ABALONE AQUACULTURE SUBPROGRAM

Preventing summer mortality of abalone in aquaculture systems by understanding interactions between nutrition and water temperature

M. Vandepeer

October 2006

FRDC Project No. 2002/200
SARDI Aquatic Sciences Publication No. RD02/0035-2

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SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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TABLE OF CONTENTS

1. NON TECHNICAL SUMMARY

2002/200	**Abalone Aquaculture Subprogram: Preventing summer mortality of abalone in aquaculture systems by understanding interactions between nutrition and water temperature**

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OBJECTIVES:

1. Induce abalone mortality and/or bloat under experimental conditions to ensure a "control" exists for subsequent experiments.
2. Induce abalone mortality and/or bloat in abalone held in laboratory and outside tanks through temperature and stocking density manipulation.
3. Produce a water quality manual for abalone.

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED TO DATE

This project has alerted the abalone industry to some of the factors that are likely to predispose abalone to summer mortality and provided them with suggestions as to actions they can take to prevent it. The production of the manual has provided the industry with a guide to water quality for abalone farming. By increasing their knowledge on water quality it will enable them to ensure their farms are running at optimal conditions. This may lead to less mortalities by reducing abalone stress and promoting a cleaner production environment.

The long production times of about 3 years for farmed abalone increases the risk associated with losses from disease or infrastructure failure. For this reason alone, there is an urgent need to address any factor that compromises health or production efficiency in abalone aquaculture systems.

Farmers often report higher mortalities of abalone over the summer months. The exact cause is unknown but is believed to be due to an increase in stress on the abalone as a consequence of warmer water temperatures and reduced water quality (elevated ammonia, lower dissolved oxygen and increased bacteria levels). Some farms have reported cases of abalone bloat characterized by dead abalone floating in tanks with swollen abdomens. Nutrition has been implicated as a causal factor in the aetiology of this disease.

A priority setting process for the abalone aquaculture sector (convened by the Abalone Aquaculture Subprogram) rated abalone health and the interactions between health, nutrition and environmental stress as one of the highest research priorities for the industry. The aim of this project was to examine these interactions in an attempt to significantly reduce the level of summer mortalities and to ensure that manufactured abalone diets do not compromise abalone health in any way.

The original objectives of the project were to induce mortality and bloat in laboratory held animals so that a control existed for further studies to investigate diet manipulation. However, mortality and/or bloat could not be induced in the first experiment as was hoped and therefore this experiment was repeated with modifications. The experiment was altered to increase stress through stocking density and water flow manipulation. This involved an inside and outside tank system. Mortalities were observed in the inside tanks across all treatments, although predominantly in the highest stocking density tanks (90% of surface area), however, only two abalone were observed to develop bloat. In comparison mortalities were observed in only two outdoor tanks (deaths occurring on the same day) with all the mortalities in one tank having bloat.

In addition to the two experiments, a mortality survey was sent out to several farms to complete over the summer period. Unfortunately only 3 farms completed and returned the survey. Several farms reported that their reason for not filling out the survey was due to the fact that they no longer had a bloat/mortality problem. Reasons attributed to this included changes in farm site and tank design. One farm, who mainly experienced mortality/bloat in their juvenile abalone, said that removing abalone from plates at a much larger size had helped. Of the farms that did return forms, one averaged 30% mortality in all 12 tanks, the second farm had much lower levels of mortality, generally below 1% in all 12 tanks and the third had low levels of mortality (< 2%) in most tanks, although it did experience high mortalities in 3 tanks (11, 14, and 51%). Bloat was observed on one farm in abalone that were exposed to air

due to a drop in water flow. Other factors said to have contributed to mortalities on this farm were 2 occurrences of oxygen supersaturation and poor water quality due to uneven tank surfaces.

The original focus of the project was to investigate the influence of diet on mortality and/or bloat, as nutrition was implicated as a causal factor. However, based on the experiments, survey reportings and numerous conversations with different farmers about mortalities on their farms during the course of the project, it became apparent that many mortality events over summer were likely to be linked to water quality and tank husbandry issues rather than nutritional composition of manufactured diets per se. Manufactured diets probably contribute to mortality and bloat being excellent sites for bacterial growth. This is a particular issue in summer due to the warmer water temperatures resulting in elevated bacterial levels and more rapid break down of the diets.

Based on the above it was decided, with endorsement from the Abalone Aquaculture Subprogram leader, that the project's planned outcomes be changed to:

1. An understanding of the effect of water quality on abalone health;
2. An understanding of the abalone's tolerance and limits to various water quality parameters;
3. An understanding of which water quality parameters are important to measure and how they should be measured.

To meet these outcomes it was viewed that the remaining objective of the project be changed to the production of a water quality manual for abalone farmers.

Further work should be aimed at gaining a better understanding of water quality on farms, particularly, the degree to which it fluctuates. The establishment of ongoing water quality assessment programs on farms for at least one complete year would be beneficial. This would help establish the variability in water quality on farms throughout the year, in particular over summer, and thus any correlation between water quality and abalone mortalities or bloat. In conjunction with water quality monitoring, farm-based experiments that investigate the effects of stocking density on tank mortality levels over summer may also help to determine the effect of stocking density on mortality and to determine the appropriate rate at which to stock so as to significantly reduce mortality. Finally, the effectiveness of adding various immune enhancing

compounds to abalone diets just prior to and over summer could be assessed to determine whether they may be beneficial in reducing mortalities.

KEYWORDS: Abalone, summer mortality, *Vibrio*, bloat

2. ACKNOWLEDGMENTS

Thanks are owed to Mr Mark Gluis, Dr Xiaoxu Li and Mr Steven Clarke for reviewing the draft copy of the final report and to Adam and Amos Abalone Foods Pty Ltd who provided the abalone diets for experiments 1 and 2. Thanks are also extended to those farmers who completed and returned the survey forms and answered numerous questions about past mortality events on their farms.

3. BACKGROUND

The Australian abalone aquaculture industry is an emerging sector with a total commercial production of approximately 300 tonne in 2004/05, worth about $12.2 million. This significant leap from previous years marks the beginning of full production capacity for some farms. Production is expected to grow at 20% per year as more farms reach commercial production levels and others increase their production targets. To ensure these potential targets are reached, both abalone health and nutrition must be optimised.

The development of cost effective diets that meet the nutritional requirements of abalone has always been a high priority for the industry as the commercial harvesting of the abalone's natural diet macroalgae, has always been considered to be problematic. Early research mainly focused on greenlip abalone (*Haliotis laevigata*) and included the determination of the abalone's requirement for nutrients (protein, amino acids, lipid type and quantity and energy) and an evaluation of the protein and energy digestibility of a range of ingredients for inclusion in manufactured diets. More recent work includes an evaluation of formulated feeds for juvenile abalone and establishing whether the nutritional requirements of the blacklip abalone are the same as greenlips and hence whether they should be fed similar manufactured diets. It is widely recognised that abalone nutrition research completed in Australia since 1995 has reduced feed costs from $5-$7.00 a kilogram to around $2.50-$3.00 a kilogram while improving growth performance (growth rate and feed conversion efficiency) significantly. All commercial farms use diets based on this research.

Despite the significant improvements in the growth performance of farmed abalone resulting from the use of manufactured feeds, it still takes 3 years for abalone to reach market size. This makes abalone aquaculture a high risk, high return industry and places an emphasis on the maintenance of abalone health for the entire production cycle. Farmers often report higher mortalities of abalone over the summer months. The exact cause is unknown but is believed to be due to an increase in stress on the abalone as a consequence of warmer water temperatures and reduced water quality (elevated ammonia, lower dissolved oxygen and increased bacteria levels). Some farms have reported cases of abalone bloat characterized by dead abalone floating in tanks with swollen intestines. Nutrition has been implicated as a causal factor in the aetiology of this disease. Manufactured diets contain many highly fermentable substrates that may be digested and fermented rapidly in abalone, and when combined with increases in intestinal microbial activity consistent with warmer water temperatures, may result in excess microbial gas production.

A priority setting process for the abalone aquaculture sector (convened by the Abalone Aquaculture Subprogram) rated abalone health and the interactions between health, nutrition and environmental stress as one of the highest research priorities for the industry. The aim of this project was to examine these interactions in an attempt to significantly reduce the level of summer mortalities and to ensure that manufactured abalone diets do not compromise abalone health in any way. In addition, it was planned that outcomes from this project would facilitate the development of manufactured abalone diets that increase the resistance of farmed abalone to environmental stresses thus reducing mortality rates and optimising production efficiency.

4. NEED

Abalone production times of about 3 years increase the risk associated with losses from disease or infrastructure failure. For this reason alone, there is an urgent need to address any factor that compromises health or production efficiency in abalone aquaculture systems. In this instance, other reasons necessitating completion of the proposed project include:

1. As the abalone aquaculture sector has grown, it has become apparent that high mortalities can occur during the summer months as water temperatures increase. For example, in 2000, one farm in the Port Lincoln area reported stock losses of up to 50% (representative of the SA Abalone Growers Industry, pers. comm.). The deaths are not isolated to one state or one species. Similar losses over summer have also been reported for farms culturing blacklip abalone in Tasmania (Mike Wing, Tas. Tiger Abalone, pers. comm.) and in Victoria (Mark Gervis, Southern Ocean Mariculture, pers. comm.). In Tasmania abalone mortalities are associated with rapid epithelial loss and the appearance of pustule like blisters on the footsole filled with a clear liquid. All mortalities are associated with elevated levels of *Vibrio harvyi* and cannibalism usually occurs once a mortality appears. As manipulation of water temperature is not a commercially viable option in these production systems, other means must be devised to prevent losses of this magnitude. At present, farmers are addressing summer mortalities by reducing feeding rates to ensure maximum water quality. While this reduces mortalities, production levels suffer, hence other options are desirable.

2. The exact relationship between abalone mortality and decreased production levels, elevated water temperatures (stress) and nutrition is poorly understood. An understanding of these relationships will facilitate intervention to optimise health and

production.

5. OBJECTIVES

1. Induce abalone mortality and/or bloat under experimental conditions to ensure a "control" exists for subsequent experiments.
2. Examine the interaction between high levels of fermentable carbohydrate and temperature on abalone growth rates, mortality and haemocyte counts.
3. Define nutritional treatments that may alleviate the effects of increased water temperature on abalone mortality including extrusion of dietary ingredients and immune enhancing diet additives such as antioxidants and mannan oligosaccharides.
4. Apply the results of experiments 2 and 3 to black lip abalone.

6. REVISED OBJECTIVES

Due to outcomes from initial experiments the project direction was revised and the original objectives 2, 3 and 4 were not conducted. The revised objectives are outlined below:

1. Repeat from above.
2. Inducement of abalone mortality and/or bloat in abalone held in the laboratory and outside tanks through temperature and stocking density manipulation.
3. Production of a water quality manual for abalone.

7.1 Experiment 1: Inducement of abalone mortality and/or bloat under laboratory conditions

AIMS

The aim of the first experiment was to induce abalone mortality and/or bloat under experimental conditions that are similar to those on farms when mortalities and/or bloat are experienced. This experiment was required so that a control existed for subsequent experiments that focussed on looking at strategies that may alleviate or reduce mortalities and/or bloat.

METHODS

Experimental Design

A 3x3 factorial experiment involving three temperatures (20, 22 and 24ºC), as recommended by industry, and three feeding levels (not fed, fed to subsatiation and fed to excess) was conducted to determine if mortality and/or bloat could be induced. The experimental setup consisted of three replicate tanks of each feed treatment being blocked within temperature. The water temperature at the start of the experiment was 17.5°C. It was raised by 0.5°C a day until the three set point temperatures (20, 22 & 24°C) had been reached.

Tank and water system

The tanks used were 27 x 17 x 17 cm in dimensions and the water level was 12.5 cm deep. This resulted in a tank volume of 5.8 L. The tanks were on a flow through water system with a flow rate of approximately 300-500 mL/min. The seawater was filtered to 30 µm by primary sand filters and then to 10 µm using secondary composite sand filters. An additional 50 µm filter was added to each water line (hot, ambient and cold) prior to them being mixed in the header tanks above the tanks. This was to ensure the fine taps linked to each tanks didn't block and reduce flow. Temperature was maintained at +/- 1ºC of the desired temperature.

Abalone

Ten abalone (30 mm greenlips) purchased from South Australian Abalone Developments (SAABDEV) were randomly allocated to each tank. The diet fed to the abalone was a commercial diet that had been used by industry during times when mortality and bloat was observed on their farms. Abalone were inspected daily for mortality and bloat and during this time oxygen and temperature were recorded. Abalone were initially fed and tanks cleaned by siphoning every second day. The subsatiation feeding level was 1.3g/tank (1% of their body weight per day). At this level, no feed was found remaining in the tanks on the subsequent cleaning day. The excess feeding level was greater than 2% of their body weight per day. At this level feed was always found remaining in tanks on the subsequent cleaning day.

RESULTS

The experiment commenced on April 25th. It took until the 8th of May for the set point temperatures to be reached. During this time 2 abalone died (Figure 7.1-1). It is assumed that their deaths were a result of transport and handling stress rather then temperature/diet induced stress. Neither of the abalone had bloat.

No more deaths were observed until the 17th of May when one abalone was found dead in the 20°C and one in the 22°C fed to excess treatments (Figure 7.1-1). Again, neither showed signs of bloat. Another abalone was found dead with no signs of bloat on the 20th of May in the 20°C, no food treatment (Figure 7.1-1). To reduce the water quality in the tanks and to increase abalone stress, tank cleaning was reduced to once a week instead of every 2nd day starting from the 21st of May. It was hoped that this might increase bacterial levels and induce bloat. The feed was observed to have developed a white growth on it by the end of the week (possibly a bacterial film), and it was evident it had gone anoxic due to the odour emitted when siphoning it out of the tanks and the black colouration underneath the food. Water samples (100 µL) taken from the tanks held at 24°C were plated to test for the presence of *Vibrio* sp. No colonies were found on the plate of water taken from the control tank (no abalone) but all other tanks contained numerous *Vibrio* colonies.

Due to a fault with the temperature control system, tank temperatures in the 24°C tank system went to 26°C and cycled 10 times from 26 to 12°C (in 15 minutes) over a four day period (Friday May 31st – Monday June 3rd). The fault was rectified on the Monday and the temperature was reset at 26°C instead of 24°C as no abalone were found to have died despite this stress. Since the last deaths recorded on the 20th of May it was not until the 7th of June

that another 2 abalone were found dead (without bloat) (Figure 7.1-1). Both were in the fed to excess treatments, one held at 22°C and the other at 26°C.

It was not until the 16th of June, 13 days after the stress event and being held at 26°C, that abalone started to gradually die in the 26°C tanks (Figure 7.1-1). This occurred at the rate of 1-3 per day across all treatments (no feed, subsatiation and fed to excess). The food in the tanks of the abalone held at 26°C did not appear to be eaten as there were no faecal pellets in the tanks as could be seen in the tanks of abalone held at 20 and 22°C. In addition, in the tanks held at 20 and 22°C there was no food left in the subsatiation treatments by the 2nd day but all pellets remained in the 26°C fed to subsatiation treatments even after 1 week.

The results to date were discussed with industry representatives. From this discussion a summary of factors that were thought to be contributors to bloat was made. These included:

- Switching diets (from starved to manufactured feed, from one brand of feed to another and from macroalgae to manufactured feed)
- Combinations of diet change and temperature increase
- High stocking density
- Poor water quality

Based on the above it was decided that the commercial diet being fed to the abalone should be changed. The feeding of the new diet commenced on the 17th of June. In addition it was decided that abalone that had been in the starved treatment should start being fed to see what effect this had.

On the 24th of June a mass mortality of the abalone in the 20°C tanks occurred as a result of changing over the 50 μm filter on the hot water line (Figure 7.1-1). The filters, one each for the hot, cold and ambient water lines, had been changed every fortnight. Usually the filters were changed after the tanks had been siphoned but on this day the filter for the hot water line was changed before siphoning. Upon changing the filter and turning the water back on, there was a surge in flow that caused the food sitting on the bottom of the tanks to be stirred up and foul the water. Immediately all the abalone became agitated and started twisting back and forth as they have been observed to do when subjected to temperature shock. The temperature of the tanks was checked and all tanks were at their correct set points of (20, 22 & 26°C).

Strangely, the abalone in the 22 and 26°C eventually settled back down but many of the abalone in the 20°C turned on their backs and 10 minutes later 22 of them had died. Only one abalone was found dead in the 22°C tanks and one in the 26°C tanks. Because the tanks were cleaned once a week, the food had gone black and was anoxic underneath. As a result of the deaths due to the anoxic food being stirred up in the tanks, feeding and cleaning every 2nd day was conducted as had been done previously.

On the 27th of June, 10 days after starting feeding the new diet, the first incidence of bloat was recorded in one abalone from the 22°C fed to excess treatment (Figure 7.1-1). The abalone was taken to Dr Ruth Reuter, IDEXX Veterinary Pathology Services, Adelaide for histopathology. A needle was inserted into the intestine where gas bubbles were present. Results showed that the bacteria were a mixture of gram negative rods which varied from smaller bacilli (*Vibrio* sp.) to thin long filamentous forms suggesting *Flexibacter* organisms. The *Vibrio* sp. isolated was *V. hollisae* which is recognised as a cause of diarrhoea in humans and has public health implications. *Flexibacter* has been associated with disease, and has been seen on occasion in abalone (Dr Ruth Reuter pers. comm.). It can cause problems in water temperatures as low as 15 °C, however, overcrowding, low dissolved oxygen, high organic loads and temperatures above 20 °C tend to be ideal for this organism (Dr Ruth Reuter, pers. comm.). Dr Jeremy Carson (Principal Microbiologist, Fish Health Unit, Dept. of Primary Industries, Water & Environment, Launceston) was contacted and asked about the likelihood of either bacteria being the cause of bloat in abalone. Apparently neither of them are known to be producers of gas through fermentation. Gram positive rod bacteria are the type likely to have the capacity to produce gas (Dr Jeremy Carson, pers. comm.).

From the 28th of June till the end of the experiment on the 19th of July the only deaths that occurred were in the 26°C tanks. These occurred at a rate of 1-2 per day.

The dissolved oxygen levels in the tanks ranged between 5.5-6 mg/L for the 20°C tanks, 5 - 5.5 mg/L for the 22°C tanks and 4.5-5 mg/l for the 24°C (& later 26°C) tanks (Figure 7.1-2 a,b & c).

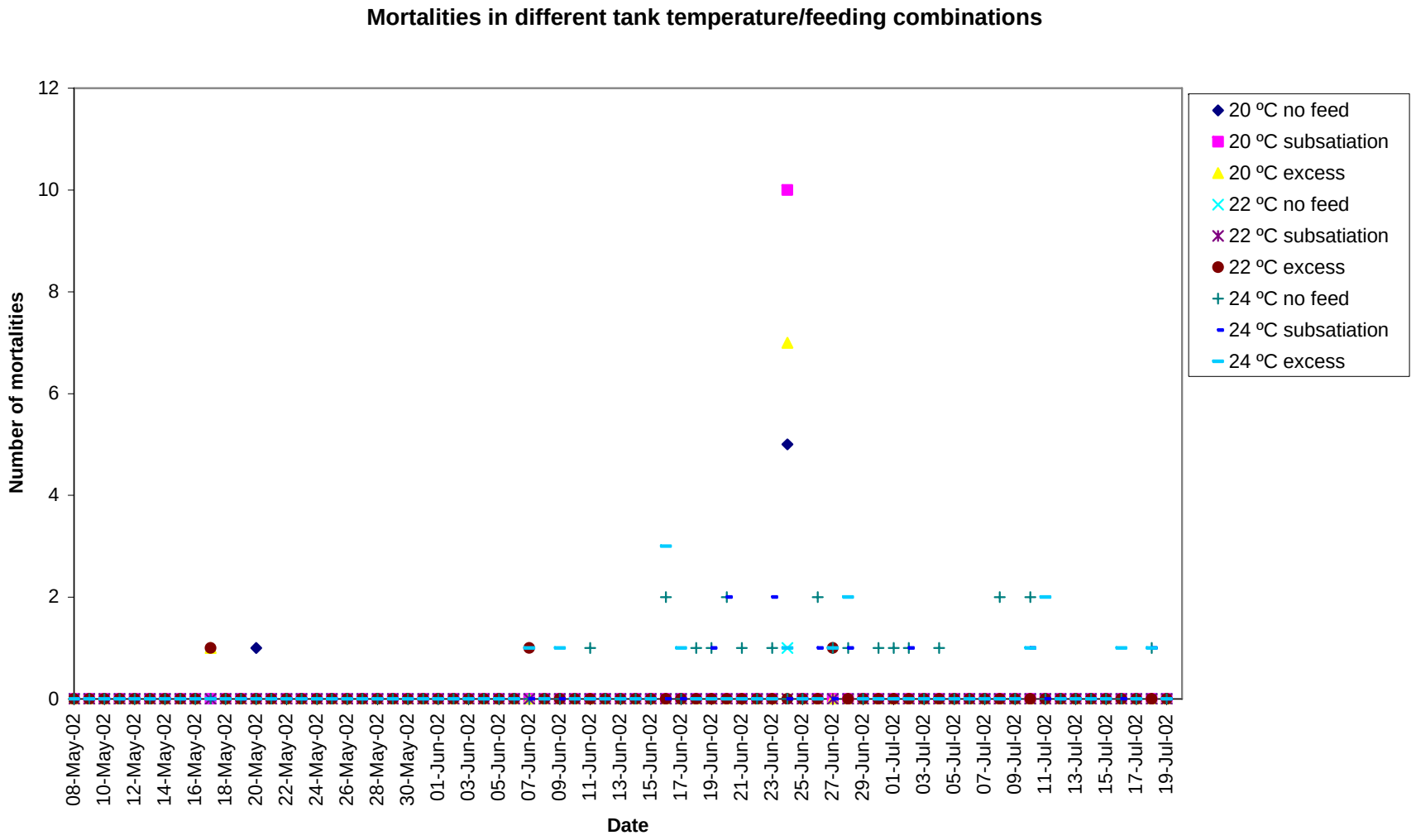
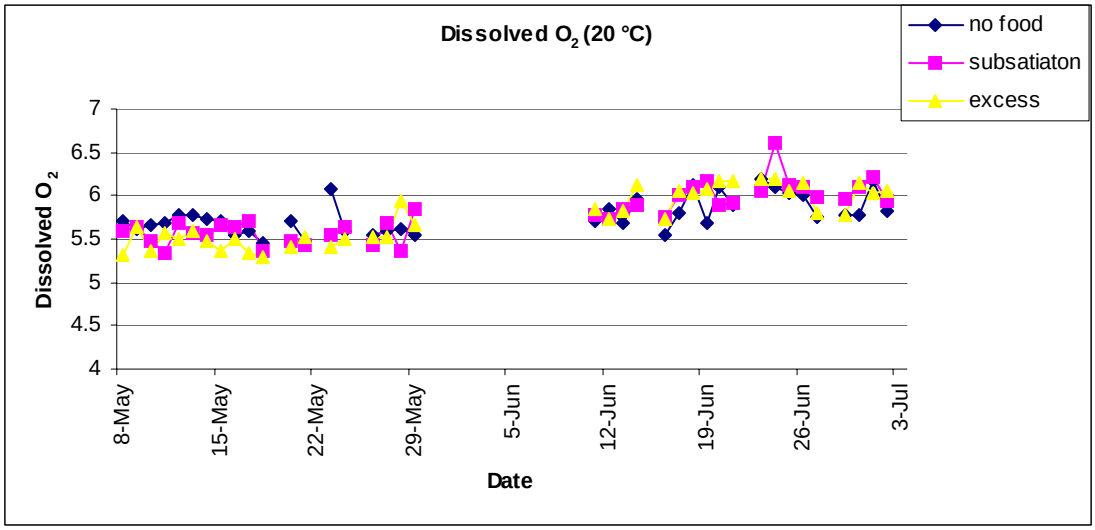
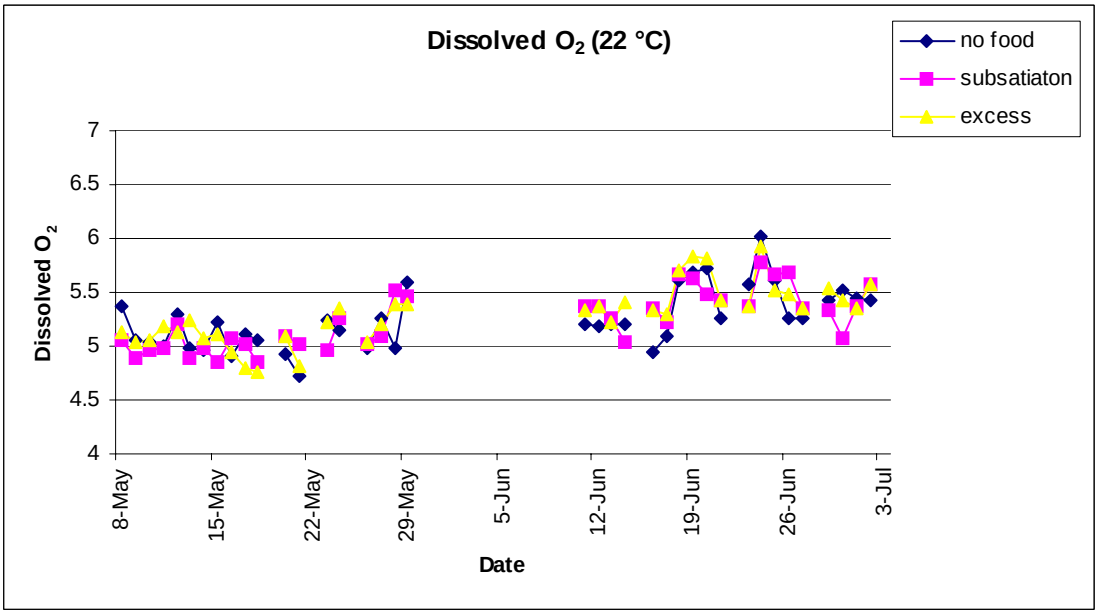


Figure 7.1-1 Total mortalities occurring in each temperature and feeding level treatments each day from the 8th May till the 19th of July. Values are sum of mortalities from 3 replicate tanks for each treatment.

a



b



c

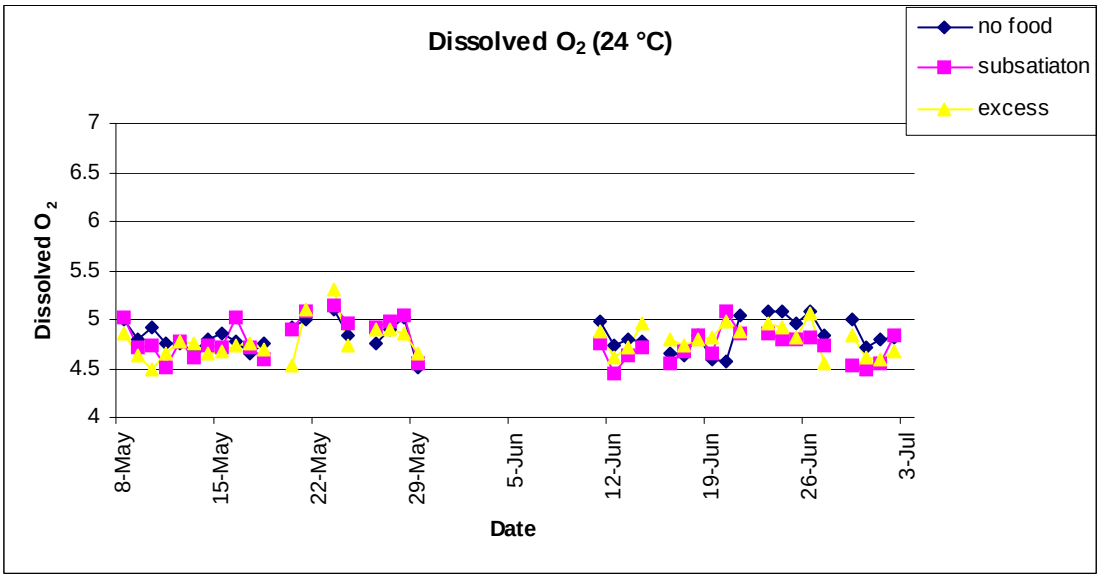


Figure 7.1-2 Dissolved oxygen (mg/l) in (a) 20°C (b) 22°C (c) 24°C tanks for each of the three feeding levels. It should be noted that the 24°C tanks were increased to 26°C on June 3rd.

DISCUSSION

Bloat and mortality were unable to be induced in abalone held in laboratory tanks that were set at the same temperature farm tanks experience at times when high levels of mortality and bloat have been observed. These results indicate that stress due to high water temperature and low oxygen is not enough to induce bloat or mortality in abalone fed the same diets as those used by farmers over summer. These findings in themselves are of significant importance to our understanding of the summer mortality problem and its causal factors. It is possible that these factors may contribute to animal stress and compromise health but that some other factor/s is required to induce mortality.

The fact that in the tanks held at 20 and 22°C there was no food left in the subsatiation treatments by the 2nd day, but all pellets remained in the 26°C fed to subsatiation treatments even after 1 week, indicates that the deaths in the 26°C tanks were likely to be due entirely to temperature stress rather than any temperature/nutrition interaction. This is not surprising as Gilroy and Edwards (1998) found the 50% critical thermal maxima for *H. laevigata* to be 27.5°C. In comparison Madigan et al. (2000) found the detachment temperature to be 27.6°C and the 50% critical thermal maxima to be 29.5°C for *H. laevigata*. Although death could be eventually induced in abalone when the temperature was increased to 26ºC, abalone industry representatives report that problems occur on farms at lower temperatures than this. Bloat and mortality have reportedly occurred over summer when water temperatures have been approximately 22 – 23ºC. Thus the deaths that occurred in this experiment when the water temperature was increased to 26°C are not representative of conditions on farms.

It is possible the deaths that occurred on the 24th of June as a result of changing over the 50 µm filter on the hot water line were a result of hydrogen sulfide poisoning. The hydrogen sulfide would have been released from the black and decaying feed when it was stirred up due to the surge in water flow that occurred when turning the system back on after changing the filter. Low levels of hydrogen sulfide (0.1 ppm) are stressful to prawns (Apud et al., 1985) while 0.5-10 ppm have resulted in fish kills (Langdon, 1998). Hydrogen sulfide inhibits aerobic respiration via its reversible binding of mitochondrial cytochrome *c* oxidase (Julian et al., 1998). The question remains however, as to why only the abalone in the 20°C tanks died? Not surprisingly, due to their warmer temperature, the 22 and 26°C tanks have lower dissolved oxygen than the 20°C tanks (Figure 1.1). Temperature and external oxygen levels have been clearly linked to such factors as heart rate, blood pressure, and resultant oxygen uptake (Nimura & Yamakawa, 1989;

Russell & Evans, 1989). In the quiescent period, tissue exposure to toxicants may be reduced as a result of lower transfer across the gills and lower tissue perfusion rates (Hindrum et al., 2001). Physiological differences in the abalone in the 22 and 26°C tanks as a result of lower oxygen levels compared with the 20°C tanks may well have resulted in a reduced effect of the hydrogen sulfide on the abalone in these tanks compared with the abalone in the 20°C tanks.

The results from this experiment raise the question as to why only one abalone developed bloat when large mortalities and occurrences of bloat have occurred on farms during summer when the water temperatures were the same as in this experiment and the abalone were fed the same diets. The mortalities that occurred in the experiment were nearly all in the tanks held at 26°C which is at the extreme of their upper limit for survival and thus to be expected. Despite this, 40 out of an original 90 abalone were still alive after 39 days of being held at this temperature. As mentioned previously, incidences of bloat and mass mortality on farms have occurred at much lower temperatures (20 - 24°C). One stress factor that could be responsible and that was not replicated in the current experiment is stocking density. There were only 10 abalone per tank (1,232 cm^2 in surface area) in this experiment compared with farms where abalone are in close contact with one another. This may play an important role in the proliferation and transfer of bacteria. It is also possible that the levels or types of bacteria in the tanks used within SARDI are much lower or of a different type to those in tanks on farms. As described in the methods section, the water at SARDI is filtered to 30 μm by primary sand filters and then to 10 μm using secondary composite sand filters. On commercial farms, due their enormous pumping requirements, they are unable to filter their water as at SARDI. The filtration used at SARDI would stop fine particulate silt and particulate organic matter entering into the system. Several studies have documented the association of pathogenic species of *Vibrio* with silt, organic matter and zooplankton (Kaneko and Colwell, 1973 & 1978; Huq et al., 1984; Hood et al., 1984; Nair et al., 1988). Thus the filtration used at SARDI may reduce the levels of "pathogenic bacteria" entering the tank system explaining the low mortalities and levels of bloat despite stress from high water temperature and low oxygen levels. To induce disease the animal needs to be stressed and a pathogen present. If there is no pathogen, the animal will not get diseased regardless of whether it is stressed and its immune system weak.

7.2 Experiment 2.1: Inducement of abalone mortality and/or bloat in abalone held in laboratory and outside tanks through temperature and stocking density manipulation

AIMS

In order to identify the cause of summer mortalities and bloat on farms and assess treatments that may alleviate this, it must first be demonstrated that mortality and/or bloat can be induced in abalone held in the laboratory. Although this was the objective of the 1st milestone, mortalities and bloat did not occur as expected. As the subsequent milestones could not be investigated until mortalities/bloat can be induced, the first experiment was repeated with changes made to the experimental design in an attempt to induce mortality/bloat.

METHODS

The changes made to the experimental design were based on discussions held with farmers at and also after the annual abalone aquaculture workshop. One factor reiterated by many farmers as a contributor to mortalities was stocking density. Several farmers stated that the only difference between some tanks that had suffered mortalities over summer compared with others that had not, was higher stocking rates, all other factors including diet, size and age class being the same. Another factor that was mentioned by Daryl Evans, chairman of the SA Abalone Grower's Association, as affecting summer mortality on farms is flow rate. Based on these comments the experimental design was modified to take into account variation in both stocking density and flow rates.

Tank systems

Two sets of experimental tanks were set up, one inside and one outside (see Figures 7.2-1 and 7.2-2). The reason for this is that it was thought that absence of natural sunlight may possibly have been the reason for the lack of mortality observed in experiment one. Sunlight encourages the growth of biofilms in tanks that may increase bacterial levels and reduce water quality. Because stocking densities as high as 90% were used the tanks were modified from those used in experiment one. This was so less abalone would be required to stock the tanks. Although the tank dimensions were similar to those used in experiment one (27 x 17 x 17 cm – experiment 1 vs 27 x 18.5 x 30 cm – experiment 2), the tank volume was reduced so that the water was just covering the tops of the abalone (about 2.5 cm deep) as is the situation with the slab and raceway tanks used on commercial farms. In experiment one the water level was 12.5 cm deep resulting in

a tank volume of 5.8 L, the new modification meant the tank volume was only 1.5 L. Thus in experiment one there were only 10 abalone in 5.8 L of water compared with up to 32 (for the 90% stocking rate) in 1.5 L in experiment two.

Inside Experimental Design

A 3 x 3 factorial design was used consisting of 3 temperatures (20, 22 & 24ºC) and 3 stocking densities (50, 70 & 90%). Each temperature x stocking density treatment was replicated three times by randomly allocating them to a system of 30 tanks. All tanks were on a flow through system with flow rates at 600 mL a minute. This equated to 24 tank water exchanges per hour. Flow rates were checked once per week. The seawater supplying the tanks was filtered to 30 μm by primary sand filters and then to 10 μm using secondary composite sand filters. Tanks were monitored on a daily basis for mortalities and bloat.

Statistical analyses

The effect of temperature and stocking density on the percentage of original abalone still alive at the end of experiment was analysed by two way analysis of variance (JMP_{IN}, version 4.0.3, SAS Institute Inc.).

Outside Experimental Design

The same tanks were used outside as those used inside however, they had shade cloth over the top of them (90% cover) and 5mm rubber matting with a reflective coating wrapped around them for insulation (Figure 7.2-2) . A 3 x 2 factorial design was used consisting of 3 stocking densities (50, 70 & 90%) and 2 flow rates (600 and 1200 mL per minute). Each stocking density by flow rate treatment was replicated three times by randomly allocating them to a system of 20 tanks. Unlike the inside tanks, tank temperature was not manipulated but was the ambient temperature. Temperature from one of the high and one of the low flow rate tanks was logged for the duration of the experiment. Flow rates were checked once per week. In comparison to the inside tank system the seawater supplying the outside tanks was filtered to 30 μm by primary sand filters only. Tanks were monitored on a daily basis for mortalities and bloat.

(7.2-1)

(7.2-2)

Figure 7.2-1; Inside tank system.
Figure 7.2-2; Outside tank system.

Abalone

The greenlip abalone used to stock the tanks (inside and outside) were 40 mm in length and obtained from SAABDEV. Five of the abalone were sent to IDEXX Veterinary Pathology Services, Adelaide, for examination of the gut bacteria prior to starting the experiments.

Diets, feeding and cleaning

All abalone in both the inside and outside tanks were fed a manufactured diet supplied by Adam and Amos Abalone Foods Pty Ltd. The abalone were originally fed 4% of their body weight every 2nd day. This was later decreased to 2% of their body weight every 2nd day. Tanks were also cleaned every 2nd day in the morning prior to feeding in the afternoon.

***Vibrio* monitoring**

The outlet water from the outside tanks was monitored each week from the end of December until early February for *Vibrio* levels. Water samples were taken in sterile 14 mL McCartney bottles on the day the tanks were due to be cleaned, prior to cleaning. After collection 100 μL from each sample was pipetted onto TCBS plates (Oxoid) and incubated overnight at 24ºC. The following day the number of colonies were counted.

RESULTS

Pathology of abalone prior to starting the experiments

Gram stains from the gut of three of the five abalone assessed showed very light growth of a gram negative bacterium. The isolate was the same from all three abalone. Gram stains from the guts of the other two abalone examined showed no bacteria, however, culture of the guts produced light growth of a gram negative bacterium. The pathology results from the gut reported that low numbers of gram negative bacilli occurred in the gut of three of the abalone but there was no associated inflammation.

Inside tanks

The abalone were placed into the tanks and the experiment started on the 17th of November 2002 and terminated on the 5th February 2003. Mortalities and occurrences of bloat in the inside tanks during the experimental period are listed in Figure 7.2-3. In addition a summary of the

number of abalone remaining in each tank at the end of the experiment and percentage of original number remaining is shown in Figure 7.2-4.

It can be seen that considerable mortality occurred between the 19th of November up until mid December across all temperatures and stocking densities. After mid December the mortality levels decreased to only two to three abalone per day until the end of the experiment. Stocking density was found to significantly affect abalone survival ($p<0.05$) whilst temperature did not ($p>0.05$). The majority of the mortalities occurred in the highest stocking density tanks which averaged only 51.1% (SE ± 4.36) survival. As was expected the lowest mortality levels occurred in the lowest stocked tanks which averaged 73.1% (SE ± 4.45) survival. Further analyses of the data by Tukey HSD tests to compare means revealed that the significant difference in survival for stocking density resulted from a significant difference between the 50 and 90% stocked tanks ($p<0.05$). No significant differences were found between either the 70 and 90% or the 50 and 70% stocked tanks and no temperature x stocking density interaction was observed ($p>0.05$).

Many of the abalone that died between the 19th of November and mid December had abnormal foot morphology including blisters, loss of foot epithelium and patchy discolouration of the epithelium. During the period of peak mortalities three sick abalone were taken to IDEXX Veterinary Pathology Services for examination. One of the abalone had mild oedema of the foot with small foci of inflammation and necrosis associated with numerous small bacilli. The second abalone had a large abscess in the foot associated with numerous bacteria that were also widespread through other tissues. The third abalone had numerous bacilli through the tissues but no apparent lesions associated with them. The pathologist reported that there was reasonable evidence to suggest that the abalone were affected by *Vibriosis*. Microbiological examination was also done on the gut, haemolymph and foot of all three abalone. No difference was observed in the amount or type of bacteria in the gut, haemolymph or foot. Moderate growth of gram negative bacilli was reported for all three. Although the species was not identified, the microbiologist reported that compared with the five healthy abalone sent in at the start of the experiment, the *Vibrio* species of the sick abalone differed in morphology from those isolated from the healthy abalone and thus were likely to be a different species.

Only two abalone developed bloat during the experimental period. Both were in the 22°C treatment, one from the 70%, the other from the 90% stocked tanks (Figure 7.2-3). The abalone that developed bloat on the 14th of January was sent to IDEXX Veterinary Pathology Services for examination. The gram stain of the gut showed that there were occasional gram positive

cocci and occasional gram negative bacilli. Culture of the gut produced heavy growth of *Vibrio alginolyticus*.

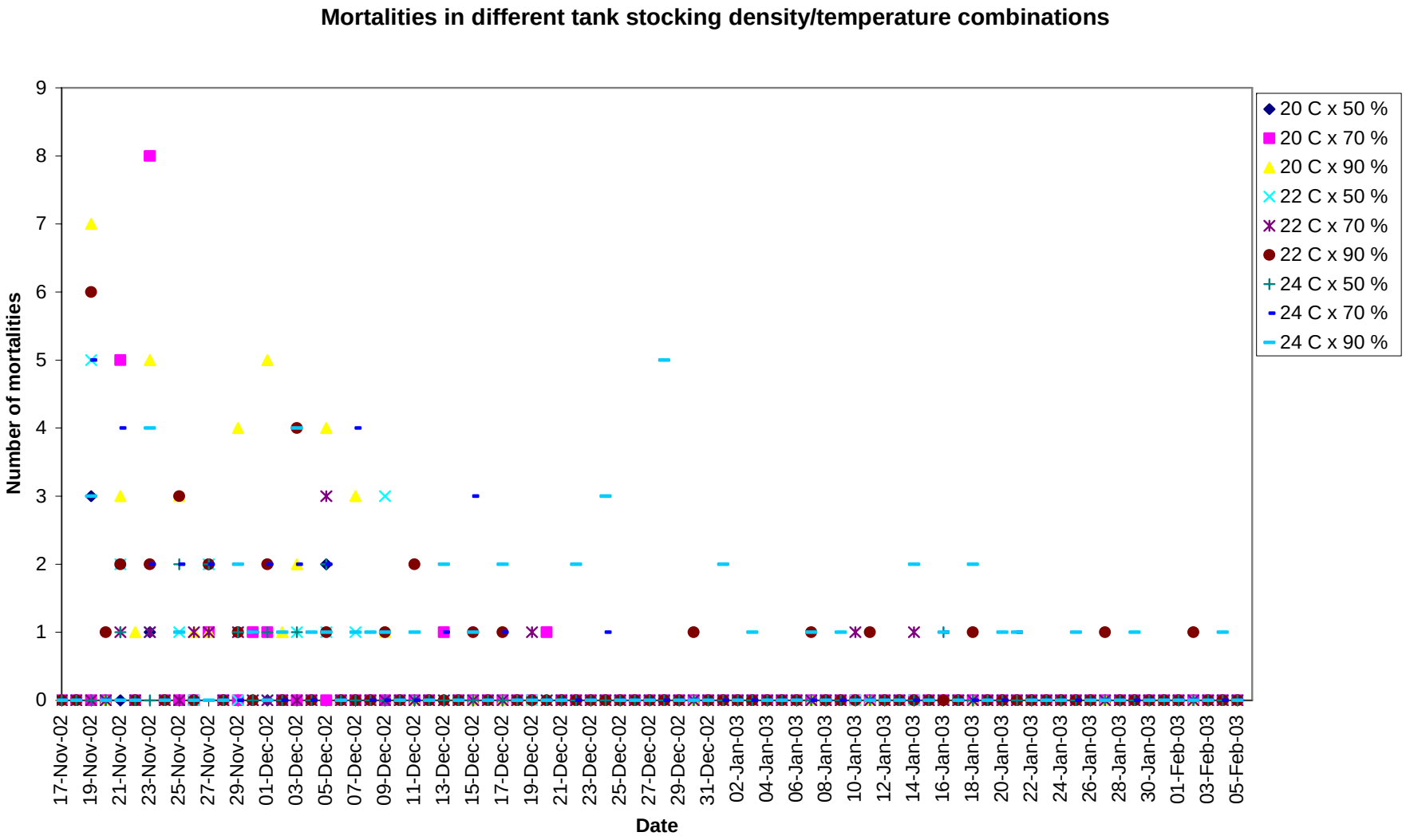


Figure 7.2-3 Total mortalities occurring in each temperature and stocking density treatment each day from the 17th November till the 5th of February. Values are sum of mortalities from 3 replicate tanks for each treatment

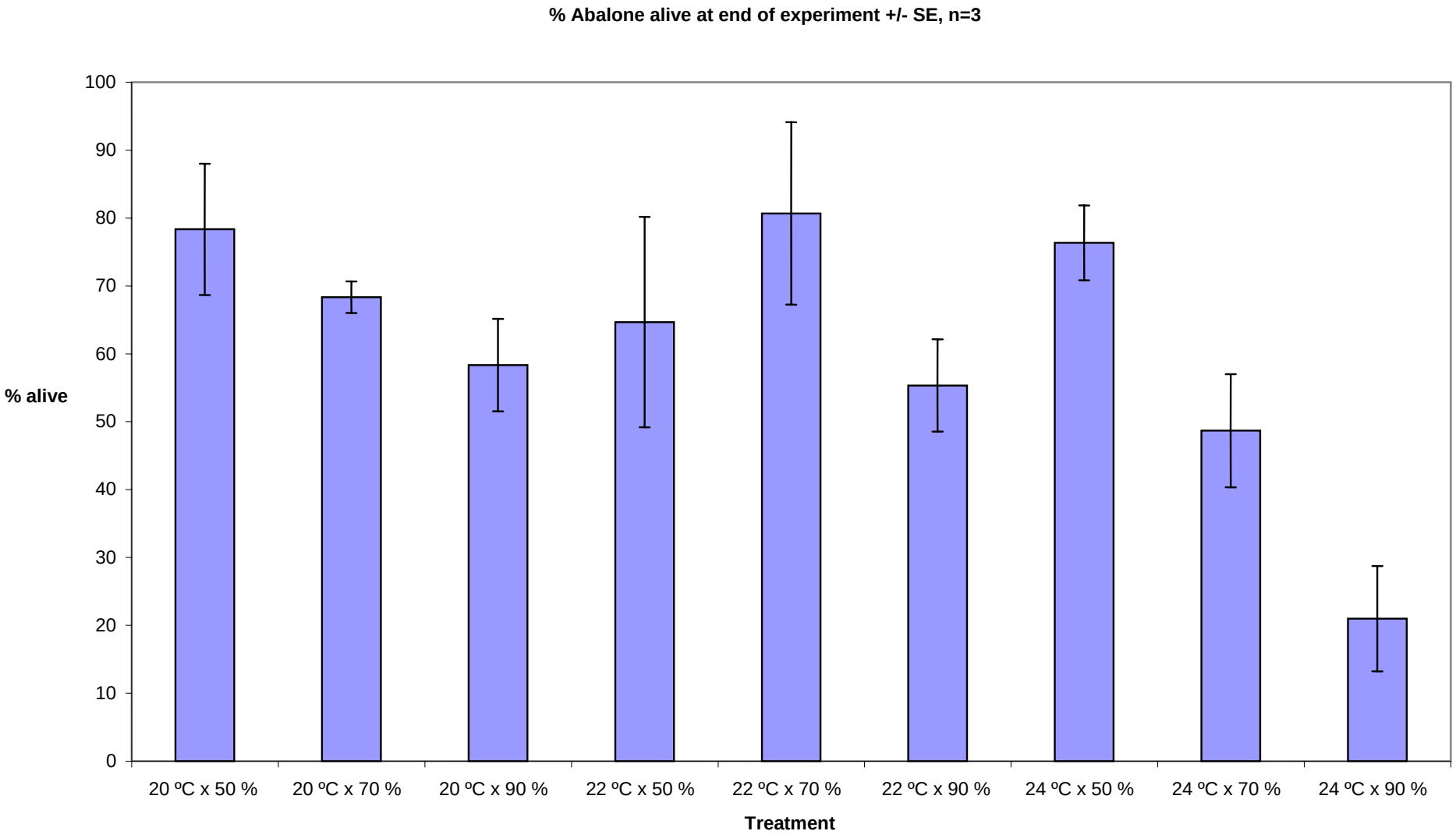


Figure 7.2-4 Percentage of abalone remaining alive in each temperature/stocking density treatment at the end of the experiment.

Outside tanks

The abalone were placed into the tanks and experiment started on the 14th of November 2002. Unfortunately due to a pipe splitting on the weekend of 16th of November resulting in no tank water the majority of the abalone died. A second lot of abalone were acquired on the 27th and the experiment restarted. It was terminated on the 17th of February 2003. Mortality and bloat for each tank treatment over time can be seen in Figure 7.2-5. Changes in ambient water temperature for both the high and low flow rate tanks are provided in Figure 7.2-6. The levels of *Vibrio* in the outside tanks outlet water over time can be seen in Figure 7.2-7.

Unlike the inside tanks, very few mortalities occurred in the outside tanks with the exception of the 15th day of December on which 7 abalone died in one of the 50% stocking rate slow flow tanks and 13 abalone died in one of the 70% stocking rates/slow flow tanks (Figure 7.2-5). All 7 of the abalone in the 50% stocked/slow flow tanks had bloat. Unfortunately as the mortalities occurred on a weekend the abalone were unable to be taken to IDEXX Veterinary Pathology Services for pathological and histological examination.

It can be seen that *Vibrio* levels ranged from as low as 0 colonies per mL for the control tanks with no abalone to as high as 11,200 colonies per mL in one of the 90% stocking density tanks. No clear pattern emerged with regard to stocking density, flow rate and *Vibrio* counts. Counts were highly variable between weeks for the same tanks.

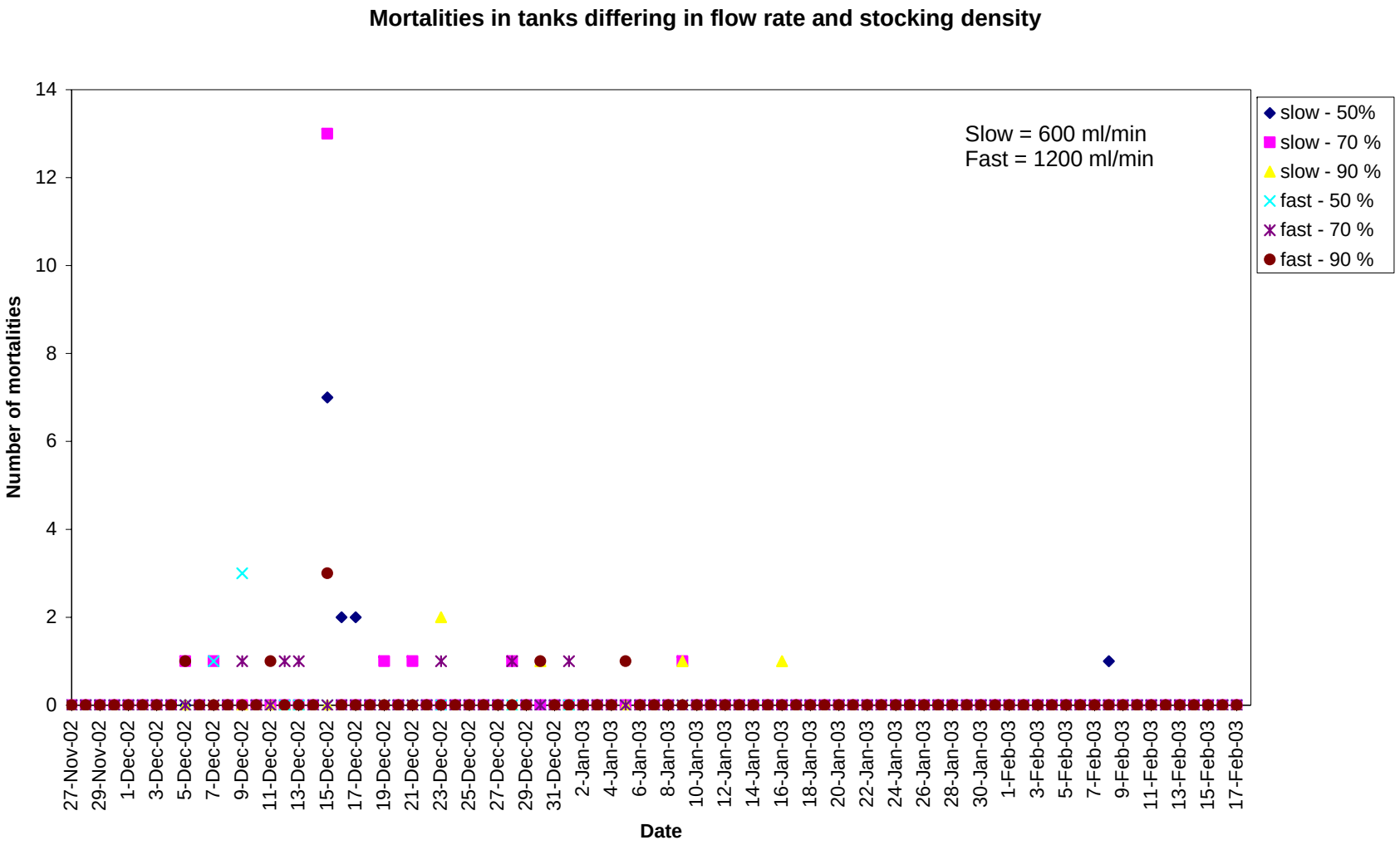


Figure 7.2-5 Total mortalities in different flow rate and stocking density combination treatments each day from 27th November to 17th February. Values are sum of mortalities from 3 replicate tanks for each treatment.

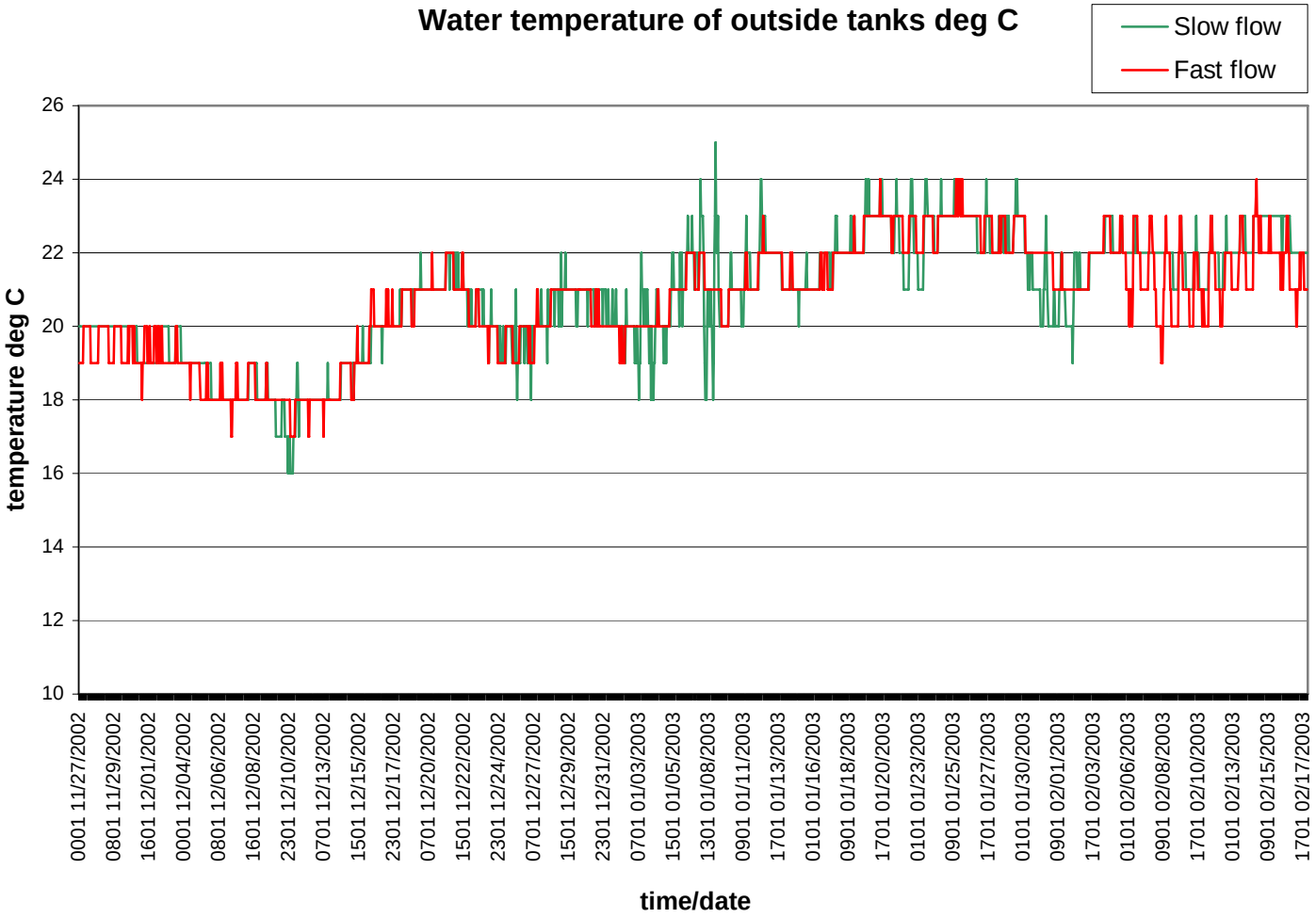


Figure 7.2-6 Water temperature of outside tanks (slow and fast flow rates) between 27th of November 2002 and 16th of February 2003.

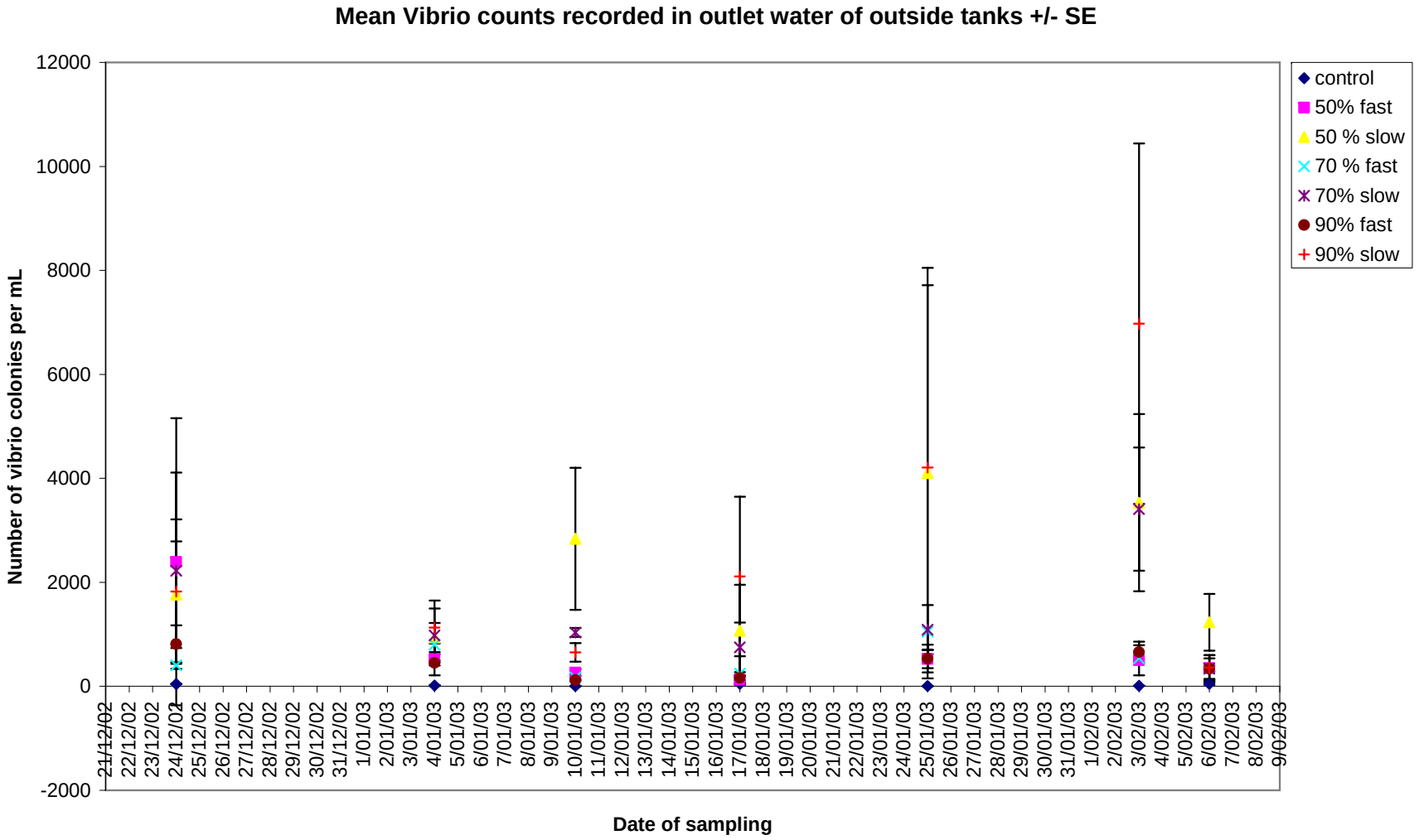


Figure 7.2-7 Mean vibrio counts recorded in outlet water of outside tanks, n=3 except for 50% fast flow on the 25/01, 6/02; 70% slow flow on the 4/01,6/02; 70% slow flow on 6/02 and 90%fast flow on the 4/01 and 25/01 where n=2 as the plates was unable to be read due to an overgrowth of colonies.

DISCUSSION

Inside tanks

The initial pathology of the abalone prior to commencing the experiments showed that they were in good health. The mortalities that occurred in the inside tanks between the 19th of November up until mid December were likely to have been caused by the high initial feeding rates and possibly stress from initial handling. For the first couple of days of the experiment the tanks were fed at 4% of the abalone's body weight. As the abalone were only fed this amount every 2nd day this equated to being fed 2% of their body weight per day. This feeding regime has been used in previous experiments with the deeper tank system. The stocking density of the deeper system has always been 10 abalone per tank (≈20% coverage). The modification of the tank system to shallow water (2.5 vs 12.5 cm deep) and higher stocking densities (50, 70 & 90% tank surface coverage compared with only 10 abalone per tank) resulted in the abalone being surrounded by and in constant contact with feed and faeces. High levels of bacteria are naturally associated with feed and faeces as they are a nutrient source and therefore the abalone would have been in close contact with bacteria. Some bacteria (ie. *Vibrio* sp.) produce cytotoxins that can disrupt the integrity of the epithelial cell membrane and may incidentally lead to considerable inflammation and necrotic change in the surrounding mucosa (Bishop, 1982). This is likely to have been the reason for the inflammation and necrosis observed in the foot of the abalone in this study. The feeding levels were reduced to 2% of body weight every 2nd day on the 19th of November 2002.

Similar pathological findings to those in this study (exfoliation of pedal, epipodial and pallial epithelium on otherwise healthy animals) and large numbers of bacteria on the surface of the foot were reported in a study investigating the morbidity of intensively cultured red abalone (Elston and Lockwood, 1983). They also attributed the mortality to *Vibriosis* – specifically due to *Vibrio alginolyticus*. Li et al. (1997) studied the histology and ultrastructure of pustule (blisters) disease in abalone *Haliotis discus hannai*. They claim the pustule forms on the surface of the foot and then extend into the foot of the infected abalone. The pustules were attributed to *Vibrio fluvialis* -II (Li et al., 1999). A study examining the extracellular products of *Vibrio fluvialis* – II found them to contain amylase, gelatinase, lipase, casease and hemolysin. They attributed the many pustules in the foot of the abalone to the enzymes produced by *V. fluvialis* II dissolving the foot tissue (Li et al., 1999).

It is not surprising that the majority of the mortalities occurred in the 90% stocking density tanks as the higher the stocking rates, the greater the amount of food and faeces in the tank and thus

the higher the bacterial load. In addition, the higher the stocking density, the closer the abalone are in contact with one another and thus the easier the transmission of bacteria. The fact that water temperature did not have a significant effect on survival indicates that despite high temperatures, mortalities could be reduced over summer by significantly reducing stocking densities. Obviously this would be dependent on whether spare tanks were available and weighing up the cost of running more tanks versus losses incurred through mortalities.

The fact that bloat occurred in just 2 abalone in the inside experimental tanks and mainly in one tank in the outside experimental tanks indicates that it is doubtful that bloat is merely a result of an interaction between the composition of the manufactured diet and warmer water temperatures. If this were so then it would be expected that bloat would be observed in more tanks and in a greater number of abalone. The occurrence of bloat in only two abalone is difficult to explain, however, similar results were recorded in the previous experiment in which only one abalone was observed to have developed bloat. Several farmers have also reported unusual occurrences of bloat whereby they have found bloat in only one tank or only in a few abalone within a tank and not in others. Proliferation of bacteria in the gut is usually dependent on the ability of the microorganisms to adhere to the epithelial cell surface. This ability is in turn dependent upon two genetically determined properties i.e. the possession by the bacterium of adhesive antigenic groupings and the presence of appropriate receptor sites on the gut epithelial cell of the host (Walker, 1982). Receptor sites on the surface of the host epithelial cell are genetically determined (at least in pigs) (Walker, 1982). It is possible that this is also the situation with abalone. If this were so then some abalone may have more receptor sites than others in their gut for the attachment of bacteria. This could explain occurrences where individual or only a few abalone within a tank develop bloat.

It is likely that bloat is caused by a change in the type (i.e. aerobic vs. anaerobic) and number of bacteria in the abalone gut. A change in bacterial type, from aerobic to anaerobic would obviously occur through a change in oxygen levels in the gut. Changes in gut oxygen levels could be caused directly by a drop in tank oxygen levels or indirectly through stasis in gut content.

Gut stasis resulting in altered gut bacterial composition and quantity in abalone could result from exotoxins produced by pathogenic bacteria such as *Vibrio*. Heavy growth of *Vibrio hollisae* was observed in the gut of the abalone that developed bloat in the previous experiment whilst heavy growth of *Vibrio alginolyticus* was observed in the gut of one of the abalone that developed bloat in this experiment. Interestingly, both of these species cause gastroenteritis in humans (Lowrie and Borneman, 1999). Some toxins produced by bacteria are able to paralyse the villi and normal

motility of the intestine is interfered with. In humans, propulsive episodes in the gut serve to repel the oral migration of the colonic bacterial flora and prevent small intestinal bacterial overgrowth (Thompson and Wingate, 1985). If normal peristalsis is inhibited a profuse bacterial flora may develop in the areas of stasis (Tabaqchali and Booth, 1985). *Vibrio cholerae* is capable of markedly altering intestinal motility in experimental animals producing bursts of irregular, caudally migrating myoelectric activity (Mathias et al., 1976). Such a change or disturbance in the gut environment would allow for the colonisation of allochthonous microbial populations that are able to survive in anaerobic conditions. Hydrogen and methane are the byproducts of anaerobic metabolism and unlike carbon dioxide which is the byproduct of aerobic metabolism, are not soluble in the tissues and thus can accumulate (Lowrie and Borneman, 1999). This may explain the development of bloat in abalone.

Gut stasis and hence change in bacterial type and quantity in the gut could also occur when abalone are anaesthesised (i.e. with benzocaine) due to the anaesthetic slowing bodily functions, such as heart pumping rate, which is believed to be associated with movement of gut contents (Edwards et al., 2003). The author has heard reports from farmers that abalone fed in the morning and then anaesthetised in the afternoon have developed bloat the next day (Jim Morrison, pers. comm.). It would be wise to ensure that whenever situations exist whereby gut motility in abalone is impeded (and thus an anaerobic condition is created in the gut) the abalone have no food in their gut as this would obviously support bacterial proliferation and potentially induce bloat.

As stated previously, change in the type (i.e. aerobic vs. anaerobic) and number of bacteria in the abalone gut may also be a result of a direct change in oxygen levels in the gut due to a decrease in tank oxygen levels with a rise in water temperature. Pump failures would further exacerbate this situation. When ambient oxygen levels drop to a critical level abalone begin to reduce their oxygen consumption linearly with oxygen content in the water (Jan and Chang, 1983). If the oxygen levels in the water drop low enough the abalone may start to undergo anaerobic respiration (Jan and Chang, 1983; Wells and Baldwin, 1995). This would result in low oxygen levels in the gut of the abalone and potentially a change in bacterial type. In addition to anaesthetics and bacterial toxins, low oxygen levels could affect heart pumping rate. A study by Nimura and Yamakawa (1989) showed how the heart rate of small abalone *Sulculus supertexta* decreased with decreasing ambient oxygen concentration. If movement of gut contents in abalone is controlled by heart pumping rate as suggested by Edwards et al. (2003) then a decrease in heart rate with decreased ambient oxygen levels could lead to stasis of gut contents, bacterial proliferation and consequently bloat.

Due to the effect of temperature on dissolved oxygen levels and the potential implication of low oxygen levels in the aetiology of bloat, greater attention should be paid to tank oxygen levels, particularly during summer.

Outside Tanks

As can be seen from Figure 7.2-6 the water temperature fluctuated from 16 to 25°C during the experimental period. In comparison to the inside tanks relatively few mortalities occurred in the outside system. The most likely reason for this is the fact that unlike the inside tanks which started off being fed at 4% of the abalone's body weight every 2nd day, and then changed to 2% of the abalone's body weight every 2nd day, the outside abalone were only ever fed at 2% of the abalone's body weight every 2nd day. This is because the outside experiment started later than the inside one and observations from the inside experiment revealed that feeding 4% of body weight every 2nd day was too high for the modified tank design. Thus the initial overfeeding in the inside tanks may have caused the greater number of mortalites in comparison to the outside tanks.

Interestingly several mortalities did occur in two tanks during the experiment, both on the same day and in one of the tanks (slow flow – 50% stocking density) all the mortalities had bloat (Figure 7.2-5). The other tank with mortalities was also a slow flow tank but with 70% stocking density. Although the flows of the tanks were monitored and adjusted to ensure they remained at the desired rates it was hard to keep them consistent as line pressure varied during each day. The variability was not consistent between tanks but varied depending where along the main line the tanks inlet was situated. Thus it is possible the reason that both of the tank mortalities occurred on the same day was due to a drop in pressure and consequently flow to these two tanks, although this was not observed at the time the mortalities were observed. Why only one of the tanks developed bloat is again uncertain and difficult to explain particularly given it was in one of the lowest stocking density tanks.

***Vibrio* levels**

Vibrio levels in open seawater are approximately 30 colonies or less per mL (Dr Jeremy Carsons, pers. comm.). The *Vibrio* levels found in the open seawater in this study are in agreement with this (see levels in outlet water of the control tanks, Figure 7.2-7). The highest level of *Vibrio* recorded in this study was 12,700 colonies per mL (90% stocking density slow flow, Figure 7.2-

7). This is comparable with levels recorded on one commercial abalone farm that reported *Vibrio* levels as high as 12,000 colonies per mL.

Due to the low number of mortalities observed and the highly variable *Vibrio* levels within tanks, no pattern could be established between *Vibrio* levels and tank mortalities. Monitoring levels of *Vibrio* per se, may not be a very useful predictor of mortality events as there are many species of *Vibrio* and not all are pathogenic, the majority probably living in harmony with the abalone. The *Vibrio* species that was isolated in high numbers in bloated abalone in this experiment was *Vibrio alginolyticus*. This does not necessarily mean that *V. alginolyticus* was the cause of death/bloat in the abalone. To determine this the *V. alginolyticus* isolated from the dead abalone would need to be cultured and inoculated into healthy abalone to see if they developed the same symptoms. It should be mentioned however that *V. alginolyticus* has been attributed to pathogenesis of red abalone (Elston and Lockwood, 1983) and the South African abalone (Dixon et al., 1991) and does occur in the alimentary tracts of marine animals (Dixon et al., 1991). In addition, Dixon et al. (1991) reported that the digestive gland of the abalone became distended as was found in this current study. It was hypothesised by Dixon et al. (1991) that the stressful conditions of capture and transport and the forced weaning onto a new diet combined to create suitable condition for the infection of the abalone by *V. alginolyticus*. *V. alginolyticus* readily colonises the substratum, which allows for intimate contact with the foot of the abalone. It also rapidly colonises the musculature of the foot if there is any injury to the integument. Once the foot is invaded the bacteria colonise the vascular system and the nerve fibre sheaths (Dixon et al., 1991). Based on the above facts it is likely that *V. alginolyticus* was responsible for the deaths and bloat observed in the abalone in this experiment.

7.3 Experiment 2.2: National abalone farm summer mortality survey

AIMS

The aim of the national abalone farm summer survey was to gain a greater understanding of the level of mortality on commercial abalone farms over summer and the factors that contribute to it by getting farmers to answer various questions regarding feeding, cleaning, tank flow rates, etc and to obtain data on tank temperatures and mortalities over the summer.

METHODS

A questionnaire (see Excel and Word files in Appendix 3) was sent out to 10 abalone farms around Australia to be filled out over the summer. Each farm was contacted prior to sending out the survey to explain its nature and ensure they were prepared to be involved. The survey contains questions regarding tank feeding and cleaning regimes, stocking densities and flow rates. It also asked the farmer to keep a record of tank temperatures, mortalities and bloat for November, December, January, February and March.

RESULTS/DISCUSSION

Of the 10 farms that were sent the survey only 3 completed and returned the forms. The data is summarised in Tables 7.3-1, 7.3-2 & 7.3-3. One of the farms averaged 30% mortality in all 12 of the tanks (4 replicates of 3 size classes) it monitored from the 19th of November till the 13th of March. Temperature on this farm ranged from 17 – 25ºC. No bloat was observed during that time in any of the tanks. The total mortality for the 12 tanks was 4,128 abalone.

The second farm had much lower levels of mortality, generally below 1% for all of the 12 tanks monitored between the 28th November and the 3rd of February. Temperature was lower, ranging from 16 – 22.3ºC. Some mudworm was observed and 46 abalone developed bloat. Of the abalone that developed bloat 45 were from the 8 mm weaning size class. The majority of the deaths were also from this size class (471). The total mortality for the 12 tanks monitored on this farm was 737 abalone.

The respondent from the second farm provided a list of observations regarding bloat occurrences on their farm. They reported that with regard to abalone less than 15 mm in size, held in round weaning tanks, they tend to congregrate around the top of the standpipe and a small percentage will crawl out daily. These abalone usually get bloat. Also, if the water flows are

low, abalone will congregate under the spray bar where water flow is highest, and due to low water movement over them, they can become loose and turn upside down. These abalone may develop bloat if not replaced into an area of adequate water supply. Apparently abalone held in pipetanks (15-45 mm in length) rarely get bloat, except under the following circumstances:

- If tanks become overstocked, food or silt that is not cleaned out will decompose between cleaning periods and abalone that eat this can develop bloat.
- Pipes are cleaned up to one hour. During this time at least some of the abalone are out of water as not all of the pipe is completely full during cleaning. If the air temperature is over 25°C and tanks are not flushed regularly, abalone can be exposed to the air for long periods and bloat can occur.

The third farm in general had low levels of mortality (< 2%) in each of its tanks, however it did experience high mortalities in 3 tanks (14, 11 and 51%). These could be attributed to events whereby water quality was affected, including 2 incidences of oxygen supersaturation and one incidence where there was a drop in water flow causing the abalone to be exposed. In addition, this farm reported that due to uneven pouring of some of its concrete tanks one of the tanks (the one with 51% mortality) was replaced as the uneven surface created 'dead patches' in the tank where no water exchange occurred. Only 3 incidences of bloat were recorded. The temperature on this farm ranged from 17 – 22.3ºC. Its total mortality for all 12 tanks was 14,303 abalone.

Two of the farms that did not return the survey said their reason for doing so was because of the low level of mortality they experienced during the survey period. One of the farms said that although it was a very hot summer for them, much hotter then last year, and despite having much higher stocking densities and anaesthestising the abalone during warm days, they had much lower mortalities than previous years. They attributed this to a change in farm site. This new site apparently has significantly less silt and suspended solids. They also changed their tank design, which although similar in shape to their old design has a new non porous surface made from epoxy resin. Apart from the reduced levels of suspended solids and new tank surface all other factors on this farm remained the same (i.e. same feeding regime, same diets, same cleaning regime).

The other farm that did not return the survey because of low summer mortalities during the survey period previously experienced very high levels of mortality and bloat in its juvenile abalone (< 1 year old). They attributed their significantly reduced levels of mortality during the survey period to the fact that the juvenile abalone were much bigger this year at the start of

summer compared with previous years. The abalone were removed off the plates at a larger size (12 mm this year compared with 8 mm in previous years). Apart from that all other factors on the farm were the same as in previous summers (i.e. same stocking densities and flow rates, same temperature, same diets, same amount of silt). They did however report that their cleaning regime was poorer than last year. They did experience one incidence of bloat which they attributed to human error causing food to be left in the tanks for much longer than normal.

Table 7.3-1 Summary data from farms for 20-35 mm size class abalone

	Farm 1	**Farm 2**	**Farm 3**
Monitoring period	7th Nov 02 – 12th March 03	19th Nov 02 - 13th March 03	28th Nov 02 – 16th March 03
Species	Greenlip	Greenlip	Blacklip
System	Slab	Raceway	Pipetank
Stocking density at start of monitoring period (% tank surface area covered)	40 %	70%	65 %
Ave stocking density at end of monitoring period (% tank surface area covered)	70 % (one tank 50 %)	50%	Not stated
Flow rate	108-192 l/min	13.75 l/min	43 l/min
Amount Fed	1 % BW/day	2 % BW/day	3 % BW every 2nd day
Frequency of feeding	6 days per week	6 days per week (not fed when temp > 24 °C)	Every 2nd day
Temperature range	17.0-22.3°C	16.5-25°C	16.5-22°C
Temperature average	19.7°C	20.9°C	19.4°C
Mortality (total for 4 tanks)	7064	1496	29
Ave mortality per tank	One tank with 4672 morts the others averaged 797	374	7
Bloat occurrence (total for 4 tanks)	0	0	0

Table 7.3-2 Summary data from farms for 40-55 mm size class abalone

	Farm 1	**Farm 2**	**Farm 3**
Monitoring period	7th Nov 02 – 12th March 03	19th Nov 02 - 13th March 03	29th Nov 02 – 15th March 03
Species	Greenlip	Greenlip	Blacklip
System	Slab	Raceway	Pipetank
Ave stocking density at start of monitoring period (% tank surface area covered)	40% (one tank 75%)	75-80 %	50 %
Ave stocking density at end of monitoring period (% tank surface area covered)	70 %	55%	Not stated
Flow rate	144-222 l/min	13.25 l/min	43 l/min
Amount Fed	1 % BW/day	2 % BW/day	3 % BW every 2nd day
Frequency of feeding	6 days per week	Every day except Sunday & when temp > 24 °C	Every 2nd day
Temperature range	17.0-22.3°C	16.5-25°C	17-21.7°C
Temperature average	19.7°C	20.9°C	19.5°C
Mortality (total for 4 tanks)	2469	1281	238
Ave mortality per tank	One tank with 1616 morts, the others averaged 284	320	60
Bloat occurrence (total for 4 tanks)	1	0	1

Table 7.3-3 Summary data from farms for 60-80 mm size class abalone, except Farm 3 which monitored 8mm (6 month old) abalone

	Farm 1	**Farm 2**	**Farm 3**
Monitoring period	7th Nov 02 – 12th March 03	19th Nov 02 - 13th March 03	28th Nov 02 – 3rd Feb 03
Species	Greenlip	Greenlip	Blacklip
System	Slab	Raceway	Round weaning tank (2 m diameter)
Ave stocking density at start of monitoring period (% tank surface area covered)	44 %	92.5%	10 %
Ave stocking density at end of monitoring period (% tank surface area covered)	58 %	65%	Not stated
Flow rate	126-168 l/min	12.5 l/min	5.5 l/min
Amount Fed	1 % BW/day	2 % BW/day	Water temp < 19°C = 1 % BW/day Water temp >19°C = 0.5 % BW/day
Frequency of feeding	6 days per week	Every day except Sunday & when temp > 24 °C	Fed daily
Temperature range	17-22.3°C	16.5-25°C	16.0-22.3°C
Temperature average	19.7°C	20.9°C	19.1°C
Mortality (total for 4 tanks)	4770	1351	470
Ave mortality per tank	1192	338	118
Bloat occurrence (total for 4 tanks)	2 (from 1 tank)	0	45
Ave no of bloated abalone per tank	<1	0	11

7.4 Abalone Water Quality Manual

AIMS

The original focus of the project was to investigate the influence of diet on mortality and/or bloat, as nutrition was implicated as a causal factor. However, based on the results from laboratory experiments, survey reports and conversations with different farmers about mortalities on their farms during the course of the project, it became apparent that many mortality events over summer were more likely to be linked to water quality and tank husbandry issues than nutritional composition of manufactured diets per se. It is likely that manufactured diets probably contribute to mortality and bloat, not through a negative interaction between their component ingredients and the abalones' gut, but merely by encouraging microbial growth as they are highly dense sources of nutrients. Subsequently, it was viewed that the focus of the project should change from nutrition to water quality.

Whilst several studies have been conducted to investigate abalones' response to various water quality parameters, the findings have been published in a range of formats including journal articles, proceedings of workshops and books. As such, the information is not very accessible to farmers. It was agreed, with endorsement by the Abalone Aquaculture Subprogram Leader, that it would be beneficial to summarise this information in a manual that could be used by farmers. The aims of the manual were to provide farmers with;

- An understanding of the effect of water quality on abalone health;
- An understanding of abalone's tolerance and limits to various water quality parameters;
- An understanding of which water quality parameters are important to measure and how they should be measured.

RESULTS

See Appendix 5.

CONCLUSION

The major water quality parameters on farms that can affect abalone health, and that farmers need to be aware of, include dissolved oxygen, ammonia, bacteria, total dissolved gases, water temperature, suspended solids, salinity and pH. Of the water quality parameters, only oxygen, ammonia, bacteria and total dissolved gases can be partially managed within current abalone farming systems whilst water temperature, suspended solids, salinity and pH are beyond the capacity of current farm systems to control.

With regard to temperature, the optimum level for growth of both greenlip and blacklip abalone has been determined as well their upper temperature tolerances. It should be pointed out however, that the interaction between water temperature and other water quality parameters is not known. As water temperature increases, the amount of dissolved oxygen present in water decreases and yet abalones' requirement for oxygen increases due to increased metabolic rate. In addition, the proportion of total ammonia that exists in the toxic un-ionised form (NH_3) as opposed to the ionised from (NH_4^+) also increases at elevated water temperatures. Thus, it is possible the upper temperature tolerances of both greenlip and blacklip abalone may be lower than those values reported in the literature. Further studies are required to investigate the interaction between water temperature and other water quality parameters and how they affect abalone health. Another compounding factor is that studies on red abalone, *H. rufescens,* have shown that the optimal temperature for growth changes with size. This may also be true for the Australian species and also requires investigation as it is likely that if their optimal temperature for growth changes with size, then so will their tolerance levels to different water quality parameters.

The effect of oxygen and ammonia on abalone performance have also been investigated. The results from the oxygen requirement study indicated that the growth potential of abalone will not be achieved in conditions that are at less than 100% saturation. This is particularly important during periods of elevated water temperature when high growth rates can be achieved to maximize farm productivity. When interpreting the data on the effects of oxygen and ammonia on growth and survival, farmers' need to take into account that the research was conducted at water temperatures that were optimal for growth of *H. laevigata* (mean 17.8 – 18.3 ºC). Greater growth reductions and mortality impacts would be expected as water temperatures rise to those experienced by farms during summer, therefore the information from these studies is probably conservative and should only be used as a guide.

Whilst no investigations have been done to investigate the effect of sediment level in the water and abalone health, it should be kept in mind that bacteria, including pathogenic species, attach to particles. Thus when oceanic waters are stirred up and high silt levels come through in the inlet pipes it is likely that bacterial levels will increase. Higher levels of attention may need to be paid to cleaning tanks and ensuring no feed or faeces are lying around during these times.

Obviously farmers will need to record some baseline measurement to determine if their water quality is within the ranges reported for optimum growth of abalone. There is a range of equipment available to do this ranging from simple and inexpensive to sophisticated automated instruments that can be programmed to log recordings at set time intervals. It is recommended that periodic intensive sampling be conducted to identify the variability in water quality and the most important times to sample. Routine monitoring should then be set up based upon the outcomes of the intensive investigation. As a minimum, it is recommended that 2 or more representative tanks for each size category of abalone across the farm be routinely monitored. Influent and effluent DO, water temperatures, feed inputs, uneaten feed and mortality should be recorded.

Whilst temperature, suspended solids, salinity and pH are beyond the capacity of current farm systems to control, there are steps that can be taken to improve water quality on farms. Some recommendations include:

- Assessing the potential benefits of an oxygenation system to operate during periods of elevated water temperature.
- Reviewing recommendations for optimal stocking density during the most stressful times of the year. This should be done for each type of system used and each size class stocked in order to provide the best available water quality during periods of elevated water temperature.
- Exploring options to increase water flow after feeding (i.e. at night).
- Increasing attention to farm hygiene particularly during summer including disinfection of cleaning brooms between tanks; daily removal of mortalities, uneaten feed and faeces, and consideration of the use of water brooms or other alternatives.
- Reducing stocking density through selective harvest of larger animals leading up to summer and restocking of smaller cohorts before summer.

- Adopting night feeding or delayed feed addition (6 - 8 pm) and feed at 2 - 4 day intervals during periods of elevated water temperature.
- Monitoring uneaten feed to adjust feeding to minimum inputs particularly over summer.

Recommendations for future research on the effects of water quality on abalone health include:

- Conducting trials on commercial abalone farms to measure potential benefits from improved water treatment i.e. mechanical filtration (silt removal) and disinfection (lower levels of incoming bacteria).
- Investigating the effects of interactions of major water quality parameters (i.e. dissolved oxygen and ammonia) at elevated water temperatures similar to those experienced over summer.
- Measuring the interaction of oxygen consumption rate and elevated water temperature for a range of size classes of greenlip and blacklip abalone.
- Measuring the metabolic oxygen demand for a range of size classes of greenlip and blacklip abalone over a range of water temperatures.
- Investigating the relationship between temperature preference and size class of abalone.
- Measuring mantle cavity circulation flow rates against external water velocity for both greenlip and blacklip. If possible measure water quality parameters sampled from within the mantle cavity.
- Investigating the use of multiple inlet locations on culture units to increase turbulence and water velocity along the full length of raceways, slabs and troughs.
- Investigating further tank design improvements that combine high water velocity systems to improve mantle cavity flow, variable flow rate systems to allow feed addition.
- Investigating the use of recirculating aquaculture systems to culture abalone in a controlled environment.
- Determining size class and stocking density relationships for abalone grown at elevated water temperatures.
- Designing and assess non-physical cleaning methods to remove waste and uneaten feed to provide added bacterial control/management over summer.
- Determining an effective measure of stress in abalone to detect the point of increase at elevated water.
- Assessing the effect of immune enhancing feed additives such as probiotics over the summer period.

8. GENERAL DISCUSSION

Elevated mortality levels are regularly observed by many Australian abalone farmers over the summer period. Sometimes during these mortality events farmers report that the abalone have swollen abdomens which they have termed 'bloat'. No pattern has been observed in these incidences of bloat that provide an indication to any underlying cause. They do not occur with every mortality event, nor do all abalone in a tank develop bloat. At the time of conception of this project many farmers believed bloat was due to an interaction between components of manufactured feeds and the elevated water temperatures. As such this project was developed to investigate the interaction between nutrition and water temperature.

It became apparent early on in the project that that mortality and bloat in abalone is not simply a result of an interaction between water temperature and diet. Mortality was difficult to induce even after elevating the water temperature to 26°C, the upper limit of abalone's tolerance level. This is further supported by the observation of one of the farms that participated in the monitoring survey that although they had a very hot summer, much hotter than the previous year, they had much lower mortalities than previous years.

Repeating the mortality experiment with increased stocking density and water flow manipulation, as recommended by the Abalone Subprogram, produced mixed results. Whilst mortalities were observed in the inside tanks across all treatments, mortalities were only observed in two of the outdoor tanks (deaths occurring on the same day). The occurrences of bloat were equally as inconsistent with only two abalone from separate tanks developing it in the inside tank, and all the mortalities in one of the outside tanks developing it. Farmers have reported similar observations of only one or a few isolated incidences of bloat in tanks. As stated in section 7.2, proliferation of bacteria in the gut is usually dependent on the ability of the microorganisms to adhere to the epithelial cell surface. This ability is in turn dependent upon the presence of appropriate receptor sites on the gut epithelial cells of the host. Thus it is possible some abalone have more receptor sites on the surfaces of their gut cells than others and thus are more prone to bloat than others.

Based on the mortalities that occurred in the second inside experiment where temperature and stocking density were manipulated, it is clear that stocking density has a greater affect on mortality than water temperature. Stocking density was found to significantly affect abalone survival whilst temperature did not. The higher the stocking density, the greater the amount of

manufactured feed that each tank will require and the greater amount of waste that will be in each tank. This correlates to a much higher nutrient loading in the tank compared with other tanks with lower stocking density. As a result it would be expected that bacterial numbers (including pathogenic bacteria) would be greatest in the higher stocking density tanks. In addition, the potential for disease transmission would be much greater because the abalone are in closer proximity to each other. Thus, destocking tanks is one way farmers can try and reduce mortality. Whether this is economically viable needs to be assessed by individual farms with potential losses due to abalone deaths weighed up against the costs of destocking.

Whilst water temperature per se will not directly cause mortality (unless outside an animal's physiological tolerance limits) as observed in this study, elevated temperatures, in addition to high stocking density, promote bacterial growth. For example, the optimum temperature for the growth of most *Vibrio* is in the 20-45°C range. Thus, when water temperatures start approaching 20°C on farms, bacterial levels will start increasing. Although farmers have little ability to manipulate water temperature, keeping tanks clean as possible and removing all uneaten feed as well as faeces will help reduce bacterial growth as it is removing sources of nutrients that bacteria need to grow.

It should be kept in mind that although the presence of a pathogen is required for disease to occur, disease rarely results from simple contact between the host (abalone) and potential pathogen. Factors such as excessive crowding, high water temperatures and poor water quality not only promote bacterial growth but they also cause stress to the abalone. Stress events are usually present before animals become sick. Stress triggers a chain of events within animals such as:

- Increases in blood sugar. Stored sugars, such as glycogen are metabolized. This creates an energy reserve which prepares the animal for an emergency action.
- Osmoregulation is disrupted because of changes in mineral metabolism. This disruption requires that extra energy be used to maintain osmoregulation.
- Respiration increases, blood pressure increases,

Thus, as abalone try to adjust to the stress imposed upon them they use up energy reserves. During this period they may look and act normal. If the stress is not removed, their energy reserves become depleted and they become "exhausted." At this phase their ability to resist disease organisms, with which they are in constant contact, is severely compromised and subsequently they may become sick or die. Incidences of stress events on farms that have

resulted in abalone mortalities and bloat include oxygen supersaturation and reduced water flow as reported in the farm survey responses (Section 7.3). Control or correction of stressors on farms is essential if farmers wish to reduce incidences of mortality and bloat. For this reason it is critical that farmers monitor water quality parameters, particularly during summer, and ensure they fall within optimum levels.

In conclusion, mortality/bloat events on farms are likely to be a result of a complex interaction between abalone, their environment, and pathogens. It is possible to have pathogens present but for abalone not become sick or die providing their environment is optimal and they are not under any stress such as physical (handling) or chemical (water quality). At the same time, it is also possible to have poor water quality but for abalone not to become sick if there is no pathogen present. For example, one farm reported in their response that although they had a very hot summer, much hotter then the previous year, and despite having much higher stocking densities and anaesthestising the abalone during warm days, they had much lower mortalities than previous years. The only thing that was different was that they had changed their farm site and the new site apparently had significantly less silt and suspended solids. It is possible the reduction in mortality was due to the fact that bacterial levels in their tanks had dropped owing to the significantly less silt and suspended solids in their water, even despite the fact there was equivalent or greater stress to the abalone than at the previous site. It is the combination of presence of pathogens and stress caused by poor environmental conditions that will result in mortality. Thus, the key to reducing or controlling mortality on farms is to keep stress and bacteria levels (potential pathogens) to a minimum. This can achieved by ensuring water quality, as outlined in the manual in this report, is maintained at all times.

9. BENEFITS AND ADOPTION

Groups that will benefit from this research and the benefits they will gain are:

1. Abalone growers

Benefits:
1) Reduction in summer mortality of abalone and thus an increase in the number of abalone surviving to market size. This is through a greater awareness of how water quality affects abalone health, knowledge of abalone's tolerance and limits to various water quality parameters and knowledge on how to monitor water quality.

2. Abalone diet manufacturers

Benefits:
1) An increase in sale of manufactured diets as a consequence of the decrease in abalone mortality on farms.

10. FURTHER DEVELOPMENT

To gain a better understanding of the water quality on farms, in particular, the degree to which it fluctuates, the author sees benefit in establishing ongoing water quality assessment programs on farms for at least one complete year. This would help to establish the variability in water quality on farms throughout the year and whether a decline in water quality over summer is responsible for the increased mortality on the farms.

In conjunction with water quality monitoring on farms, farm based experiments that investigate the effects of stocking density on tank mortality levels over summer may also help to determine the effect of stocking density on mortality and what rate to stock at to significantly reduce mortality levels.

In addition, the effectiveness of various immune enhancing dietary additives could be assessed. Abalone that have been fed diets containing commercially available immune enhancing additives could be subjected to physiological challenges (i.e. heat stress combined with *Vibrio* sp) and various immune parameters measured (i.e. antimicrobial activity). Comparison of the immune

system in the abalone fed the control diets (no additives) against those fed the dietary additives this would establish whether the various additives available are of any benefit to abalone health.

11. PLANNED OUTCOMES

The original planned outcomes for the project included:

1. A cost-effective manufactured abalone feed that maintains abalone health and survival during periods of elevated water temperature;
2. A range of ingredients suitable for use in manufactured abalone feeds that maintain abalone health and intestinal condition;
3. Identified feed additives that increase abalone resilience to environmental stresses;
4. An understanding of the interactions between abalone health, water temperature, stress and nutrition and remedial actions to optimise health and production efficiency.

Due to the change in the objectives of the project the outputs and planned outcomes differ from those in the original proposal. The new planned outcomes include:

1. An understanding of the effect of water quality on abalone health
2. An understanding of the abalone's tolerance and limits to various water quality parameters
3. An understanding of which water quality parameters are important to measure and how they should be measured.

The production of the water quality manual has addressed these planned outcomes.

12. CONCLUSION

This project was initiated in 2001 due to commercial abalone farmer's concerns about high levels of mortality over the summer months. At the time abalone bloat was often associated with the mortalities with reports of dead abalone floating in tanks with swollen abdomens. Nutrition was implicated as a possible causal factor of the mortalities and so the initial objectives were focused on the interaction between nutrition and mortality/bloat as outlined below:

1. Induce abalone mortality and/or bloat under experimental conditions to ensure a "control" exists for subsequent experiments.

2. Examine the interaction between high levels of fermentable carbohydrate and temperature on abalone growth rates, mortality and haemocyte counts.
3. Define nutritional treatments that may alleviate the effects of increased water temperature on abalone mortality including extrusion of dietary ingredients and immune enhancing diet additives such as antioxidants and mannan oligosaccharides.
4. Apply the results of experiments 2 and 3 to black lip abalone.

Mortality and/or bloat were unable to be induced in the first experiment and therefore this experiment was repeated with modifications in place of the second objective. Based on recommendations by abalone farmers after presentation of the results at the 9th Annual Abalone Aquaculture Workshop the experiment was altered to include stocking density and water flow manipulation. This involved an inside and outside tank system. Mortalities were observed in the inside tanks across all treatments, although predominantly in the 90% stocking density tanks, however, only two abalone were observed to develop bloat. In comparison mortalities were observed in only two of the outdoor tanks (deaths occurring on the same day) and in one of the tanks all the mortalities had bloat.

In addition to the experiment a mortality survey was sent out to commercial abalone farms to complete over the summer period. Only 3 farms completed the survey that was sent out. Several farms reported that their reason for not filling out the survey was due to the fact that they no longer had a bloat/mortality problem. When asked what they thought had changed to cause this reduction in bloat/mortality the reasons that were cited were 1) A change in farm site and tank design, the new site apparently having significantly less silt and suspended solids. 2) The other farm use to experienced very high levels of mortality and bloat in its juvenile abalone (< 1 year old). They attributed their significantly reduced levels of mortality experienced this summer to the fact that they removed the juvenile abalone off the nursery plates at a much larger size than in previous years (12 mm compared to 8 mm). This farm also reported that all other factors on their farm had remained the same as in previous summers (i.e. same stocking densities and flow rates, same temperature, same diets, same amount of silt). Of the farms that did not formally take part in the survey, tank mortality levels ranged from < 1% to as high as 50%. Bloat was observed on one farm in abalone that were exposed to air due to a drop in water flow. Other factors that contributed to mortalities on this farm were 2 occurrences of oxygen supersaturation and poor water quality due to uneven tank surfaces.

The original focus of the project was about investigating the influence of diet on mortality and/or bloat because nutrition was implicated as a causal factor. However, based on the

experiments, survey reportings and numerous conversations with different farmers about abalone mortalities on their farms during the course of this project, it became apparent that many of the mortality events over summer were likely to be linked to water quality and tank husbandry issues. These included factors such as interruption to tank water flow, oxygen supersaturation events, food being left too long in tanks etc, rather than nutritional composition of manufactured diets per se. Manufactured diets probably contribute to mortality and bloat through merely being excellent sites for bacterial growth, this being a particular issue in summer due to the warmer water temperatures resulting in elevated bacterial levels and more rapid break down of the diets. Given that the abalone are under multiple stresses during summer, especially in terms of water temperature and bacterial challenge, then any trigger event such as cessation in water flow, drop in oxygen level etc. is likely to result in mortalities. For this reason, extra diligence should be paid to water quality and tank husbandry.

Phone calls were made to several farmers asking questions in regard to their knowledge of water quality requirements for abalone, with the majority reporting they did not know oxygen or ammonia upper and lower limits. Based on the above it was decided, with endorsement from the Abalone Aquaculture Subprogram leader, that the project's planned outcomes be changed to:

1. An understanding of the effect of water quality on abalone health
2. An understanding of the abalone's tolerance and limits to various water quality parameters
3. An understanding of which water quality parameters are important to measure and how they should be measured.

To meet these outcomes it was viewed that the remaining objective of the project be changed to the production of a water quality manual for abalone farmers. Although several studies have been conducted and published on the effects of different water quality parameters on abalone growth and survival they are scattered throughout different scientific papers and theses which are not easy to access by farmers and also not published in a simple, easy to read style as has been done for fish. The water manual for abalone produced as part of this project discusses water quality monitoring on abalone farms, provides a review of the effects of major water quality factors on farmed abalone including temperature, dissolved oxygen, ammonia, total dissolved gases and hydrogen sulphide, and discusses options for improvement of water quality on farms.

13. REFERENCES

Apud, F., J. H., Primavera & P. L Torres. 1983. Farming of Prawns and Shrimps. Aquaculture Department, SEAFDEC, Tigbauan, Iloilo, Phillipines, Extension Manual No. 5, 3rd edition. p. 59.

Bishop, R.F. 1982. Spectrum of infectious agents in acute diarrhoea. In: Gastroenterology 2. Small Intestine. V.S. Chadwick & S.F. Phillips (eds.). Butterworth Scientific. pp. 319 – 331.

Dixon, M.G., T. Hecht & C.R. Brandt. 1991. Identification and treatment of a Clostridium and *Vibrio* infection in South African abalone, *Haliotis midae* L. *Journal of Fish Diseases.* 14: 693-695

Edwards, S., D. Geraghty & J. Ralph. 2003. Pharmalogical and histological assessment of gut muscle movement in blacklip abalone, *Haliotis rubra* (Leach). *Aquaculture Research.* 34: 383-388.

Elston, R. & G. S. Lockwood. 1983. Pathogenesis of *vibriosis* in cultured juvenile red abalone, *Haliotis rufescens* Swainson. *Journal of Fish Diseases.* 6: 111-128.

Gilroy, A. & J. S. Edwards. 1998. Optimum temperature preference for growth of Australian abalone: preferred temperature and critical thermal maximum for blacklip abalone, *Haliotis rubra* (Leach), and the greenlip abalone, *Haliotis laevigata* (Leach). *Aquaculture Research.* 29: 481-485.

Hindrum, S. M., C. M. Burke, S. J. Edwards & D. R. Johns. 2001. Effects of combined exposure to elevated ammonia and low dissolved oxygen levels in greenlip (*Haliotis laevigata* Donovan) and blacklip (*H. rubra* Leach) abalone. 1. Growth and mortality data from simulated systems failure. *Journal of Shellfish Research.* 20 (1): 679-684.

Hood, M., G.E., Ness, G.E. Rodrick and N.J. Blake. 1984. The ecology of *Vibrio cholerae* in two Florida estuaries. *Vibrios* in the environment. R.R. Colwell (ed.). John Wiley & Sons, USA. pp. 398-409.

Huq, A., E.B. Small, P.A. West and R.R. Colwell. 1984. The role of planktonic copepods in the survival and multiplication of *Vibrio cholerae* in the aquatic environment. *Vibrios* in the environment. R.R. Colwell (ed.). John Wiley & Sons, USA. pp. 521-534.

Jan, R. &. K. Chang. 1983. The oxygen consumption by formosan abalone, *Haliotis diversicolor supertexta* Lischke, during decline of ambient oxygen. *Bull. Inst. Zool., Academia Sinica.* 22(1): 43-48.

Jan, R., K. Shao & K. Chang. 1981. A study of diurnal periodicity in oxygen consumption of the small abalone (*Haliotis diversicolor supertexta* Lischke). *Bull. Inst. Zool., Adademia Sinica.* 20(1): 1-8.

Julian, D., W. E. Dalia, & A. J. Arp. 1998. Neuromuscular sensitivity to hydrogen sulfide in the marine invertebrate *Urechis caupo*. *The Journal of Experimental Biology.* 201: 1393-1403.

Kaneko, T. & R.R. Colwell. 1973. Ecology of *Vibrio parahaemolyticus* in Chesapeake Bay. *Bacteriol.* 113: 24-32.

Kaneko, T. & R.R. Colwell. 1978. The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. *Microbial Ecology*. 4: 135-155.

Langdon, J. 1998. Investigation of fish kills: I. History and Causes. Fish Diseases. Refresher Course for Veterinarians. Proceedings 106, 23-27 May, 1998. p. 179.

Li, T., J Xiang., R. Liu, M. Ding, P. Shi & S. Wang. 1999. Studies on bacteriophage control pustule disease of abalone *Haliotis discus hannai*. *Oceanologia et Limnologia Sinica*. 30(4): 374-380.

Lowrie and Borneman, 1999. A survey of marine microbes. http://www.animalnetwork.com/fish2/aqfm/1999/may/features/1/default.asp

Madigan, S., D. Lehmann, & S. Clarke. 2000. Determination of critical thermal maximum for four species of South Australian abalone; *Haliotis laevigata*, *H. scalaris*, *H. roei* and *H. cyclobates*. A report by the South Australian Research and Development Institute. 13 pp.

Mathias, J.R., G.M. Carlson, A.J. DiMarino, G. Bertiger, H.E. Morton & S. Cohen. 1976. Intestinal myoelectric activity in response to live *Vibrio cholera* enterotoxin. *J. Clin. Invest.* 58: 91-96.

Nair, G.B., B.L. Sarkar, S.P. De, M.K. Chakrabarti, R.K. Bhadra and S.C. Pal. 1988. Ecology of *Vibrio cholerae* in the freshwater environs of Calcutta, India. *Microbial Ecology*. 15(2): 203-215.

Nimura, Y. & H. Yamakawa. 1989. Oxygen uptake rate and heart rate of small abalone (*Sulculus supertexta*) as related to the ambient oxygen concentration. *Nippon Suisan Gakkaishi*. 55: 1869.

Tabaqchali, S. & C. Booth. 1985. Bacterial Overgrowth. In: Disorders of the small intestine. C. Booth & G. Neale (eds.). Blackwell Scientific Publications. pp249-298.

Thompson, D.G. & D.L. Wingate 1985. Motility and its disorders. In: Disorders of the small intestine. C. Booth & G. Neale (eds.). Blackwell Scientific Publications. pp 78-91.

Russell, C. W. & B. K. Evans. 1989. Cardiovascular anatomy and physiology of the black lip abalone (*Haliotis ruber*). *J. Exp. Biol.* 252:105-117.

Walker, P.D. 1982. Interactions among micro-organisms and the host. In: Gastroenterology 2. Small Intestine. V.S. Chadwick & S.F. Phillips (eds.). Butterworth Scientific. pp. 142 – 161.

Wells, R.M.G. & J. Baldwin. 1995. A comparison of metabolic stress during air exposure in two species of New Zealand abalone, *Haliotis iris* and *Haliotis australis*: implications for the handling and shipping of live animals. *Aquaculture*. 134: 361-370.

14. APPENDIX 1: Intellectual property

The intellectual property developed in this project is shared between the South Australian Research and Development Institute and the Fisheries Research and Development Corporation as defined in section of C7 of the project application.

15. APPENDIX 2: Staff

Principal Investigator: Meegan Vandepeer

Co-investigators: Dr Robert van Barneveld

Production of water quality manual: Wayne Hutchinson

16. APPENDIX 3: Summer Mortality Survey Forms

Preventing summer mortality of abalone in aquaculture systems by understanding interactions between nutrition and water temperature

Project Investigators: Meegan Vandepeer & Robert van Barneveld

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Abalone Health Survey

Objectives: This survey is part of the FRDC summer mortality project managed by the Abalone Aquaculture Subprogram. The aim of the survey is to obtain as much information as possible on the mortality/bloat problems experienced by farms over the coming summer period. By gathering information from as many farms as possible on stocking densities, water flow rates, temperature, feeding rates and mortality it is expected that a greater understanding of the causal factors responsible for the problem of summer mortality on farms will be identified. This will provide us with valuable information that can be used to direct our future research efforts.

Survey details: The survey consists of a series of questions to be filled out daily over November, December, January, February and March. The daily questions for each tank include information on:

- tank temperature,
- number of mortalities,
- number of animals with bloat,
- whether the tank was cleaned,
- whether the animals were fed, and
- tank silt level.

In addition there is a summary sheet assigned to each tank regarding the abalone size, species, tank flow rate, tank size etc. Although it would be ideal for every tank on the farm to be monitored, I realise this is not practically possible. Instead, the survey requests that details be recorded for 12 tanks on each farm over the summer period. These 12 tanks will include 4 replicate tanks from three animal size classes (20-35, 40-55 & 60-80 mm) to be picked at random by the farms.

Additional Information

The survey requests that details be filled out daily for 12 tanks over the summer period, however, farmers will obviously be monitoring all their tanks over this time. I would appreciate it if farms could notify me by phone, fax or email (see above) if they experience mortalities or bloat in any other tanks providing details such as:

- Whether the mortalities/bloat occurred in all size class or just one size class
- Whether they were isolated to only one or two tanks or many tanks
- If they were in just one or two tanks, what is peculiar to those tanks, ie,. were they fed a different diet?, were they shifted recently?, did they have higher stocking densities than other tanks?, did they have a different flow rate? etc.

Abalone Pathology

If farms send sick or dead abalone to a pathologist for examination, I would also appreciate it if a copy of the pathology results be sent to me. As *Vibrio* has been hypothesised as one of the possible causes of summer mortality it would be useful for the examination to include analysis of the bacterial levels and main bacteria type (to species level) in the gut, on the foot and also in the haemolymph of the abalone. A list of the fish pathology labs in each state is provided below:

South Australia

IDEXX Veterinary Pathology Services
33 Flemington St
Glenside SA
Ph (08) 8372 3777
Free Call 1800 882 515
(Contact person – Peter Philips)

Tasmania

Aquaculture & Fisheries Institute Fish Health Unit
Kings Meadows TAS
Ph (03) 6336 5389
(Contact person – Judith Handlinger)
Email: Judith.Handlinger@dpiwe.tas.gov.au

Victoria

Natural Resources and Environment Unit
Victorian Institute of Animal Science
475-485 Mickleham Road,
Attwood VIC 3049
(Contact person - Malcolm Lancaster)
Head, Disease Investigations
Email: malcolm.lancaster@nre.vic.gov.au
Ph: 03 9217 4200 Fax: 03 9217 4199

Or

Alistair Brown
Aquatic Veterinary Services
69 Harrison St
East Brunswick VIC 3057
ph: 03 93998199 or A/H 03 93860783
mobile: 0438 356153

Western Australia

Fisheries Department of Western Australia
Fish Health Section
GPO Box 1400
Perth WA 6001
(Contact person - Brian Jones)
Senior Fish Pathologist

Ph: 08 9368 3649 Fax: 08 9474 1881

Email bjones@agric.wa.gov.au

Confidentiality of information

The information provided in these surveys by farms will remain confidential to the project's principal and co-investigator and the Subprogram leader. No information will be released regarding farm identities and the mortalities experienced by individual farms.

Farm Questionnaire - FRDC Summer Mortality Project (please fill out a separate sheet for each of the 12 tanks)

1. Tank ID:
2. Abalone Size Class - please circle (20-35mm) (40-55mm) (60-80mm)
3. Abalone age:
4. Abalone Species:
5. At the start of data recording what % of tank surface area was stocked:
5. At the end of data recording (March 2003) what % of tank surface area was stocked:
6. Number of abalone in tank:
7. Indoor or outdoor tank:
8. Type of tank (ie slab, raceway, pipe, maze etc) & tank dimensions & volume of water:

9a. Tank flow rate (litres/sec):

9b. Tank exchange rate (time it takes for total volume of tank to be replaced):

10. Diet type (size and brand) that abalone were fed on during the time of recording measurements, please state if you changed diets and what diet you changed to:

11. How is your food stored? (ie in a refrigerated room, tin shed, in the light or in the dark) How hot does the room get where it is stored? How long is the food stored for?

12. For those that have used antibiotic feed during incidences of mortalities was it effective in clearing up/reducing your mortalities?

13. Feeding Regime for tank. How much were the abalone fed per day (% of body weight)? How often were the abalone fed?
(ie If you fed at 2% of their body weight per day did you feed every day or did you feed every three days and give them enough food each time that it equated to them being fed at 2% per day.

*****_The information provided on this sheet will remain confidential to the project investigators and Abalone Subprogram leader. No data will be published relating farm names and abalone mortalities over summer. Once completed please return the sheets to Meegan Vandepeer c/o SARDI Aquatic Sciences Centre, PO Box 120, Henley Beach, SA 5022. Please call me on (08) 8200 2466 if you have any queries. Thankyou!_

FRDC Summer Mortality Project - *One of these sheets needs to be filled out for each of 12 tanks (4 replicate tanks chosen at random for three different size classes of abalone (20-35, 40-55 & 60-80mm). Once completed please return the sheets to Meegan Vandepeer c/o SARDI Aquatic Science Centre, PO Box 120, Henley Beach SA 5022. If you have any queries please phone me on (08) 8200 2466. Thankyou.*

TANK ID [ ]

Date	**Tank temp** degrees C	**No of abs in tank with bloat**	**No of dead abs** (bloat plus others)	**If you cleaned the tank this day put a "C"**	**If the tank was fed this day put a "F"**	**Tank Silt level (High, Medium, Low or none)**
01-Nov-02						
02-Nov-02						
03-Nov-02						
04-Nov-02						
05-Nov-02						
06-Nov-02						
07-Nov-02						
08-Nov-02						
09-Nov-02						
10-Nov-02						
11-Nov-02						
12-Nov-02						
13-Nov-02						
14-Nov-02						
15-Nov-02						
16-Nov-02						
17-Nov-02						
18-Nov-02						
19-Nov-02						
20-Nov-02						
21-Nov-02						

17. APPENDIX 4: Pathology results from IDEXX

From: Ruth Reuter 08 8372 3700 To: Fax#08 8200 2481 Date: 2/07/2002 Time: 2:16:42 PM Page 1 of 1

IDEXX VPS VET LAB

IDEXX VETERINARY PATHOLOGY SERVICES

PO BOX 445
GLENSIDE SA 5065
Phone: 08 8372 3700
Fax: 08 8372 3777

VET: VANDEPEER
OWNER: SARDI
CASE #:

CLINIC: SARDI AQUATIC SCIENCES
GAS ABALONE Y
RECEIVED: 27/06/2002 PRINTED: 02/07/02

CLINIC NO: A309*
ANIMAL ID:
LAB NO: V202681

HISTORY: Held at 22° C for 2 + month. This one was on one commercial diet, changed to another brand about 2 weeks ago. Semolina was base – not digested. Rich in nutrient. About 30% crude protein. ?Soluble fibre/mps. Slow down in gut movement. Bloat – gas bubbles in gut. Float off side of tank.

GROSS PATHOLOGY: The specimen submitted consisted of one abalone with apparent gas bubble formation in the intestine. Cut sections of abalone were taken for histopathology.

HISTOPATHOLOGY: Sections of the abalone exhibited marked dilation of vessels, with oedema of the adjacent connective tissue. The gut sections were dilated and mostly empty of food, but filled with large numbers of mixed bacteria. Collections of bacteria were also present on and under the surface epithelium, and in clumps through the foot muscle.

DIAGNOSIS: Gut dilation, oedema.

COMMENTS: Although there were large numbers of bacteria present, and the gut epithelium had detached, the remainder of the tissue appeared well preserved, suggesting that this was not due to post mortem change. A range of bacteria was seen. Special stains are being done to identify the types if possible, and a further report will follow when these have been completed.

The large numbers of bacteria on the surface of the animal would suggest that the bacterial count in the water was also high.

Ruth E. Reuter
DVM, Dip Vet Path, Dip ACVP, PhD, MACVSc
Specialist Veterinary Pathologist

VET: VANDEPEER
OWNER: SARDI
CASE #:

CLINIC: SARDI AQUATIC SCIENCES
GAS ABALONE Y
RECEIVED: 27/06/2002 PRINTED: 01/07/02

CLINIC NO: A309*
ANIMAL ID:
LAB NO: V202681

MICROBIOLOGY

SPECIMEN: Needle Aspirate Fluid (From gut)

GRAM: No bacteria seen.

CULTURE: Moderate growth of gram negative bacilli [1].

FURTHER TO PREVIOUS REPORT: Isolate [1] has been identified as *Vibrio hollisae*.

This is a final result.

SENSITIVITY	1	2	3	4
Penicillin				
Ampicillin				
Amoxycillin				
Methicillin				
Clavulox	S			
Cloxacillin				
Ticarcillin				
Erythromycin				
Tylosin				
Tilmicosin				
Lincomycin				
Clindamycin				
Lincospectin	R			
Neomycin	R			
Streptomycin				
Apramycin				
Gentamicin				
Framycetin				
Tetracyclines	R			
Doxycycline	R			
Chloramphenicol	S			
Co-trimoxazole	S			
Su/Trimethoprim				
Polymyxin				
Novobiocin				
Cephalothin				
Enrofloxacin				
Other f-quinolones				

IDEXX VETERINARY PATHOLOGY SERVICES

VET LAB

PO BOX 445
GLENSIDE SA 5065
Phone 08 8372 3700
Fax 08 8372 3777

VET: VANDEPEER
OWNER: SARDI
CASE #:

CLINIC: SARDI AQUATIC SCIENCES
GAS ABALONE Y
RECEIVED: 27/06/2002 PRINTED: 05/07/02

CLINIC NO: A309*
ANIMAL ID:
LAB NO: V202681

FURTHER TO PREVIOUS REPORT

HISTORY: Held at 22° C for 2 + month. This one was on one commercial diet, changed to another brand about 2 weeks ago. Semolina was base – not digested. Rich in nutrient. About 30% crude protein. ?Soluble fibre/mps. Slow down in gut movement. Bloat – gas bubbles in gut. Float off side of tank.

GROSS PATHOLOGY: The specimen submitted consisted of one abalone with apparent gas bubble formation in the intestine. Cut sections of abalone were taken for histopathology.

HISTOPATHOLOGY: Sections of the abalone exhibited marked dilation of vessels, with oedema of the adjacent connective tissue. The gut sections were dilated and mostly empty of food, but filled with large numbers of mixed bacteria. Collections of bacteria were also present on and under the surface epithelium, and in clumps through the foot muscle.

DIAGNOSIS: Gut dilation, oedema.

COMMENTS: Although there were large numbers of bacteria present, and the gut epithelium had detached, the remainder of the tissue appeared well preserved, suggesting that this was not due to post mortem change. A range of bacteria was seen. Special stains are being done to identify the types if possible, and a further report will follow when these have been completed.

The large numbers of bacteria on the surface of the animal would suggest that the bacterial count in the water was also high.

Ruth E. Reuter
DVM, Dip Vet Path, Dip ACVP, PhD, MACVSc
Specialist Veterinary Pathologist

LAB NO: V202681

ADDENDUM 5/07/2002:

Special stains have now been completed on this animal. The bacteria seen were a mixture of Gram negative rods, which varied in morphology from smaller bacilli suggesting *Vibrio* species, to thin, long filamentous forms suggesting *Flexibacter* organisms.

Flexibacter has been associated with disease, and has been seen on occasion in abalone. It can cause problems in water temperatures as low as 15° C, however overcrowding, low dissolved oxygen, high organic loads and temperatures above 20° C tend to be ideal for this organism. It can be a difficult organism to culture, however I believe a molecular probe has been developed by Jeremy Carson. It might be of value to discuss this with him in association with your studies., to see what involvement, if any, these bacteria have with the bloating problem.

Ruth E. Reuter
DVM, Dip Vet Path, Dip ACVP, PhD, MACVSc
Specialist Veterinary Pathologist

VET: VANDEPEER
OWNER: SARDI
CASE #:

CLINIC: SARDI AQUATIC SCIENCES
GAS ABALONE Y
RECEIVED: 27/06/2002 PRINTED: 29/06/02

CLINIC NO: A309*
ANIMAL ID:
LAB NO: V202681

MICROBIOLOGY

SPECIMEN: Needle Aspirate Fluid

GRAM: No bacteria seen.

CULTURE: Moderate growth of gram negative bacilli [1].

COMMENT: Further identification and sensitivities to follow.

SENSITIVITY	1	2	3	4
Penicillin				
Ampicillin				
Amoxycillin				
Methicillin				
Clavulox				
Cloxacillin				
Ticarcillin				
Erythromycin				
Tylosin				
Tilmicosin				
Lincomycin				
Clindamycin				
Lincospectin				
Neomycin				
Streptomycin				
Apramycin				
Gentamicin				
Framycetin				
Tetracyclines				
Doxycycline				
Chloramphenicol				
Co-trimoxazole				
Su/Trimethoprim				
Polymyxin				
Novobiocin				
Cephalothin				
Enrofloxacin				
Other f-quinolones				

DS

IDEXX LABORATORIES

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www.idexx.com.au

ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES **Clinic No:** A0309
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5 **Species:** Other
Date Received: 15/11/02 **Date Printed:** 18/11/02 **Lab Number:** 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
1+ Gram negative bacilli
CULTURE
Light growth of a *gram negative organism.* [1]

bar Sample ID - Abalone 4
Isolate [1] as abalone 1.
Further identification in progress.
INTERIM REPORT.
Daniela Signoriello. Microbiologist.

Laboratory Locations

ADELAIDE:
33 Flemington Street
Glenside SA 5065
PO Box 445
Glenside SA 5065

BRISBANE:
3 Overend Street
East Brisbane QLD 4169
PO Box 1119
Coorparoo DC QLD 4151

MELBOURNE:
Unit 124, 45 Gilby Road
Mt Waverley VIC 3149
Locked Bag 15
Mt Waverley VIC 3149

SYDNEY:
The Metro Centre
Unit 20, 38-46 South Street
Rydalmere NSW 2116
PO Box 227
Rydalmere NSW 2116

IDEXX ADELAIDE 08 8372 3777 P.3

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ABN 31 063 154 332

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 18/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
2+ Gram negative bacilli
CULTURE
Light growth of a gram negative organism. [1]

Sample ID - Abalone 3
Isolate [1] as Abalone 1.
Further identification in progress.
INTERIM REPORT.
Daniela Signoriello. Microbiologist.

Laboratory Locations

ADELAIDE:
33 Flemington Street
Glenside SA 5065
PO Box 445
Glenside SA 5065

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ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 18/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
No bacteria seen.
CULTURE
Light growth of *a gram negative organism.* [1]

Abalone - 2
Isolate [1] as Abalone 1.
Further identification in progress.
INTERIM REPORT.
Daniela Signoriello. Microbiologist.

Laboratory Locations
ADELAIDE:
33 Flemington Street
Glenside SA 5065
PO Box 445
Glenside SA 5065

BRISBANE:
3 Overend Street
East Brisbane QLD 4169
PO Box 1119
Coorparoo DC QLD 4151

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www.idexx.com.au

ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 18/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
No bacteria seen.
CULTURE
A few colonies of *a gram negative organism.* [1]

Sample ID -Abalone 1 Further identification and sensitivities in progress.
INTERIM REPORT.
Amir Al-Obaidi (Microbiologist)

Laboratory Locations
ADELAIDE:
33 Flemington Street
Glenside SA 5065
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ies Tue 19 Nov 2002 01:57:18 PM CST Page 1 of 1

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www.idexx.com.au

ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 19/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
No bacteria seen.
CULTURE
A few colonies of *a gram negative organism.* [1]
----------ANTIMICROBIAL SENSITIVITY REPORT----------

	Isolate 1
tracycline	R

Sample ID -Abalone 1 Further identification in progress.
INTERIM REPORT.
Amir Al-Obaidi (Microbiologist)
Corrected: 19/11/2002 11:51 SUSCEPT: : A gram negative organism. » New result(s)

18 Nov 02 06:43p IDEXX ADELAIDE 08 8372 3777 p.1

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ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES **Clinic No:** A0309
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5 **Species:** Other
Date Received: 15/11/02 **Date Printed:** 18/11/02 **Lab Number:** 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
1+ Gram negative bacilli
CULTURE
Bacteria not isolated after 24 or 48 hours.

Sample ID - Abalone 5
FINAL REPORT.
Daniela Signoriello. Microbiologist.

18 Nov 02 06:43p IDEXX ADELAIDE 08 8372 3777 p.2

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ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 18/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
1+ Gram negative bacilli
CULTURE
Light growth of *a gram negative organism.* [1]

Sample ID - Abalone 4
Isolate [1] as abalone 1.
Further identification in progress.
INTERIM REPORT.
Daniela Signoriello. Microbiologist.

18 Nov 02 06:43p IDEXX ADELAIDE 08 8372 3777 p.3

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www.idexx.com.au

ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 18/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
2+ Gram negative bacilli
CULTURE
Light growth of a *gram negative organism.* [1]

Sample ID - Abalone 3
Isolate [1] as Abalone 1.
Further identification in progress.
INTERIM REPORT.
Daniela Signoriello. Microbiologist.

Laboratory Locations

ADELAIDE:
33 Flemington Street
Glenside SA 5065
PO Box 445
Glenside SA 5065

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Rydalmere NSW 2116

18 Nov 02 06:43p IDEXX ADELAIDE 08 8372 3777 p.4

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www.idexx.com.au

ABN 31 063 151 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 18/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
No bacteria seen.
CULTURE
Light growth of *a gram negative organism.* [1]

Abalone - 2
Isolate [1] as Abalone 1.
Further identification in progress.
INTERIM REPORT.
Daniela Signoriello. Microbiologist.

18 Nov 02 06:44p IDEXX ADELAIDE 08 8372 3777 p.5

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www.idexx.com.au

ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 18/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
No bacteria seen.
CULTURE
A few colonies of *a gram negative organism.* [1]

Sample ID -Abalone 1 Further identification and sensitivities in progress.
INTERIM REPORT.
Amir Al-Obaidi (Microbiologist)

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www.idexx.com.au

ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES **Clinic No:** A0309
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5 **Species:** Other
Date Received: 15/11/02 **Date Printed:** 19/11/02 **Lab Number:** 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
No bacteria seen.
CULTURE
A few colonies of *a gram negative organism.* [1]

----------ANTIMICROBIAL SENSITIVITY REPORT----------	
	Isolate 1
.tracycline	R

Sample ID -Abalone 1 Further identification in progress.
INTERIM REPORT.
Amir Al-Obaidi (Microbiologist)
Corrected: 19/11/2002 11:51 SUSCEPT: : A gram negative organism. » New result(s)

Laboratory Locations

ADELAIDE:
33 Flemington Street
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Rydalmere NSW 2116
PO Box 227

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www.idexx.com.au

ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVI **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 21/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
No bacteria seen.
CULTURE
A few colonies of *Vibrio sp.* [1]

----------ANTIMICROBIAL SENSITIVITY REPORT----------

	Isolate 1
etracycline	R

Sample ID -Abalone 1
Isolate [1] was H2S negative.
FINAL REPORT.

Results to Megan Vandepeer. Results to Megan Vandepeer.

Laboratory Locations

ADELAIDE:
33 Flemington Street
Glenside SA 5065
PO Box 445
Glenside SA 5065

BRISBANE:
3 Overend Street
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PO Box 227

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ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 21/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
No bacteria seen. →
CULTURE
Light growth of *Vibrio sp.* [1]

Abalone - 2
ate [1] as Abalone 1. – H2S negative

FINAL REPORT.
Daniela Signoriello. Microbiologist.
Attention Results to Megan Vandepeer.

IDEXX
LABORATORIES

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ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 21/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
2+ Gram negative bacilli
CULTURE
Light growth of *Vibrio sp.* [1]

Sample ID - Abalone 3
ate [1] as Abalone 1. H_2S negative

FINAL REPORT.
Daniela Signoriello. Microbiologist.
Attention results to Megan Vandepeer.

IDEXX
LABORATORIES

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lab–melbourne@idexx.com

ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVI **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 21/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
1+ Gram negative bacilli
CULTURE
Light growth of *Vibrio sp.* [1]

Sample ID - Abalone 4
ate [1] as abalone 1. H2S negative

FINAL REPORT.
Daniela Signoriello. Microbiologist.
Attention Results to Megan Vandepeer.

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ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 21/11/02

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
1+ Gram negative bacilli
CULTURE
Bacteria not isolated after 24 or 48 hours.

Sample ID - Abalone 5
AL REPORT.
Daniela Signoriello. Microbiologist.

Laboratory Locations

ADELAIDE:
33 Flemington Street
Glenside SA 5065
PO Box 445
Glenside SA 5065

BRISBANE:
3 Overend Street
East Brisbane QLD 4169
PO Box 1119
Coorparoo DC QLD 4151

MELBOURNE:
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Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 14/01/03 **Date Printed:** 15/01/03

Clinic No: A0309
Species: Other
Lab Number: 03 - 1012411

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
Occasional Leucocytes
Occasional Gram positive cocci
CULTURE
Bacteria not isolated after 24 hours.

Further incubation in progress.
INTERIM REPORT.

Amir Al-Obaidi Microbiologist

Laboratory Locations

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Glenside SA 5065

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Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVIC **Animal ID:** ABALONE 5
Date Received: 14/01/03 **Date Printed:** 17/01/03

Clinic No: A0309
Species: Other
Lab Number: 03 - 1012411

MICROBIOLOGY

SPECIMEN: Fish
GRAM STAIN
Occasional Leucocytes
Occasional Gram positive cocci
Occasional Gram negative bacilli
CULTURE
eavy growth of *Vibrio alginolyticus* [1]

----------ANTIMICROBIAL SENSITIVITY REPORT----------

	Isolate 1
Tetracycline	R

FINAL REPORT.

Amir Al-Obaidi Microbiologist

Laboratory Locations

ADELAIDE:
33 Flemington Street
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From IDEXX Laboratories Tue 18 Feb 2003 05:05:28 PM CST Page 1 of 1

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ABN 31 063 154 352

Veterinarian: SARDI AQUATIC SCIENCES
Clinic: SARDI AQUATIC SCIENCES
Owner: SARDI AQUATIC SERVI **Animal ID:** ABALONE 5
Date Received: 15/11/02 **Date Printed:** 18/02/03

Clinic No: A0309
Species: Other
Lab Number: 02 - 1005926

Histopathology Report Form LAB-76 Issued: 05.12.97 Revision: 0 Page 1 of 1

HISTORY: As per original attachment.

GROSS PATHOLOGY: Cassette A 1, Cassette B 2, Cassette C 3, Cassette D 4, Cassette E 5.

HISTOPATHOLOGY: Abalone 1: The intestine of this animal contained scattered small, occasionally-bent, gram-negative bacil. here was no associated inflammation.

Abalone 2: Bacteria were not detected in the intestine of this animal.

Abalone 3: **Low numbers of gram-negative bacilli were present within the intestine of this animal. There was no associated inflammation.**

Abalone 4: Bacteria were not detected in the intestine of this animal.

Abalone 5: Low numbers of gram-negative bacilli were detected in the intestine of this animal. There was no associated inflammation.

COMMENTS: Megan, Sorry these have taken so long to get to you. They got put on the back burner while urgent work was done, but then got buried until just recently when I rediscovered them! I hope this sort of report is what you were looking for. Peter P.

Peter Phillips BVSc (Hons) PhD Veterinary Pathologist

18. APPENDIX 5: Water Quality: Effects and management on abalone farms

WATER QUALITY: EFFECTS AND MANAGEMENT ON ABALONE FARMS

Wayne Hutchinson and Meegan Vandepeer

SARDI Aquatic Sciences
Publication Number RD02/0035-3

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

PRIMARY INDUSTRIES AND RESOURCES SA

Government of South Australia

Australian Government
Fisheries Research and Development Corporation

WATER QUALITY: EFFECTS AND MANAGEMENT ON ABALONE FARMS

Wayne Hutchinson

Meegan Vandepeer

SARDI Aquatic Sciences

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Approved by: John Carragher

Signed: 

Date: September 2004
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Circulation: Public Domain

CONTENTS

SECTION 1: WATER QUALITY MONITORING ON ABALONE FARMS

1.1 WATER QUALITY AND GROWTH

To date efforts to improve growth of cultured abalone has concentrated on nutrition and the production of artificial feeds that fulfill the requirements of abalone and have physical attributes suited to farming systems. It is possible that sub-optimal water quality conditions within current abalone farming systems may also affect the growth performance of cultured animals as in some instances the growth rates reported in wild abalone are greater than those achieved in farming systems (Shepherd and Triantafillos, 1997; Haaker *et al*, 1998). Faster growth rate of wild abalone is suggested to be due to natural environmental conditions and diet availability not yet provided in farming systems (Steinarsson and Imsland, 2003).

A number of reviews and specific papers have been prepared dealing with the effects of water quality parameters on green-lip abalone, *Haliotis laevigata*, and black-lip abalone, *Haliotis rubra* (Freeman, 2001; Harris, 1999a and 1999b; Huchette *et al*, 2003; Gilroy and Edwards 1998; Hahn, 1989). Of relevance to management on commercial farms are the potential effects of these parameters on growth and survival of cultured animals, and the ability of seawater supply systems to minimise potential problems that may be associated with water quality.

Major water quality parameters can only be partially managed within current abalone farming systems. These parameters include:

- Dissolved oxygen
- Ammonia
- Bacteria
- Total dissolved gases

Other parameters that interact with water quality, or cause direct effects on abalone, that are beyond the capacity of current farm systems to control include:

- Water temperature
- Suspended solids
- Salinity
- pH

1.2 TREATMENT OF SEAWATER SUPPLIES TO ABALONE FARMS

All abalone farms visited, and others known to the authors, use unfiltered raw seawater for all on-growing operations. Apart from coarse screens (15 – 20mm mesh) on each seawater intake pipe, no mechanical filtration or other water treatments are undertaken before delivery to

culture tanks. Additional filtration using combinations of sand filters, cartridge filters and ultraviolet disinfection is only used for seawater delivered to hatchery and nursery sections on farms.

1.3 WATER QUALITY MONITORING ON FARMS

Firstly it is important to identify the purpose of water quality monitoring on commercial abalone farms. Monitoring water quality should not be confused with monitoring of system operation that is undertaken to provide alarms in the event of malfunction, breakdown or interruptions to power supply etc. Water quality monitoring should be undertaken routinely from strategic locations across production facilities in order to:

- Monitor performance of the water supply and distribution system with respect to provision of optimal water quality to stock.
- Identify seasonal and diurnal (i.e. daily) trends and separate these from fluctuations within the system that may be the result of other changes that can be explained.
- Assess benefits of changes to system operation or husbandry practices.
- Assist farm operators to identify potential problems.

Monitoring of water quality parameters is generally regarded as essential for aquaculture operations and frequency and the scope of monitoring activities usually increase in importance as the intensity of stocking of culture systems increases.

Farms visited recorded relatively little water quality data on a routine basis and those that did failed to present these data in a format that allowed easy evaluation of emerging long term (i.e. seasonal or annual) or short term (i.e. daily/diurnal) trends. Although some water quality monitoring does occur, records are mostly stored in hard copy format and are not routinely entered onto electronic databases that can be readily used for matters that may help improve farm operations and/or identify problems. It is clear that a usable water quality monitoring and reporting system would benefit abalone farm management and allow analysis of trends between environmental parameters, growth, mortality and feed usage to be undertaken to improve farm operations and reduce production costs.

Typically water quality monitoring concentrates on variable parameters that may change as a result of stocking, feeding, environmental changes and water supply. These include dissolved oxygen (DO), ammonia, suspended solids, pH and carbon dioxide. The concentrations of these parameters may change quickly and a routine monitoring program will be required to detect these changes.

1.3.1 Scale of monitoring activities

Ideally a water quality monitoring program should commence as a series of intensive sampling programs designed to identify water quality variables that are changing within the culture system. These changes may be:

Spatial

These include changes that occur within culture units (i.e. tanks, troughs or raceways) or between different sections of the farm; or

Temporal

These include changes that occur across a period of time (i.e. seasonal or daily changes).

Identification of the type of water quality changes occurring within a farm will require a series of 24 hour programs that involve sampling at short time intervals (i.e. every 1-2 hours) from a number of similar tanks across the farm, sampling of selected tanks for each stock category, and sampling at a number of locations within each tank. This intensive sampling program should reveal variations occurring within production tanks throughout a typical day attributed to factors such as feeding activity and diurnal variability of the incoming water supply.

As a minimum this intensive water quality monitoring should incorporate:

- Continuous (i.e. at least every hour) logging of DO and water temperature of the seawater supply at a central location before distribution across the farm.
- Two hourly recording of influent (inflow) and effluent (outflow) DO, water temperature, pH and ammonia taken from selected tanks.

After the intensive program has been completed a more routine program can be devised that targets the parameters identified as being significant and variable. This monitoring program should also ensure that samples are taken at times that reflect maximum or minimum levels of these parameters that may impact upon stock within the culture system (i.e. DO and ammonia are likely to be critical in early the hours of the morning due to the effects of night time feeding and/or night time respiration by abalone, algae and bacteria).

1.3.2 Routine water quality monitoring

Routine water quality records should be taken in order to compile a data set for each farm. Due to the large number (i.e. 100's) of tanks used for abalone farming it does not appear practical to monitor all tanks so selection of representative tanks will need to be considered. It is suggested that records should be maintained for 3 or more representative tanks for each stock category of abalone across the farm. These records should include:

- Daily measurement of inlet and outlet DO, water temperature and pH.
- Weekly sampling of ammonia levels and pH in the early morning and mid-afternoon of a day when feeding occurs. Although more frequent sampling may be beneficial, the time required and reagent costs will prohibit more frequent monitoring unless a problem is revealed that requires more intensive investigation.
- Weekly measurement of the water flow rate entering each monitored tank.

A sample water quality monitoring sheet is provided (Appendix 1) that may be modified to suit specific requirements of individual farms.

To assist interpretation of water quality data other records should be kept that monitor stock within tanks and husbandry practices undertaken. These records may include:

- A record of the initial tank stocking density and total number of animals.
- Daily count of mortalities with provision to deduct these from the initial count of stocked animals.
- Daily recording of the amount and timing of feed inputs and the amount of uneaten feed remaining from the previous feeding.
- Routine *Vibrio* species counts from the seawater supply.
- Monthly sub-sampling of tanks from each stock category to estimate growth of abalone and feed utilisation efficiency.

It is critical that these data be interpreted routinely for any benefit to be realised. To allow this, these data need to be transferred routinely (i.e. daily or weekly) from farm water quality records sheets to a database maintained on a computer. This data needs to be subsequently compiled into a descriptive report, preferably in a graphical format. This report should be presented to those responsible for farm management in a format that allows visualisation and comparison of parameters recorded. This could be achieved using an MS Excel® based spreadsheet or one of the more specialised commercial software products available (e.g. Abalone Assist®) for farm record keeping and management. Regardless of the data base used, it is important for farm managers to delegate data collection and compilation responsibilities to reliable staff and take the time to consult the compiled data.

1.4 WATER QUALITY MONITORING METHODS

There is a wide range of options for testing water quality parameters available for use on abalone farms. Types of testing devices include colorimetric methods, electronic and non-electronic instruments.

1.4.1 Non-electrical instruments

Manual measurement of some water quality parameters can be undertaken using non-electronic devices such as thermometers, salinity refractometers and hydrometers.

1.4.2 Colorimetric methods

Water analysis products based upon colorimetric methods are available in kit form that provide rapid analysis of a range of selected parameters without the need for knowledge of analytical chemistry. These test kits use colour change as a result of a chemical reaction to indicate the concentration of the desired water parameter. A range of portable water quality test kits developed for, or transferable to, the aquaculture industry use some form of colorimetric method, including:

1.4.2.1 Test strips

Test strips are available for a range of parameters including pH, hardness, nitrate, nitrite and chlorine. Test strips are simply dipped in the water sample and indicate concentration by comparison of final colour to a set of colour standards. These strips offer advantages of convenience and simplicity and are useful to identify concentration to a scale of resolution required to quickly test parameters. Cost per test is relatively high which may preclude routine use for large numbers of samples.

1.4.2.2 Test kits

Single parameter test kits containing packaged reagent buffers and catalysts are available for a wide range of water quality parameters (e.g. ammonia, nitrite, nitrate, pH, chlorine, iron, copper). After addition of prepared reagents to the water sample the concentration of the parameter tested is determined by comparison of the final colour of the sample solution with a colour chart or comparator device provided. Test kits are produced by companies such as Hanna, Merck, Hach, YSI, Lovibond and LaMotte.

1.4.2.3 Multiple parameter kits with spectrophotometers

More sophisticated kits include packaged reagents and a spectrophotometer device to provide more precise measurement of the concentration of many parameters. Spectrophotometers are microprocessor controlled and are programmed with procedures and time sequences for each test. Examples of these test kits include Merck Spectroquant™, Hach Portable

Spectrophotometer, Hanna Instruments Photometer, YSI Photometers and LaMotte Electronic Aqualab.

Spectrophotometry is widely used for analysis of the concentration of chemicals and is based upon the absorption of light by a coloured solution. For water analysis, reagents are added to the sample that react specifically with the chemical being measured. A spectrophotometer is used to measure the colour intensity of the final solution. Within the spectrophotometer the transmission of monochromatic light is set at the wavelength at which light is most strongly absorbed by the chemical being measured. The principle followed is that the intensity of transmitted light decreases with increasing concentration of a coloured solution. Generally two samples are measured for each water sample with one designated as the test sample and the other used as a blank to which all reagents are added for the test except the one required to produce the colour used for measurement of the chemical being tested. This allows the colour or turbidity in the test sample to be accounted for when determining the final concentration. Modern water analysis kits that include a portable spectrophotometer include timed programs, alarms and packaged reagents for a range of tests as selected by the operator. These devices will also conduct all calculations required to determine the final concentration based upon readings taken from test and blank samples.

1.4.3 Portable electronic instruments

Electronic water quality monitoring instruments are available to measure a wide range of parameters including, DO, pH, temperature, salinity conductivity, total dissolved solids, total dissolved gases, ammonia, turbidity, water flow, oxidation/reduction potential (ORP) etc. These units can be bench top laboratory instruments or rugged portable units. These instruments generally comprise a single sensor or multiple sensors fitted to a meter that will display the level of the parameter being tested. Sensors use the presence of a variable environmental parameter to induce a concentration dependant change in electrical potential across a selectively permeable membrane. This change is amplified using electronics within the sensor housing to provide a 4-20 mA output to the meter. The meter interprets the strength of the electrical current received and produces a visual display of the concentration of the parameter being tested.

Aquaculturalists generally use hand held portable meters and these can be either single or multiple parameter units. Modern hand held meters will incorporate features that provide compensation for water temperature interactions with the parameter being tested. Options will also be provided to display the parameter tested in a number of units or interpretations. For

example dissolved oxygen can generally be displayed as mg/L or % saturation. There is a wide range of water testing instrument manufacturers, including;

Eutech Instruments http://www.eutechinst.com/

Greenspan http://www.greenspan.com.au/

Hanna Instruments http://www.hannainst.com.au/

Horiba http://www.horiba.com/

Hydrolab http://www.hydrolab.com/

In-Situ Inc. (Troll) http://www.in-situ.com/

Merck WTW Meters http://www.merck.com.au/

Oakton http://www.eutechinst.com/

OxyGuard http://www.oxyguard.com/

Royce Instruments http://www.royceinst.com./

TPS http://www.tps.com.au/

Yellow Springs Instruments http://www.ysi.com./

YeoKal http://www.yeokal.com.au/

Some advanced hand held units will provide the capacity to test up to 9 parameters simultaneously and incorporate the ability to deploy the sensor for use as a logger for months at a time. Dedicated electronic water quality logging devices are also available. These include small temperature loggers (i.e. T-Tec, iButtons or thermochron™ http://www.t-tec.com.au/ or www.maxim-ic.com/products/ibutton; Hastings Data Loggers Tinytag® range www.hdl.com.au and Oakton®) and remote set loggers for water temperature, salinity etc. These small and relatively inexpensive devices may be beneficial for monitoring diurnal water temperature variations in a range of tanks or points in the water supply and discharge system across the farm.

1.4.4 Continuous monitoring and control systems

Intensive production industries use process control technology to monitor, control and improve system performance, leading to increases in growth and competitiveness.

Intensive aquaculture systems often install centralised systems for continuous monitoring of critical water parameters that are measured by analogue inputs such as DO, pH and temperature. A range of equipment is also available to provide process control capabilities to

aquaculture operations. These include Oxyguard™, Innotech®, Sinergia™, Point Four™, and Single Chips Inc. Contact details of some local suppliers of this equipment are provided (Appendix 2).

These systems can be installed to provide a range of functions including:

- Monitoring for alarm purposes only that may be as simple as water flow switches connected to automatic phone dialers.
- Monitoring and logging of water quality parameters to indicate problems, to assess conditions provided to stock and evaluate system performance.
- Expanded capability of the system to provide monitoring and control of water quality parameters when equipment is installed that can vary these parameters within the culture system (i.e. oxygenation, heating/cooling systems, pumping systems etc) and other system components (i.e. auto feeders, lighting etc).

The time consuming nature of this sampling and data recording supports the installation of continuous monitoring equipment wherever this is practical. The most important water quality parameters to be measured by these systems are dissolved oxygen and water temperature. Some modern DO probes incorporate a temperature probe used to determine oxygen saturation (%) from DO measurements recorded (mg/L). Other sensors that may be used include pH, water flow, water level, pressure and ORP (redox). Sensors are output devices that interface with a metering/monitor unit (with visual display) or transmitter (no visual display). Each meter/monitor or transmitter unit provides a specified number of channels that determine the number of sensors that can be connected. Meters may include additional features such as built-in data logging and PC connectivity to allow storage of data and download to a PC to capture and to provide real time observation of readings and view data directly. A range of software is available to set up, visualize and modify the monitoring system from a central or remote computer. Provision of relay output options for control of switching devices such as alarms, solenoid valves, and blowers is also a common feature.

Examples of the increasing complexity that can be provided by continuous monitoring systems for a conceptual farm of 240 individual culture units (tanks, raceways or troughs) are presented (Figures 1 – 4). These representations show the most basic configuration (Figure 1) that includes a single monitoring point located at a common water supply point. This may be a DO and/or a water temperature sensor, or a simple water flow switch. The next representation (Figure 2) includes additional sensors installed to monitor DO and/or water temperature at the furthest points within the water distribution system. Both of these configurations will not reveal any information on water quality changes within individual

culture tanks. This sophistication can only be achieved if sensors are mounted at both the inlet and outlet of culture tanks (Figure 3).

When monitoring systems are being planned for abalone farms a number of considerations should be accommodated. These include:

- The design should allow easy expansion of system capacity (i.e. additional sensors, alarms etc).
- The large number of individual tanks used to culture abalone and how best to provide representative monitoring of all types of farm stock or tank types using the least number of sensors.
- Unit cost of equipment and installation will generally diminish with size of the system.
- Installation of systems in locations that provide accessibility for service and calibration of sensors and minimize exposure to harmful environmental impacts for support equipment (i.e. meters, monitors, transmitters, cabling etc).

Location of sensors will be based largely upon the intended use of the monitoring and control system. Sensors should be installed at strategic or more critical locations across the farm such as:

- At points that are closest and furthest from the supply pumps.
- In the tanks most likely to have the highest stocking density or oxygen demand.
- In tanks identified for monitoring that are representative of similar tanks across the farm (i.e. similar size categories of stock or those receiving different culture procedures being assessed by management etc).

An extension of monitoring is control where metered inputs are used to change the operation of components within the system. Control systems will require additional input/output units that allow the system to register input from contact switching or activate switch output functions that can be incorporated into an aquaculture system. In combination with monitoring, these systems are used to provide some degree of control over components within aquaculture systems that effect water quality such as oxygenation systems (Figure 4); actuated valves and solenoids, or varying the number or speed of pumps.

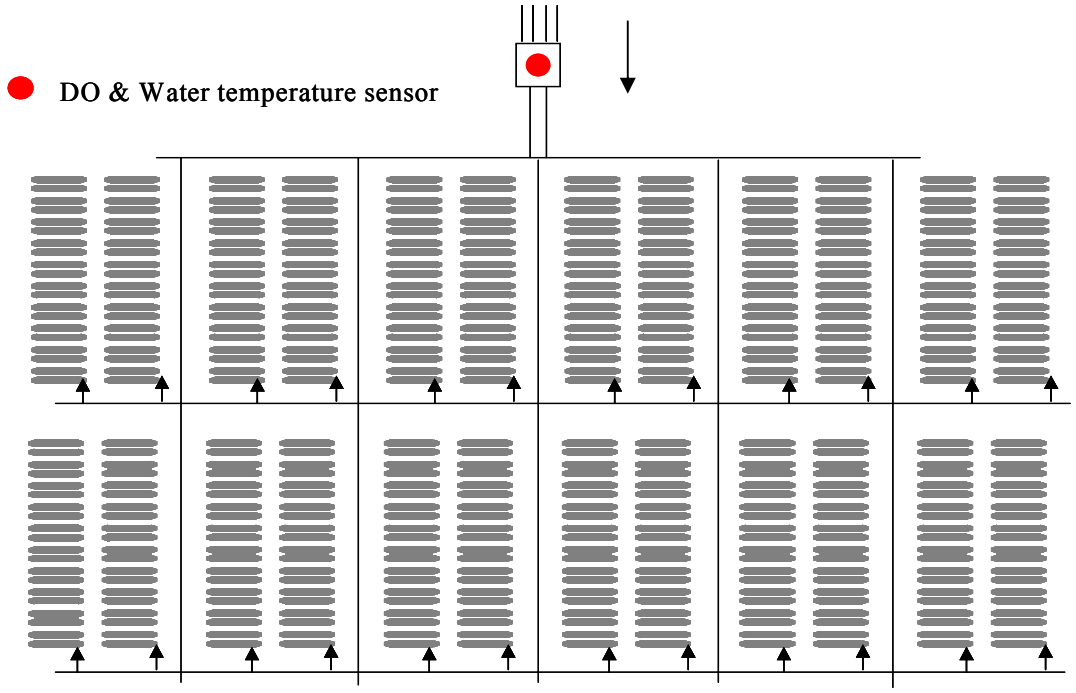

Figure 1. Representation of a simple continuous water quality monitoring system for an abalone farm. Monitoring of DO and water temperature at a common inlet location before distribution across the farm.

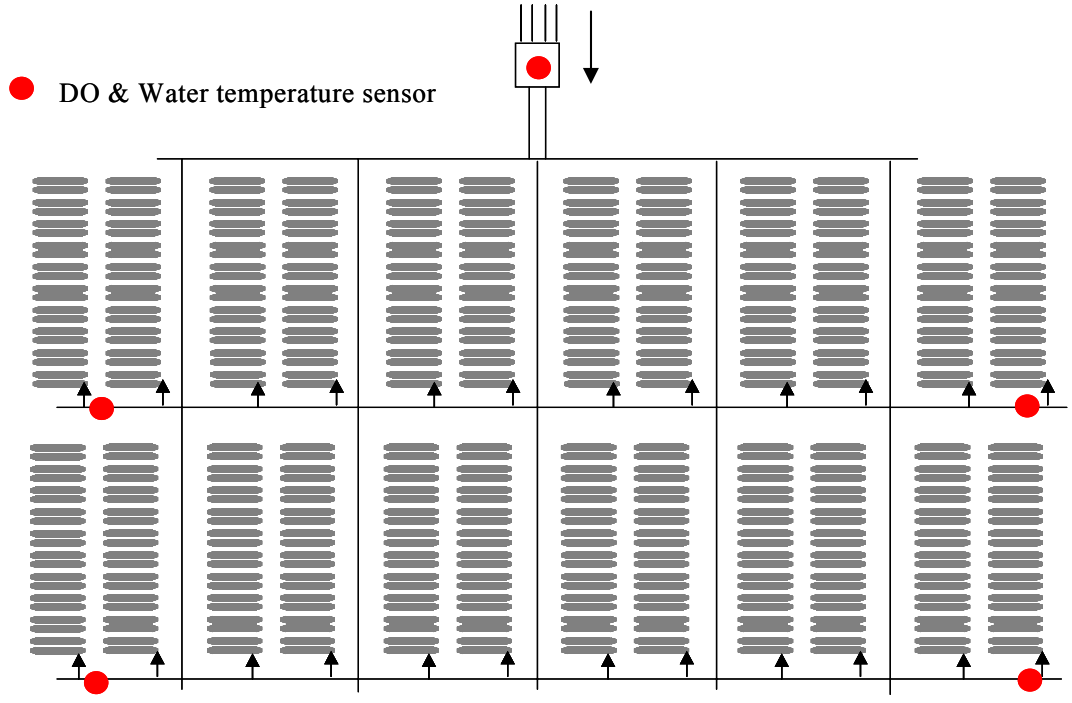

Figure 2. Representation of a continuous water quality monitoring system for an abalone farm. Monitoring of DO and water temperature at a common inlet location with water flow sensors at furthest supply points in the distribution system across the farm.

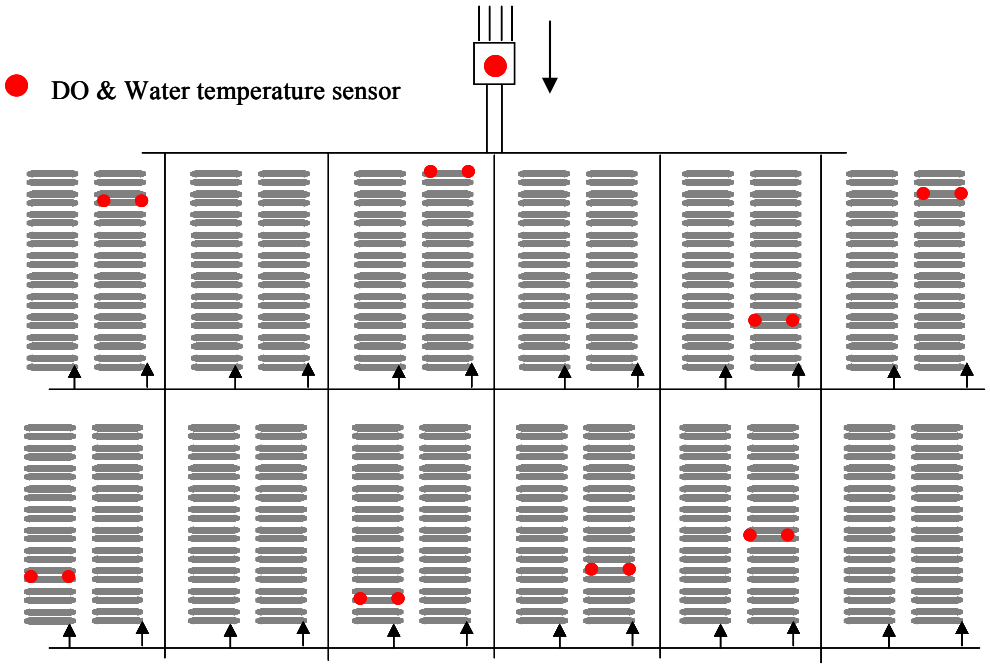

Figure 3. Representation of a continuous water quality monitoring system for an abalone farm. Includes monitoring of DO and water temperature at a common inlet location and at the water inlet and outlet of 8 individual tanks. This representation could cover 2 tanks to be monitored from each of 4 size categories of stock.

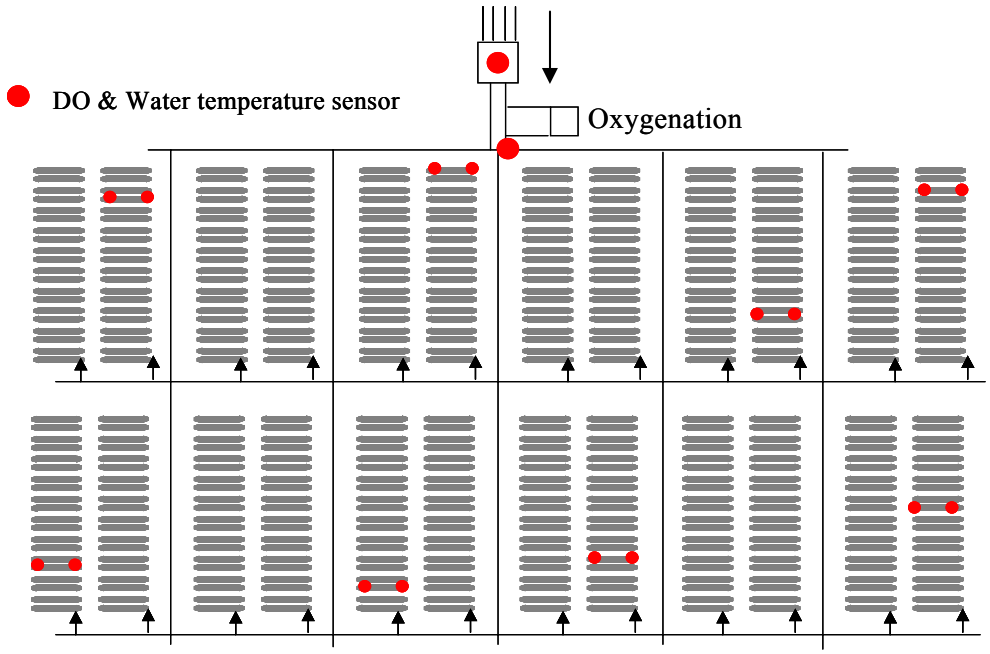

Figure 4. Representation of a continuous water quality monitoring system for an abalone farm that incorporates oxygenation. Includes monitoring of DO and water temperature before and after oxygenation; and at the water inlet and outlet of 8 individual tanks. This representation could cover 2 tanks to be monitored from each of 4 size categories of stock.

SECTION 2: REVIEW OF THE EFFECTS OF MAJOR WATER QUALITY FACTORS ON FARMED ABALONE

2.1 WATER TEMPERATURE

Summary

- Water temperature affects a wide range of physiological functions in ectotherms such as abalone.
- Management of operational variables is the only option currently available to farms to minimise mortality during periods of elevated water temperature.
- Optimum water temperatures for greenlip and blacklip abalone have been investigated and are as follows;

Species	Author	Optimum temp. (^{o}C)	Temperature (^{o}C) when adverse behaviour observed	50% Critical thermal max. (^{o}C)
H. rubra	Gilroy and Edwards (1998)	16.9	21.0	26.9
H. laevigata	Gilroy and Edwards (1998) Madigan *et al* (unpublished, 2000)	18.9	22.5	27.5 28.4

- There is evidence that abalone may exhibit size dependant temperature preference.
- No studies have been conducted on abalone to investigate the effects of interactions between elevated water temperature and varying levels of other water quality parameters.
- The interaction between disease and water temperature requires further investigation.

Commercial aquaculture seeks to maximise growth and when food and oxygen are not limiting water temperature is the primary factor that can affect this objective. The water temperature available to onshore flow-through abalone farms is dictated by the flow through of ambient seawater and there are no viable options to provide control of this variable. As such, farm management can only hope to obtain an annual growth "window" over months of the year in which ambient seawater temperatures are favourable within upper and lower thermal tolerance limits for the species selected. As warmer water temperatures promote growth, farms will tend to be sited at locations that may expose animals to their upper thermal tolerance limits. Management of operational variables is the only option currently available to farms to minimise mortality during periods of elevated water temperature.

2.1.1 General effects of water temperature on physiology of aquatic animals

After oxygen, water temperature is the most important water quality factor that affects the growth and well being of cultured aquatic organisms, as these are ectotherms (i.e. cold blooded) that have their body temperature regulated by the external environment. As a result, many critical metabolic processes are temperature dependent and the rate of growth and metabolism of aquatic organisms will change in response to water temperature (Table 1).

Table 1. Range of physiological and other affects attributed to water temperature.

Factors that increase at elevated water temperature	Factors that decrease at elevated water temperature
Metabolic rate Oxygen consumption Gill irrigation rate Osmoregulatory demand Feed consumption Waste production Biological oxygen demand Rate of bacterial replication	Dissolved oxygen available in water

Water temperature also has an impact on a range of other water quality parameters. As water temperature increases, the amount of dissolved oxygen present in water decreases. At higher water temperatures the greater metabolic rate and oxygen demand, combined with reduced oxygen solubility, will require an increased gill irrigation rate. The proportion of total ammonia that exists in the toxic un-ionised form (NH_3) as opposed to the ionised from (NH_4^+) also increases at elevated water temperatures but pH has a greater effect on the equilibrium established.

2.2.2 Water temperature tolerance of cultured abalone

It is understood that upper temperature tolerance of fish is often within a few degrees of the preferred temperature and the optimal temperature for growth is generally very close to this (Jobling, 1981). Behavioral thermoregulation will dictate that motile organisms such as abalone will congregate within a narrow water temperature range defining their temperature preference (Diaz *et al*, 2000). Gilroy and Edwards (1998) state “in general, abalone have a conservative thermal response and little tendency to adapt to chronically altered thermal

environments". The same authors refer to Gilchrist (1995) who suggests that it is not unusual for organisms to be adapted to narrow thermal ranges and show relatively small differences in thermal preference. From these studies it appears that an "ideal" abalone farm would seek to provide a relatively narrow water temperature "window" around the optimal temperature in order to maximise growth. Of interest to this report is the effect of exposing abalone to extended periods during which water temperatures exceed that preferred by the species selected for culture.

Jobling (1981) divided the temperature responses of fishes into tolerance, resistance and preference with the upper boundary of the resistance zone represented by the critical thermal maximum (CTM). Experimentally the CTM can be determined for abalone by increasing water temperature by 1^{o}C per hour and recording the temperature at which individuals lose attachment from a vertical surface (Hines *et al*, 1979). The 50% CTM is calculated from a regression of data covering the temperature when first and final detachment of abalone is recorded (Madigan *et al*, 2000).

The optimal temperature for growth of *H. laevigata* is 18.9 ^{o}C while *H. rubra* prefer 16.9 ^{o}C with 50% CTM recorded as 27.5 ^{o}C and 26.9 ^{o}C respectively (Gilroy and Edwards, 1998). In this study detachment of abalone started at approximately 25.1 ^{o}C for *H. laevigata* and 24.3^{o}C for *H. rubra* indicating that animals start to experience a significant degree of temperature stress at, or less than, these levels. Behavioural responses (i.e. 180^{o} turns of the shell and lifting the shell to expose the mantle) reported in this study and discharge of mucous starting at 22.5 ^{o}C for *H. laevigata* and 21.0 ^{o}C for *H. rubra* also support this suggestion.

A more recent study of four abalone species found in South Australia (Madigan *et al*, 2000 unpublished) presents data that shows that local *H. laevigata* may be more tolerant of elevated water temperatures than those used by Gilroy and Edwards (1998) that were collected from the North and North East coast of Tasmania. In this study the 50% CTM for animals collected from Gulf St Vincent and Spencer Gulf was 0.9 ^{o}C higher at 28.4 ^{o}C suggesting that the SA stocks of this species may be adapted to higher water temperatures than those in Tasmania. Apart from the 50% CTM being higher, there appears to be a narrow upper temperature tolerance interval between the points of first and final detachment in the SA stock as all animals fell from plates within an 1.9 ^{o}C interval compared to over a 5.5 ^{o}C interval in the Tasmanian study. These results of Madigan *et al* (2000) are in agreement with studies conducted on *H. cracherodii* (Hines *et al*, 1980) that recorded an extremely abrupt response to heat stress where after lethal temperatures were reached the difference between 0 and 100% mortality was recorded over a range of less than 1.0 ^{o}C.

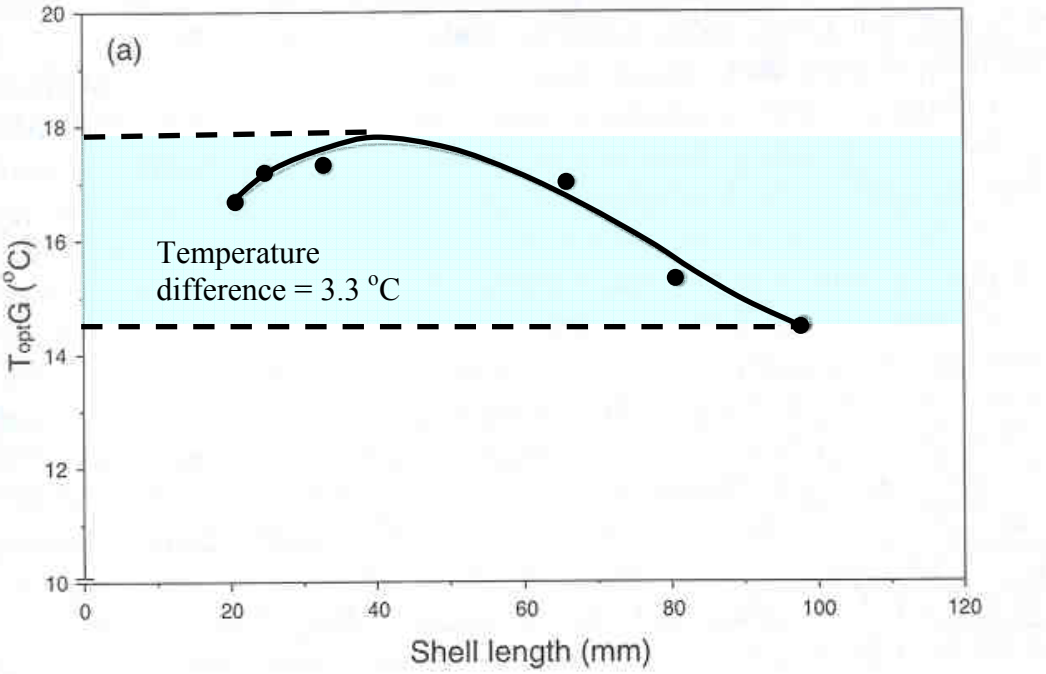

Figure 5. Change in optimum temperature with size of *H. rufescens* (Steinarsson and Imsland, 2003).

Compounding these findings are results of trials on red abalone, *H. rufescens* that have shown that the optimal temperature for growth ($T_{opt.G}$) of this species changes with size (Figure 5). In these studies abalone at 21 mm were found to prefer 16.7 °C and $T_{opt.G}$ increased to a predicted peak of 17.8 °C for 44.2 mm abalone, then declined to $T_{opt.G}$ of 14.5 °C for mature 98 mm abalone (Steinarsson and Imsland, 2003). Gilroy and Edwards (1998) optimal temperature studies were conducted using 79.3 and 17.2 mm *H. rubra* and 84.5 and 10.8 mm *H. laevigata*. Madigan *et al* (2000) used similar sized (82.8 and 9.2 mm) *H. laevigata*. This phenomena has been reported in Australia for scallops (Heasman *et al*, 1996) and *H. rubra* (Heasman *et al*, 2001). Steinarsson and Imsland (2003) are of the opinion that "variation in temperature optimum and growth potential may be a physiological trait common to many marine fish and shellfish species". If size dependant temperature preference occurs then this may have implications for management approaches for different size classes being grown on abalone farms.

2.2.3 Water temperature and mortality on farms

Summer water temperatures experienced between mid January until early February on abalone farms on Boston Point may reach as high as 24 °C for periods of weeks and may reach peaks of 25 °C to 27 °C at Louth Bay. These elevated water temperatures are expected

to directly affect growth performance of abalone as they are beyond those that have been shown to induce behavioural changes in *H. laevigata* (22.5 ^{o}C) and are approaching temperatures shown to result in detachment of abalone in short term critical thermal preference trials (Madigan *et al*, 2000; Gilroy and Edwards, 1998). These behavioural and physiological effects may be further exacerbated by a combination of feeding and tank management practices, bacterial activity and interactions between water quality parameters.

Data recorded at South Australian Seafoods (SAS) abalone farm on Boston Point (Figure 6) over the summers between 2000 - 2002 show that maximum water temperatures at this location did not exceed 24 ^{o}C and there was annual variability in both duration of periods of elevated water temperatures and the maximum levels achieved. Elevated water temperatures occurred for a longer period and reached greater levels during the 2000 - 2001 season at which time mortality was high. Water temperatures did not reach the same levels during either the 2001 - 2002 or 2002 - 2003 season. Over the 2002 - 2003 summer season, management concentrated on implementation of husbandry practices and farm operations that provide optimal conditions for abalone over periods of elevated water temperature. Results show that mortality was reduced to approximately 5% across the farm although this corresponds to a relatively mild season compared to that experienced in 2000-2001. This result suggests that improved farm practices over summer may reduce the level of mortality in this location.

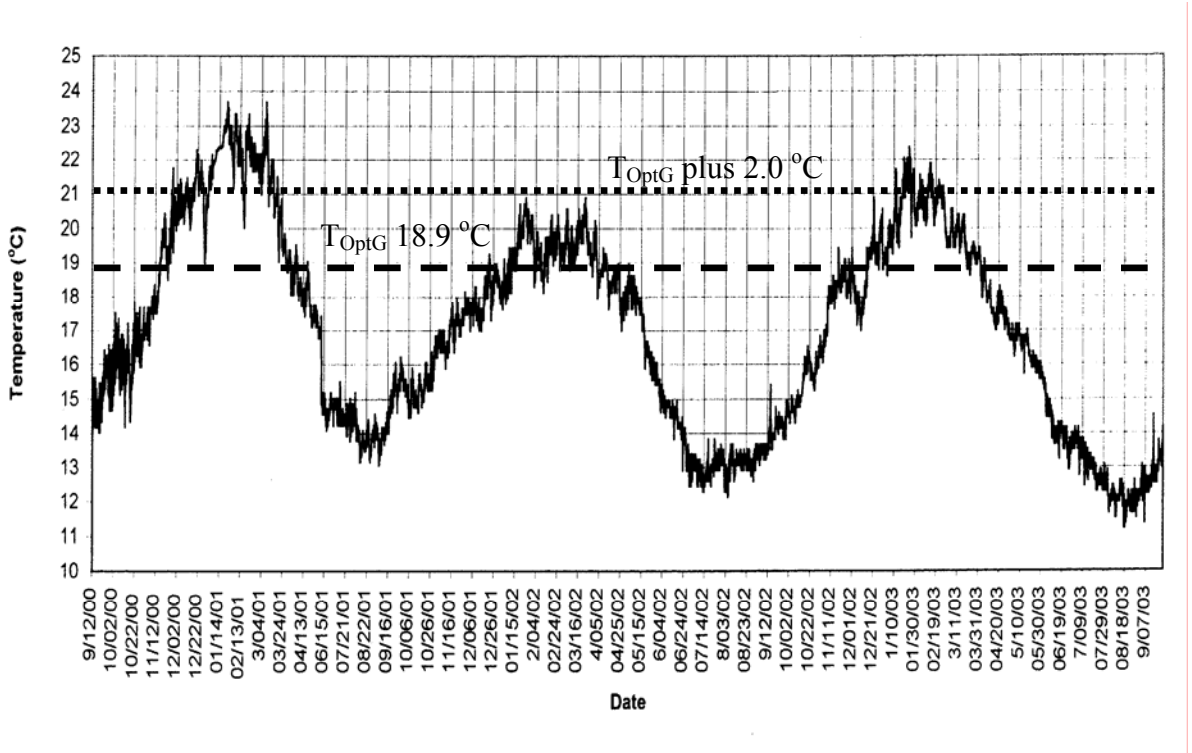

Figure 6. Intake seawater temperature recorded at Southern Australian Seafoods (SAS) abalone farm at Boston Point between Sept 2000 and September 2003.

The situation experienced by abalone farm operators South Australian Abalone Developments (SAABDEV) at Louth Bay near Port Lincoln is different. In this location seawater is withdrawn from a depth of 6-8m within a relatively shallow bay and consequently water temperature and silt load are elevated periodically in this low energy environment where reduced water movement occurs. Water temperature may reach 27 ^{o}C for short intervals within the farm on some days and after these instances mortality has been observed to increase in the following 1-2 days. Mortality data compiled with water temperature data over the 2002 - 2003 summer at this location show that mortality began to increase when water temperatures rose to 20 ^{o}C (late November) and higher mortalities occurred at temperatures above this level (mid December until late January). Abalone farming operations at this location ceased in 2004 but may recommence.

Another consideration is the timing of farm operations to avoid handling of stock during period of elevated water temperatures. It may be prudent for farm managers to accept that all major abalone handling operations need to be completed before a date in late spring/early summer. After this time they should increase attention given to husbandry operations that ensure that optimal water quality conditions are maintained during periods of elevated water temperature.

2.2 SUMMER MORTALITY

Significant mortality events have been experienced by a number of commercial abalone farms during summer with one farm reporting up to 25 - 35% mortality across all size classes over the 2002 - 2003 summer season. Abalone bloat has also been reported over this period characterised by animals floating with swollen guts (Vandepeer, 2003). *Vibrio sp*. in particular *V. alginolyticus* and *V. hollisae* have been implicated as the possible cause of mortalities and bloat in experiments undertaken by Vandepeer (2003) to simulate summer mortality. These species of bacteria can be pathogenic and prefer water temperatures greater than 20 ^{o}C. Farm data relating mortality and water temperature (Figure 7) suggests that there is an increase in mortality when water temperatures exceed 20 ^{o}C.

In Taiwan mass mortalities of small *Haliotis diversicolor supertexta* during the warmer season have been attributed to a withering syndrome that has symptoms such as abscess or ulcers in the mantle, shrunken foot muscle, discolouration of the epipodium (tissue circling the foot), reduced activity and inability to adhere to surfaces (Huang *et al*, 2001). Investigations have confirmed that *Vibrio parahaemolyticus* and *Vibrio alginolyticus* are the causative agents in these mortalities. The optimal temperature for culture of this abalone species

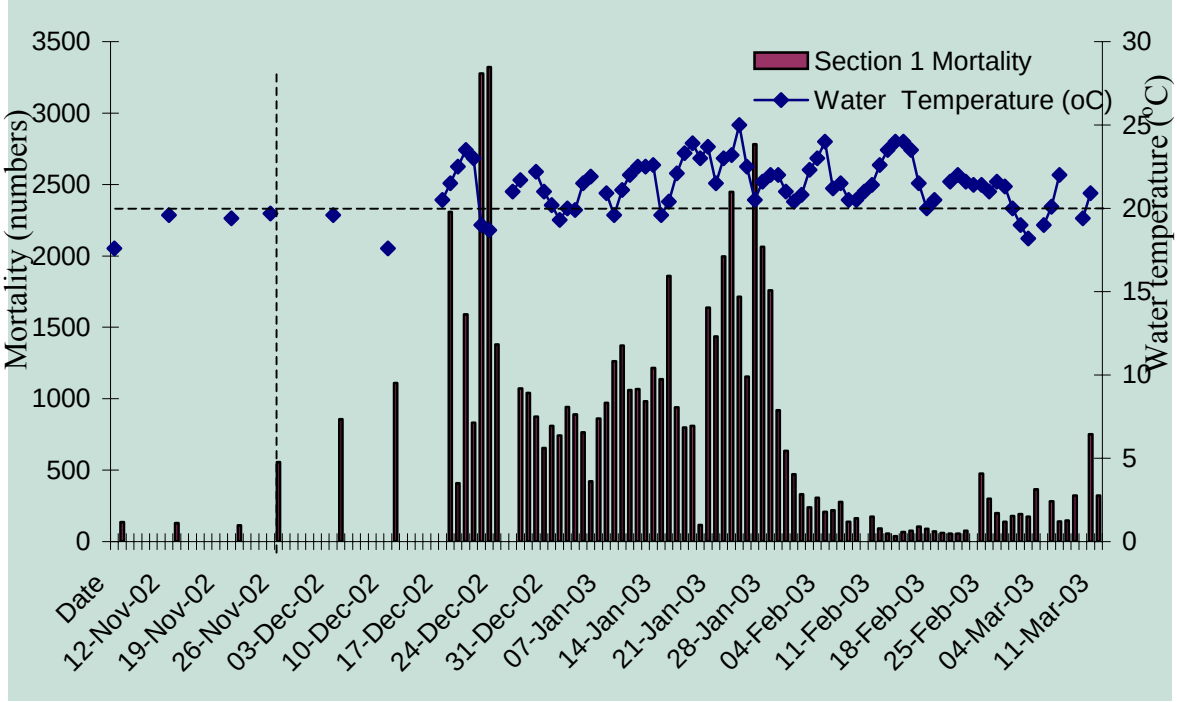

Figure 7. Intake seawater temperature and mortality from one section of raceways at South Australian Abalone Developments (SAABDEV) abalone farm, Louth Bay between November 2002 and mid March 2003.

is between 23°C–28°C. Experiments demonstrated that the virulence of these *Vibrio* species was greater when water temperature exceeded 28 °C. At the higher water temperature a much lower dosage of bacterial cells killed the abalone suggesting that at this temperature *Haliotis diversicolor supertexta* are stressed and more susceptible to vibriosis (Lee *et al*, 2001). Mass mortalities of European abalone *Haliotis tuberculata* have been reported for several years since 1997 in the natural environment and on a commercial farm. This mortality has been attributed to *Vibrio carchariae* and is related to an increase in water temperature above 17 °C (Nicolas *et al*, 2002). Clearly these findings show that there is an increased incidence of mortality of abalone due to *Vibrio* species that are triggered at elevated water temperatures.

All farms visited report incidences of dead abalone as "irregular" across culture units with mortality occurring in “patches” often downstream from dead animals. In some instances these are thought to be associated with irregularities in the floors of slab tanks that create areas with lower water flow.

Increased bacterial numbers associated with elevated silt load have also been suggested to be involved in summer mortality. It is possible that water quality interactions may also be contributing including various interacting effects of water temperature, dissolved oxygen and ammonia.

2.3 DISSOLVED OXYGEN

Summary

- Dissolved oxygen (DO) is the most important water quality parameter for culture of aquatic organisms.
- The amount of DO decreases with increases in water temperature, salinity and altitude.
- The amount of water delivered to abalone culture systems (i.e. flow rate) must be sufficient to supply both the oxygen requirements of animals and prevent the accumulation of wastes.
- Seasonal water temperature variations induce changes in dissolved oxygen availability in water supplied throughout the year.
- Growth of *H. laevigata* has been shown to be reduced by 5% (EC_5) at DO levels of 7.36 mg/L (95.6% saturation relative to control at 7.70 mg/L) and by 50% (EC_{50}) at 5.91 mg/L (76.7% saturation relative to control).
- In addition to consumption by animals being grown, the dissolved oxygen demands within a culture system will be affected by water temperature, feeding practices, size of animals and the biological decomposition of wastes.
- Abalone can tolerate dissolved oxygen supersaturation.
- The use of oxygenation appears to be a viable option for abalone farms to increase DO levels during summer. There is a point at which the cost of oxygenation will become cheaper than the costs associated with pumping more water.

2.3.1 General effects of dissolved oxygen on the physiology of aquatic animals

The amount of DO available to aquatic organisms decreases with increases in water temperature, salinity and altitude. For abalone farming the most significant impact will be the relationship between DO and water temperature (Appendix 3) with DO in water reducing as temperature increases. Dissolved oxygen (DO) is the most important water quality parameter for culture of aquatic organisms as it can affect growth through limiting the scope for respiration of aerobic animals (Brett, 1979). In addition, the energy cost incurred for oxygen conforming animals to maintain oxygen consumption in reduced DO conditions can also reduce growth (Ingerson and Geddes, 1995; cited in Harris *et al*, 1999).

Oxygen is difficult to extract from water and aquatic animals achieve this by exposing a large gill surface area to the environment in order to absorb oxygen across a complex of blood capillary membranes arranged within fine gill lamellae. Fish are able to enhance the volume of water and thus oxygen available to this absorption system by actively pumping water through the gills using the mouth and gill covers to induce pumping in conjunction with their movement through water (Choat, 1988). This mechanism is not available to abalone and they

must rely on water flow through the mantle cavity to make oxygen available to the gills and to remove metabolic byproducts such as ammonia.

A consequence of exposing the gills directly to water for gas exchange and maintenance of osmotic balance is that this apparatus also functions as a very efficient radiator of heat so that blood returning from the gills will ensure that internal body temperature reflects the ambient water temperature (Choat, 1988).

2.3.2 Seawater supply and dissolved oxygen

Flow-through culture systems that predominate on abalone farms in Australia rely on water supply to deliver the oxygen requirements of animals and prevent the accumulation of wastes. The amount of water delivered to culture systems (i.e. flow rate) must be sufficient to supply both of these needs. To ensure growth rates are maximised, it is generally assumed that DO content of water should be kept at saturated levels (100%). The 100% saturation level is the maximum amount of oxygen (mg/L) that can be dissolved at the prevailing conditions of water temperature, salinity and barometric pressure. Appendix 3 provides saturation levels (mg/L) of dissolved oxygen at sea level for a range of water temperature and salinity conditions.

Most abalone farms in southern Australia are sited in locations with access to oceanic seawater with salinity that generally ranges from 35‰ - 38‰ with water temperatures ranging from 11 ºC – 24 ºC, although extremes may be experienced for short periods (i.e. weeks). Under these conditions dissolved oxygen levels will vary between 8.83 mg/L (11 ^{o}C and 35 ‰) in winter, down to 6.89 mg/L (24 ^{o}C and 35‰) in summer. Under the same water flow conditions this seasonal change represents a 1.94 mg/L (22.0%) difference in oxygen availability from water delivered at the peaks of summer and winter.

2.3.3 Effects of low levels of dissolved oxygen on abalone

Farms inspected reported that DO at outlets could be as low as 80 – 85% saturation with one farm recording DO as low as 4.0 mg/L (56% saturation of seawater at 22^{o}C). No indication was given how long these reduced DO conditions prevailed. It is expected that these levels of reduced DO will lead to reduction in growth if they were to persist and further reductions would increase stress to animals and ultimately contribute indirectly, or directly, to mortality.

Harris *et al* (1999) conducted trials to investigate the effect of chronic exposure of juvenile *H. laevigata* to reduced levels of DO over a 77 day period. Results showed that growth as measured by whole wet body weight was reduced by 5% (EC_5) at DO levels of 7.36 mg/L

(95.6% saturation relative to control at 7.70 mg/L) and by 50% (EC_{50}) at 5.91 mg/L (76.7% saturation relative to control). Survival in these trials was not significantly reduced at levels of 63% saturation or below, although mortality was approximately 10% greater at both 81% and 73% saturation compared to the control abalone at 100% saturation. These results suggest that the growth potential of abalone will not be achieved in conditions that are at less than 100% saturation. This is particularly important during periods of elevated water temperature when high growth rates can be achieved to maximize farm productivity.

The research by Harris *et al*. (1999) was conducted at temperatures that are optimal for growth of *H. laevigata* (mean 17.8 – 18.3 ºC). Greater growth reductions and mortality impacts would be expected as water temperatures rise to those experienced by farms during summer. Of interest in these investigations was the use of warm water, similar to that experienced on farms during peak summer temperatures (23 – 25 ºC), to relax and detach the animals prior to data collection and stocking of respirometers suggesting that *H. laevigata* do not behave normally following abrupt exposure to these water temperatures.

2.3.4 Dissolved oxygen demands within the culture system

It is suspected that there are cumulative effects on dissolved oxygen levels that occur within flow through systems due to the influences of a number of environmental and operational factors.

2.3.4.1 Effect of water temperature on oxygen consumption

As water temperature increases, so does the respiration rate of abalone within the culture system. The only information located on the effect of water temperature on oxygen consumption is for *H. discus hannai*. Uki and Kikuchi (1975) in Hahn (1989) showed that there was a linear increase in oxygen consumption with increasing water temperature from 8.3 to 20.0 ^{o}C, but there is a change in the relationship after this point (Figure 8) suggesting that the maximum rate of oxygen consumption for the species has been reached. It is suggested that 20 ^{o}C represents the highest temperature experienced by this species. Oxygen consumption of *H. discus hannai* was also measured by Bi *et al* (2000) over water temperatures from 12ºC to 28ºC. These results also show an increasing oxygen consumption with increasing water temperature. No similar studies have been conducted for either *H. laevigata* or *H. rubra* but it is expected that oxygen consumption in both species will increase as water temperature increases.

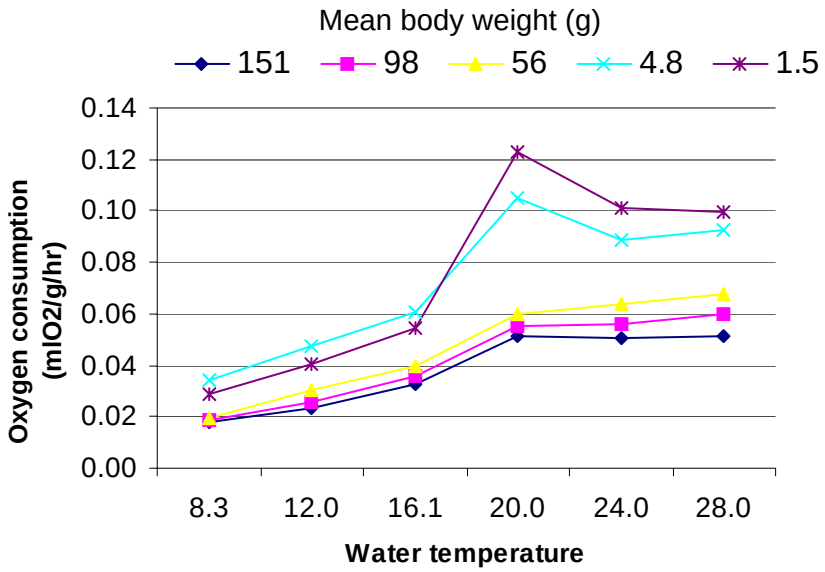

Figure 8. Average oxygen consumption of different size (g) abalone (*H. discus hannai*) over a 24 hour period (graph prepared from data of Uki and Kikuchi (1975) presented in Hahn (1989).

2.3.4.2 Size of animals in culture units

Smaller animals will generally consume much greater amounts of oxygen per unit of body weight such that a similar biomass of small abalone will consume far greater amounts of oxygen than larger animals stocked in a raceway receiving the same water flow rate. This relationship can be seen (Figure 8) from studies on *H. discus hannai* conducted by Uki and Kikuchi (1975) in Hahn (1989).

In addition, Jan and Chang (1983) concluded that *Haliotis diversicolor supertexta* adopted a size dependant approach to oxygen regulation (Figure 9). In this study animals of less than 78.2 g regulated consumption as DO declined to a critical content (Cc) that varied with size, after which consumption fell as DO content of the surrounding seawater declined. Animals larger than 78.2 g showed no capacity for oxygen regulation and were classed as oxygen conformers with their consumption reducing in a linear manner as DO declined. If this is the case with *H. laevigata* it may be of relevance to aquaculture as larger animals may not able to maintain oxygen consumption as DO content declines in the seawater supplied to the culture units. Smaller animals may have an ability to maintain oxygen consumption over a range of reduced DO conditions after which they will switch to being oxygen conformers.

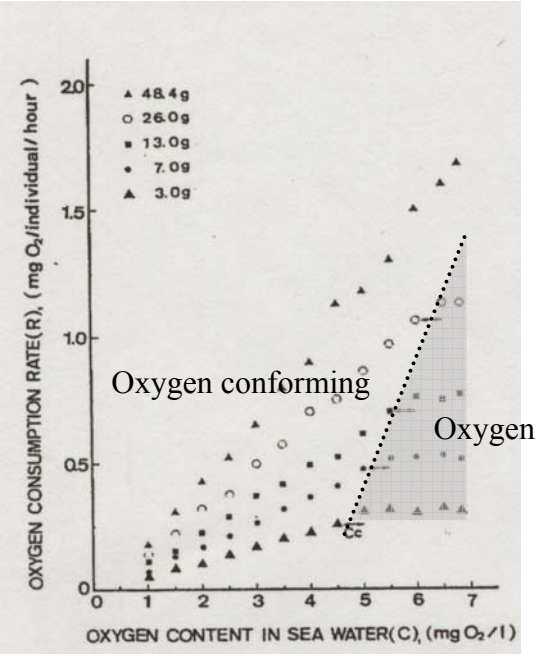

Figure 9. The oxygen consumption of different weight groups of abalones (*Haliotis diversicolor supertexta*) in declining oxygen content of seawater at 23 ^{o}C and 35‰ (from Jan and Chang, 1983).

2.3.4.3 Changes in oxygen demand due to feeding practices

Most feeding activity of abalone occurs after dusk and before midnight. Fleming (1996) recorded most activity of juvenile greenlip abalone between 8:00 pm and midnight with a subsequent decline to 8.00 am. It is likely that there will be a related increase in oxygen consumption due to feeding activity and digestion. Uki and Kikuchi (1975) in Hahn (1989) also found a circadian rhythm in oxygen consumption for *H. discus hannai* that increased from dusk until midnight and decreased from midnight to midday indicating that the most active feeding period for this species was early in the dark period.

There will be an increase in oxygen demand following feeding due to consumption required for digestion of feed and absorption by the animals. It is not uncommon for this metabolic oxygen demand to be more than double that of the maintenance levels of oxygen consumption by animals. Metabolic oxygen demand following evening feeding may reach peak levels during the night that correspond with times when a reduced level of DO is available from inflowing seawater due to night time depletion by phytoplankton respiration. This interaction should be investigated through the use of sequential DO measurements or installation of meters and probes with logging capability.

With respect to restricted feeding of abalone it should be noted that the oxygen consumption rate of starved abalone (*Haliotis diversicolor supertexta*) has been shown to increase by 20% during the night (Jan and Chang, 1983) which was in agreement with the 20% increase in

consumption at night recorded for other species (*H. sieboldii*, *H. gigantea* and *H. discus hannai* (Uki and Kikuchi cited in Jan *et al* 1981). Such an increase in oxygen consumption could be of significance during summer when feeding is restricted or no feeding occurs for extended periods (i.e. days).

2.3.4.4 Biological oxygen demand (BOD)

Bacteria will consume relatively large amounts of oxygen during decomposition of wastes (i.e. uneaten feed, organic matter and faeces) within the system. If uneaten feed and faeces are allowed to accumulate DO consumption by bacteria may equate to a significant additional oxygen demand within the system.

2.3.5 Effects of high levels of dissolved oxygen (supersaturation)

Hahn (1989) reported that adult abalone became intoxicated at oxygen concentrations equivalent to more than 203% saturation. Loipersberger (1996) reported no mortality for settled *H. laevigata* after 4 days confinement in vials sealed at 300% saturation and did not drop below 150% saturation. The same author maintained 30mm animals over 22 days at 100%, 200% and 300% saturation and reported no mortality in the high DO tank and observed a shift in feeding patterns with abalone in the high oxygen tanks spending most of the time away from shelters with increased feeding behaviour. Harris (1999a) reported no significant difference in growth or survival for juvenile *H. laevigata* maintained at 117% oxygen saturation over a 77 day period compared to controls maintained at 100% saturation.

From these results it is clear than abalone can tolerate levels of oxygen supersaturation that would be required to maintain optimal DO conditions across all culture tanks on a farm. This could be achieved by the use of whole farm oxygenation systems to allow adjustment of influent DO concentrations or water flow rates to provide 100% DO saturation at the outlet of tanks.

It has been demonstrated that growth rates of salmonids cultured in supersaturated water at 20 – 25^{o}C can significantly exceed growth at optimal temperature for these species (15.5 ^{o}C). suggesting that the lack of oxygen at elevated temperatures may reduce growth (Forteath, 1988). There may be opportunities to increase growth performance of abalone at elevated water temperatures with supersaturation DO.

2.3.6 Oxygenation

Preliminary appraisal of oxygenation systems for abalone farms suggests that this is a viable option to improve water quality over periods of elevated water temperature during which a number of factors compound to reduce the amount of oxygen available to animals. These factors include:

- Reduced oxygen saturation of seawater at elevated temperatures.
- Higher respiration rates of abalone.
- Increased oxygen demands due to digestion and degradation of uneaten feed and faeces when feeding occurs at elevated water temperatures.
- Increased water use demands across the farm.

Oxygenation would best be achieved through installation of on site liquid oxygen storage with oxygen introduced to the seawater supply at a single point through a highly efficient saturation device (i.e. greater than 90% transfer efficiency). This would allow all sections of the farm to receive seawater with greater than 100% saturation with the flow set to achieve 100% dissolved oxygen saturation at outlets to each culture unit. The use of diffusers in tanks is impractical for this application due to the minimal transfer efficacy that is possible due to the low water depth of culture tanks. Any section of the farm that did not require supersaturated water (i.e. hatchery) could be supplied from take offs before the oxygen saturation point.

2.3.6.1 Estimating the cost of adding oxygen

Assume that a farm with a water temperature of 22 ^{o}C, salinity of 35‰ and using 500 L/sec of seawater wishes to elevate DO levels by 2.0 mg above saturation. Under these conditions this will be achieved if the DO of the incoming water increases from 7.134 to 9.134 mg/L (an increase of 28%). The associated cost would be:

Oxygen to be added	= 500L/sec x 2.0 mg/L
	= 1000 mg/sec
	= 1.0 g/sec
	= 3600 g/hr
	= 86,400 g/day
	= 86.4 kg/day
Allow for 90% transfer efficiency	= 96 kg/day

1 kg liquid oxygen converts to 738 L

96 kg oxygen required per day	= 70.85 m^3 liquid oxygen
Approximate cost of oxygen	= \$2.50 per m^3
Approximate cost per day	= \$177.12
Costs for extra 2.0 mg/L for 100 days	= \$17,712

Storage vessel charges will be in the order of \$900 - \$1,100 per month. Additional costs may be incurred for purchase or rental of the oxygen dissolver equipment, and operating and maintenance. Initial establishment costs are likely to include the cost of the saturation device and associated plumbing and oxygen monitoring and control and alarm systems with costs varying in relation to the complexity of the system and operating features desired.

2.4 AMMONIA

Summary

- Ammonia is the major waste product in intensive aquaculture systems.
- Ammonia is excreted passively through the gills by aquatic animals.
- Ammonia in water can exist as non-toxic ionised (NH_4^+) or highly toxic un-ionised ammonia (NH_3) determined by pH and to a lesser extent water temperature.
- Alkaline pH strongly favours the formation of toxic un-ionised ammonia so effects of ammonia are more significant in seawater (standard pH = 8.0 - 8.2).
- Harris *et al* (1998) demonstrated a 5% reduction in growth (EC_5) of juvenile *H. laevigata* at free ammonia nitrogen - FAN (un-ionised NH_3) levels greater than 0.041 mg/L with a 50% reduction at FAN levels greater than 0.158 mg/L.
- Huchette *et al* (2003) demonstrated an EC_5 for juvenile *H. rubra* of 0.006 mg FAN/L.
- Management of ammonia toxicity in flow through abalone aquaculture systems can be achieved by:
  - Increasing water flow rates.
  - Removal of decomposing organic matter (i.e. faeces, uneaten feed).
  - Feed management to avoid overfeeding.
  - Adjustment of stocking density.

2.4.1 Ammonia in aquaculture systems

Ammonia is the major waste product in intensive aquaculture systems. It is produced through metabolism of feed required to meet demands for growth, maintenance and activity.

Ammonia is excreted passively through the gills by aquatic animals where it is quickly diluted in the natural environment but may accumulate to toxic levels in more intensive aquaculture systems.

Ammonia in water can exist as non-toxic ionised (NH_4^+) or highly toxic un-ionised ammonia (NH_3) and the relative proportions (mole fraction) of these forms is primarily determined by the prevailing pH and to a lesser extent water temperature (Table 2). The toxicity of ammonia is more significant in seawater (standard pH = 8.0 - 8.2) as the proportion of the toxic un-ionised form of ammonia increases greatly as pH becomes more alkaline. For example, the percentage of ammonia existing in the un-ionised form in seawater at 20°C will increase by over 700% as pH increases from 8.0 to 9.0 (Table 2).

Un-ionised ammonia is toxic to aquatic organisms and finfish develop gill lesions due to chronic exposure to levels >0.02 mg/L. It is possible that chronic exposure to un-ionised ammonia may also act indirectly as animals affected may ultimately display reduced growth and survival particularly if gills are damaged to the extent that their function is impaired and exposed to bacterial infection (Forteath, 1988).

Table 2. Comparison of the effect of water temperature and pH on the percentage change in mole fraction of un-ionised ammonia (based on data from Creswell, 1993.)

Water Temperature	pH					% Change	
	7.8	8.0	8.2	8.3	9.0	pH 8.2-8.3	pH 8.0-9.0
20 oC	0.0136	0.0215	0.0336	0.0419	0.1798	20%	736%
25oC	0.0195	0.0305	0.0475	0.0591	0.2394	20%	685%
% Change 20 -25	30.3%	29.5%	29.3%	29.1%	24.9%		

2.4.2 Ammonia considerations for abalone culture systems

Harris *et al* (1999) conducted a range of trials on juvenile *H. laevigata* held at 18 ºC. These trials demonstrated a 5% reduction in growth at free ammonia nitrogen - FAN (un-ionised NH_3) levels greater than 0.041 mg/L with a 50% reduction at FAN levels greater than 0.158 mg/L. These figures equate to total ammonia nitrogen (TAN) levels of 1.6 mg/L and 6.29 mg/L respectively, that would be obtained from using a standard ammonia test kit at 18 °C and pH 8.1. Huchette *et al* (2003) found free ammonia nitrogen (FAN = NH_3) to be significantly higher under hides used in culture of *H. rubra*. In this study FAN increased significantly as water progressed through a series of tanks at a high stocking density (170 individuals/m^2) and increased faster in the high stocking density treatment compared to a

lower stocking density treatment (85 individuals/m^2). FAN was negatively correlated with growth and total ammonia nitrogen (TAN) was positively correlated with the total number of animals upstream. From this trial the chronic ammonia exposure causing a 5% reduction in growth (EC_5) was deduced to be 0.006 mg FAN/L and it was estimated that each juvenile (37 ± 6 mm) produced 0.011 ± 0.002 μmol TAN/g/hr. Observations by these authors suggest that ammonia levels do vary with the time between tank cleaning and the amount of accumulated uneaten food and faeces.

Edwards *et al* (1997) demonstrated increased oxygen consumption by juvenile (20 – 80 mm) *H. laevigata* that were chronically exposed to elevated levels of ammonia (to 0.5 mg NH_3-N/L) over 2-3 months. Oxygen consumption was up to 188% greater than the control value and it is suggested that this results from greater energetic cost of ion pumping at the gills.

These studies confirm that ammonia has the potential to significantly reduce the growth of abalone, particularly as the density of culture increases. These studies confirm that management of ammonia toxicity in flow through aquaculture systems can be achieved by:

- Increasing water flow rates to dilute ammonia concentrations.
- Removal of decomposing organic matter (i.e. faeces, uneaten feed).
- Feed management to avoid overfeeding.
- Adjustment of stocking density.

2.4.3 Nitrite

Nitrite is rarely a problem in flow through seawater systems as high levels of the chloride ion compete with nitrite for uptake across the gills of aquatic animals. Hence the level of nitrite that is regarded as toxic in seawater is much higher than in freshwater. Nitrite toxicity may be of concern for abalone farming where they are cultured in recirculation systems for grow out or prior to processing or live transport. Appropriate water exchange and progressive stocking to allow complete biological filter function can be used to limit build up of nitrite in these systems.

2.5 OTHER WATER QUALITY PARAMETERS

2.5.1 Hydrogen sulphide

Hydrogen sulphide (H_2S) is highly toxic to aquatic animals and forms through anaerobic decomposition of thick deposits of organic matter. The proportion of un-ionised H_2S and

ionized HS^- and S^{2-} is regulated by pH with proportion of toxic H_2S decreasing with increasing pH (Lawson, 1995). Abalone are very susceptible to H_2S and reduced growth has been recorded at levels of 0.05 mg/L (Olin, 2000). Due to the high toxicity of H_2S any detectable level should be considered to be a hazard (Lawson, 1995).

Accumulation of decomposing organic matter in delivery lines, stagnated pipe sections and accumulation of uneaten feeds in culture tanks are likely locations for production of H_2S in abalone culture systems. Control of H_2S in culture systems is generally through minimizing locations that allow accumulation of organic matter and subsequent anaerobic decomposition as thick layers develop. Plumbing layouts installed on abalone farms inspected provide the capacity for periodic use of inlets at opposite ends of tanks. This provides the potential for build up of H_2S in relatively long pipe sections (20 m) that are stagnant for extended periods (days-weeks). Plumbing to all tanks should include the ability to easily discharge anoxic water for 1-5 minutes before directing water into tanks following periods during which pipes have not been in use. Better placement of valves could assist this by allowing closure of supply lines with minimal retention of water when pipe sections are not in use.

2.5.2 Total dissolved gases

Supersaturation is often overlooked as a cause of mortality in aquaculture systems that rely on pumping water. Gas bubble disease that may cause significant mortality in fish is attributed to the uncompensated hyperbaric pressure of dissolved gases that form bubbles in tissues (Colt, 1984). Total dissolved gases other than oxygen that are implicated in this problem include nitrogen and to a lesser extent carbon dioxide. The most common cause of supersaturation of seawater is air being sucked into the delivery system on the suction side of pumps through minor leaks in pipes, fittings or flanges. Given the large pumps and multiple flanged joints used in abalone farming, supersaturation due to minor air leaks would not be unexpected.

A study on the effect of gas supersaturation on juvenile (12 mm ± 1 mm) red abalone, *Haliotis rufescens*, in which gas supersaturation was achieved by introducing compressed air into seawater within a pressure chamber was conducted by Leitman (1992). Results showed that with each gas level from 100, 105, 110 and 115% there was an incremental decrease in growth rate. Abalone lost weight at or above 120% saturation. Apart from one animal, the only mortalities recorded in this study were in treatments at or greater than 120% saturation with cumulative mortality over the duration of the trial reaching 100% after 48 days, 72 days and 90 days for the 143%, 130% and 120% treatments respectively. No similar studies have been conducted for *H. laevigata* or *H. rubra*.

Gas supersaturation is generally minimised through the use of either packed column type or vacuum degassers that typically lower levels of total gas pressures to <102%. No degassing systems are used on the abalone farms inspected. Slab tank systems used at South Australian Mariculture (SAM) Abalone and SAS may achieve some degassing through spray bars at the entry points and during dumping cycles and the high surface area of moving water exposed to air in these systems. More efficient degassing could only be achieved on commercial farms through installation of large purpose built packed columns or vacuum degassers. This could only be justified if some causal link could be established between supersaturation and mortality or reduced growth performance.

Measurement of total dissolved gases can be achieved using a saturometer that will measure total gas pressure from which the quantity of dissolved nitrogen can be determined.

2.6 STRESS

Summary

- Poor nutrition and water quality will subject animals to stress that will adversely affect growth and predispose animals to disease.
- Stress can be acute due to a high level of threat exerted over a short period of time; or chronic due to a low level challenge over an extended period.
- Lacoste *et al* (2001a,b) recorded an increased load of pathogenic bacteria and higher mortalities in stressed infected oysters compared to unstressed infected animals. Similarities between molluscan immune systems suggest a similar relationship would be found with abalone.
- Management and husbandry operations on abalone farms need to avoid or minimize all potential stressors particularly during periods in which animals are subject to elevated water temperatures.

In culture conditions poor nutrition and water quality will subject animals to stress that may adversely affect growth and predispose animals to disease. It is important that abalone farmers be able to determine when animals are under stress. It is difficult to assess the degree that abalone are stressed as they display few behavioural characteristics prior to death (Haldane, 2002). Stress can be acute due to a high level of threat exerted over a short period of time, or chronic due to a low level challenge over an extended period (weeks, months) or constantly. The effect of stress on aquatic animals generally results in increased susceptibility to disease due to immune suppression, reduced growth or mortality.

A number of studies have been conducted that have attempted to measure stress in abalone or have investigated the stress response mechanism of abalone. Haldane (2002) used a number of biochemical indicators to measure stress in *H. laevigata*. These studies measured the

response of these indicators to stress exerted through exercise and exposure to elevated water temperature.

Haldane (2002) concluded that muscle pH (decreased consistently and significantly with exercise and temperature stressors) and haemolymph glucose (increased significantly with temperature stress) had the most potential for assessing stress in this species. This author recommended further studies would be required to determine stable baselines for these indicators for a variety of stressors.

Baldwin *et al* (1992) also demonstrated that exercise decreased pH in the foot muscle of *H. iris* and attributed this to activation of anaerobic glycolysis in the foot muscle. This may be significant to abalone farmers as meat toughness increases and texture deteriorates as foot muscle pH declines.

It has been demonstrated that catecholamines (e.g. noradrenaline and dopamine) exert immunosuppressive effects on haemocytes in oysters (*Crassostrea gigas*) and has demonstrated an increased load of pathogenic bacteria and higher mortalities in stressed infected animals compared to unstressed infected animals (Lacoste *et al*, 2001a, 2001b). Malham *et al*, 2003 demonstrated an increase in noradrenaline and dopamine in the haemolymph of stressed *H. tuberculata* and suggests that levels of catecholamines may reflect the stress status of abalone. These authors suggest that in response to stress, animals may downgrade immune function to allow bioenergetic resources to be redirected towards other higher priority physiological functions such as increased respiration, oxygen uptake, and glycolysis and contend that stress induced immunomodulation is a common phenomenon in molluscs.

The farm management implications of this research are that stressors such as elevated water temperature and sub-optimal levels of DO (and interactions of these) may induce immunosuppression in abalone that will predispose animals to infection by pathogenic bacteria (*Vibrio* sp) implicated in summer mortality and bloat (Section 6). This predisposition will be further enhanced through poor feed management and other husbandry practices that increase stress during the summer period.

2.7 OPERATIONAL VARIABLES AND WATER QUALITY MANAGEMENT

2.7.1 Stocking density

Stocking density has the ability to significantly effect water quality through the oxygen consumption required to support the biomass stocked and the ammonia produced as a result of feeding. The number of animals in each culture unit will also impact indirectly through their physical presence that will affect water flow characteristics within tanks and the ability of feed to be flushed from the system.

Moore and Hone (1995) conducted trials on *H. laevigata* at stocking densities ranging from 25% - 150% available surface area and concluded that high densities suppress growth and best yield per tank was recorded at 75% - 100% effective cover although this may not provide the best economic return. Of interest was the observation that weight was not affected by density as much as length with a higher meat to shell ratio recorded for abalone cultured at high stocking densities. Maquire *et al* (1996) reported a decrease in growth equivalent to 39% for abalone stocked at 300 per tank (70L aquaria) compared to 100 per tank. Huchette *et al* (2003) compared growth of *H. rubra* at high (170 animals/m^2) and low (85 animals/m^2) densities in raceway tanks (2.7 x 0.5 x 0.25m) with water flowing through five tanks connected in series. Results demonstrated significant decreases in growth with increasing initial size, stocking density and free ammonia nitrogen (FAN) levels. The authors suggest that competition for space affected growth to a larger degree than the other factors and small animals performed better than larger ones. Other studies on abalone have also demonstrated reduction in growth as density increases. Huchette *et al* (2003) summarised these studies and reported growth reductions of 14 to 52% attributed to a 2 – 60 fold increase in stocking density, although no obvious correlation between growth and density could be found.

Clearly the interactions between water quality, stocking density, tank design and management are not well understood and stocking density is not an easy factor to alter during the culture of this attached animal. It is recommended that the optimal stocking density should be reviewed and allowances made to provide best available water quality during the most stressful times of the year for each type of system used and each size class stocked. Currently this is achieved on farms by using water flow rates that produce less than 10% reduction in dissolved oxygen levels between inlet and outlet points.

2.7.2 Water flow and water velocity

With industry development culture systems have progressed away from deep tanks to culture within long lengths of closed pipes, to shallow raceway tanks, to convoluted raceways (maze tanks), to shallow slab tanks with tippers that appear to be current system of choice. It would be fair to conclude that tank design is a "work in progress" with further modifications being explored on all farms although there is currently a convergence towards the “slab tank” design. It is also apparent that different tank types are better suited for culture of different sized abalone so farms may be comprised of sections with differing tank types.

Two of the three commercial abalone farms visited are primarily using variations of the slab tank design (20m x 2.5m). In these inlet spray bars are located across tanks at each end to allow direction of water flow to be reversed periodically. At any time water is delivered from one spray bar only. Operation of slab tanks is generally based upon an assumption that the water flow of 2.5 L/sec/tank is required. This requires a total farm pumping capacity in the order of 500 L/sec (1.8 ML/hr) for a 200 slab tank farm plus additional water required for nursery and hatchery sections. Expected water velocity (cm/sec) at a range of water depths (mm) and flow rates (L/sec) for 2.5m slab tanks is provided (Table 3). A water flow of 2.5 L/sec/tank across a 2.5m wide slab tank with 25mm water depth provides a water velocity of 4 cm/sec.

Raceway tanks (15 - 20m x 0.3m) have a single inlet spray bar fixed at one end with flows in the order of 15 L/min/tank (0.25 L/sec/tank). Expected water velocity (cm/sec) at a range of water depths (mm) and flow rates (L/sec) for 300mm wide raceways is provided (Table 4). A water flow of 0.25 L/sec/tank across a 300mm wide slab tank with 25mm water depth provides a water velocity of 3.3 cm/sec.

Table 3. Range of water velocities (cm/sec) expected at different combinations of water depth (mm) and water flow (L/sec) in 2.5m wide slab tanks.

Tank width (mm) 2500

Depth	Water flow (L/sec)														
(mm)	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
5	8.0	12.0	16.0	20.0	24.0	28.0	32.0	36.0	40.0	44.0	48.0	52.0	56.0	60.0	64.0
10	4.0	6.0	8.0	10.0	12.0	14.0	16.0	18.0	20.0	22.0	24.0	26.0	28.0	30.0	32.0
15	2.7	4.0	5.3	6.7	8.0	9.3	10.7	12.0	13.3	14.7	16.0	17.3	18.7	20.0	21.3
20	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0	10.0	11.0	12.0	13.0	14.0	15.0	16.0
25	1.6	2.4	3.2	4.0	4.8	5.6	6.4	7.2	8.0	8.8	9.6	10.4	11.2	12.0	12.8
30	1.3	2.0	2.7	3.3	4.0	4.7	5.3	6.0	6.7	7.3	8.0	8.7	9.3	10.0	10.7
35	1.1	1.7	2.3	2.9	3.4	4.0	4.6	5.1	5.7	6.3	6.9	7.4	8.0	8.6	9.1
40	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0
45	0.9	1.3	1.8	2.2	2.7	3.1	3.6	4.0	4.4	4.9	5.3	5.8	6.2	6.7	7.1
50	0.8	1.2	1.6	2.0	2.4	2.8	3.2	3.6	4.0	4.4	4.8	5.2	5.6	6.0	6.4

Table 4. Range of water velocities (cm/sec) expected at different combinations of water depth (mm) and water flow (L/sec) in 300mm wide raceway tanks.

Tank width (mm) 300

Depth	Water flow (L/sec)			
(mm)	0.25	0.50	0.75	1.00
5	16.7	33.3	50.0	66.7
10	8.3	16.7	25.0	33.3
15	5.6	11.1	16.7	22.2
20	4.2	8.3	12.5	16.7
25	3.3	6.7	10.0	13.3
30	2.8	5.6	8.3	11.1
35	2.4	4.8	7.1	9.5
40	2.1	4.2	6.3	8.3
45	1.9	3.7	5.6	7.4
50	1.7	3.3	5.0	6.7

2.7.3 Mantle cavity flow

Based on discussions with farm operators and reviewing the available literature it is apparent that considerable attention has been directed at water quality within tanks and little to the water quality within the mantle of the abalone within the tanks. Abalone are mostly sedentary animals with their paired gill structure housed within a shell that has relatively little capacity for water exchange with the surrounding water. In comparison, fish are able to move through water and have an operculum and mouth function that provides open transfer of high volumes of water across respiratory surfaces of the gill. In effect, the water retained within the mantle cavity of an abalone can be regarded as being relatively isolated and partially independent of the external water body.

Voltzow (1983) cited in Tissot (1992) suggests that respiratory pores or "tremata" are not exclusively exhalent structures and showed in *H. kamtschatkana* that they induce a passive circulation of water through the mantle cavity in response to the dynamics of water flowing over the shell surface. It is proposed that variations in shell structure between abalone species has evolved in response to water movement that causes drag forces and pressure gradients between tremata to promote mantle cavity circulation and the author raises the hypothesis that abalone are energetically dependant on induced mantle cavity flow (Tissot, 1992). The significance of this for abalone farming is that suitable water velocities need to be provided to induce sufficient mantle cavity circulation to allow optimal performance of the species selected for culture in Australia.

Tissot (1992) studied induced water flow through the mantle cavity over a range of external flow velocities for several abalone species with different shell sculpture indices and tremata

elevation. Of significance from this study, is that black abalone, *H. cracherodii* have a sculpture index (=1) similar to smooth shelled *H. laevigata* and apart from the black colouration of the shell they are visually very similar in appearance and profile (Figure 8). This similarity suggests comparable water flow characteristics between these two geographically isolated species. Black abalone were found to experience little induced mantle flow at low water velocities but had elevated induced mantle flow rates at higher velocities (Figure 9) that approximated those of their natural habitat (> 50 cm/sec). By comparison, red abalone, *H. rufescens,* that have a greater shell sculpture index (=2) similar to *H. rubra,* with larger more elevated tremata, were able to induce a two-fold increase in mantle circulation at low external velocities (5 – 15 cm/sec). The author concludes that species with large elevated tremata (such as *H. rubra*) are more efficient at promoting mantle cavity circulation at low external velocities and can maintain this in a wider range of habitats than species with small un-elevated tremata (such as *H. laevigata*). The significance of this morphological feature to abalone farming is the suggestion that higher flow rates may be required in tanks used to farm *H. laevigata* and this species may not be as efficient at oxygen removal in low flow situations and thus more susceptible to poor performance in reduced DO conditions. Reduced mantle circulation may also effect tolerance to other water quality parameters if this species is physiologically dependant on higher external water velocities.

Put in perspective, slab tanks using 2.5 L/sec/tank provide water velocity of 4 cm/sec based on a 2.5 m wide slab with 25 mm water depth. Raceway tanks using 15 L/min equate to a water velocity of 3.3 cm/sec. Both water velocities are well below that at which smooth shelled black abalone (*H. cracherodii*), similar to *H. laevigata*, display a significant increase in induced mantle flow. Farm water velocities are closer to those that have been shown to induce increased mantle flow (between 5 – 10 cm/sec) in red abalone, *H. rufescens* that has similar shell sculpture index to *H. rubra*. It is expected that physiological adaptation to high water velocities may include the amount of gill respiratory area and tolerance to internal water quality related to mantle cavity flushing rates. Culture of abalone at water velocities below those to which they are morphologically and physiologically adapted is likely to lead to sub-optimal growth performance.

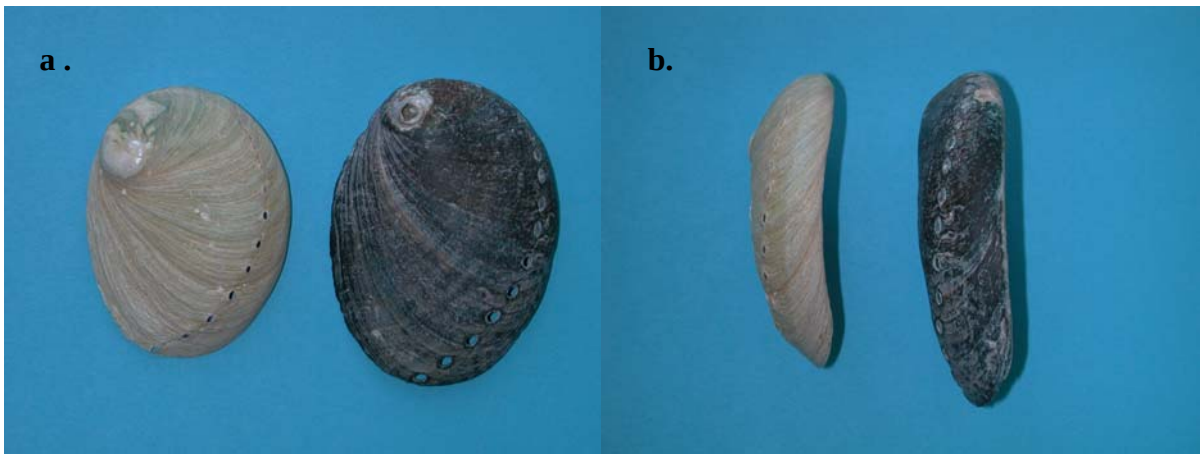

Figure 8. Comparison of shells of *H. laevigata* (left) and *H. cracherodii* (right)
a. Top view. b. Side profile

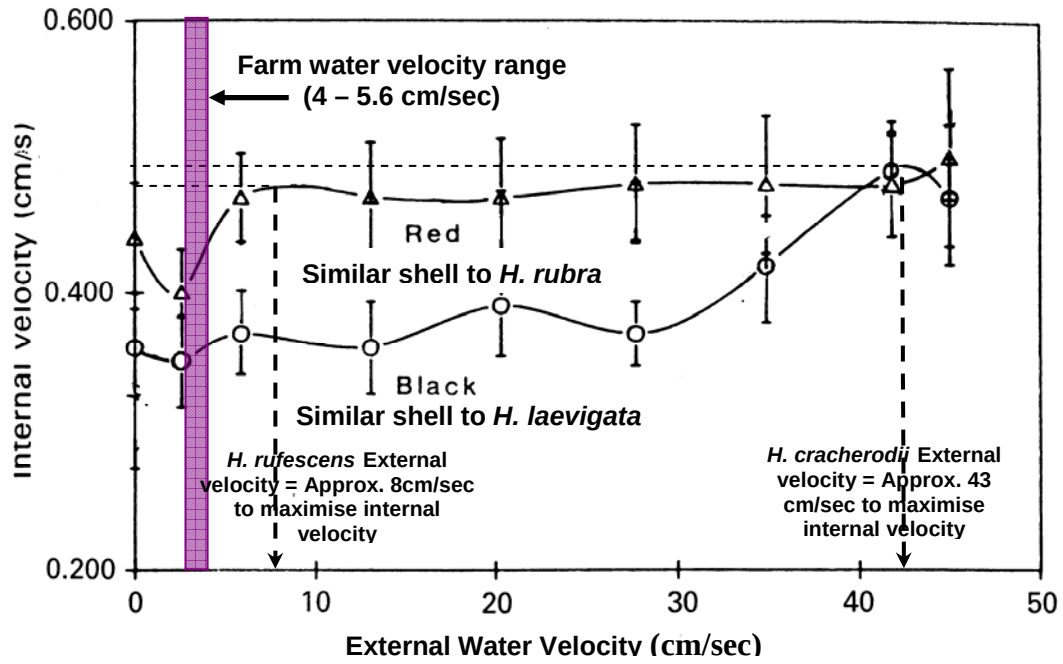

Figure 9. Induced mantle flow recorded by Tissot (1992) for *H. cracherodii* (black abalone) and *H. rufescens* (red abalone) related to shell types of species farmed and current water velocities used on farms in SA.

The water velocities used on farms are in accordance with those demonstrated to promote feed consumption by *H. laevigata* in studies by Higham and Hone (1996) and Fleming *et al* (1997). In both these trials a linear increase in feed consumption was recorded across the range of increasing water velocities between 0.7 – 5.8 cm/sec and 0.42 – 11.95 cm/sec

respectively. Subsequent trials indicated that specific growth rate increased with water flow rate to a peak at 7.6 cm/sec in canoe type raceways and 4.4 cm/sec in PVC guttering type experimental tanks. It was suggested that at high flow rates abalone may not be able to react quickly enough to trap artificial feed Higham *et al* (1998).

The observation that farmed abalone tend to migrate towards the water inlet point in tanks is thought to be attributed to the availability of the cleanest and highest DO content of water at this point. An alternative explanation supported by the studies reported suggests that this behaviour may be attributed to abalone having a preference for higher water flow rates or turbulence that induce better mantle cavity flow. This is supported by observations that abalone grow better in effluent pipes and within open drains collecting outflow from many tanks across the farm. In both situations water velocity will be far greater than that within individual tanks. If the water velocity currently used for farming abalone is not sufficient it would be expected that animals may respond by moving towards the point of highest water velocity in tanks. In addition the turbulence induced at inlets will effectively be improving diffusion of dissolved gases by disruption of boundary layers that may be inhibiting gas and waste transfer at low water velocities.

It is apparent that current water velocities are selected to accommodate characteristics of feed and costs of water movement. Improvements in growth performance would be expected if alternative higher velocity systems were shown to be required after measurement of induced mantle flow rates in local species. It is recommended that induced mantle flow be measured for both *H. laevigata* and *H. rubra* in order to assess the suitability of water velocities currently used on abalone farms.

To date systems used rely on water flow typically from one inlet location to provide a number of functions, including:

- Provision of oxygen.
- Removal of wastes.
- Allow artificial feed to be retained.
- Stimulate feeding activity.

Optimal performance of abalone may not be achieved unless some of these functions can be provided using specific components. This approach advocates design of systems and operation directed towards providing the environmental requirements of abalone rather than adapting farm practices to the limitations of the system. This could be achieved through specific methods to provide each requirement, such as:

- The use of oxygenation systems to increase the DO level of inflow water.

- Development of methods to increase water velocity within tanks. This will require attention to feed retention that may be possible using variable flow with low flow (i.e. current levels) over feeding periods. Economical low head methods to increase water velocity will largely determine the possibilities of this approach.
- Waste removal should form the basis of water flow requirements. This has already been identified with installation of tippers as a separate component to address this specific problem. Other solutions may be possible.

2.7.4 Feeding and water quality

The feeding strategy adopted by abalone farms has the capacity to impact upon water quality. Discussions with farm managers confirm that feed is generally added to tanks during the mid-late afternoon. During the night abalone feed and excess feed and wastes flush from tanks or are washed out using brooms assisted by periodic increases in water flow using tippers on slab tanks. The amount of feed fed will be related to the size of the animals and water temperature following guidelines provided by feed manufacturers and experience of farm managers.

Typically, abalone are fed 2-3 times per week or every 2 days. Maguire *et al* (1996) demonstrated that feeding every 2 days and 4 days improved growth of *H. laevigata* relative to controls fed daily. Water quality available to abalone will be optimized if feeding is restricted to every 2 – 4 days followed by removal of excess feed the day following feeding, although correct feed rates should ensure that no more than 5% should remain the following morning (pers. comm. Tom Hyde, Farm Manager SAM Abalone). This schedule is in agreement with observations that only 26 – 31% of abalone fed every second day were moving and presumably seeking feed between 8 pm – 12 midnight suggesting that restricted feeding at this level does not affect consumption (Fleming, 1996).

It would be expected that some improvement in the culture environment, growth performance and survival could be achieved through the use of night feeding, careful management of feed inputs, frequent removal of wastes and mortalities, and better feed storage particularly over the summer period. Gains achieved through better growth and reduced mortality over the productive summer period should be assessed to determine if these compensate for additional labour inputs required to achieve any improvements.

2.7.5 Feed storage

Storage of feeds on farms was also observed to be inadequate for an expensive feed. Abalone feeds contain fat (<3%) and vitamins that will be subject to deterioration at shed temperatures that could be up to 50^{o}C. Maguire *et al* (1996) reported a 43.8 % reduction in growth due to use of feed that had been subjected to heat treatment at 110ºC for 48 hours. It is likely that some improvement in growth may be possible if better conditions for feed storage are implemented.

2.7.6 Farm hygiene

Discussion with farm mangers suggests some interactions between summer mortality, and farm operations. There is a possibility for tank to tank transfer of disease through the use of brushes and brooms to clean slabs, raceways and tanks, although it appears that abalone may be predisposed to infection by some other factor. The use of alternative methods for cleaning tanks, particularly when water temperatures exceed 20^{o}C should be evaluated. One method that may be beneficial is the use of "water brooms" or pressure cleaners to reduce transfer between tanks although potential for aerosol transfer of disease between tanks would need to be minimised.

Maguire *et al* (1996) attributed an increase in growth when feeding every 2 – 4 days to reduced daily disturbance. In this trial tanks were completely drained for cleaning. Daily drainage of tanks produced a 17.6% reduction in growth when compared to daily siphoning. This effect was not observed with tanks drained every 2 - 4 days for cleaning.

2.8 OPTIONS FOR IMPROVEMENT OF WATER QUALITY

2.8.1 Silt/particulates and bacteria association

Currently raw unfiltered seawater is used for abalone farms in South Australia and links have been identified between disease causing bacteria and sediment loads in water. Monitoring of seawater supplied to the SAABDEV site at Louth Bay during windy months (October - November) showed an increase in *Vibrio* sp. levels associated with increased suspended solids at this time (pers. Com Mark Gluis). A seasonal study on the occurrence of *Vibrio* sp. in Chesapeake Bay by Kaneko and Colwell (1978) revealed a cyclical pattern whereby their numbers in the water and in sediment decreased during winter and then peaked during summer. It was suggested that *V. parahaemolyticus,* a pathogenic species, were released from

sediment into the water column during summer by wind and wave action whereby they then proliferated after becoming associated with plankton (Kaneko and Colwell, 1978). Some of the bacteria would be expected to be brought back to the bottom via attachment to fragments of plankton returned to the bottom via sedimentation. Thus bacteria in the sediment increase not only due to growth but as a result of sedimentation as well. *V. cholerae,* another toxic species, has been shown to readily attach to a number of substrates including chitin, cellulose, glass and $CaCO_3$, plankton and plants (Hood *et al*. 1984). It also can survive unattached in the water column as a free living form. However, experiments by Hood *et al*. (unpublished data) revealed that the viability of *V. cholerae* is reduced in seawater in the absence of particulates. A study by Jun-yi *et al*. (2003) showed that the higher the organic sediment level in a pond, the lower the survival rate of the abalone.

2.8.2 Mechanical filtration

The direct use of pumped raw seawater is the most economical but least managed system to supply water to abalone culture tanks. The most basic water treatment available to improve water treatment on abalone farms includes the use of mechanical filtration and disinfection (ultraviolet or ozone).

If additional water treatment is regarded as beneficial the system installed will either need to retain the pressure (head) within current distribution systems, or alternatively raw seawater will need to be pumped to a header tank or pond for short term storage before non-pressurised water treatment and distribution to culture tanks. Analysis of particle size distribution in seawater would need to be undertaken for selection of the appropriate level of filtration required. Increased bacterial numbers associated with elevated silt load have been suggested to be involved in summer mortality and deposition of silt in culture tanks is a common occurrence. Silt particle sizes range between 20μm – 50μm (Egna and Boyd, 1997) and removal can be achieved with mechanical filtration as typical filters can remove particles in this size range. Additional benefit may be provided through reduction of fouling organisms in the culture systems.

2.8.3 Pressurised water treatment options

These systems require a pressure head to operate. Examples for mechanical filtration options that could be considered on abalone farms include commercial rapid sand filters (RSF) that are physical filters, and hydroclones that rely on subjecting suspended solids to centrifugal acceleration that makes them separate (i.e. centrifugal separation). Filtration of silt particles in

the desired range of 20µm – 50µm is readily achievable using RSF with capacity of these units dependant on water flow rates and suspended solids load in the seawater supply. Final filtration components would need to be preceded by coarse primary screens (automated self cleaning) to remove or preclude weed, shells etc. RSF or hydroclones are available in multiple units to provide the filtration capacity and system operational flexibility required (i.e. number of pumps in operation). RSF will introduce a head loss reducing water flow and increasing power consumption. Hydroclones will require a small underflow of water to be discharged continuously to remove separated solids. The high volumes of seawater used on abalone farms will require installation of commercial filtration equipment and appropriate design specifications to be determined by water treatment engineers.

2.8.4 Non-pressurised water treatment options

A pre-requisite for these treatment and delivery systems is a structure such as a header tank or dam to conserve head pressure after delivery from the seawater intakes. Passive or gravity driven flow can then be used throughout the treatment and delivery systems. This configuration would need to be considered during initial design of the farm.

This approach to water treatment may involve the use of a settlement basin of appropriate size to match flow volume requirements with a suitable retention time to take advantage of passive sedimentation (e.g. 500 L/sec would require 1.8 ML capacity pond to allow one hour retention). Larger sized ponds may also provide the benefit of back up storage for a limited duration (hours) if power or pump failures occurred.

Micro-screen filtration (e.g. drum filters or disc filters) is an option for low pressure filtration of high water volumes to a relatively fine degree (20µm is possible).

A disadvantage of non-pressurised systems is the need for larger diameter plumbing to deliver water to tanks. The extra cost of this may be partly offset through the use of cheaper lower pressure rated pipe and fittings and reduced pumping costs compared to pressurised treatment options.

2.8.5. Disinfection of water

2.8.5.1 Ozone

Ozone is an option to provide either disinfection or sterilization of incoming seawater but a relatively large generator would be required. For a 500 L/sec farm using a typical ozone dose of 0.05 - 0.1 mg/L this would equate to a system capable of producing 90 – 180 g/hr or 2.16 -

4.32 kg/day. Residual ozone is always of concern, particularly in closed delivery systems such as those utilized on abalone farms. To overcome this, a typical ozone disinfection system will require an ozone contact vessel followed by a method for degradation of residual ozone (i.e. granular activated carbon filter, extended holding, degasser or high intensity UVc light).

The ideal water supply configuration for use of ozone on abalone farms would include the use of a header tank or dam from which all culture tanks would receive water supply by gravity flow. In effect the header tank could function as an ozone contact tank. This will allow ozonation of water entering the header tank under pressure and provide 10 – 20 minute contact time before discharge to a degasser with subsequent distribution of water to the farm. Ozone will improve effectiveness of mechanical filtration if installed before this component as apart from disinfection (bacteria, viruses and algae) it will also assist by degradation and flocculation of dissolved organics.

Ozone can be added via a venturi system as water enters the contact tank or through a fine diffuser in the contact tank. Ozone dose is generally maintained at a desired concentration by use of an oxidation reduction potential (ORP) probe located after the degasser. The ORP probe measures redox potential in millivolts and is connected to a meter and controller that is in turn connected to the ozone generator. It is recommended to maintain an ORP reading within a set range that is generally 300-350 mv for seawater (Carson, 1997). Due to the wide range of ozone treatment equipment available it is not possible to provide a useful estimate of the cost associated with this method of water treatment. Any estimate will need to include the costs of the additional equipment that needs to be installed as part of this type of treatment method. These items include:

- Air drying or oxygen generation.
- ORP monitoring and control of ozone dose.
- Ozone contact vessel/s.
- Method of residual ozone removal prior to use (i.e. degassing, activated carbon filtration).

2.8.5.2. Ultra-violet disinfection

Ultraviolet filtration can provide economical disinfection of high volumes of seawater. The current water supply systems used on abalone farms would enable easy installation of inline pressurised UV units. Efficacy of UV disinfection is positively related to increasing water clarity, as a good kill rate requires irradiation to penetrate through the water. Particles in water will also provide a barrier to irradiation. As no fine filtration of seawater is undertaken it would be expected that large units would be needed and the efficacy of disinfection would not be high at times when water clarity was reduced. Frequent routine cleaning of lamp sheaths

would be required to remove bacterial films and other biofouling. The large UV units required for such an application would generally be available with manual or automated lamp wipers to assist with this maintenance requirement. Again there is a wide range of manufacturers of ultraviolet treatment equipment and it is not possible to provide a useful estimate of the cost associated with this method of water treatment.

The advantages to be gained from the use of mechanical filtration or disinfection need to be demonstrated. A significant benefit would need to be shown to justify the large capital cost to install and maintain the water treatment equipment required.

2.9 WATER TEMPERATURE CONTROL

Cooling costs can be estimated based upon the need for approximately 1.16 kW to cool 1.0 KL of seawater by 1 ^{o}C per hour. For a farm using 500 L/sec flow this equates to a cooling energy requirement of approximately 2000 KW/hr/^{o}C that could be provided using commercial refrigeration equipment with between 330 – 400 KW/hr power usage (approximately $50 - $60.00/hour cost). It may be possible to reduce some of the energy demand through use of outflow water to partly cool incoming seawater. Regardless, the high installation and operating costs prohibit this option in flow through abalone culture systems.

2.10 ALTERNATIVE WATER SOURCES

The possible use of bore water at each farm location should be thoroughly investigated. It would be expected that seawater from coastal bores at approximately 20m should provide a relatively constant water temperature (19 – 21 ^{o}C). Volume available may be limited and this may require a number of bores to be installed to provide the total flow required. World wide, many high volume flow-through based aquaculture facilities utilise bore water as the sole supply. As no dissolved oxygen will be contained, the use of bore water needs to incorporate a degassing/aeration system and possibly an oxygenation system. Another advantage of bore water is that it should be free of bacteria.

2.11 FEED ADDITIVES

There are a number of additives that can be incorporated into feed that may assist abalone to tolerate adverse water quality conditions. These include;

- Immunostimulants such as beta glucans, mannan oligosaccharides and nucleotides that propose to enhance the function of the immune system.

- Probiotics to promote the colonization of beneficial bacteria in the gut to out compete or suppress the growth of pathogenic bacteria.
- Compounds that bind iron (ie. lactoferrin) are another additive that could be investigated as bacteria need iron for their survival and binding up free iron should inhibit their growth. The effectiveness of these additives should be assessed on farms over the summer period.

2.12 RECOMMENDATIONS

- Improve water quality monitoring and reporting across farms by undertaking periodic intensive sampling to identify variable parameters and most important times to sample. Initiate routine monitoring based upon outcomes of the intensive investigation. As a minimum, routinely monitor selected representative tanks (2 or more) for each size category of abalone across the farm. Influent and effluent DO, water temperatures, feed inputs, uneaten feed and mortality should be recorded.
- Enter water quality and mortality data weekly and report this graphically for comparison against previous records (i.e. last week/s and previous seasons).
- If possible install DO and water temperature logging at a common water supply point, at key distribution point and at inlet and outlets of selected representative tanks.
- Assess potential benefits of an oxygenation system to operate during periods of elevated water temperature.
- Review recommendations for optimal stocking density during the most stressful times of the year. This should be done for each type of system used and each size class stocked in order to provide the best available water quality during periods of elevated water temperature.
- Explore options to increase water flow after feeding (i.e. at night).
- Increase attention to farm hygiene particularly during summer including disinfection of cleaning brooms between tanks; daily removal of mortalities, uneaten feed and faeces, and consideration of the use of water brooms or other alternatives.
- Reduce stocking density through selective harvest of larger animals leading up to summer and restocking of smaller cohorts before summer.
- Adopt night feeding or delayed feed addition (6 - 8 pm) and feed at 2 - 4 day intervals during periods of elevated water temperature.
- Monitor uneaten feed to adjust feeding to minimum inputs particularly over summer.
- Store food in cool locations or preferably in a cold room.
- Investigate the availability of bore water at each farm location.

- Undertake an analysis of the potential of water reuse or recirculating systems for abalone culture.

2.13 FUTURE RESEARCH

- Trials conducted on commercial abalone farms to measure potential benefits from improved water treatment i.e. mechanical filtration (silt removal) and disinfection (lower levels of incoming bacteria).
- Investigate effects of interactions of major water quality parameters (i.e. dissolved oxygen and ammonia) at elevated water temperatures similar to those experienced over summer.
- Measure the interaction of oxygen consumption rate and elevated water temperature for a range of size classes of greenlip and blacklip abalone.
- Measure the metabolic oxygen demand for a range of size classes of greenlip and blacklip abalone at range of water temperatures.
- Investigate the relationship between temperature preference and size class of abalone.
- Investigate the relationship between disease and water temperature.
- Measure mantle cavity circulation flow rates against external water velocity for both greenlip and blacklip. If possible measure water quality parameters sampled from within the mantle cavity.
- Investigate the use of multiple inlet locations on culture units to increase turbulence and water velocity along the full length of raceways, slabs and troughs.
- Investigate further tank design improvements that combine high water velocity systems to improve mantle cavity flow, variable flow rate systems to allow feed addition.
- Investigate the use of recirculating aquaculture systems to culture abalone in a controlled environment.
- Determine size class and stocking density relationships for abalone grown at elevated water temperatures.
- Design and assess non-physical cleaning methods to remove waste and uneaten feed to provide added bacterial control/management over summer.
- Determine an effective measure of stress in abalone to detect the point of increase at elevated water temperatures (i.e. catecholamines or phenoloxidase). This measure should be validated on wild abalone sampled *in situ.*
- Assess the effect of immune enhancing additives, probiotics and iron binding compounds in manufactured diets over the summer period.

REFERENCES.

Baldwin J, Wells R M G, Low M and Ryder J M (1992). Tauropine and D-lactate as metabolic stress indicators during transport and storage of live Paua (New Zealand abalone) (*Haliotis iris*). *J. Food. Science* 57 (2) 280 – 282.

Bi Y, Jiang S, Lui H, Xue K and Dong J (2000). Effect of temperature and weight on oxygen consumption rate and ammonia excretion rate of *Haliotis discus hannai*. *Chin. J. App. Environ. Biol.* 6 (5) 444 -446.

Brett J R (1979). Environmental factors and growth. In: Hoar W S, Randall D J and Brett J R (ed's.), Fish Physiology Vol VIII, Bioenergetics and growth. Academic Press.

Choat J H (1988). The aquatic environment. Pp 135 – 141 In: Fish diseases - Refresher course for veterinarians, Proceedings 106. Post Grad. Comm. Vet. Sci. University of Sydney.

Colt J (1984). Computation of dissolved gas concentrations in water as functions of temperature, salinity and pressure. *Am. Fish Soc. Spec*. Publ., 14.

Creswel R L (1993). Aquaculture desk reference. Van Nostrand Reinhold, New York.

Diaz F, del Rio-Portilla M A, Sierra E, Aguilar M and Re-Araujo A D (2000). Preferred temperature and critical thermal maxima of red abalone *Haliotis rufescens*. *Journal of Thermal Biology* 25: 257 – 261.

Egna H S and Boyd C E (1997). Dynamics of pond aquaculture. CRC Press Boca Raton, New York.

Fleming A (1996). Does feed availability influence consumption? Pp 55 – 58 In: Hone P W (ed.), Proceedings of the 3rd Annual Abalone Aquaculture Workshop, August 1996, Port Lincoln. SARDI Adelaide.

Fleming A, Hone P and Higham J (1997). The effect of water velocity on consumption and growth of greenlip abalone in tanks. Proceedings of the 4th annual abalone aquaculture workshop. SARDI Adelaide.

Freeman K A (2001). Aquaculture and related biological attributes of abalone species in Australia - A review. Fisheries Western Australia. Research Report No 128.

Forteath N (1988). Aquaculture. Pp 145 – 164 In: Fish diseases - Refresher course for veterinarians, Proceedings 106. Post Grad. Comm. Vet. Sci., University of Sydney.

Gilchrist G W (1995). Specialists and generalists in changing thermal environments. I. Landscapes of thermal sensitivity. *American Naturalist* 146: 252-270.

Gilroy A and 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 laevigata* (Leach). *Aquaculture Research* 29: 481 – 485.

Haaker P L, Parker D O, Barsky K C and Chun C S Y (1998). Growth of red abalone, *Haliotis rufescens* (Swainson), at Johnsons Lee, Santa Rosa Island, California. *J. Shellfish. Res*. 17: 747 – 753.

Hahn K O (1989). Biotic and abiotic factors affecting the culture of abalone. Pp 113 – 134. In: Handbook of culture of abalone and other marine gastropods. CRC Press Inc, Boca Raton, Florida

Haldane C (2002). Measuring stress in greenlip abalone (*Haliotis laevigata*) using biochemical indicators. Honors thesis, Flinders University of South Australia.

Harris J O (1999a). Chronic effects of adverse water quality on the greenlip abalone, *Haliotis laevigata* Donovan. Ph. D. Thesis, University of Tasmania.

Harris J O (1999b). Environmental requirements of Australian abalone. *Aust-Asia Aquaculture* Aug/Sept pp 51 – 53.

Harris O H; Maguire G B; Edwards S J and Johns D R (1999). Low dissolved oxygen reduces growth rate and oxygen consumption rate of juvenile greenlip abalone, *Haliotus laevigata* Donovan. *Aquaculture* 174: 265 – 278.

Heasman M P, O'Connor W A and Frazer A W J (1996). Ontogenetic changes in optimal rearing temperatures for the commercial scallop, *Pecten fumatus* Reeve. *J. Shellfish. Res*. 15: 627 – 634.

Heasman M P, Diemar, J A, Savva N M, Brand C P, Chick R C, Worthington D G and Gibson P T (2001). Effect of temperature on growth on four age classes of blacklip abalone *H. rubra*. Proceedings of the 8th annual abalone aquaculture workshop. SARDI Adelaide.

Higham J, Hone P, Clarke S, Baudinette R and Geddes M (1998). The effect of flow on growth in juvenile greenlip abalone, *Haliotis laevigata* (Donovan). Proceedings of the 5th annual abalone aquaculture workshop. SARDI Adelaide.

Higham J and Hone P (1996). Preliminary results on the effect of water movement on feed consumption. In: Hone P W (ed.), Proceedings of the 3rd Annual Abalone Aquaculture Workshop, August 1996, Port Lincoln. SARDI Adelaide.

Hines A, Anderson S and Brisbin M (1979). Heat tolerance In the black abalone *Haliotis cracherodii* Leach, 1814: Effects of temperature fluctuation and acclimation. *The Veliger* 23 (2) 113 – 118.

Hood M, Ness G E, Rodrick G E and Blake N J (1984). The ecology of *Vibrio cholerae* in two Florida estuaries. Pp 398-409 In: Colwell R R (ed.), *Vibrios* in the environment. John Wiley & Sons, USA.

Huang C-Y, Liu P-C and Lee K –K (2001). Withering syndrome of the small abalone, *Haliotis diversicolor supertexta* is caused by *Vibrio parahaemolyticus* and associated with thermal induction. *Z. Naturforsch* 56c: 898 – 901.

Huchette S M H, Koh C S and Day R W (2003). Growth of juvenile blacklip abalone (*Haliotis rubra*) in aquaculture tanks: effects of density and ammonia. *Aquaculture* 219: 457 – 470.

Huguenin, J. & J. Colt. (1989). Design and operating guide for aquaculture seawater systems. Elsevier.

Ingerson T I and Geddes M C (1995). Respiration and survival of yabbies, *Cherax destructor* Clark, during hypoxia and the effect of hypoxia on growth in the laboratory. *Freshwat. Crayfish* 10: 221 – 229.

Jan R-Q, Shao K-T and Chang K-H (1981). A study of the diurnal periodicity in oxygen consumption of the small abalone (*Haliotis diversiclor supertexta* Lischke). *Bull. Inst. Zool. Academia Sinica* 20 (1) 1-8.

Jan R-Q and Chang K-H (1983). The oxygen consumption of the Formosan abalone *Haliotis diversiclor supertexta*, during decline of ambient oxygen. *Bull. Inst. Zool. Academia Sinica* 22 (1) 43- 48.

Jobling M (1981). Temperature tolerance and the final preferendum - rapid methods for the assessment of optimum growth temperatures. *J. Fish. Biol* 19: 439 – 455.

Kaneko T and Colwell. R R (1978). The annual cycle of *Vibrio parahaemolyticus* in Chesapeake Bay. *Microbial Ecology* 4: 135-155.

Lawson T B (1995). Fundamentals of aquaculture engineering. Chapman & Hall.

Lacoste A, Malham S K, Cueff A, and Poulet S A (2001a). Stress and stress induced neuroendocrine changes increase susceptibility of juvenile oysters (*Crassostrea gigas*) to *Vibrio splendidus*. *Appl. Environ. Microbiol*. 67: 2304 – 2309.

Lacoste A, Malham S K, Cueff A, and Poulet S A (2001b). Stress as a trigger factor of juvenile oyster summer mortalities. *Appl. Environ. Microbiol*. 67:2304 – 2309.

Lee K-K, Liu P-C, Chen Y-C and Huang C-Y (2001). The implications of ambient temperature with the outbreak of vibriosis in cultured small abalone *Haliotis diversicolor supertexta* Lischke. *Journal of Thermal Biology* 26: 585-587.

Leitman A (1992).. The effects of gas supersaturation on the behaviour, growth and mortality of red abalone, *Haliotis rufescens* (Swainson). Pp 75 – 85 In: Shepherd S A, Tegner M J and del Proo G (ed's.), Abalone of the World – Biology, fisheries and culture. Fishing News Books.

Loipersberger M (1996). The effect of enhanced oxygen levels on abalone survival and feeding behaviour. Proceedings of the 3rd Annual Abalone Aquaculture Workshop, August 1996, Port Lincoln. SARDI Adelaide.

Lu J Y, Sun B L and Huang K (2002). Effects of different rearing modes on controlling out-breaking epidemic disease in Haliotis diversicolor under intensive culture. *Journal of Fisheries Sciences of China* 9(2): 147-151.

Madigan S, Lehmann D and Clarke S (2000). Determination of critical thermal maximum for four species of South Australian abalone; *Haliotis laevigata*, *H. scalaris*, *H roei* and *H. cyclobates*. Unpublished SARDI Report.

Maguire G B, Hindrum S, Johns D, Dunstan G A and Cropp M (1996). Effects of tank drainage frequency on growth of juvenile greenlip abalone, *Haliotis laevigata.* Proceedings of the 3rd Annual Abalone Aquaculture Workshop, August 1996, Port Lincoln. SARDI Adelaide.

Maguire (1995). Malham S K, Lacoste A, Gelebart F, Cueff A and Poulet S A (2003) Evidence for a direct link between stress and immunity in the mollusk *Haliotis tuberculata*. *J. Exp. Zoology* 295A: 136 144.

Moore M and Hone P (1995). Results of varying stocking density on growth and yield per unit tank area. Proceedings of the 1st and 2nd annual abalone aquaculture workshops.

Nicolas J L, Basuyaux O, Mazurie J and Thebault A (2002). *Vibrio carchariae*, a pathogen of the abalone *Haliotis tuberculata. Diseases of Aquatic Organisms* 50: 35-43.

Olin P (2000). Abalone culture In: Stickney R R (Editor) Encyclopedia of aquaculture John Wiley & Sons Inc.

Shepherd S A and Triantafillos L (1997). Studies on southern Australian abalone (genus *Haliotis*) XVII. Chronology of *H. laevigata. Moll. Res*. 18: 233 – 245.

Steinarsson A and Imsland A K (2003). Size dependent variation in optimal growth temperature of red abalone (*Haliotus rufescens*). *Aquaculture* 224: 353 – 362.

Tissot B N (1992). Water movement and the ecology and evolution of the Haliotidae. Pp 34 – 45 In: Shepherd S A, Tegner M J and del Proo G (ed's.), Abalone of the World – Biology, fisheries and culture. Fishing News Books.

Vandepeer M (2003). Inducement of abalone mortality and/or bloat in abalone held in laboratory and outside tanks through temperature, flow rate and stocking density manipulation. Proceedings 10th Abalone Farming Workshop, Port Lincoln. SARDI Adelaide.

Vandepeer M and Van Barnveld R (2002). Preliminary report on the project "Preventing summer mortality of abalone in aquaculture systems by understanding interactions between nutrition and water temperature". Proceedings 9th Abalone Farming Workshop, Port Lincoln. SARDI Adelaide.

APPENDIX 1: FARM WATER QUALITY MONITORING DATA SHEET

WATER QUALITY DATA RECORDS

TANK No. and LOCATION _____ **STOCK IDENTIFICATION** _____

STOCK DENSITY _____

DAILY DATA		Dissolved Oxygen (% Saturation)		Water Temperature (^{o}C)			WEEKLY DATA	Ammonia Outlet	Water Flow Rate	
DATE	Time	Inlet	Outlet	Inlet	Outlet	Comments	Recorded By	(mg/L)	L/min	Recorded By

APPENDIX 2: LOCAL SUPPLIERS OF CONTROL AND MONITORING SYSTEMS FOR AQUACULTURE.

Technolab Marketing Pty Ltd
72 Browns Road
Kingston
Tasmania 7050
Phone: 03 6229 7437
Facsimile: 03 6229 2748

Supply and installation of Oxyguard™ product range of sensors, transmitters, monitors, data loggers and software developed for aquaculture.

Innotech Control Systems Australia
Cnr Miles Platting Road & McKechnie Drive
Eight Mile Plains
Queensland 4113
Phone: 07 3841 1388
Facsimile: 07 3841 1644

Supply and installation of Innotech® control systems including Genesis Controller and peripherals.

APPENDIX 3: SATURATION LEVELS OF DISSOLVED OXYGEN IN WATER

Saturation levels of dissolved oxygen in water at varying levels of salinity and temperature at sea level (Huguenin and Colt, 1989).

TABLE 2.8
AIR SOLUBILITY OF OXYGEN (mg/l) IN SEAWATER, 0–40 g/kg

Temp. (°C)	Salinity (g/kg)								
	0	5	10	15	20	25	30	35	40
0	14.621	14.120	13.636	13.167	12.714	12.277	11.854	11.445	11.051
1	14.216	13.733	13.266	12.815	12.378	11.956	11.548	11.154	10.773
2	13.829	13.364	12.914	12.478	12.057	11.650	11.256	10.875	10.507
3	13.460	13.011	12.577	12.156	11.750	11.356	10.976	10.608	10.252
4	13.107	12.674	12.255	11.849	11.456	11.076	10.708	10.352	10.008
5	12.770	12.352	11.947	11.554	11.175	10.807	10.451	10.107	9.774
6	12.447	12.043	11.652	11.272	10.905	10.550	10.206	9.872	9.550
7	12.139	11.748	11.369	11.002	10.647	10.303	9.970	9.647	9.335
8	11.843	11.465	11.098	10.743	10.399	10.066	9.744	9.431	9.128
9	11.559	11.194	10.839	10.495	10.162	9.839	9.526	9.223	8.930
10	11.288	10.933	10.590	10.257	9.934	9.621	9.318	9.024	8.739
11	11.027	10.684	10.351	10.028	9.715	9.412	9.117	8.832	8.556
12	10.777	10.444	10.121	9.808	9.505	9.210	8.925	8.648	8.379
13	10.537	10.214	9.901	9.597	9.302	9.017	8.739	8.470	8.210
14	10.306	9.993	9.689	9.394	9.108	8.830	8.561	8.300	8.046
15	10.084	9.780	9.485	9.198	8.921	8.651	8.389	8.135	7.888
16	9.870	9.575	9.289	9.010	8.740	8.478	8.223	7.976	7.737
17	9.665	9.378	9.099	8.829	8.566	8.311	8.064	7.823	7.590
18	9.467	9.188	8.917	8.654	8.399	8.151	7.910	7.676	7.449
19	9.276	9.005	8.742	8.486	8.237	7.995	7.761	7.533	7.312
20	9.092	8.828	8.572	8.323	8.081	7.846	7.617	7.395	7.180
21	8.914	8.658	8.408	8.166	7.930	7.701	7.479	7.262	7.052
22	8.743	8.493	8.250	8.014	7.785	7.561	7.344	7.134	6.929
23	8.578	8.334	8.098	7.867	7.644	7.426	7.214	7.009	6.809
24	8.418	8.181	7.950	7.725	7.507	7.295	7.089	6.888	6.693
25	8.263	8.032	7.807	7.588	7.375	7.168	6.967	6.771	6.581
26	8.113	7.888	7.668	7.455	7.247	7.045	6.849	6.658	6.472
27	7.968	7.748	7.534	7.326	7.123	6.926	6.734	6.548	6.366
28	7.827	7.613	7.404	7.201	7.003	6.810	6.623	6.441	6.263
29	7.691	7.482	7.278	7.079	6.886	6.698	6.515	6.337	6.164
30	7.558	7.354	7.155	6.961	6.772	6.589	6.410	6.236	6.066
31	7.430	7.230	7.036	6.846	6.662	6.483	6.308	6.137	5.972
32	7.305	7.110	6.920	6.735	6.555	6.379	6.208	6.042	5.880
33	7.183	6.993	6.807	6.626	6.450	6.278	6.111	5.948	5.790
34	7.065	6.879	6.697	6.520	6.348	6.180	6.017	5.857	5.702
35	6.949	6.767	6.590	6.417	6.248	6.084	5.924	5.768	5.617
36	6.837	6.659	6.485	6.316	6.151	5.991	5.834	5.681	5.533
37	6.727	6.553	6.383	6.218	6.056	5.899	5.746	5.597	5.451
38	6.619	6.449	6.283	6.121	5.963	5.810	5.660	5.513	5.371
39	6.514	6.348	6.186	6.027	5.873	5.722	5.575	5.432	5.292
40	6.412	6.249	6.090	5.935	5.783	5.636	5.492	5.352	5.215

Based on Benson and Krause, 1984.