpolation. Other variables for labour, electricity and infrastructure were not included in this basic sales gain analysis, as these costs were common between slab tanks for each feeding strategy throughout the on-farm trial.

Information provided in the table is derived from:

- The Great South Waters on-farm trial (Table 5.3, Chapter 5).
 - Feed inputs for each feeding strategy treatment.
 - Biomass gain for each feeding strategy treatment.
 - FCR for each feeding strategy treatment
 - Trial duration (561 d; 18.3 months)
- AAGA members
 - Abalone farm-gate value (\$25 kg⁻¹)
 - Standard (30.0% CP) and high protein (35.0% CP) diets used.
 - Cost premium of the high protein diet was estimated at ~\$100 tonne⁻¹ or 4.8%.
- The cash contribution to research and development from AAGA and ASCRC (\$300,000.00) is derived from the AAGA ASCRC abalone diet development project (2010/736).

The model has also included the facility to calculate the annual improvements in sales revenue (after feed costs) for a 200 tank farm and on a \$ m⁻² of slab tank year⁻¹ basis.

A3.3 Results

The use of the high protein diet feeding strategy with hybrid abalone over the 561 d on-farm trial at Great Southern Waters resulted in a 100.2 kg increase in biomass gain per slab tank (Table A3.1a). This biomass gain was achieved using an additional 7.1% increase in feed input (including freight) valued at \$158 slab tank⁻¹ trial⁻¹ (Table A3.1a). Combined, a 9.5% increase in sales revenue (after feed costs) valued at \$2,347 slab tank⁻¹ trial⁻¹ was attained by feeding the high protein diet (Table A3.1a). When interpolated back to a 12 month period, this resulted in an increase in sales revenue, valued at \$1,527 slab tank⁻¹ y⁻¹, achieved with a 7.1% increase in feed input (including freight) valued at \$104 slab tank⁻¹ y⁻¹ (Table A3.1b). This equated to an increase in sales revenue of \$30.54 m⁻² slab tank area y⁻¹, which was

achieved with an additional high protein feed input cost of \$2.07 m⁻² slab tank area y⁻¹ (Table A3.1c).

Table A3.1. a, b, c, d. Financial benefits of feeding the high protein diets compared to the standard protein diet to hybrid abalone during the Great South Waters on-farm trial.

Table A3.1a. Financial outcomes of the Great Southern Waters on-farm trial (per 50 m² slab tank)				
GSW diet feeding treatment (CP = crude protein)	Standard protein 30% CP	High protein 35% CP	Difference	% Difference
Culture unit				
- Slab tank area (m ²)	50	50		
Time-frame				
- Days	561	561		
- Months	18.3	18.3		
Yield per slab tank (18.3 months)				
- Farm-gate price (\$ kg ⁻¹)	\$ 25	\$ 25		
- Biomass gain	1081.70 (kg)	1181.90 (kg)	100.20 (kg)	9.3%
Sale value	\$ 27,043	\$ 29,548	\$ 2,505	9.3%
Estimated feed cost (\$ kg⁻¹)	\$ 2.10	\$ 2.20	\$ 0.10	4.8%
Feed input costs (18.3 months)				
- FCR (kg fed / kg biomass gain)	0.985	0.921		
- Feed input	1065.30 (kg)	1088.80 (kg)	23.50 (kg)	2.2%
- Cost of feed inputs	\$ 2,237	\$ 2,395	\$ 158	7.1%
Sale value after feed costs (18.3 months)	\$ 24,805	\$ 27,152	\$ 2,347	9.5%
Table A3.1b. Annualised feed & sales per 50 m² slab tank				
GSW diet feeding treatment (CP = crude protein)	Standard protein 30% CP	High protein 35% CP	Difference	% Difference
Feed input cost (calculated to one year)¹	\$ 1,464	\$ 1,567	\$ 104	7.1%
Sale value after feed costs (calculated to one year)	\$ 16,139	\$ 17,666	\$ 1,527	9.5%
Table A3.1c. Annualised benefits per square metre				
Additional feed input cost of high protein diet (per m² per year)				\$2.07
Annual farm increase in sales for high protein diet treatment after feed costs (per m² per year)				\$30.54
Ratio of return (food cost to sales increase)				1:14.8
Table A3.1d. Your R&D money is recovered on a 200 slab tank farm within 12 months				
Cash contribution to R&D from AAGA and AS CRC				\$ 300,000
Annual farm increase in sales after high protein feed costs	50 m² slab tanks = 200			\$ 305,373
R&D return in the first year (%)				102%

¹ Annual increased sales revenue after feed costs (\$ tank⁻¹ y⁻¹) were calculated using straight line interpolation.

A3.4 Conclusions and Recommendations

The biomass gain achieved using the high protein diet feeding strategy contributed significantly to the model (Table A3.1a). While additional feed input volumes and costs only contributed a very small proportion to the total operating costs in this model (Table A3.1a).

The cost premium of the Skretting high protein diet was only \$100 tonne⁻¹, or 4.8% more than the standard protein Skretting diet (Table A3.1a). There are large financial gains (9.5% y⁻¹) to be achieved by using the high protein diet feeding strategy for the culture of hybrid abalone in a commercial setting (Table A3.1a, b). An example of the annual farm increase in sales revenue is also provided in Table A3.1b. Based on this simplistic model, the adoption of the high protein diet feeding strategy with hybrid abalone on a farm using two hundred 50 m² slab tanks will give a \$305,373 increase in annual sales revenue, after feed costs (Table A3.1d). This outcome indicates an excellent return on the initial research and development investment by the AAGA and ASCRC of \$300,000 for this project (Table A3.1d). We recommend that this feeding strategy is adopted by AAGA members.

Appendix 4. Abalone on-farm grow-out trial manual.

Abalone on-farm grow-out trial manual

**David A.J. Stone, Matthew S. Bansemer
and
James O. Harris**

Project No. 2010/736



**AUSTRALIAN
SEAFOOD
COOPERATIVE
RESEARCH CENTRE**

November 2014

XX 2014

Title: Abalone on-farm grow-out trial manual.

Australian Seafood Cooperative Research Centre (ASCRC) Project: Development of formulated diets for cultured abalone (2010/736).

Client Contact Details

Name: Australian Abalone Growers' Association (AAGA)
Attention: Nick Savva
Address: 171 Bevic Road, Clarence Point, TAS, 7270
Email: admin@abtas.com.au
Telephone: (03) 6383 4115
Facsimile: (03) 6383 4117

Principal Investigator Associate Professor David A.J. Stone
Position: Subprogram Leader Aquaculture Nutrition and Feed Technology
Address: South Australian Research and Development Institute (SARDI) Aquatic Sciences Centre, 2 Hamra Avenue, West Beach, SA, 5022
Email: david.stone@sa.gov.au
Telephone: (08) 8207 5350
Facsimile: (08) 8207 5481

Co/Investigator Dr James Harris
Position: Senior Lecturer
Address: School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, 5001
Email: James.Harris@flinders.edu.au
Telephone: (08) 8201 5974
Facsimile: (08) 8201 3015

Table of Contents

A4.1 Introduction	XV
A4.1.1 Potential of multi-diet feeding strategies	XV
A4.1.2. Purpose of the on-farm manual	XV
A4.2 Aim	XVII
A4.3 Materials and Methods	XVII
A4.3.1 Overview of on-farm experimental design	XVII
A4.3.2 Dedicated farm staff for on-farm trial	XIX
A4.3.3 Experimental animals	XIX
A4.3.4 Experimental system	XIX
A4.3.4.1 <i>Allocation of treatments to culture tanks</i>	XIX
A4.3.4.2 <i>Preparation of culture tanks</i>	XXI
A4.3.5 Stocking of trials	XXI
A4.3.5.1 <i>Example of systematic interspersion for stocking of trials</i>	XXII
A4.3.5.2 <i>Tagging procedures (optional)</i>	XXIII
A4.3.6 Feeds, feed storage and feeding	XXIII
A4.3.6.1 <i>Feeds</i>	XXIII
A4.3.6.2 <i>Feed storage</i>	XXIII
A4.3.6.3 <i>Feeding</i>	XXIV
A4.3.7 Sampling procedures for weight checks and harvesting	XXIV
A4.3.7.1 <i>Labelling of bags for sample collections</i>	XXIV
A4.3.7.2 <i>Monthly weight checks</i>	XXV
A4.3.7.2.1 <i>Method to sample abalone at weight checks</i>	XXV
A4.3.7.3 <i>Three monthly weight checks</i>	XXVI
A4.3.7.4 <i>Harvesting at 9 months to reduce stocking density</i>	XXVI
A4.3.7.5 <i>Re-stocking at 9 months with reduced stocking densities</i>	XXVII
A4.3.7.5.1 <i>Method to randomly select abalone from middle 1/3 of culture tank for restocking</i>	XXVIII
A4.3.7.6 <i>Harvesting at 18 months at the completion of the trial</i>	XXVIII
A4.3.8 Summary of data collection and staff allocation for the trial	XXIX
A4.3.8.1 <i>Summary for stocking</i>	XXIX
A4.3.8.2 <i>Summary for monthly weight checks</i>	XXX
A4.3.8.3 <i>Summary for three monthly weight checks</i>	XXX
A4.3.8.4 <i>Summary for intermediate stocking density reduction harvest</i>	XXX
A4.3.8.5 <i>Summary for intermediate re-stocking</i>	XXXI
A4.3.8.6 <i>Summary for final harvest</i>	XXXI
A4.3.9 Calculation of growth performance and feed efficiency indices	XXXII
A4.3.10 Environmental monitoring of experimental systems	XXXII
A4.3.11 Tank cleaning and mortality monitoring and reporting	XXXIII
A4.3.12 Statistical analyses	XXXIV
A4.4 Acknowledgements	XXXV
A4.5 References	XXXVI

A4.6 Appendix A4.1	XXXVII
A4.6.1 Tagging method	XXXVII

List of Tables

Table A4.1. Water quality variables and measurement frequencies for on-farm trial tanks.	XXXIII
---	--------

List of Figures

Figure A4.1. Flow diagram of the timing of stocking, sampling and harvest events used for the 18 month on-farm trials.	XVIII
Figure A4.2. An example of random or systematic allocation of the different feeding strategy treatments to the culture tanks.	XX
Figure A4.3. Description of the location for sample collection at monthly and 3 monthly weight checks.	XXVI
Figure A4.4. Diagram of the area used (middle third of culture tank) to randomly collect abalone for re-stocking for second phase of experiment.	XXVIII

A4.1 Introduction

A4.1.1 Potential of multi-diet feeding strategies

Multi-diet feeding strategies, aimed at meeting differences in protein requirements of an animal at different seasonal temperatures and ontogenic stages of development, are commonly used throughout the grow-out cycle to improve productivity for a range of farmed terrestrial and aquatic organisms. Once Australian abalone have been weaned from the nursery and transferred to their land-based grow-out systems, production relies predominantly on the use of a single diet, which may contain a crude protein level within a relatively narrow range (~27 - ~30%). This range of protein is based on some excellent earlier research by Coote et al. (2000), for juvenile greenlip abalone of one size class (25 mm initial shell length [SL]) at one water temperature (20°C). There is evidence that demonstrates marked difference in the growth performance and feed utilisation of related abalone species of different size classes in relation to changes in dietary protein level (Britz and Hecht 1997; Bautista-Teruel and Millamena 1999). Temperature has also been demonstrated to have a significant effect on growth rate of abalone (Gilroy and Edwards 1998; Steinarsson and Imsland 2003).

Results from the laboratory experiments (conducted within the parent AAGA ASCRC project at SARDI Aquatic Science Centre) investigating the interactions of dietary protein level, water temperature and animal size class, demonstrated differences in the growth performance and feed efficiency of post-weaned sub-juvenile (<6 month-old), juvenile (1-year-old) and sub-adult (2-year-old) greenlip abalone (*Haliotis laevis*) in relation to changes in dietary protein level and water temperature (Stone et al. 2013, Chapter 2; Bansemmer et al. 2015, Chapter 3). In summary, very little growth occurred for each size class of abalone at the lower water temperature of 14°C, regardless of the dietary protein level. However, the growth performance and feed utilisation of 1-year-old greenlip abalone at warmer water temperatures was improved by using 31 to 34% dietary protein. In contrast, there was little to no gain by increasing the protein level above 27% for the larger two year old abalone, regardless of water temperature. The results suggest that there is considerable scope to incorporate a multi-diet feeding strategy, targeting dietary protein level manipulation, into the production cycle of abalone in land-based culture in Australia, or for that matter, elsewhere.

Results from the laboratory based experiment were distributed to AAGA members and representatives of the three participating Australian abalone feed companies. The information was used to form the basis for the formulation of diets with varying dietary protein levels for the on-farm multi-diet feeding strategy trials (Stone et al. 2013, Chapter 5).

A4.1.2. Purpose of the on-farm manual

In summary the manual will be distributed to AAGA members and the purpose of the manual is as follows:

- Use manual for on-farm grow-out trials in the current AAGA ASCRC project;
- To ensure the on-farm trials are conducted in a controlled manner between farms; and
- Minimise variation as much as possible to get best value out of the experiments.

Following on from the laboratory experiments, the next phase of the project plans to run, concurrently, six on-farm trials to test the hypothesis that abalone productivity will be increased by utilising a multi-diet feeding strategy in a production situation. During the initial

stages of project development it was brought to our attention that results from previous on-farm trials, carried out by AAGA members investigating the growth potential and feed utilisation of abalone in relation to dietary alterations, have been quite variable and difficult to interpret with confidence (Personal communication, Justin Fromm, Executive Officer, Australian Abalone Growers' Association). In order to address these concerns, it was decided that we should develop an on-farm feed trial manual for the upcoming land-based abalone trials to standardise methods, as much as practically possible, across the association. By no means do we claim to be able to completely eliminate intra or inter-farm variations within and between experimental results. However, the implementation of this manual should help to minimise on-farm variations, enhance the consistency and reliability of results and improve confidence to make informed management decisions based on the outcomes of the on-farm trials. The manual was developed in collaboration with AAGA members, particularly the managers of the six farms originally participating in the on-farm trials, and representatives from each of the three participating Australian abalone feed companies.

The original six participating AAGA farms were:

- Coastal Seafarms Holding Pty. Ltd., 66 Snapper Point Rd, Allestree, Victoria, 3305; (Great Ocean Road, South West Victoria, Western District). Contact: Mr Tim Rudge (Greenlip).
- Great Southern Waters Pty Ltd, 366, The Esplanade, Indented Head, Victoria, 3223, Australia. Contact: Mr Anton Krsinich and Ms Lucy Saunders (Hybrid).
- Kangaroo Island Abalone Pty. Ltd., North Coast Road, Kangaroo Island, PO Box 898, Kingscote South Australia, 5223. Contact: Mr David Connell (Greenlip).
- Southern Ocean Mariculture Pty. Ltd., RMB 2068 Princes Highway, Port Fairy, Victoria, 3284, Contact: Mr Mark Gervis and Mr Hamish Ebery, (Hybrid). Out
- Abtas Marketing Pty. Ltd. PO Box 216, Beaconsfield, Tasmania, 7270. Contact: Mr Nick Savva (Hybrid). Out
- SAM Abalone Pty. Ltd., Boston Point, Port Lincoln, South Australia, 5606. Contact: Mr Tom Hyde and Mr Steve Martin (Greenlip). Out

The three participating Australian abalone feed companies are:

- Aquafeeds Australia (Formerly Adam and Amos Abalone Foods Pty. Ltd.) PO Box 1029, 18 Simper Crescent Mount Barker, South Australia, 5251. Contact: Joel Scanlon. Out
- Eyre Peninsula Aquafeeds Pty. Ltd., 44 Donegal Rd, Lonsdale, South Australia 5606. Contact: Dr Tom Coote or Mr Kym Heidenreich.
- Skretting Australia, 26 Maxwells Road, Cambridge, Tasmania, 7170; PO Box 117, Rosny Park, Tasmania, 7018, Australia. Contacts: Dr Matthew Bransden and Dr Rhys Hauler.

During the development of this manual we undertook farm visits to the six participating AAGA facilities. During these visits we discussed:

- The suitability of various replicated experimental production systems to be used in the on-farm trials;
- Animal availability and stocking arrangements;
- Typical strategies currently employed to run feed trials;
- Logistics associated with stocking and running of the on-farm trials; and
- Practices that could be altered to improve the outcomes of the upcoming on-farm trials.

Visits to the participating facilities were enlightening, with respect to the commercial pressures associated with conducting controlled replicated trials. Trials require a large amount of time and dedicated resources, while still ensuring the day to day operation of a profitable production facility. The visits also revealed large differences in tank designs, environmental parameters, water quality management and monitoring, stock management

strategies, feeding strategies, stock handling and weighing and measurements procedures and data recording practices. The provision of dedicated staff to run the studies also presents a practical problem.

The manual has been developed taking into account all of the variables. The information contained in this manual will form the basis of the methods used in the 18 month on-farm trials at participating AAGA members' facilities described in the parent AAGA ASCRC Project "Development of formulated diets for cultured abalone". The on-farm trials will investigate the growth performance of abalone using a combination of dietary treatments comprised of newly formulated sub-juvenile and juvenile grow-out diets versus the growth performance of abalone fed the grow-out diet alone across the normal production cycle.

A4.2 Aim

The aim of this manual is to provide a framework of methods to standardise the running of the AAGA ASCRC project on-farm trials investigating the potential of multi-diet feeding strategies in a commercial setting (Chapter 5).

A4.3 Materials and Methods

A4.3.1 Overview of on-farm experimental design

The aim of the on-farm trial is to compare the on-farm growth performance of greenlip or hybrid abalone using the current "single protein level" grow-out diet grow-out strategy vs. a "high protein" / "low protein" grow-out diet combination. The experiment will follow the procedure displayed in Figure A4. 1.

The feed companies will use the information from the laboratory-based experiment (Stone et al. 2013, Chapter 2) to formulate diets of appropriate protein levels at different water temperatures for juvenile and sub-adult abalone for the multi-diet feeding strategy trial. The actual diet formulations and ingredient composition will be up to each company to decide on. Test diets from the feed companies are to be allocated to separate farms (one greenlip producer and/or one hybrid producer). Each farm will use these diets to test two feeding strategies (two treatments):

- Feeding Strategy 1. Use current method of one protein level for entire grow-out cycle.
- Feeding Strategy 2. Sequential use of "high protein" / "low protein" grow-out diet combination.

Please note the Principal Investigator will only be responsible for analysing the results obtained for each respective Feeding Strategy from the AAGA ASCRC sanctioned feed trial. However, farmers may carry out their own comparisons of trial results with farm production results in their own time.

Each feed company will provide the experimental diets for both Feeding Strategies to two farms at 50% cost. The other 50% feed cost will be considered as an in-kind contribution to the current AAGA ASCRC project by the abalone company. A minimum of four commercial tanks will be used on each farm for both the single diet feeding strategy (control) and the "high protein" / "low protein" grow-out diet combination feeding strategy treatments (eight culture tanks farm⁻¹). The trials will run for a period of 18 months with sampling carried out as described in Figure A4.1. Growth performance, feed efficiency, survival, meat yield, and productivity economics will be measured.

The Principal Investigator will be designating a feed company to each farm. The decision will be made based on feedback provided to the Principal Investigator, following extensive consultation with farm managers and feed company representatives.

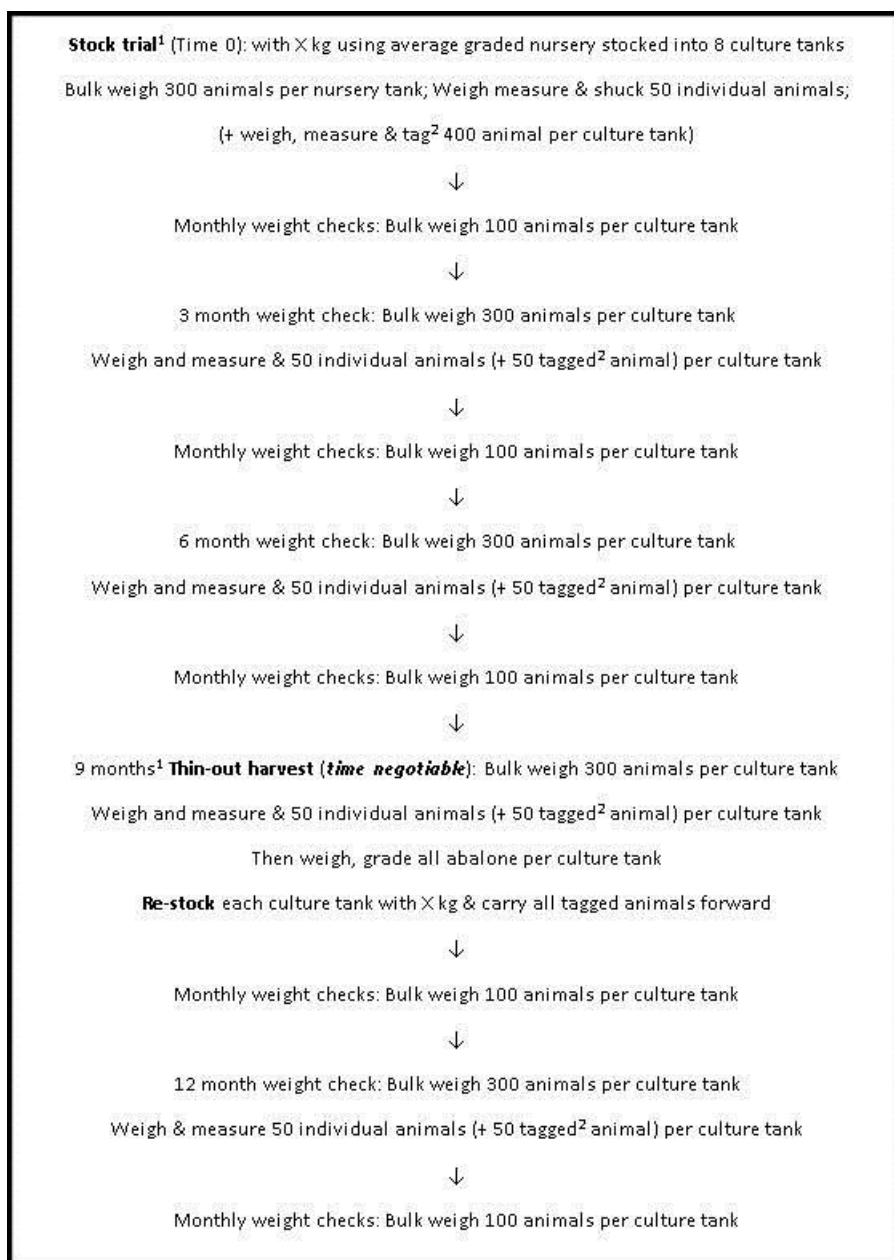


Figure A4.1. Flow diagram of the timing of stocking, sampling and harvest events used for the 18 month on-farm trials.

¹ The stocking and thinning harvest dates are negotiable.

² Tagging is optional.

A4.3.2 Dedicated farm staff for on-farm trial

Each farm must have a Trial Manager and technician dedicated to running the trial. The Trial Manager will act as the main point of contact for the AAGA ASCRC project Principal Investigator and feed company representatives. The Trial Managers will be responsible for setting up and running the trial. Their duties, in conjunction with the technician, will include organising, overseeing, and participating in all of the following procedures:

- Stocking activities;
- Tagging activities (optional);
- Weighing of feeds;
- Feeding;
- Sampling;
- Alteration of feed rates in collaboration with feed company representative and the Principal Investigator;
- Environmental monitoring;
- Data collection;
- Data entry in Excel spreadsheets;
- Data transfer to Principal Investigator;
- Harvesting the trial at completion;
- Reporting of results to Principal Investigator and feed company representative;
- If any trial related event out of the ordinary occurs, the Trial Manager should contact the Principal Investigator and the feed company representative immediately; and
- Any query related to the project should be directed to the Principal Investigator.

A4.3.3 Experimental animals

The abalone used in each of the farm trials should ideally be sourced straight from nursery stock (weaning method is optional), be from the same year class and of typical average quality available at the time of stocking. They should be graded (medium grade preferable) prior to being stocked into the experimental culture tanks. It would be desirable for the grading to take place at the same time as stocking. A feeding history and a description of the culture tanks that they come from, and the environmental conditions in which they were held in, prior to stocking into the on-farm trials will be required.

A4.3.4 Experimental system

A4.3.4.1 Allocation of treatments to culture tanks

The tank designs used between farms for the trials will differ; however, each separate farm trial will require a minimum of eight identical culture tanks which will be typical of production tanks used on farm. The abalone will be stocked into four replicate culture tanks per feeding strategy treatment. If more tanks are available, and management wish to use them, then the replication will be such that the treatments contain equal numbers of identical culture tanks. When selecting the location of the eight culture tanks several very important points apply:

- The culture tanks for each feeding strategy should be systematically (recommended) or randomly allocated (Figure A4. 2);
- In no case should the culture tanks for the same feeding strategy be conveniently placed in blocks of four next to each other;
- Bear in mind that they should be exposed to similar light and noise levels;

- They should be located in an area away from a doors etc. to minimise disturbances; and
- All tanks are to be clearly labelled with a sign that contains the following information:
 - The experimental code (AAGA/CRC feed trial);
 - The feeding strategy (1, single or 2, multiple);
 - The experiment tank number (1 through to 8); and
 - The replicate number (replicate 1 through to 4).

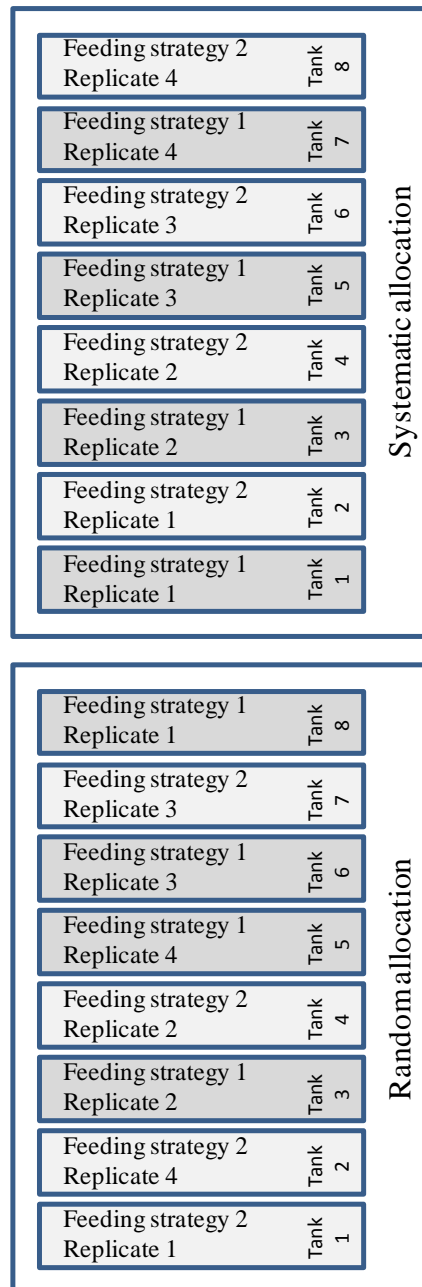


Figure A4.2. An example of random or systematic allocation of the different feeding strategy treatments to the culture tanks.

A4.3.4.2 Preparation of culture tanks

Prior to the commencement of the trial all designated culture tanks should be:

- Emptied;
- Cleaned;
- Disinfected; and
- Free from fouling.

Please note: The cleaning and disinfection should be done in advance to ensure that no chemical residues will harm the newly stocked abalone. Other important points to consider are:

- Screens should be fitted at the end of each culture tank to prevent the escape of live abalone and to collect any dead abalone or shells that may be swept from the tank with the water flow;
- The water inflow point should also be designed to preclude escape by abalone;
- The water inflow rates to each culture tank should be similar to those used for normal production;
- The water inflow rates should be equal between all experimental tanks;
- The water inflow rates should be checked and adjusted on all culture tanks on at least a weekly basis;
- The water levels within culture tanks should be standardised; and
- Tipper cleaners should be set to run at the same rate and frequency for all culture tanks, regardless of stocking density differences.

A4.3.5 Stocking of trials

Please refer to Figure A4.1 in Section A4.3.1 for the flow diagram of the timing of stocking, sampling and harvest events used for the on-farm trials. The aim of the stocking exercise is to ensure that each identical culture tank receives the same initial biomass (weighed to the nearest g) of medium graded abalone.

- It is extremely important to recognise that to ensure the optimum opportunity to detect differences due to dietary feeding strategies over the course of the trials, culture tanks should be stocked with a total initial biomass that ensures that **growth will not be limited by stocking density** when we reach the first harvest thinning event at **9 months (thinning date negotiable)**.
- The actual stocking density will vary between farms as culture tank dimensions and management practices differ. However, it is essential that the weight of all abalone stocked into each culture tank is equal (weighed to the nearest g).
- Each farm will use its own anaesthetic and handling methods at stocking.
 - Trial Managers to report the specific details of handling, etc. to the Principal Investigator.
- It is recognised that on each farm it will be necessary to use animals from several different spawning batches of the same year class to provide sufficient animal numbers to stock the minimum of eight culture tanks required for the trial. In this case the systematic interspersation (see example in section A4.3.4.1) of animals from each nursery tank throughout all of the designated trial culture tanks will be required to avoid bias during stocking.
- Grading of animals should be performed at this point, and should be carried out on a tank by tank basis. For each nursery tank all medium grade abalone should be withheld in an aerated holding tank, supplied with flow through water, until the grading of that tank is completed. The graded abalone should then be drained for at least 10 minutes, to remove excess water prior to weighing.

- The drained, graded animals should be weighed and divided equally, using the method of systematic interspersion (see example in section A4.3.4.1), between all culture tanks designated for the feed trial.
- Concurrently, for the determination of average initial animal weight, three separate randomly selected sub-samples (100 abalone per sub-sample) of medium graded abalone, from each nursery tank, should be weighed to the nearest g. This data will be pooled to provide initial starting weight for the trial.
- Additionally, 50 randomly selected medium grade animals (preferably with representatives evenly and randomly sampled from each nursery tank) should also be individually weighed (± 0.1 g), measured (nearest mm) and have their shell removed and weighed (± 0.1 g), and meat frozen at -20°C for analysis of biochemical composition.
 - These animals will provide initial data for individual stocking weight, length, meat weight, shell weight, condition index and biochemical composition.
- **Tagging optional** (Figure A4.1, Section A4.3.5.2, Appendix A4.1): Tag up to 400 randomly selected animals from each culture unit.
 - These animals should be individually weighed (± 0.1 g) and measured (nearest mm).

A4.3.5.1 Example of systematic interspersion for stocking of trials

The stocking exercise will require that we use medium graded sub-juvenile abalone from multiple nursery tanks and distribute them evenly, with equal initial biomass, amongst eight culture tanks. This method of stocking will need to be used at every farm participating at the AAGA ASCRC Project on-farm feed trials.

Please note: The numbers of animals and nursery tanks, and the stocking density used in this instance will differ from those required on each farm and are used in this exercise to serve as an example to explain the method of systematic interspersion. For example:

- We plan to stock 50.0 kg abalone in each of the eight trial culture tanks, using medium graded abalone from 16 nursery tanks;
- To complete the stocking this will require a total of 400 kg of medium graded abalone;
- Let's say the each nursery tank contains 25 kg of abalone in the required medium size grade; and
- We will need 25 kg of medium size graded abalone from 16 nursery tanks.

Therefore, to stock using systematic interspersion we use the following procedure:

- A sub-sample of 3 kg of medium graded animals from one nursery tank should be randomly collected, weighed to the nearest g and systematically allocated to each culture tank, on a rotational basis, so that the animals from each nursery tank are evenly distributed throughout each of the eight culture tanks.
- As each nursery tank is emptied we move on to the next nursery tank and repeat the procedure until all of the culture tanks contain 50 kg (to the nearest g) of graded animals.
- To ensure that the culture tanks stocked last do not always receive the animals handled the longest; the order of distribution from the nursery tanks to the culture tanks should be reversed for each subsequent nursery tank.
- At the completion of the task each culture tank should contain the same amount of abalone, i.e. 50 kg (to the nearest g) of medium graded abalone which is made up of an equal portion of medium graded abalone from each of the nursery tanks.

A4.3.5.2 Tagging procedures (optional)

Please note: This procedure is optional. Once the abalone have reached sufficient size (either at stocking or at 3 month weight check) there will be up to 400 abalone tagged in each culture tank. The shell length and weight of these animals will be measured (nearest mm), weighed (± 0.1 g) and recorded.

Tagging of abalone will be done in accordance with the methods described in Appendix A4.1.

A4.3.6 Feeds, feed storage and feeding

A4.3.6.1 Feeds

The feed companies will use the information from laboratory-based experiment (Stone et al. 2013, Chapter 2) to formulate diets of appropriate protein levels at different water temperatures for juvenile and sub-adult abalone for the multi-diet feeding strategy trial.

- The actual diet formulations and ingredient composition will be up to each company to decide on.
- If sufficient AAGA farms are available to participate in the project the test diets from one feed company are to be allocated to two separate farms (this decision will be made by the Principal Investigator):
 - One greenlip producer; and
 - One hybrid producer.
- Each farm will use these diets to test two feeding strategies (two treatments):
 - Feeding Strategy 1. Use current method of one protein level for entire grow-out cycle.
 - Feeding Strategy 2. Sequential use of “high protein” / “low protein” grow-out diet combination.
- The formulation of the feeds will be kept under strict confidence, with only the respective feed company and Principal Investigator having access to the dietary formulation, excluding the vitamin and mineral premix.
 - Formulations of the vitamin mineral premixes will be held in confidence by each feed company.

A4.3.6.2 Feed storage

Food will spoil if improperly stored. It is important to store feeds correctly throughout the trials. The spoilage may not be detectable to the naked eye. Spoiled food:

- May contain mycotoxins that will be harmful to abalone;
- Be of reduced nutritional value and will limit the growth of abalone; and
- May be a vector for the transmission of disease to abalone.

Food storage prior to feeding: To ensure that feed spoilage does not impact on the trial results all trial feeds should preferably be stored in a cool room at all times prior to weighing for feeding. Failing this, feed should be stored in a cool dry place away from rodents, and out of direct sunlight.

Food storage at feeding: the maximum quantity of feed for one week’s operation may be transferred from the feed storage area to the weighing point and held in a cool dry area protected from rodent activity.

Weekend feeding or weighing feed in advance: Feed for each culture tank that is weighed out in advance and not fed directly, (e.g. weekend feeds) must be placed in a plastic bucket with a lid and stored in a cool dry place.

A4.3.6.3 Feeding

Strict control of feeding is essential to ensure the success of all on-farm feed trials in this project. The following procedures must be adhered to strictly by the farm Trial Manager and technician in order to ensure an accurate outcome from each trial.

- All information related to feeding will be made available to the Principal Investigator by the respective feed company representative and the farm Trial Manager and technician.
- For each on-farm trial, each feed company representative will provide the Trial Manager with the recommended:
 - Feed rates (% body weight d^{-1} ; or agreed alternate method);
 - Feeding frequencies (number of feeds d^{-1}); and
 - Feeding times (time of day).
- Feeding should be such that animals are fed to a slight excess at each feed.
- Feeding may be based on the percentage of the total biomass within each tank or an alternative method that is agreed upon by the Principal Investigator.
- The feed rate may vary between treatments (feeding strategies), this will be dependent on the feed company's recommendations, but WILL NOT vary between tanks within the same feeding strategy treatment.
- A feed scoring system may be implemented by each feed company. The details will be made available to the Trial Manager, technician and Principal Investigator.
- Biomass records for each culture tank will be kept and updated following monthly weight checks, taking into account the loss of mortality weights.
- All feed will be weighed individually (nearest g), for each tank and recorded daily.
- In the advent of any feed not being offered to a culture tank on a given day the following list of tasks should be carried out immediately:
 - The feed must be weighed to the nearest g;
 - Recorded;
 - A written reason for withholding the feed must be recorded in the trial diary; and
 - An email message must be sent to the feed company representative and Principal Investigator on the day to explain why the feed was withheld.

A4.3.7 Sampling procedures for weight checks and harvesting

Please refer to Figure A4.1 in Section A4.3.1 for the flow diagram of the timing of stocking, sampling and harvest events used for the on-farm trials. A description of what is required for each sampling event is provided below. In section A4.3.8, a summary of the staffing required and the data and samples to be collected are also provided for each type of sampling event.

A4.3.7.1 Labelling of bags for sample collections

Any samples collected throughout the trials at each sampling event must be stored in clearly labelled (indelible ink permanent marker) bags, or containers, according to the prescribed methods within this manual. The bag must be labelled with the following information:

- The farm name;
- The experimental code (AAGA/CRC feed trial);
- The date collected;
- The event collected from;

- The sample type;
- The diet feeding strategy (Feeding strategy 1 or Feeding strategy 2);
- The experiment tank number (1 through to 8);
- The replicate number (replicate 1 through to 4); and
- The initials of the person collecting the sample.

It will be essential to analyse diets that are used throughout each farm trial. In order to do so we will need to regularly collect and store sub-samples. A 200 g sample of each new batch of each trial diet that is used must be randomly collected and placed into a clearly labelled (indelible ink using a permanent marker) bag and stored frozen and be made available to Principal Investigator. The bag must contain the following information:

- The experimental code (AAGA/CRC feed trial);
- The farm name;
- Feed company name;
- Feeding strategy (Feeding strategy 1 or Feeding strategy 2);
- Diet type;
- Diet code; and
- Date diet was supplied to farm.

A4.3.7.2 Monthly weight checks

At each monthly weight check farm staff will sample the following:

- The bulk weight (to nearest g) for 100 randomly selected individual abalone from each culture tank will be collected (refer to section A4.3.7.2.1) on a monthly basis, and recorded.

This information will be used, in conjunction with mortality data, to update the biomass within tanks and update the feed rates for all individual culture tanks on a monthly basis.

A4.3.7.2.1 Method to sample abalone at weight checks

The 100 abalone for the monthly sampling, or 300 abalone for the 3 monthly sampling, will be collected by chipping from the same positions from each culture tank using the following method:

- A transect line will be drawn across the culture tank a point 1/3 of the distance along the culture tank (Figure A4.3);
- For the monthly weight checks 50 abalone will be randomly selected in an area across this line;
- For the 3 monthly weight checks 150 abalone will be randomly selected in an area across this line;
- Another transect line will be then drawn across the culture tank a point that is 2/3 of the distance along the culture tank (Figure A4.3);
- Then from this line, and using the same methods as previously described, a further 50 abalone or 150 abalone, will be randomly sampled;
- No sampled abalone will be returned to the experiment.

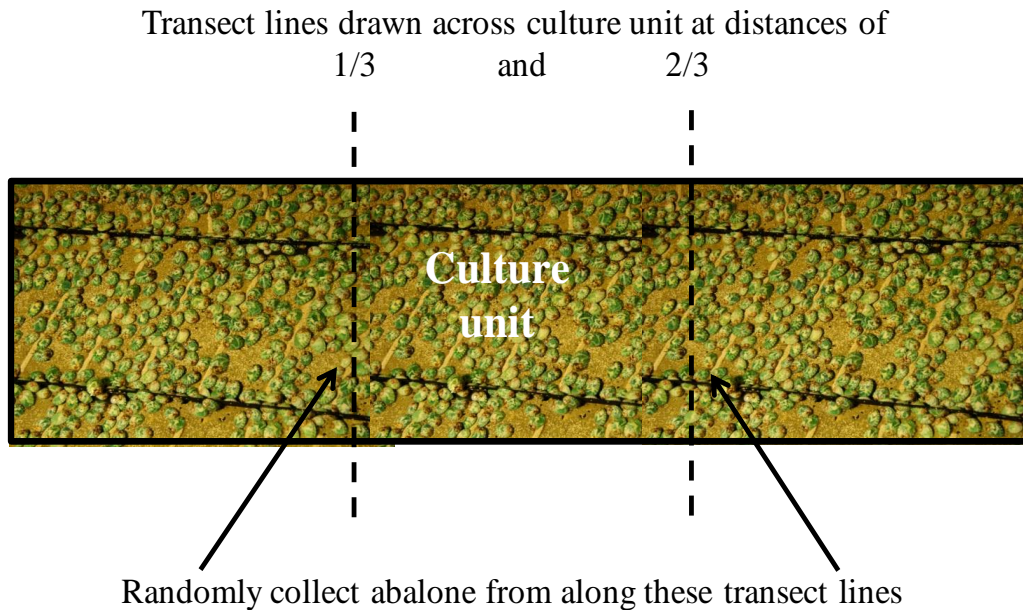


Figure A4.3. Description of the location for sample collection at monthly and 3 monthly weight checks.

A4.3.7.3 Three monthly weight checks

At each 3 monthly weight check, farm staff and project staff will sample the following:

- 300 abalone will be bulk weighed (to the nearest g) at every 3 month time point from each culture tank. The abalone will be collected in the same manner as used for the monthly weight checks (refer to section A4.3.7.2.1).
- Then a sub-sample of 30 of these abalone in the first half of the study, or 15 in the second half of the study, will be randomly selected and individually weighed (± 0.1 g), measured (nearest mm) and shucked for the determination of meat and shell weight (± 0.1 g), and then bagged, clearly labelled and frozen for analysis at a later date.
 - In the advent that the experiment is terminated due to unforeseeable circumstances the tissue samples may be used for the analysis of body biochemical composition.
- Concurrently, a further 20 abalone from each culture tank during the first part of the experiment, or 35 abalone during the second part of the experiment will be randomly selected from the group of 300 bulk weighed abalone and be individually weighed and measured and integrated back into regular farm production.
 - This will result in collecting length weight data for 50 animals from each culture tank at each 3 monthly weight check.
- The remaining animals from each culture tank will be removed from the experiment and integrated back into regular farm production.
- **Tagging optional:** at each 3 monthly weight check 50 tagged animals per culture tank will be randomly selected and weighed (to the nearest 0.01 g), measured (to the nearest mm), and shucked for the determination of meat and shell weight, and then bagged, clearly labelled and frozen for analysis at a later date.
- The weight information from all of these samples will be used, in conjunction with mortality data, to update the biomass within tanks and update the feed rates for all individual culture tanks on a 3 monthly basis.

A4.3.7.4 Harvesting at 9 months to reduce stocking density

At the 9 month point of the 18 month feed trial, the tank biomass will be reduced to avoid stocking density dependent growth limitations. At this stage all animals will be removed from

each culture tank and a graded sub-sample of this group will then be returned to their respective (same) tanks described in section A4.3.7.5. The harvesting will be done using methods in accordance with each farms management practices. The specific details of which will be reported to the Principal Investigator. This work will be carried out by farm and project staff.

During the process of harvesting to reduce stocking density the following will occur:

- 300 abalone from each culture tank will be randomly collected and bulk weighed (to the nearest g).
 - The abalone will be collected in the same manner as used for the monthly weight checks (refer to section A4.3.7.2.1).
- A sub-sample of 30 of these abalone will be randomly selected and individually weighed (± 0.1 g), measured (nearest mm) and shucked for the determination of meat and shell weight (± 0.1 g), and then bagged, clearly labelled and frozen for analysis at a later date;
- Concurrently, a further 20 individual abalone, from the group of 300 bulk weighed abalone from each culture tank, will be randomly selected and individually weighed and measured and integrated back into regular farm production.
 - This will result in collecting length and weight data for 50 individual abalone from each culture tank
- The remaining 270 animals from each culture tank will be removed from the experiment and integrated back into regular farm production.
- **Tagging optional:** all tagged animals per culture tank will be collected and individually weighed (± 0.1 g) and measured (nearest mm).
 - Additionally, 30 of these tagged animals will be randomly selected and shucked for the determination of meat (± 0.1 g) and shell weight (± 0.1 g), and then bagged, clearly labelled and frozen for analysis at a later date.
 - All remaining tagged animals will be kept alive and be taken forward into the next phase of the experiment.
- Concurrently, all abalone from each culture tank will be harvested, bulk weighed, in small batches (to minimise damage), and graded in preparation for re-stocking.
- Any mortalities will be collected from each culture tank, counted, recorded, and discarded.

A4.3.7.5 Re-stocking at 9 months with reduced stocking densities

Following grading the experiment will be restocked and ran for a further 9 months. The re-stocking procedure will carried out in accordance with individual farm management practices and will adhere to the following stipulations:

- As for the initial stocking event, it is extremely important to recognise that to ensure the optimum opportunity to detect differences due to dietary feeding strategies over the course of the remaining 9 months of the trials, culture tanks should be stocked with a total initial biomass that ensures that **growth will not be limited by stocking density** when we reach the final harvest event at **18 months**.
- The actual stocking density will vary between farms as culture tank dimensions and management practices differ.
 - However, it is essential that the weight of all abalone stocked into each culture tank (x kg culture tank⁻¹) is equal (weighed to the nearest g).
- The biomass used for restocking will be from:
 - A randomly selected sample from the middle third of each tank (Figure A4.4 and Section A4.3.7.5.1);
- Abalone will be stocked back into their respective tanks;
- **Tagging optional:** all of the remaining tagged animals will be returned to their respective tank and incorporated into the new biomass at re-stocking.

- Concurrently, 300 abalone from each newly stocked culture tank will be randomly collected, prior to being placed back into their tank and be bulk weighed (to the nearest g).
- The feed rates will be re-calculated based on the new tank biomasses.
- The animals will be returned to their respective diets 24 hours after restocking
- The excess animals from each culture tank will be integrated back into normal farm operations.

A4.3.7.5.1 Method to randomly select abalone from middle 1/3 of culture tank for restocking

The biomass for re-stocking the second phase of the trial should be composed of randomly selected animals that have been harvested from within the area of each culture unit as displayed in Figure A4.4. The suggested method would be as follows:

- Knocking out a representative 1/3 of the tank would proceed as follows:
 - Anaesthetic would be applied to the tank in the usual fashion (as per regular farm practices);
- Once the animals are anaesthetised the central third of the tank will firstly be removed working equally from the central line outwards in both directions (towards the inlet and outlet) until the required biomass for the new trial tank is achieved;
- Following the removal of the initial third the remainder of the animals can be removed from the tank and processed to gain a total biomass for each tank.

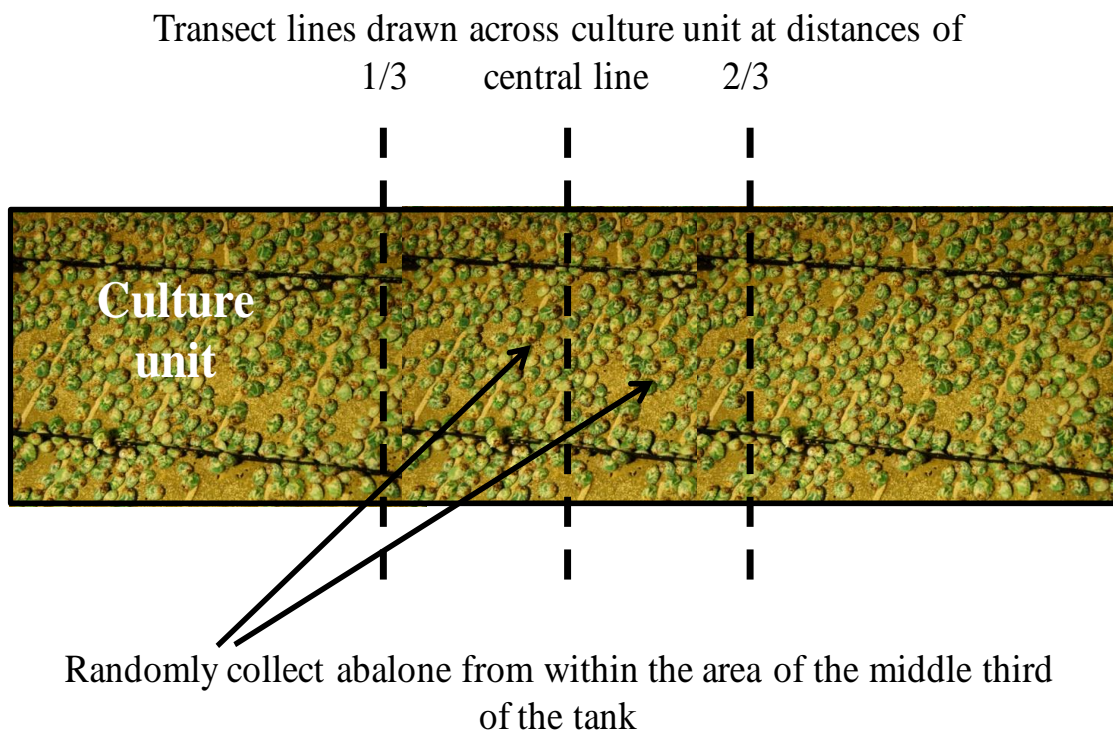


Figure A4.4. Diagram of the area used (middle third of culture tank) to randomly collect abalone for re-stocking for second phase of experiment.

A4.3.7.6 Harvesting at 18 months at the completion of the trial

At the 18 month point the trial will be completed. The tanks will be harvested in accordance with individual farm practices and will adhere to the following stipulations:

- All abalone from each individual culture tank will be bulk weighed (to the nearest g) for the determination of total biomass.
- Any mortalities will be collected from each culture tank, counted, recorded, and discarded.
- Then for the determination of final average individual animal weight for each culture tank, three randomly selected sub-samples (100 abalone per sub-sample) of abalone, from each culture tank, will be bulk weighed to the nearest g.
 - Concurrently, a further 30 individual abalone (10 from each group of 100 per sub-sample) from will be randomly selected from each culture tank and weighed (± 0.1 g), measured to the nearest mm, shucked and the shell (± 0.1 g) and meat (± 0.1 g) weighed, then placed in clearly labelled bags, and frozen for subsequent analyses of biochemical composition.
 - Concurrently, a further 20 individual abalone (10 from each group of 100 per sub-sample) will be randomly selected from each tank, weighed (± 0.1 g) and measured (nearest mm), and integrated back into regular farm operations.
 - The information derived from these 50 animals will provide final data with respect to individual weight, length, meat weight, shell weight, condition index and biochemical composition.
- **Tagging optional:** all of the remaining tagged abalone will be collected and weighed and measured to the nearest 0.1 g and mm, respectively.
 - Additionally, 30 of these tagged animals will be randomly selected and shucked for the determination of meat and shell weight (nearest 0.1 g), and then bagged, clearly labelled and frozen for analysis at a later date.
- The remaining abalone in each culture tank will then be size graded and recorded.
- At the completion of the harvesting process, once all data has been collected, all living abalone may then be integrated back into regular farm operations.

A4.3.8 Summary of data collection and staff allocation for the trial

Please refer to Figure A4.1 in Section A4.3.1 for the flow diagram of the timing of stocking, sampling and harvest events used for the on-farm trials.

Animal weight and size data will need to be collected during the following events of the trial:

1. Stocking;
2. Monthly weight checks;
3. Three monthly weight checks;
4. Intermediate harvest to reduce stocking density (9 months);
5. Intermediate re-stocking with reduced stocking densities (9 months); and
6. Final harvest (18 months).

A4.3.8.1 Summary for stocking

Staffing: farm staff and project staff

Data and samples required:

- Initial size grade for experiment at stocking;
- Then from a sub-sample of 50 randomly selected medium grade animals collected from nursery tanks prior to stocking we will need to record:
 - Initial average individual weights;
 - Initial average individual shell lengths;
 - Initial average individual shell weights;
 - Initial average individual meat weights; and
 - Freeze meat samples for biochemical analyses.
- Initial biomass stocked into each culture tank.

A4.3.8.2 Summary for monthly weight checks

Staffing: farm staff

Data and samples required:

- Bulk weight data for 100 randomly selected individual abalone from each culture tank.
- Monthly feed input (as fed) for each culture tank.
- Mortality number, and estimated weight from each culture tank.

A4.3.8.3 Summary for three monthly weight checks

Staffing: farm staff and project staff

Data and samples required:

- 3 monthly feed input (as fed) into each culture tank.
- Bulk weight for 300 randomly selected individual abalone from each culture tank.
 - A sub-sample of 30 abalone from each culture tank in the first half of the trial, or 15 in the second half of the trial, will be randomly selected, weighed, measured and shucked and stored frozen in freezer in clearly labelled bags.
 - Then another 20 abalone in the first half of the trial, or 35 in the second half of the trial, will be weighed and measured and integrated back into regular farm operations.
 - Bulk weight.
- We need to record and collect the following for:
 - Average individual weights;
 - Average individual shell lengths;
 - Average individual shell weights;
 - Average individual meat weights; and
 - Bag and clearly label and freeze meat samples for biochemical analyses.
- Mortality number and estimated weight from each culture tank.
- **Tagging optional:** 50 tagged abalone will be randomly selected, weighed (± 0.1 g), measured (nearest mm) and shucked and weighed (± 0.1 g), and stored frozen in freezer in clearly labelled bags.

A4.3.8.4 Summary for intermediate stocking density reduction harvest

Staffing: farm staff and project staff

Data and samples required:

- Total biomass of each culture tank at 9 months;
- The size grade for each culture tank at 9 months;
- Total feed input (as fed) into each culture tank at 9 months;
- Average individual weights based on the bulk weight of 300 abalone for each culture tank at 9 months:
 - A sub-sample of 30 individual abalone from each culture tank will be randomly selected, weighed, measured and shucked and stored frozen in freezer in clearly labelled bags; and
 - Then another 20 individual abalone will be weighed and measured and integrated back into regular farm operations.
- From this we will need to record and collect:
 - Average individual weights;
 - Average individual shell lengths;
 - Average individual shell weights;

- Average individual meat weights; and
- Bag and clearly label and freeze meat samples for biochemical analyses.
- Mortality number and estimated weight from each culture tank.
- **Tagging optional:**
 - All individual weight and length data from tagged abalone; and
 - 50 tagged abalone will be randomly selected, weighed (± 0.1 g), measured (nearest mm) and shucked and weighed (± 0.1 g), and stored frozen in freezer in clearly labelled bags.

A4.3.8.5 Summary for intermediate re-stocking

Staffing: farm staff and project staff

Data and samples required:

- The total re-stocked biomass of each culture tank.
 - This will come from a randomly selected sample from the middle 1/3 of each culture tank; or
 - Be made up of a proportion of each size class.
- The size grade for the re-stocked abalone for each culture tank at 9 months.
- The average individual weights based on the bulk weight of 300 of the re-stocked abalone for each culture tank.

A4.3.8.6 Summary for final harvest

Staffing: farm staff and project staff

Data and samples required:

- Total final biomass for each culture tank;
- Final size grade of abalone from each culture tank;
- Total feed input (as fed) into each culture tank;
- Mortality number, shell length, and estimated weight for each culture tank;
- Final bulk weight of 300 randomly selected abalone from each culture tank;
 - A sub-sample of 30 individual abalone from each culture tank will be randomly selected, weighed, measured and shucked and stored frozen in freezer in clearly labelled bags; and
 - Then another 20 individual abalone will be weighed, measured and integrated back into normal farm production.
- From this we will need to record and collect:
 - Average individual weights;
 - Average individual shell lengths;
 - Average individual shell weights;
 - Average individual meat weights; and
 - Bag and clearly label and freeze meat samples for biochemical analyses.
- **Tagging optional:**
 - All individual weight and length data from tagged abalone; and
 - 50 tagged abalone will be randomly selected, weighed (± 0.1 g), measured (nearest mm), shucked and weighed (± 0.1 g), and stored frozen in freezer in clearly labelled bags.

A4.3.9 Calculation of growth performance and feed efficiency indices

Performance indices for each tank will be calculated as follows:

- Stocking density = Biomass (kg) / tank area (m²)
- Biomass gain = (final weight + Σ mortality weight) – (initial weight + Σ mortality replacement weight);
- Specific growth rate (SGR) = (ln final weight - ln initial weight) x 100 / time (d);
- Apparent feed intake = amount feed offered per culture tank;
- Apparent economic feed conversion ratio (FCR) = amount of dry feed offered (g) / biomass gain (g);
- Apparent protein efficiency ratio (PER) = wet weight gain g / protein offered g;
- Apparent energy efficiency ratio (EER) = wet weight gain g / energy offered MJ;
- Condition factor (CF) = 5575 x (weight [g] / length [mm]^{2.99}) (Britz and Hecht, 1997).

From each trial the protein, lipid, ash, moisture and energy content of each diet (information provided by each company) and initial and final abalone will be calculated and the following indices will be derived:

- Apparent protein deposition (PD%) = Final soft body protein content – initial soft body protein content x 100 / protein offered.
- Apparent energy deposition (ED%) = Final soft body energy content – initial soft body energy content x 100 / energy offered.

The dry matter leaching loss of each diet from each study will be determined in triplicate over a period of 1, 6 and 16 h by SARDI staff at SARDI Aquatic Sciences Centre, West Beach, South Australia.

A4.3.10 Environmental monitoring of experimental systems

Throughout the studies the minimum environmental variables and measurement frequencies for the on-farm trial tanks are outlined in Table A4.1. Please note that all water quality variables in the culture tanks should be measured 2 h after feeding.

Table A4.1. Water quality variables and measurement frequencies for on-farm trial tanks.

Water quality variable	Measurement frequency ^{1,2}
Culture tank water inflow rate (L min ⁻¹)	Once a week for all tanks
Dissolved oxygen (mg L ⁻¹ and % saturation)	Daily in at least 2 tanks per feeding strategy (preferably all tanks)
pH	Daily in at least 2 tanks per feeding strategy (preferably all tanks)
Salinity (g L ⁻¹)	Weekly in at least 2 tanks per feeding strategy or more regularly in the face of an obvious environmental event such as heavy rainfall (preferably all tanks)

¹To reduce sampling bias during environmental monitoring of water quality, the two tanks selected from each dietary feeding strategy should be alternated on a daily basis and the tank number recorded.

²Water quality measurements should all be measured 2 h after feeding.

- The measurement of the farm intake water supply for the same water quality variables at the same frequencies and times described in Table A4.1 would also be desirable.
- A measurement of light intensity
 - Measured on a bright cloudless day, at a similar location at each culture tank.
 - This should be carried out at least once during the trial; however, measurements at the mid-point of each season throughout the trial would be desirable.
 - The Principal Investigator can organise to bring a light meter out to each farm.
- The measurement of ammonia (NH₄⁺/NH₃ mg L⁻¹) will not be necessary as adequate water exchange rates and tank cleaning procedures would be provided to maintain safe levels of this compound throughout each trial.

A4.3.11 Tank cleaning and mortality monitoring and reporting

The mortalities for each separate culture tank will be collected from each tank at every tank clean:

- Cleaning twice weekly during winter;
- Cleaning three times weekly during summer;
- Cleaning will also occur during the harvest event.

The mortalities will be:

- Counted;
- Recorded;
- Then discarded;
- An average weight will be assigned to each mortality based on the average weight from the previous weight check for the given culture tank;
- Feed rates will be adjusted on a weekly basis on Monday mornings using this data.

A4.3.12 Statistical analyses

All statistical analysis of the results from all of the on-farm trials will be carried out by the Principal Investigator.

- In this project we will be testing the performance of the different feeding strategies in a commercial situation.
- This will be done on all participating farms at once, with two different types of abalone
 - Greenlip abalone.
 - The hybrid ('tiger') of the greenlip and the blacklip abalone.
- The results from each farm trial will be analysed separately.
 - As each feed company's diets are assigned to only one greenlip farm and/or one hybrid farm.
- During this project no direct comparisons will be drawn between the performances of the diets of any different feed companies for the AAGA by the Principal Investigator.
- Results will be presented as initial sample means and standard deviations, and tank means and standard deviations.

A4.4 Acknowledgements

We would like to acknowledge Mr Justin Fromm and Mr Dan Machin of AAGA, Mr Nick Savva of Abtas Marketing Pty. Ltd. and AAGA, Mr Tim Rudge of Coastal Seafarms Holding Pty. Ltd., Mr Anton Krsinich and Ms Lucy Saunders of Great Southern Waters Pty Ltd, Mr David Connell of Kangaroo Island Abalone Pty. Ltd., Mr Tom Hyde of SAM Abalone Pty. Ltd., and Mr Mark Gervis of Southern Ocean Mariculture Pty. Ltd., and other members of the AAGA for their input into the development of this manual. I would also like to thank Joel Scanlon of Adam and Amos, Kym Heidenreich and Dr Tom Coote of Eyre Peninsula Aquafeeds, Dr Rhys Hauler and Dr Matthew Bransden of Skretting Australia for their input into the development of this manual. I would also like to thank ASCRC and Marine Innovation South Australia for their support.

A4.5 References

- Bansemer, M.S., Harris, J.O., Qin, J.G., Duong, D.N., Stone, D.A.J., 2011. Growth and feed utilisation of juvenile greenlip abalone (*Haliotis laevis*) in response to water temperatures and increasing dietary protein levels. *Aquaculture* 436, 13-20.
- Bautista-Teruel, M., Millamena, O., 1999. Diet development and evaluation for juvenile abalone, *Haliotis asinina*: protein and energy levels. *Aquaculture* 178, 117-126.
- Britz, P.J., Hecht, T., 1997. Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*. *Aquaculture* 156, 195-210.
- Coote, T.A., Hone, P.W., Van Barneveld, R.J., Maguire, G.B., 2000. Optimal protein level in a semipurified diet for juvenile greenlip abalone *Haliotis laevis*. *Aquaculture Nutrition* 6, 213-220.
- Gilroy, A., Edwards, S.J., 1998. Optimum temperature for growth of Australian abalone: preferred temperature and critical thermal maximum for blacklip abalone, *Haliotis rubra* (Leach), and greenlip abalone, *Haliotis laevis* (Leach). *Aquaculture Research* 29, 481-485.
- Steinarsson, A., Imsland, A.K., 2003. Size dependent variation in optimum growth temperature of red abalone (*Haliotis rufescens*). *Aquaculture* 22, 353-362.
- Stone, D.A.J., Harris, J.O., Wang, H., Mercer, G.J., Schaefer, E.N., Bansemer, M.S., 2013. Dietary protein level and water temperature interactions for greenlip abalone, *Haliotis laevis*. *Journal of Shellfish Research* 32, 119-130.
- Stone, D.A.J., Bansemer, M.S. and Harris, J.O. (2014). Final Report. Development of formulated diets for cultured abalone. Prepared by the South Australian Research and Development Institute (Aquatic Sciences), Adelaide. CRC Project No. 2010/736. 131pp.

A4.6 Appendix A4.1

A4.6.1 Tagging method

Recommended tags: FPN glue-on shellfish tag (Hallprint Pty. Ltd., Hindmarsh Valley, South Australia, Australia).

- The shell length and weight of animals to be tagged will be recorded to the nearest mm and 0.1 g, respectively.
- Prior to tagging, compressed air is used to dry the abalone shell and then methylated spirits is dabbed on with a cotton tip to remove any algae present that would inhibit adhesion.
- Compressed air is then re-applied to ensure the shell surface is dry before Supa Glue Gel (Selleys® Quick Fix™, Selleys®, Padstow, New South Wales, Australia) is applied.
- The tag is firmly attached and then dried and secured using compressed air.
- The tagging process for each abalone should be completed within five minutes to minimize the stress on the animal.