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ABALONE ON-FARM GROW-OUT TRIAL MANUAL

PROJECT NO. 2010/736

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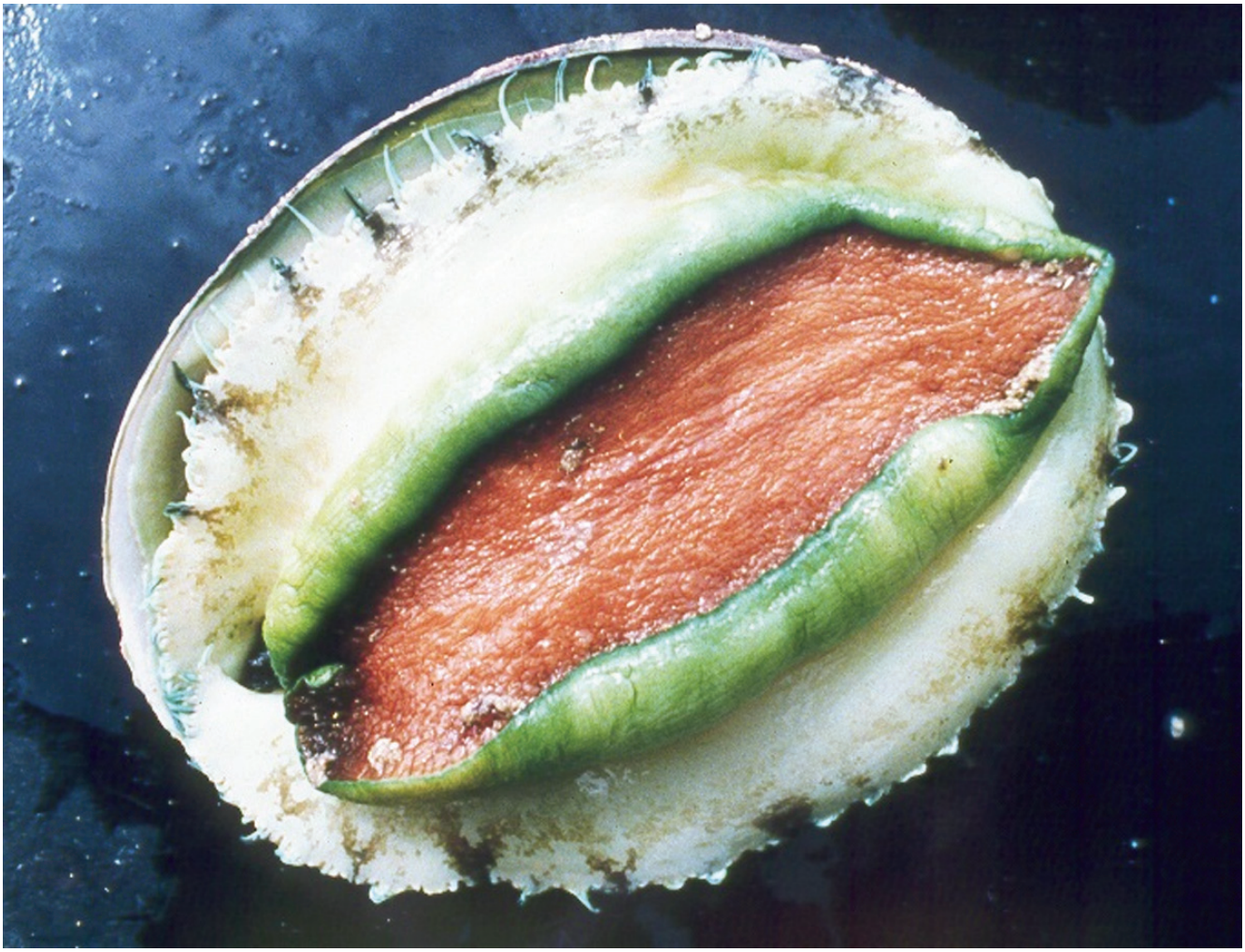
AUSTRALIAN SEAFOOD COOPERATIVE RESEARCH CENTRE



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CRC AUSTRALIA

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1. INTRODUCTION

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 AS CRC 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 laevigata) in relation to changes in dietary protein level and water temperature (Stone et al. 2013, Chapter 2; Bansemer et al. in press, 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).

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 AS CRC 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).

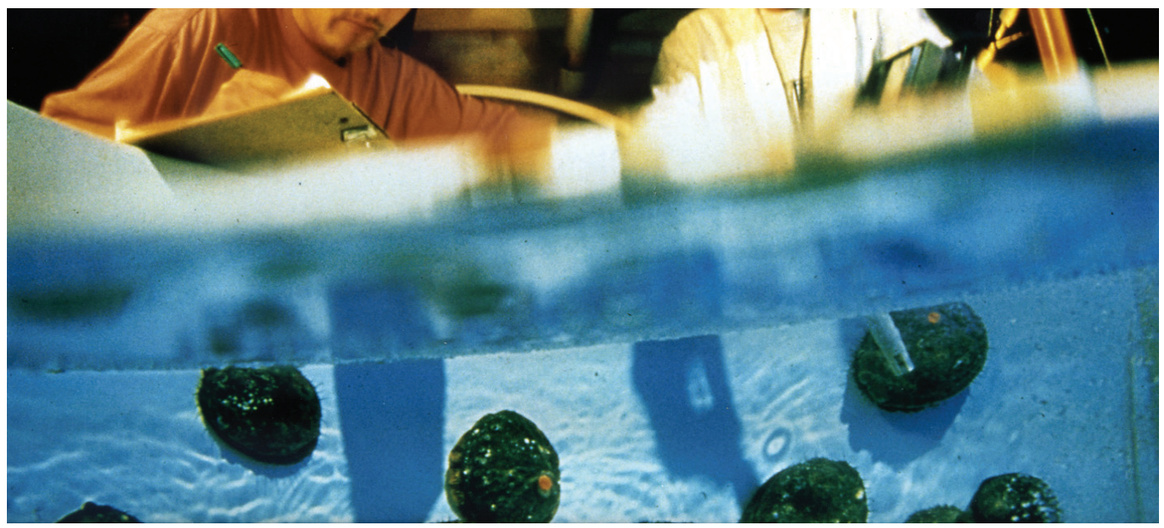
- Southern Ocean Mariculture Pty. Ltd., RMB 2068 Princes Highway, Port Fairy, Victoria, 3284, Contact: Mr Mark Gervis and Mr Hamish Ebery, (Hybrid). Out
- Abtas Marketing Pty. Ltd. PO Box 216, Beaconsfield, Tasmania, 7270. Contact: Mr Nick Savva (Hybrid). Out
- SAM Abalone Pty. Ltd., Boston Point, Port 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 & 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 & Dr Rhys Hauler.

During the development of this manual we undertook farm visits to the six participating AAGA facilitates. 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 AS CRC 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.



2. AIM

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

3 MATERIALS AND METHODS

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 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 AS CRC 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 AS CRC 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 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 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.

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 AS CRC 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.

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.

3.4 EXPERIMENTAL SYSTEM

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 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).

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.

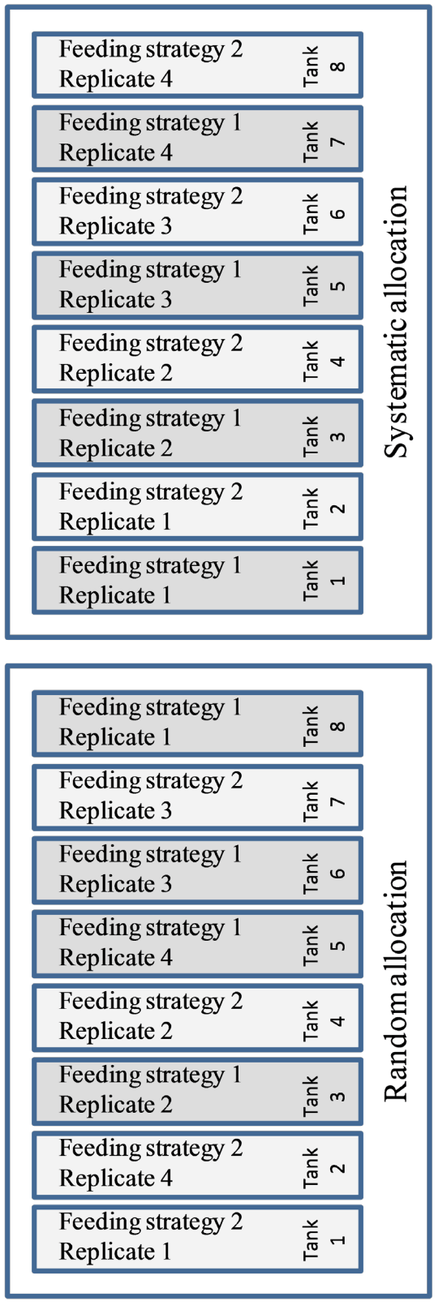


Figure 2. An example of random or systematic allocation of the different feeding strategy treatments to the culture tanks.

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 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.

3.5 STOCKING OF TRIALS

Please refer to Figure 1 in Section 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 interspersion (see example in section 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 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 1, Section 3.5.2, Appendix 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).

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.

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 1.

3.6 FEEDS, FEED STORAGE AND FEEDING

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.

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.

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.

3.7 SAMPLING PROCEDURES FOR WEIGHT CHECKS AND HARVESTING

Please refer to Figure 1 in Section 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 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.

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

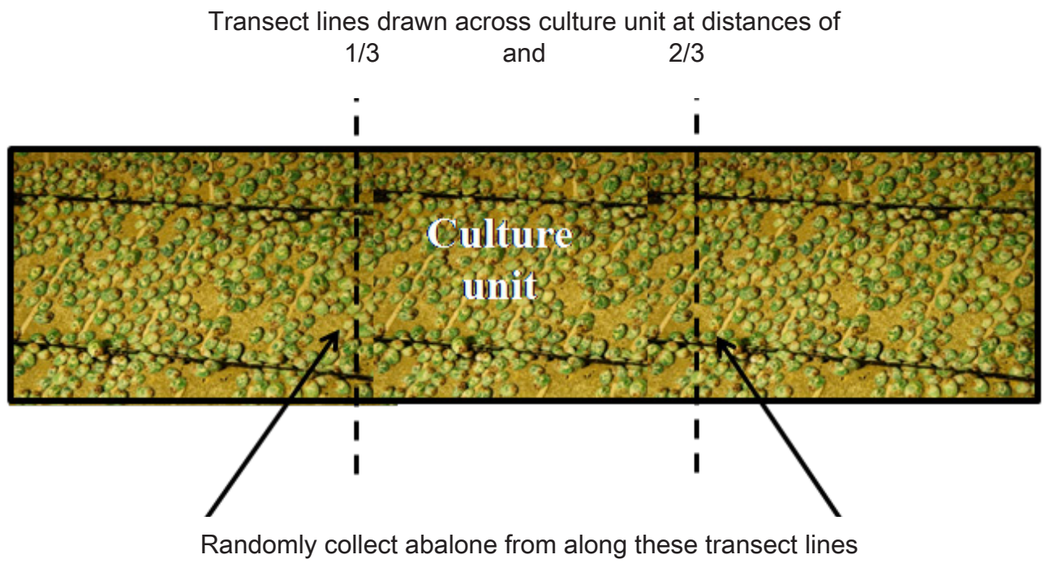


Figure 3. Description of the location for sample collection at monthly and 3 monthly weight checks.

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.

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 3.7.2.1) on a monthly basis, and recorded.

This is 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.

3.7.3. 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 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 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.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 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 event 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.

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 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 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.

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:

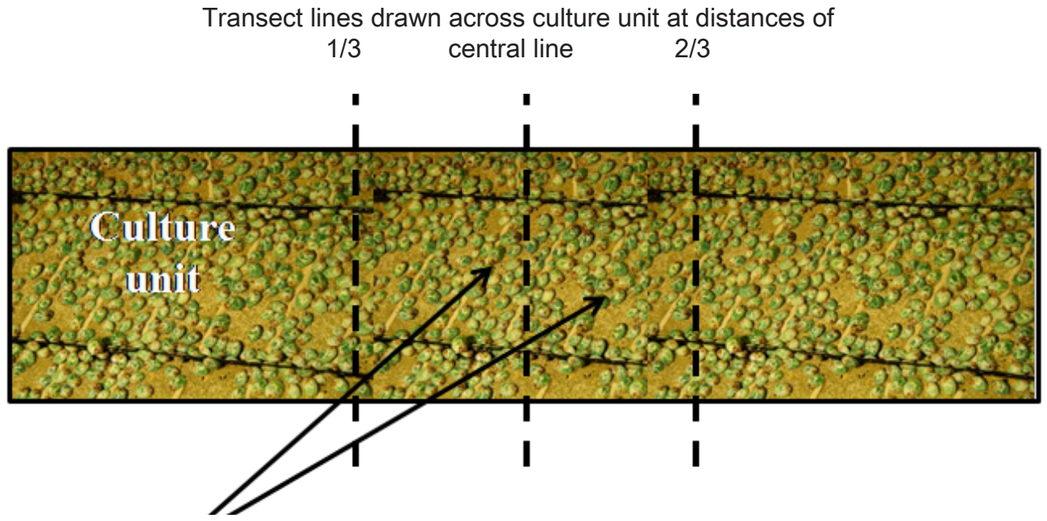


Figure 4. Diagram of the area used (middle third of culture tank) to randomly collect abalone for re-stocking for second phase of experiment.

- 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 4 and Section 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.

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 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.

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.

3.8 SUMMARY OF DATA COLLECTION AND STAFF ALLOCATION FOR THE TRIAL

Please refer to Figure 1 in Section 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).

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.

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.

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.

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.

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.

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.

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 (m^2)
- Biomass gain = (final weight + ∑mortality weight) – (initial weight + ∑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.

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 1. Please note that all water quality variables in the culture tanks should 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 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 (NH_4^+/NH_3 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.

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.

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)

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

[1]To 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.

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

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.

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6. APPENDIX 1

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 FixTM, 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.