WATER QUALITY DYNAMICS IN AN EXPERIMENTAL SERIAL-USE RACEWAY AND ITS EFFECT ON GROWTH OF SOUTH AFRICAN ABALONE, *Haliotis midae*

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ABSTRACT

An understanding of species specific water quality requirements is essential for efficient production of aquaculture products, an aspect not well documented for the land-based culture of the South African abalone, *Haliotis midae*. In order for the industry to remain competitive in international markets, efficient use of water supplies and the development of water reuse technology is needed. This study assessed the changes in water quality between tanks in a tiered serial-use raceway in relation to accumulated biomass and water flow and estimated the flow index (FI) (L h^{-1} kg^{-1}) at which growth becomes significantly affected. The effect of dietary protein level, supplementation of pure oxygen and addition of sodium hydroxide ($NaOH^-$) on water quality and fundamental production parameters in the serial-use raceways was also assessed. The serial-use raceways were used as a tool to create a range of water quality conditions at which the growth, feed conversion ratio (FCR) and condition factor (CF) of "cocktail" size (60 – 70 mm) *H. midae* could be monitored.

The metabolic activity of the abalone resulted in a deterioration in water quality between tanks in series. pH ($r^2 = 0.99$; $p < 0.001$) and dissolved oxygen concentration ($r^2 = 0.99$; $p < 0.001$) were positively correlated with flow index (pH = 7.38 $FI^{0.02}$; dissolved oxygen = 6.92 $FI^{0.04}$), while free ammonia nitrogen (FAN) ($r^2 = 0.99$, $p < 0.001$) and nitrite (NO_2^- - N) ($r^2 = 0.93$, $p < 0.001$) were negatively correlated with flow index (FAN = 8.02 $FI^{-0.71}$). Nitrite concentrations increased over time indicating colonisation of *Nitrosomonas* bacteria on the basket surfaces. A flow index of 7.2 – 9.0 L h^{-1} kg^{-1} was estimated as the minimum to avoid significant reductions in weight and shell length gain and increases in FCR values. Total ammonia nitrogen (TAN) and FAN

concentrations were significantly correlated to dietary protein (P) ($t = 6.63$, $p < 0.0001$ and $t = 6.41$, $p < 0.0001$, respectively) and flow index ($t = 5.42$, $p < 0.0001$ and $t = 3.9$, $p < 0.0002$, respectively) and could be estimated using the models TAN = 9.73 P – 110.3 log (FI), and FAN = 0.132 P – 1.10 log (FI). Mean FAN concentrations were 67 and 41 % lower in tanks fed a diet containing 22 and 26 % protein respectively, when compared to tanks fed a 33 % protein diet. Supplementation with pure oxygen (103 ± 8 % saturation) improved shell length gain ($t = 3.45$, $p = 0.026$) in abalone exposed to high FAN (2.43 ± 1.1 μg L^{-1}) and low pH (7.6 ± 0.13), relative to a treatment with no oxygen supplementation (92 ± 6 % saturation). Addition of a sodium hydroxide solution resulted in elevated mean pH in treatment raceways when compared to control raceways. The increased pH resulted in significantly higher weight gain (g $abalone^{-1}$) ($F_{1, 12} = 4.51$; $p = 0.055$) and shell length gain (mm $abalone^{-1}$) ($F_{1, 12} = 4.56$; $p = 0.054$) at an α-error level of < 5.5 %. In two trials, weight gain and shell length gain were significantly correlated to pH ($p < 0.001$), and multiple regression of pH, dissolved oxygen and FAN consistently revealed pH to be the best predictor of growth.

It is therefore suggested that decreasing pH is the first limiting water quality variable for abalone in serial-use raceways. As a decrease in water pH is linked to respiration by the abalone and subsequent increase in dissolved carbon dioxide (CO_2) concentration, future studies should examine the effects of CO_2 on *H. midae* metabolic rate, calcification rate and health. The results of this study will contribute toward our understanding of the specific water quality requirements for *H. midae* in commercial aquaculture systems, and influence the design and management procedures for abalone water reuse systems.

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PREFACE

This thesis is submitted as a collection of four manuscripts, each forming a chapter. The experimental chapters are preceded by a General Introduction and followed by a General Discussion. A degree of repetition in the introductions, methods and materials and discussion sections of each manuscript was unavoidable.

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CHAPTER 1

General Introduction

The muscular foot of abalone is one of the most sought-after and highly valued seafood products in the world. The lucrative nature of this product has lead to the development of abalone farming technology in, for example, China, Korea, Taiwan, Australia, Chile, U.S.A., Mexico, Iceland, Peru and New Zealand (Gordon & Cook 2001). *Haliotis midae* Linnaeus, also known as "perlemoen", is one of five haliotid species endemic to South African waters (Branch *et al*. 2010). *Haliotis midae* is the only local species to reach sizes > 80 mm, and it is therefore the only local species of commercial value (Hecht 1994). Currently, the culture of *H. midae* represents the largest sector of the South African mariculture industry (Jones & Britz 2006). This species occurs mainly along the Western Cape coast, with smaller populations occurring as far east as Port St Johns on the east coast (Hecht 1994). Both juveniles and adults can be found on shallow sub-littoral reefs where they feed on macroalgae such as kelp, *Ecklonia maxima* and red algae, *Porphyra capensis* (Barkai & Griffiths 1988; Branch *et al*. 2010). The commercial farming of *H. midae* was established by a concerted research and development effort between governmental research institutions and the private sector in the early 1990's (Sales & Britz 2001). Factors such as favourable coastal water quality, high product prices, declines in natural stocks due to poaching and over-fishing, an apparent insatiable foreign market and favourable exchange rates have contributed to the success of the industry (Reddy-Lopata *et al*. 2006; Troell *et al*. 2006; Vosloo & Vosloo 2006). South Africa is, however, not the only producer of cultured abalone, and remaining competitive in the market will require reductions in production costs and increases in production efficiency and volume (Loubser 2005; Vosloo & Vosloo 2006).

Haliotis midae is farmed in intensive land-based single pass flow-through systems, where water is continuously passed through tanks containing 6 – 10 abalone baskets, depending on tank size (Pers. Obs). The assumption is that continuous water flow will supply sufficient oxygen and allow for the dilution of toxic excretory products such as

ammonia and carbon dioxide (Reddy-Lopata *et al*. 2006; Wassnig *et al*. 2010; Vivanco-Aranda *et al*. 2011). *H. midae* is sensitive to changes in water quality. Thus, similarly to other abalone species (Badillo *et al*. 2007; Vivanco-Aranda *et al*. 2011), water exchange rates in commercial flow-through systems range between 800 – 2400 % per day. The pumping of large volumes of water represents a major production cost. In South Africa, the cost of electricity is set to rise by approximately 200 %, from an average of R0.33 to R0.65 kWh^{-1} over the period 2010 – 2013. During the winter months (June, July & August) when demand for electricity is high, the tariff for industrial-scale electricity users doubles. Thus, in the winter of 2013, farmers may pay as much as R1.30 kWh^{-1}. This will have severe effects on abalone farm cash-flow and profitability. Efficient use of the water pumped ashore is therefore critical to achieving high profit levels (Siikavuopio *et al*. 2004). To improve production efficiency abalone farmers require information on the relationship between water flow rate and abalone growth so that production strategies which yield the highest return can be implemented.

Land-based abalone culture systems also have a high capital outlay and thus efficient use of infrastructure and grow-out space is essential (Wassnig *et al*. 2010). Farmers therefore need to maximize production by increasing stocking densities and by using formulated diets high in protein, as these have shown to produce the fastest growth rates (Britz 1996a; Britz 1996b) and are comparable in terms of cost with naturally occurring macro-algal feed. However, diets high in protein may lead to an increase in ammonia excretion (Carter *et al*. 1998; Qian *et al*. 2001; Yang *et al*. 2002; Webb & Gatlin 2003). Therefore, at high stocking densities, the metabolic processes of the cultured abalone can result in the culture water becoming sub-optimal for growth, if the increase in biomass is not matched by an increase in water flow rate. This problem may be exacerbated during harmful algal blooms when water flow is closed off (Botes *et al*. 2003) or during system failure. Many researchers and aquaculturists combine the units of water flow rate ($L\ h^{-1}$) and biomass (kg) to determine the flow index ($L\ h^{-1}\ kg^{-1}$). This allows the determination of maximum biomass per tank at a given water flow rate, or the determination of the required water supply needs for a given biomass of stock. Abalone are slow growing and periods of sub-optimal water quality can significantly decrease production and lengthen the time to market size. There is, however, a paucity

of information regarding the effects of water quality on *H. midae* growth and health. The flow-indices at which water quality becomes "sub-optimal" and the corresponding concentrations of certain important water quality variables needs to be determined.

One of the most important factors in the design and operation of aquaculture systems is accurate knowledge of the species specific water quality requirements (Poxton & Allouse 1982; Colt 2006), an aspect which is not well documented for abalone and *H. midae* in particular. This is because research into the cultivation of *H. midae* has focused on nutrition, seed production and health (Sales & Britz 2001), while the results from water quality research may have been guarded as proprietary information or may have been deemed unnecessary due to the then relatively cheap electricity and favourable coastal water quality (Troell *et al*. 2006).

From a water quality perspective, only the determination of the 50 % critical temperature maximum (27.9 °C), optimal temperatures (12 – 20 °C) for growth (Britz *et al*. 1997) and the 36-h LC_{50} free ammonia nitrogen (FAN) (50 % mortality after 36 hours exposure) concentration have been determined for *H. midae*. The tolerance of other abalone species to selected water quality variables has been determined in toxicity experiments and in studies quantifying the effective concentration of a water quality variable that reduces growth by 5 or 50 %, (EC_5 or EC_{50}) respectively. For example, the effect of chronic exposure to FAN (NH_3) has been studied in *H. tuberculata* Linnaeus (Basuyaux & Mathieu 1999), *H. laevigata* Donovan (EC_5 = 41 µg L^{-1} and EC_{50} = 158 µg L^{-1}, respectively) (Harris *et al*. 1998), *H. rubra* Leach (EC_5 = 4–6 µg L^{-1}) (Huchette *et al*. 2003) and *H. midae* (36 h LC_{50} in 1–2 cm juveniles = 9.8 µg L^{-1}, 12.9 µg L^{-1} in 5–8 cm cocktail size abalone, and 16.4 µg L^{-1} in 10–15 cm abalone broodstock) (Reddy-Lopata *et al*. 2006). Growth-reducing effects of low dissolved oxygen levels were determined for *H. laevigata* (EC_5 = 7.36 mg L^{-1} and EC_{50} = 5.91 mg L^{-1}) (Harris *et al*. 1999b) and *H. diversicolor supertexta* Reeve (Cheng *et al*. 2004a), and the importance of nitrite (NO_2^-) was assessed in *H. tuberculata* (safe concentration <5 mg L^{-1}) (Basuyaux & Mathieu 1999) and *H. laevigata* (67 % reduction in specific growth rate at > 0.56 mg NO_2^-- N L^{-1}) (Harris *et al*. 1997). The effect of pH on *H. laevigata* (EC_5 =

7.78 and EC_{50} = 7.39) and *H. rubra* (EC_5 = 7.93 and EC_{50} = 7.37) has been determined by Harris *et al*. (1999a).

Most of this research has addressed the effects of single factors while maintaining presumably acceptable levels of other water quality variables. In production systems, however, water quality changes in a multi-factorial way as a result of the metabolic activity of the animal and bacterial activity in the water (Hargreaves 1998). Thus, findings from toxicity tests may not be applicable when estimating the effect of water quality on growth and health of aquatic animals in commercial systems (Colt 2006). Findings from toxicity experiments can also be inappropriate due to the life stage of the animal at which the toxicity levels are determined (Colt 2006). Abalone farms are generally comprised of two sections, the hatchery and grow-out system. The costs associated with the maintenance of water quality in the grow-out section contribute greatly to production costs. Thus, information on the water quality requirements for animals in this life stage will be economically beneficial (Colt 2006). As more applicable data are not readily available, water quality known to affect abalone growth in toxicity tests can be used as a guideline for system design, but such research should be followed by on-farm growth experiments.

The need for increased energy conservation, while simultaneously increasing standing stock and production makes recirculating systems or serial-use raceways attractive to farmers (Eikebrokk 1990; Piedrahita 2003; Schuenhoff *et al*. 2003). The cost and complexity of recirculation systems in terms of design and operation often makes them unsuitable for large-scale production (Eikebrokk 1990). A serial-use system creates an opportunity to reuse water as long as its quality remains in the region required for good growth. Serial-use systems are designed to use the out-flowing water of a production tank to feed one or more tanks, usually after aerating the out-flowing water before reuse. The water therefore passes through an increased cumulative biomass of animals at each successive tier in the system, resulting in deterioration in water quality (Morrison & Piper 1988; Huchette *et al*. 2003). The accumulation and removal of settleable and suspended solids represent major challenges in salmonid serial-use raceways (Summerfelt *et al*. 2004). This, however, needs to be confirmed in a serial-use raceway

for abalone. Although serial-use raceways should be tested as an alternative production system for abalone, they have the valuable characteristic of creating a range of discrete water quality conditions as the water flows from one tank to the next. Monitoring water quality and recording the growth, feed conversion ratio (FCR) and condition factor at each position within the serial-use raceway may provide valuable baseline data on the specific water quality criteria for abalone in commercial grow-out systems.

In flow-through and serial-use raceways, oxygen is generally considered to be the first limiting variable (Colt & Orwicz 1991a; Sanni & Forsberg 1996; Wassnig *et al*. 2010). Changes in water quality characteristics depend on design aspects such as aeration type or weir type (Colt & Orwicz 1991b; Eshchar *et al*. 2003; Colt *et al*. 2009) or management aspects such as species cultured, feed type and stocking density (Vergara *et al*. 1999; Qian *et al*. 2001; Webb & Gatlin 2003). Therefore, under certain conditions, ammonia or carbon dioxide (CO_2) may become limiting variables for growth (Sanni & Forsberg 1996; Colt *et al*. 2009). In an assessment of water quality on a commercial *H. midae* farm, Yearsley (2008) identified gradients in dissolved oxygen, pH and free ammonia nitrogen (FAN) between the inflow and outflow of commercial flow-through abalone tanks, in which abalone where fed a high-protein formulated diet. Free ammonia nitrogen concentrations increased between the inflow and outflow, while pH and dissolved oxygen concentrations decreased. The growth of abalone in these tanks was correlated to the change in these variables, with abalone nearest to the outflow showing the lowest specific growth rate.

Identification of the first limiting water quality variable in any aquaculture system will allow suitable changes to the components or management to be made, such that the system can operate more efficiently and produce more abalone biomass. Although *H. midae* is not currently farmed in full recirculating systems (90 % retention of water per day) on a commercial scale, identification of the first limiting variable in a serial-use raceway may contribute to the design of such systems and provide information for critically important components of the system.

When assessing water quality, it is important to study the interactive effects among variables and the mechanism by which they affect the cultured species. The variables considered important for this study were: temperature; pH; dissolved oxygen; ammonia (TAN and FAN); nitrite and suspended solids.

The metabolic rate of abalone and therefore feed consumption and growth is primarily controlled by temperature (Fry 1971, Britz & Hecht 1997). In *H. midae*, a positive linear relationship exists between growth rate and temperature in the range 12 - 20 ºC, declining sharply thereafter (Britz *et al*. 1997). Temperature has shown a similar effect on the growth of *H. rubra, H. laevigata* (Gilroy & Edwards 1998), *H. tuberculata* (Kelly & Owen 2002) and *H. rufescens* (Steinarsson & Imsland 2003). Lyon (1995) showed that ammonia excretion and oxygen consumption in *H. midae* was significantly increased at temperatures above 20 ºC. An increase in temperature also increased the toxicity of ammonia (Bower & Bidwell 1978, Thurston *et al*. 1981) and reduces the solubility of oxygen in water (Weiss 1970).

Dissolved oxygen limits growth by reducing the capacity for aerobic metabolism (Fry 1971; Harris *et al*. 1999b). In systems where excess feed and faecal waste are not removed regularly, a high biological oxygen demand may reduce the availability of oxygen to the cultured species (Losordo *et al*. 1998; Cripps & Bergheim 2000). In abalone raceways, a diurnal variation in oxygen consumption was observed by Yearsley (2008). Abalone consumed more oxygen at night which relates to their primarily nocturnal feeding patterns. In a preliminary investigation of *H. midae* physiology Lyon (1995) observed that oxygen consumption in *H. midae* increased linearly with temperature while oxygen consumption per gram body weight decreased linearly as a function of shell length.

Ammonia is the principle nitrogenous waste product excreted by abalone (Bishop *et al*. 1983, Barkai & Griffiths 1987). In aqueous solution ammonia exists in a pH, temperature and salinity mediated equilibrium between the ionized form (NH_4^+) and the un-ionized form (NH_3) (Bower & Bidwell 1978, Thurston *et al*. 1981). The sum of ionized and un-ionized ammonia is the total ammonia nitrogen (TAN) and represents

the ammonia concentration that is analytically measured in water samples (Randall & Tsui 2002). Both the ionized and un-ionized form of ammonia are toxic to aquatic organisms, however, the concentration of FAN increases as pH levels rise and it diffuses across the gill membrane (Thurston *et al*. 1981) making it of greater concern in toxicity testing.

The H^+ ion concentration of water, measured as pH, indicates the degree to which water may be acidic or basic (Covington *et al*. 1985, Losordo *et al*. 1998). In reuse systems, respiration by the cultured organism and nitrification in the biological filter can depress pH (Harris *et al*. 1999a). In aquatic environments, carbon dioxide (CO_2) produced during respiration reacts with water to form a weak carbonic acid (H_2CO_3). Carbonic acid dissociates into bicarbonate (HCO_3^-), carbonate (CO_3^{2-}) and hydrogen ions (H^+). The release of hydrogen ions decreases the pH (i.e., increases the acidity) of the water (Sanni & Forsberg 1996). Similarly, the nitrification of ammonia to nitrate by nitrifying bacteria results in the release of H^+ ions (Chen *et al*. 2006) often followed by a reduction in the buffering capacity of the water. The reduction in abalone growth at high and low pH values is attributed to alterations in kidney function, tubule and lumen size and gill hyperplasia (Harris *et al*. 1999a).

In aerobic environments, nitrifying bacteria (*Nitrosomonas* spp.) oxidize ammonia to nitrite (Tomasso 1994). Nitrite (NO_2^-) is the ionised form of nitrous acid (HNO_2) (Lewis & Morris 1986). Both forms are toxic to aquatic organisms and bio-accumulate in the extra-cellular spaces of the gills, liver, brain and muscle tissue (Jensen 2003) where they oxidise other compounds such as haemoglobin. The concentration of nitrous acid changes as a function of pH. However, at pH values above 6 its concentration is negligible (Colt 2006). Nitrous acid diffuses across the gills of aquatic organisms, while nitrite requires active transport by the chloride cells to cross the gill membrane (Lewis & Morris 1986). The competitive exclusion of nitrite ion uptake via chloride ions makes marine organisms more tolerant of high nitrite concentrations (Harris *et al*. 1997). As the formation of nitrite requires synthesis by bacteria, toxic concentrations of nitrite are more likely to occur in recirculation and serial-use systems than in flow-through systems.

In the culture environment, shear forces such as water turbulence and animal movement result in the breakdown of faeces and uneaten feed into small soluble particles (Brinker *et al*. 2005). Organic particulates in the water can compromise gill function, cause decreased feeding rate, nourish facultative pathogens and increase the biological oxygen demand (BOD) of the system (Summerfelt & Penne 2005). In *H. midae* culture high concentrations of suspended solids are thought to facilitate the infestation of abalone by the sabellid worm *Terebrasabella heterouncinata* and cause reductions in growth rate (Yearsley 2008).

Aims and objectives

The aim of this study was to quantify the effect of water quality on abalone production parameters using a serial-use raceway.

The objectives of this study were to:

- Describe the changes in water quality between tanks in an abalone serial-use raceway in relation to accumulated biomass and flow-rate;
- Determine the minimum required flow-index (L h^{-1} kg^{-1}) to avoid a reduction in growth and increase in FCR as a consequence of deteriorated water quality;
- Correlate pH, dissolved oxygen and free ammonia nitrogen to *H. midae* growth;
- Compare the findings of the experimental chapters with literature in order to estimate the first limiting water quality variable in an abalone serial-use raceway; and
- Provide suggestions for water quality management and system design on commercial abalone farms.

CHAPTER 2

Water quality in a serial-use raceway and its effect on growth of South African abalone, *Haliotis midae* Linnaeus

Introduction

Most abalone farmers use flow-through systems to provide oxygen and dilute growth-reducing metabolites. To intensify production from the available water (Piedrahita 2003; Schuenhoff *et al*. 2003) farms may use recirculating systems or implement serial-use of water. In a serial-use raceway, water flow remains constant while the cumulative biomass of the farmed animal increases with increasing water reuse. The build-up of metabolic waste degrades water quality as it passes from one tank to the next (Morrison & Piper 1988; Colt & Orwicz 1991a; Summerfelt *et al*. 2004). A serial-use system can provide an opportunity to reuse water as long as water quality does not reduce growth or compromise the health of the aquatic animal (Poxton & Allouse 1982; Colt *et al*. 2009). From a research perspective, the gradients in water quality which develop between tanks in series provide an opportunity to monitor growth under a range of different water quality conditions.

Water quality in intensive aquaculture is influenced by the population density of the aquatic species and water flow (Harris *et al*. 1998; Colt *et al*. 2009). Thus, the effect of flow index (L h^{-1} kg^{-1}) on growth and health of aquatic species in serial-use systems needs to be determined in order to improve production efficiency (Fivelstad 1988; Colt & Orwicz 1991a). This has not been done for the South African species *Haliotis midae* under farm conditions. Low flow rates may cause growth and health problems, while high flow rates imply inefficient use of water. Determining the first limiting water quality variable for growth and health for a particular system is imperative for refining its design and management, and therefore increasing its water reuse capabilities. In general, oxygen has been considered the first limiting variable in serial-use raceways

(Colt & Orwicz 1991a). However, with improvements in aeration systems and the supplementation of pure oxygen, high ammonia and carbon dioxide (CO_2) concentrations may become the primary limiting variables (Colt *et al*. 2009). First limiting variables may also be system specific, i.e., by aspects such as aeration type (Colt & Orwicz 1991b; Eshchar *et al*. 2003), weir type (Colt & Orwicz 1991b; Colt *et al*. 2009), feed (Vergara *et al*. 1999) and the species cultured (Qian *et al*. 2001).

There is a paucity of information on the effects of serial-use of water on abalone growth, feed conversion and health. Most water quality research has addressed the lethal concentration (LC50 – concentration that causes 50 % mortality in a given time period) or effective concentration (EC5 or EC50 – concentration that reduces growth by 5 or 50 % respectively) for a particular abalone species. Findings from toxicity style experiments may, however, not be appropriate for a commercial venture, as they fail to take into account the potential synergistic effects of two or more variables and the multi-factorial change in water quality in production systems (Colt 2006). LC and EC values should therefore be tested in commercial scale growth experiments.

This study describes the changes in water quality between tanks in a serial-use raceway system with seven passes in relation to cumulative biomass and water flow and estimates the flow index (L h^{-1} kg^{-1}) at which growth is reduced in 60-70 mm *H. midae*. By comparing water quality in tanks where abalone growth was reduced with data from growth and toxicity experiments on this and other abalone species, this study identifies and estimates the first limiting water quality variable in the serial-use system.

Methods and Materials

The study was conducted at HIK Abalone Farm (Pty) Ltd in Hermanus, on the south west coast of South Africa (34°26'04.35"S; 19°13'12.51"E) from September to December 2008. The abalone were approximately three years old and 64.2 ± 2.6 mm in length at the beginning of the experiment. They had been spawned in the hatchery and weaned onto the formulated diet Abfeed® (Marifeed Pty Ltd., South Africa). They had been reared in well-aerated, flow-through tanks at ambient temperatures within the

temperature range of 12–20 ºC, which has been identified as suitable for this species (Britz *et al*. 1997).

Experimental system

Three serial-use raceways each containing seven tanks in series were used (Figure 2.1). The tanks (0.9 × 0.6 × 0.6 m) were set-up at varying heights to allow for gravitational water flow. Filtered seawater (100 µm) entered the top tank of each raceway (i.e., position one) supplied from a 0.3 m^3 header tank and flowed through the remaining six tanks (i.e., positions two to seven). The header tank allowed for a constant water flow to each raceway, as tidal height could have affected the volume of water pumped from the sea. Polyvinylchloride (PVC) piping (40 mm outer diameter) was used for influent and effluent pipes between tanks. During a five-day acclimation period, additional piping provided seawater at an industry standard flow rate of 116 L h^{-1} to each individual tank. Each tank held one "oyster-mesh" basket which contained vertical plastic racks providing a surface area of 3.2 m^2. Feed was placed onto a horizontal, plastic feeder plate on top of each vertical rack, positioned 10 cm below the water surface. Aeration was provided to each tank via 20 mm PVC airlines placed horizontally 50 mm above the tank bottom. The three serial-use raceways were shaded from direct sunlight through the use of densely meshed shade cloth, and the presence of the feeder plate at the water surface.

Experimental design

As the total abalone biomass per unit water flow increased with an increase in serial-use, cumulative biomass was related to flow rate to arrive at a flow index in L h^{-1} kg^{-1} (Table 2.1). At the beginning of the experiment, 50 abalone per tank were weighed individually to the nearest 0.01 g using an electronic balance (Snowrex BBA-600) and measured to the nearest 0.1 mm from photographs using the software program, SigmaScan® Pro 5 (Systat Software Inc., San Jose, California). Length measurements were taken along the long shell axis and photographs were calibrated based on length measurements with Vernier callipers. Photographing the abalone reduced handling time and the duration for which abalone were out of the water.

Figure 2.1: The three serial-use raceways used to grow South African abalone, *Haliotis midae*, each consisting of seven tanks in series.

Each of the 21 baskets was filled with 8.1 ± 0.1 kg (standard industry stocking density for this size class) of abalone and allocated randomly to a tank position. The number of abalone in each basket ranged from 163 – 169. There was no significant difference in mean initial weight among all baskets ($F_{20, 1029} = 1.38$, $p = 0.12$).

The abalone were grown in the serial-use raceways for 101 days (normal grading interval for this size class) after which the weigh and measure process was repeated to determine the mean wet weight gain (g abalone^{-1}), shell length gain (mm abalone $^{-1}$) and condition factor (CF) of abalone in each tank. CF was determined using the equation: $CF = 5575$ ($weight\ x\ length^{-2.99}$) (Britz 1996b). As uneaten feed was not removed each day, apparent feed conversion ratio (FCR) for baskets in each tank position was calculated as FCR = (dry weight feed given / biomass gain), where biomass gain is the

difference in wet weight of abalone within a basket between the start and the end of the experimental period.

Abalone were fed Abfeed S34® (composition in g kg^{-1}: 340 g protein, 530 g carbohydrates, 1.2 g lipids) to apparent satiation at 16:00 daily from a bucket assigned to each tank. Feed was given to tanks that had less than 15 pellets left on the feeder plate using a cup that contained 64 ± 2.8 g of feed. This feeding method was developed by farm management using research by Britz *et al*. (1997), to be practical and minimise food waste. The amount of feed given over a two-week period was calculated by subtracting the end weight (bucket and remaining feed) from the full bucket weight at the start of that period. Tanks were cleaned every 14 days. This involved moving the baskets to an empty farm tank, after which the three serial-use raceways could be drained, scrubbed clean and refilled. The baskets were then returned to the same position in the serial-use raceways.

Table 2.1: Initial and final cumulative biomass (kg), initial and final flow indices (L h^{-1} kg^{-1}) and mean water flow rate (L h^{-1}) at each tank position in three abalone serial-use raceways. Tank position represents the degree of water reuse, i.e., 1 = the first tank and 7 = the last tank in series. Values are mean ± standard deviation (SD) (n=3).

	Tank position						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Initial cumulative biomass (kg)	8.2 ± 0.17	16.2 ± 0.15	24.3 ± 0.17	32.3 ± 0.15	40.4 ± 0.1	48.6 ± 0.21	56.6 ± 0.15
Final cumulative biomass (kg)	10.3 ± 0.41	20.1 ± 0.45	30.2 ± 0.31	40.1 ± 0.41	49.7 ± 0.45	59.2 ± 0.17	68.4 ± 0.49
Mean water flow (L h^{-1})	288.8 ± 1.45	288.8 ± 1.45	288.8 ± 1.45	288.8 ± 1.45	288.8 ± 1.45	288.8 ± 1.45	288.8 ± 1.45
Initial flow index (L h^{-1} kg^{-1})	34.3 ± 1.38	17.8 ± 0.20	11.9 ± 0.11	8.9 ± 0.10	7.1 ± 0.06	6.0 ± 0.06	5.1 ± 0.04
Final flow index (L h^{-1} kg^{-1})	28.0 ± 1.01	14.4 ± 0.30	9.6 ± 0.11	7.2 ± 0.10	5.8 ± 0.04	4.9 ± 0.01	4.2 ± 0.01

Water quality analysis

Temperature, water pH, dissolved oxygen concentration, percentage oxygen saturation, and the concentration of total ammonia nitrogen (TAN), suspended solid and nitrite (NO_2^--N) was monitored at the outflow of each tank twice a week. Samples were taken between 09:00h and 10:00h, to achieve the most reliable estimate of daily means, following results by Yearsley (2008) for diurnal water quality conditions in abalone tanks on this farm. Water flow measurements were done in duplicate for each raceway at each sampling time, using a 2-L graduated container and a stop watch. Water flow into the first tank of each raceway averaged 288.8 ± 1.5 L h^{-1}. Temperature and pH were measured using a pH meter (YSI Inc. Model # 60/10 FT; Yellow Springs, Ohio, U.S.A.). The pH meter was calibrated once a week using buffer solutions of pH 4, 7 and 10. Dissolved oxygen concentration and oxygen saturation were measured using an oxygen meter (YSI Inc. Model # 55D; Yellow Springs, Ohio, U.S.A.). The oxygen meter was calibrated before each recording in air-saturated seawater, and the membrane of the electrode was replaced every three weeks. TAN and nitrite concentrations were determined using the method of Solorzano (1969) and the Merck Nitrite Test Kit (Cat. no. 1.14776.0001, Merck, South Africa) respectively, with colour absorbance read through a spectrophotometer (Prim Light, Secomam, 30319 Ales, France). Absorbance was converted into the concentration of total ammonia nitrogen or nitrite using the coefficients derived from least-square linear regression standard curves (TAN, n = 15, r^2 = 0.998 using ammonium chloride to make up the concentration range, Appendix B; and nitrite, n = 10, r^2 = 0.994, using sodium nitrite, Appendix C). TAN and nitrite samples were collected in acid-washed glassware, processed immediately and kept dark after adding the reagents. The concentration of free ammonia nitrogen (FAN) was calculated using the values for TAN, temperature, pH and salinity of the respective water samples (Bower & Bidwell 1978). The concentration of suspended solids > 10 µm was determined by filtering a 2-L water sample through a 10-µm filter mesh. The filtrate was washed with distilled water onto pre-dried and weighed filter paper (Munktell, Filtrak 1291, 2–5 µm typical pore size). The filter papers were re-dried at 60ºC for 24 hours and weighed to the nearest 0.0001 g using a micro-balance (Denver Instruments). The concentration of suspended solids (mg L^{-1}) was calculated as the dry weight gain of the filter paper (mg) divided by the volume of the water sample (L).

Table 2.2: Mean concentration (± SD) of water quality variables sampled from the seawater entering the serial-use abalone culture system via a header tank.

Water quality variable	**Mean**	**Range**
Temperature	15.1 ± 1.30	11.3–17.3
pH	7.96	7.69–8.19
Dissolved oxygen (mg L^{-1})	8.45 ± 0.35	7.89–9.31
Oxygen saturation (%)	104 ± 3.7	97.3–115.3
Total ammonia nitrogen (TAN; NH_4^+) (µg L^{-1})	8.73 ± 5.14	0.60–18.6
Free ammonia nitrogen (FAN; NH_3) (µg L^{-1})	0.19 ± 0.14	0.01–0.45
Nitrite (NO_2^- - N) (µg L^{-1})	6.11± 2.09	2.10–11.40
Suspended solids (mg L^{-1})	6.06 ± 2.97	1.34–15.63

Statistical analysis

Analysis of variance (ANOVA) was used to compare means of the dependent variables wet weight gain, shell length gain, FCR and CF between tank positions, i.e., between the levels of water reuse. Assumptions of equality of variance and normality of residuals were checked with Levene's test (Levene 1960) and the Shapiro-Wilk test (Shapiro & Wilk 1965), respectively. Post-hoc differences between means of the dependent variables where compared among tank positions using Tukey's HSD test. The non-parametric Kruskall-Wallis test was used when assumptions for ANOVA testing could not be met. Water quality and growth data were modelled as a function of tank position and flow index using least-square regression analysis. When making comparisons between possible regression models, the highest t-statistic, p-value to test for significance of coefficients, F-statistic and the distribution of residuals were used as criteria to decide on the preferred model. A significance level of 0.05 was used for all tests. Values in this document are reported as mean ± standard deviation (SD), unless otherwise stated.

Results

Mean wet weight gain (g abalone^{-1}) differed depending on the level of serial-use (ANOVA; $F_{6, 14} = 13.9$, p = 0.00003), i.e., between some tank positions in the raceway. Wet weight gain was significantly lower in tank positions six and seven than in positions one to four (post-hoc test). Abalone in position one had a significantly higher mean weight gain than those in position five. Wet weight gain decreased with increasing serial-use and was positively correlated with the mean flow index (L h^{-1} kg^{-1}) (least-square regression analysis, $r^2 = 0.72$; $p < 0.001$; Figure 2.2). No significant differences in mean shell length gain (mm abalone $^{-1}$) (ANOVA; $F_{6, 14} = 1.84$, p = 0.164) were observed among tank positions. In position one, shell length gain averaged 6.7 ± 0.7 mm, and 5.1 ± 0.5 mm in position seven. No dead abalone were found in any of the tanks.

Mean CF was significantly lower in tank positions six and seven than in positions one to four (ANOVA; $F_{6, 14} = 7.19$, p = 0.0012). Mean FCR differed significantly between tank positions (Kruskal-Wallis test; $H_{6, 21} = 16.3$, p = 0.012). FCR increased as a function of tank position ($r^2 = 0.60$, $p < 0.001$) (Figure 2.2) with a mean FCR of 1.1 ± 0.2 in position one and 1.8 ± 0.5 in position seven. FCR was negatively correlated with flow index ($r^2 = 0.50$, $p < 0.001$) (Figure 2.2).

For the first two weeks of the experiment, mean feed consumption in position two (209 ± 2.6 g) was 29 % lower than the mean for all tanks (306 ± 33.9 g), and was significantly lower (Kruskal-Wallis test: $H_{6, 21} = 14.2$, p = 0.027) than for tanks in position three (348 ± 6.1 g). From week 3 onwards, there were no more significant differences in bi-weekly feed consumption among tank positions (period 2: $H = 12.1$, p = 0.059; period 3: $H = 8.7$, p = 0.192; period 4: $H = 4.3$, p = 0.630; period 5: $H = 3.9$, p = 0.693; period 6: $H = 10.0$, p = 0.125; period 7: H = 8.1, p = 0.230; period 8: $H = 9.1$, p = 0.166).

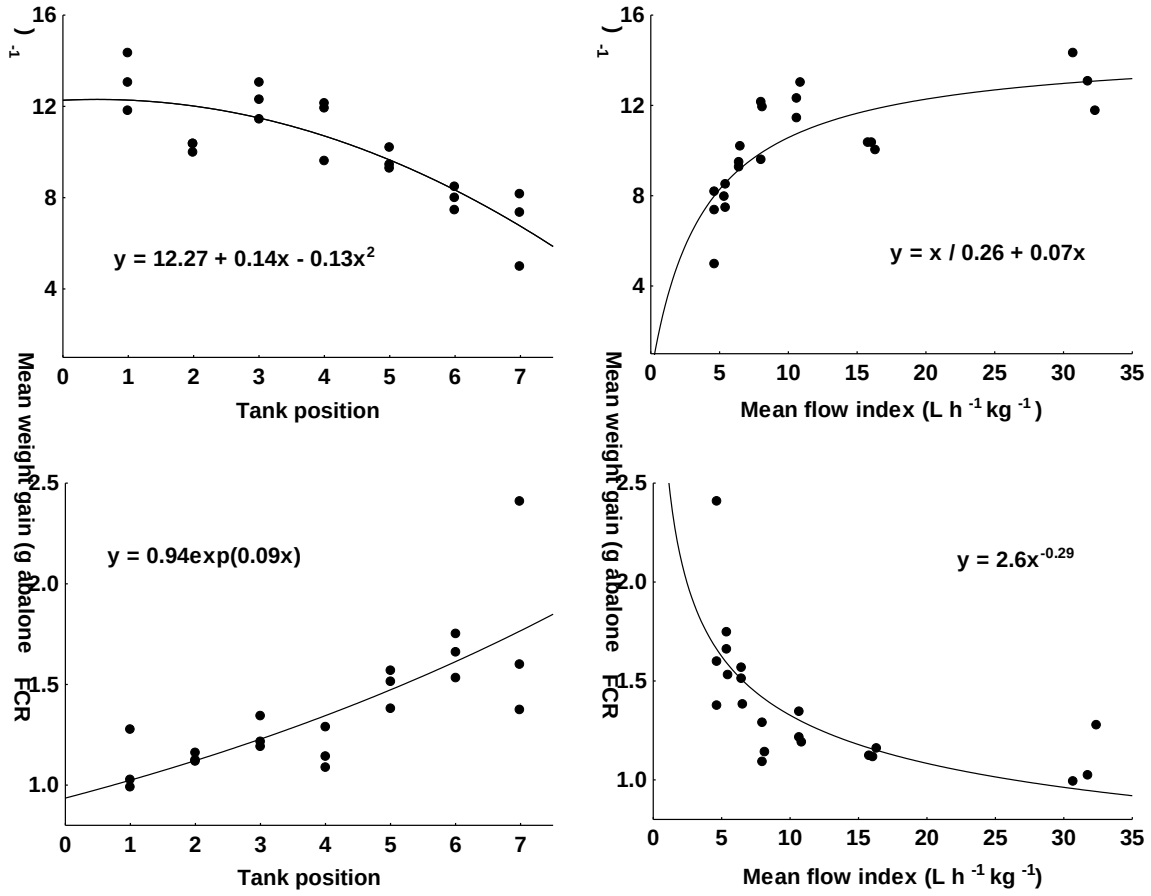


Figure 2.2: Mean weight gain (g abalone^{-1}) and feed conversion ratio (FCR) in three replicate serial-use raceways as a function of tank position and mean flow index.

Results suggest that temperature was linearly related to tank position (Linear regression, $r^2 = 0.71$, $p < 0.001$). Mean temperature in position one was 15.2 ± 1.2 °C increasing to 15.5 ± 1.3 °C in position seven. Suspended solid concentration increased from 4.2 ± 2.0 mg L^{-1} in position one to 4.8 ± 2.5 mg L^{-1} in position seven.

Repeated water use, i.e. decreased flow index reduced water pH ($r^2 = 0.99$, $p < 0.001$; Figure 2.3). The pH averaged 7.86 (range: 8.02 – 7.73) in position one and 7.60 (range: 7.75 – 7.45) in position seven. The dissolved oxygen concentration was positively correlated with flow index ($r^2 = 0.99$, $p < 0.001$; Figure 2.3). Percentage oxygen saturation displayed a similar trend to dissolved oxygen, being positively correlated to flow index ($r^2 = 0.99$, $p < 0.001$). Mean percentage oxygen saturation decreased from 99.4 ± 2.5 in position one to 91.6 ± 3.1 in position seven. Mean TAN and FAN concentration were positively correlated with tank position ($r^2 = 0.99$, $p < 0.001$; Figure 2.3) and negatively correlated with flow index ($r^2 = 0.99$, $p < 0.001$). Mean TAN was 40.7 ± 20.58 µg L^{-1} in position one and 298.1 ± 138.9 µg L^{-1} in position seven. The FAN concentration was highest in position seven (2.69 ± 1.32 µg L^{-1}). The mean NO_2^--N concentration increased exponentially from the inflow to the outflow of the serial-use raceways ($r^2 = 0.94$, $p < 0.001$) and was negatively correlated with flow index ($r^2 = 0.93$, $p < 0.001$; Figure 2.3). Nitrite concentrations increased with time, with this increase being most pronounced in the lower tank positions (Figure 2.4). Over the course of the experiment, there were cyclical variations in pH, dissolved oxygen concentration and the concentration of FAN (Figure 2.4).

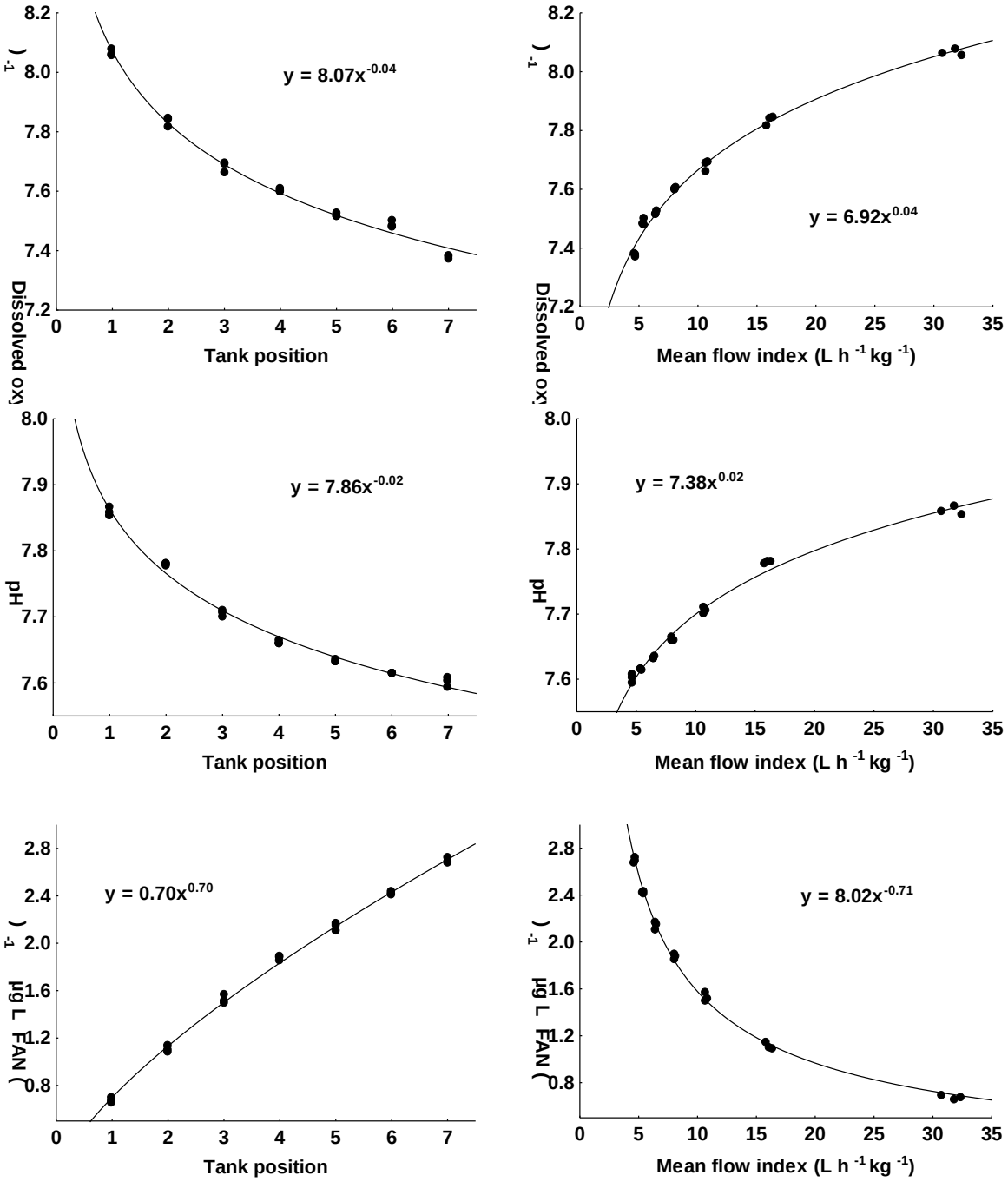
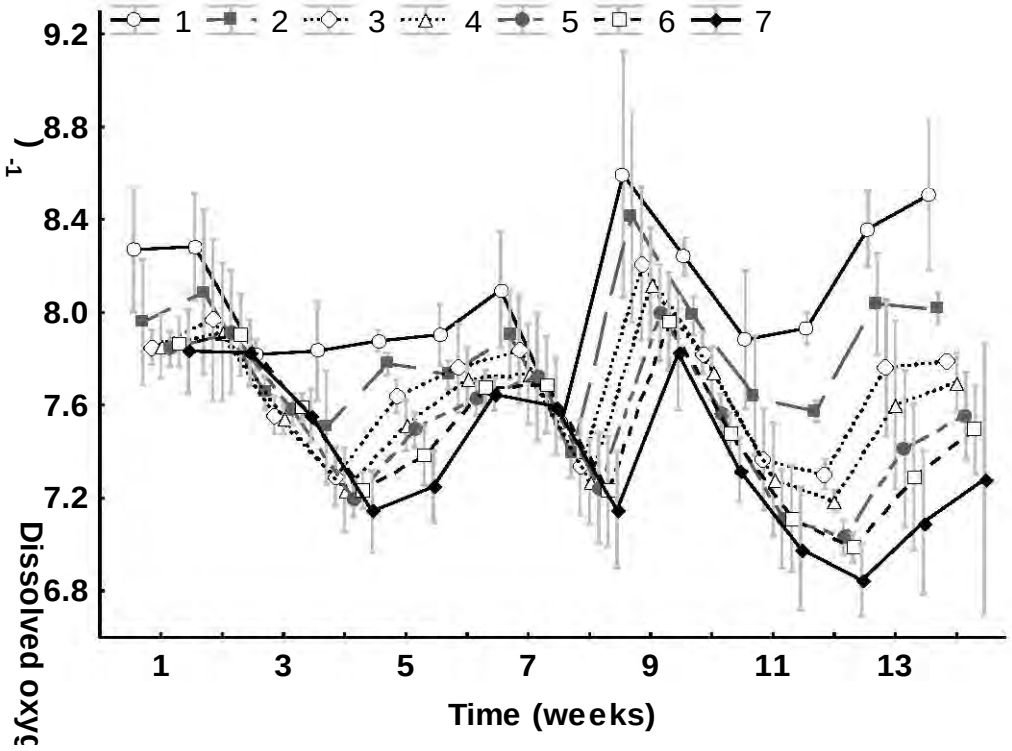


Figure 2.3: Mean concentrations of dissolved oxygen, pH and free ammonia nitrogen (FAN) of three replicate serial-use raceways over the course of 101 days, with water quality variables being modelled as a function of both tank position and mean flow index. Least-square regression models are given for each data set.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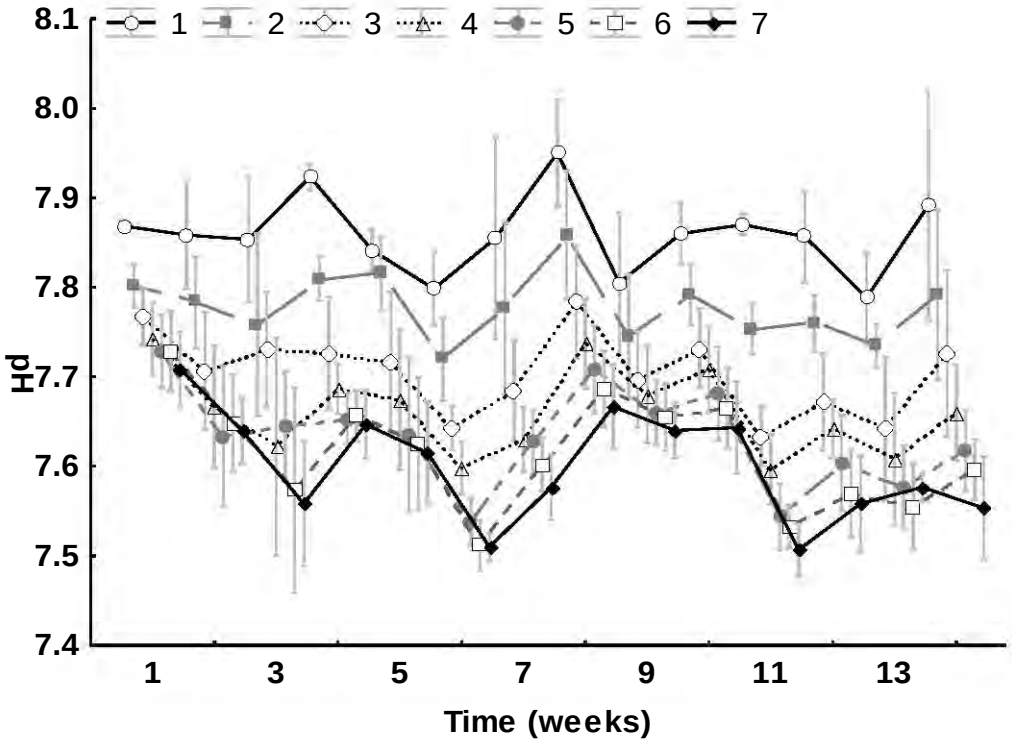
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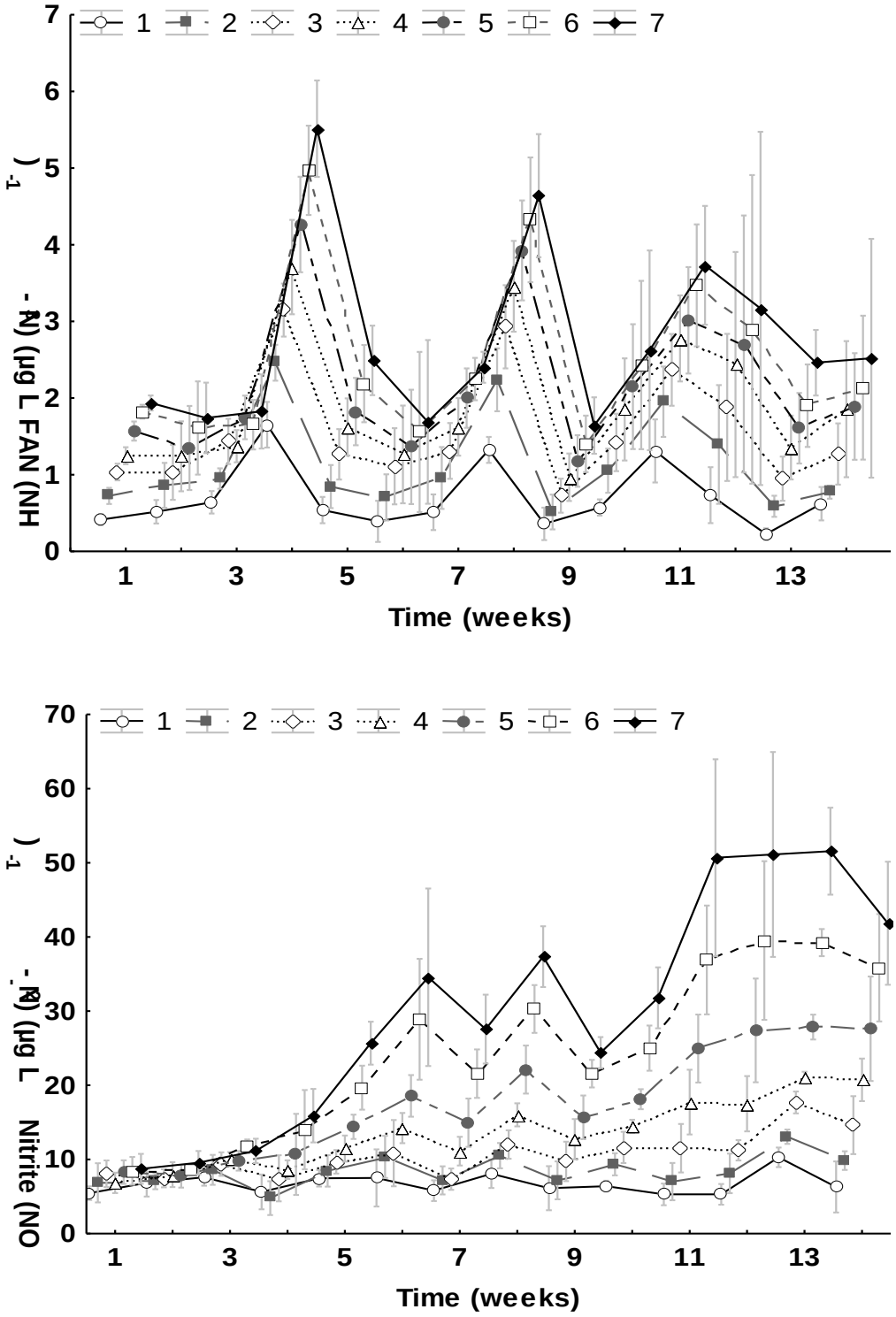


Figure 2.4: Mean weekly concentrations with 95 % confidence levels of dissolved oxygen, pH, free ammonia nitrogen (FAN) and nitrite (NO_2^- - N) concentrations over the course of the 101-day experimental period, for tank positions 1-7 of a serial-use raceway used for production of the South African abalone, *Haliotis midae*.

Table 2.3: Basic statistics for selected water quality variables from water samples taken in tank positions 1–4 and 5–7. Means were calculated on the basis that abalone growth was significantly reduced from tank position five onwards.

Positions	**Mean ± SD**	**Range**	
pH			Lower 5th percentile
1-4	7.75	7.47 – 8.02	7.59
5-7	7.61	7.44 – 7.77	7.50
Dissolved Oxygen (mg L^{-1})			Lower 5th percentile
1-4	7.80 ± 0.37	7.04 – 9.12	7.21
5-7	7.46 ± 0.35	6.60 – 8.21	6.89
Oxygen saturation (%)			Lower 5th percentile
1-4	93.1 ± 2.4	96.6 – 89.9	91.5
5-7	88.0 ± 1.2	89.4 – 86.0	87.7
Total-ammonia nitrogen (TAN) (µg L^{-1})			Upper 5th percentile
1-4	109.7 ± 75.7	7.6 – 371.6	265.8
5-7	260.1 ± 119.6	87.6 – 612.6	516.9
Free-ammonia nitrogen (FAN) (µg L^{-1})			Upper 5th percentile
1-4	1.28 ± 0.84	0.12 – 3.85	3.06
5-7	2.38 ± 1.21	0.57 – 5.75	4.90
Nitrite (NO_2^- - N) (µg L^{-1})			Upper 5th percentile
1-4	9.86 ± 4.17	2.73 – 23.07	18.55
5-7	24.07 ± 13.23	5.4 – 70.07	47.52

Discussion

Abalone growth

Water quality in the serial-use raceway became progressively degraded due to the metabolic waste from the abalone, thereby reducing weight gain in the baskets at the end of the serial-use system. Atlantic salmon (*Salmo salar* L.) fingerlings raised in a six-tier serial-use raceway showed a significant reduction in growth after third tank in series (Morrison & Piper 1988). Fivelstad *et al*. (1991) and Huchette *et al*. (2003) suggested establishing the flow rate per unit biomass at which growth reduction occurs in *S. salar* and *H. rubra*, respectively. As growth was not significantly reduced within the first four serial-passes, 7.2–9.0 L h^{-1} kg^{-1} was the lowest flow index required to prevent a growth reduction in 50–60 g *H. midae* (60-70 mm) in the serial-use raceway used in this study. Yearsley (2008) recommended flow indices in commercial flow-through tanks of 9.7–14.5 L h^{-1} kg^{-1} to avoid growth reduction in 20–30 g *H. midae*. The difference in the suggested flow indices between this study and Yearsley (2008) may be due to differences in the characteristics of the systems and the relatively high sensitivity of small abalone to poor water quality (Fallu 1991; Reddy-Lopata *et al*. 2006).

Weight gain of abalone in tank position two was low (Figure 2.2), possibly as a result of inconsistency in management. These abalone may have been subjected to more handling stress than the abalone from the other treatments, due to a support wire which made placing the baskets into this tier of the raceways difficult. The wire was subsequently removed. This may explain why the feed consumption of these animals was significantly lower for the first two weeks of the experiment, thus delaying their start to normal feeding behaviour. Abalone growth in tank positions one, three and four was consistent with that of abalone from the same batch grown in farm tanks at high flow indices (16.7–13.9 L h^{-1} kg^{-1}). Abalone in tank positions five, six and seven had higher FCR values, and it is hypothesised that these abalone required proportionally more energy to grow and maintain physiological functions such as respiration, excretion and osmoregulation due to the deteriorated water quality.

Water quality

Ambient air temperature coupled with the effect of retention time of water in the system caused mean water temperature to increase by 0.3 °C between the first and last tanks in the series. Although growth rate in *H. midae* was positively correlated with temperature within the range of 12–20 °C (Britz *et al*. 1997), this small temperature increase is unlikely to have confounded the growth results. Using a model provided by Britz *et al*. (1997), the increase in temperature down the raceways should have only increased weight gain by 0.19–0.54 g in positions five to seven. Subtracting these values from the recorded differences of 3.4, 5.1 and 6.2 g between position one and positions five, six and seven would not have altered any of the above mentioned outcomes.

Similarly, the low concentrations of suspended solids reported from this study and the small differences between the first (4.2 ± 2.0 mg L^{-1}) and last (4.8 ± 2.5 mg L^{-1}) tanks in series are unlikely to have contributed to the differences in abalone growth among tank positions. These concentrations were similar to those reported by Park *et al*. (2008) for the culture of *H. discus hannai* Ino, in a recirculating system with baffled tanks, where the total suspended solids concentration averaged 2.7 mg L^{-1} with a maximum of 7.9 mg L^{-1}. In raceways used for fish farming, movement of the fish can disturb bottom sediments thereby increasing the suspended solid concentration. As the abalone baskets were suspended within the tank, feed particles and faeces on the tank bottom could not be stirred up by the abalone. In juvenile green grouper, *Epinephelus coioides* Hamilton, exposure to a suspended solid concentration of 50 mg L^{-1} for six weeks caused only minor respiratory distress (Au *et al*. 2004). In the mussel, *Perna viridis* L., exposure to suspended solid concentrations of 0, 180, 300, 440 and 600 mg L^{-1} for 14 days, followed by 14 days recovery resulted in no significant differences in oxygen consumption or growth (Shin *et al*. 2002).

A low dissolved oxygen concentration in the culture water is a primary limiting factor for growth in aquatic organisms (Fry 1971) and the first limiting variable in flow-through systems (Colt & Orwicz 1991a). In this study, differences in weight gain were larger then EC_5 predictions for dissolved oxygen in other species, suggesting that dissolved oxygen was not the first limiting water quality variable in this serial-use

system. In abalone, a low dissolved oxygen concentration can limit growth by reducing the capacity for aerobic metabolism (Harris *et al*. 1999b), but serial-use resulted in a small decrease in dissolved oxygen concentration and oxygen saturation between the first (8.1 mg L^{-1} O_2; 99.4 %) and last tank (7.4 mg L^{-1} O_2; 91.6 %). In juvenile *H. laevigata* (10.8 g $abalone^{-1}$), weight gain was reduced by 5 and 50 % at 7.36 mg L^{-1} O_2 and 5.91 mg L^{-1} O_2, respectively, while there were no significant differences in shell growth in the range of 8.9–6.2 mg O_2 L^{-1} (Harris *et al*. 1999b). In the current trial, the mean dissolved oxygen concentration remained above the EC_{50} suggested by Harris *et al*. (1999b), however, values below the EC_5 were recorded in 36 samples (12 % of samples taken) for tanks 1–4, and 78 samples (35 % of samples taken) for tanks 5–7. For commercial flow-through tanks Yearsley (2008) observed good growth in 20–30 g *H. midae* at median dissolved oxygen concentrations of 7.0–7.2 mg L^{-1} O_2. In *H. laevigata* and *H. diversicolor supertexta*, respiration rates start to decline significantly at 4.9 mg L^{-1} O_2 and 5.5 mg L^{-1} O_2, respectively (Jan & Chang 1983; Harris *et al*. 1999b). In tank position seven, dissolved oxygen concentrations and percentage oxygen saturation were similar to the EC_5 for *H. laevigata*, but there was a 52 % decrease in weight gain relative to the values for position one, thus, it is hypothesised that other factors made a larger contribution to the observed reduction in abalone growth.

Based on a comparison of findings from this study and a review of the literature, it is suggested that low pH contributed to the reductions in growth recorded in the lower tank positions. For example, there was a reduction in pH from 7.86 to 7.6 from position one to seven. The metabolite CO_2 combines with water to form carbonic acid, the carbonic acid then dissociates into bicarbonate ions resulting in the release of H^+ ions (Sanni & Forsberg 1996). A lower pH may be beneficial in that it reduces the percentage of FAN, the toxic form of total ammonia (Bower & Bidwell 1978; Thurston *et al*. 1981), but the effect of pH on abalone health requires further studies (Harris *et al*. 1999a), due to the interactions of pH, dissolved CO_2 and alkalinity of seawater (Poxton & Allouse 1982). While pH values of 6.5–8.5 are considered safe for fish culture (Poxton & Allouse 1982; Colt & Orwicz 1991a), increased CO_2 concentrations present at a low pH reduced the growth and survival of animals with calcite shells, such as echinoderms and gastropods (Shirayama 2002). In the mussel, *Mytilus galloprovincialis*

Lamarck, reducing water pH to 7.3 using CO_2 gas caused significant reductions in growth and dissolution of the shell compared to a control treatment kept at pH 8.05 (Michaelidis *et al*. 2005). Many abalone in the current study exhibited signs of "shiny shell", which is the dissolution of the outer calcite shell layer resulting in the exposure of patches of shiny nacre. The number of abalone with these signs was not quantified. In juvenile greenlip abalone, *H. laevigata* (± 2.3 g abalone^{-1}), a significant reduction in weight and shell length gain occurred in animals exposed to pH 7.46 over 50–68 days, compared to animals exposed to pH 7.76 and pH 8.27 (Harris *et al*. 1999a). The authors estimated the EC_5 and EC_{50} at 7.78 and 7.39, respectively. In the current study, 195 pH samples (60 %) from tanks 1–4 and all pH samples taken from tanks 5–7 were below the EC_5 threshold for *H. laevigata*. Significant differences in weight gain occurred from tank position five onwards where pH values averaged 7.63, 7.61 and 7.6, respectively. These averages fall within the EC_5 and EC_{50} for growth in both *H. laevigata* and *H. rubra*, thus further supporting the hypothesis that low pH values as a result of CO_2 excretion contributed to the reduction in abalone weight gain.

However, combined changes in the concentrations of CO_2 and free ammonia nitrogen, FAN, may lead to growth-limiting conditions in serial-use systems (Fivelstad *et al*. 1991; Colt *et al*. 2009). Findings from this study suggest that the mean concentration and peaks of FAN limited abalone growth under the experimental conditions but the growth-reducing effect of FAN may have been exacerbated by low pH-values.

The excretion of ammonia by the abalone (Barkai & Griffiths 1987) and bacterial decomposition of uneaten feed and faeces (Yearsley *et al*. 2009) are hypothesised to be the main cause of the linear increase in total ammonia (TAN) concentrations with increasing water use. The linearity of the TAN increase suggests that net production of TAN was similar in all tanks. In a similarly designed study using *H. rubra*, Huchette *et al*. (2003) found that the TAN concentration was positively correlated with cumulative biomass. A high ammonia concentration in the culture water can lead to elevated ammonia concentrations in the body of aquatic organisms (Randall & Tsui 2002; Miron *et al*. 2008). Some fish species are, however, tolerant of high ammonia concentrations in the body and have developed strategies to minimise ammonia toxicity (Randall & Tsui

2002). Such strategies have not been documented for abalone. Russo & Thurston (1991) suggested that reduced growth and feed uptake were recorded at concentrations of 2–150 µg L^{-1} FAN. In the range of 40–400 µg L^{-1} FAN, fish exhibited anaemia, gill and kidney degeneration, and reduced disease resistance. Abalone seem to be very sensitive to FAN (Harris *et al*. 1998; Huchette *et al*. 2003; Reddy-Lopata *et al*. 2006). The EC_5 for weight gain at pH of 7.95–8.17 was estimated at 41 µg FAN L^{-1} for *H. laevigata* (32 mm) and 4–6 µg FAN L^{-1} for *H. rubra* (15–65 mm) (Harris *et al*. 1998; Huchette *et al*. 2003). In tank positions one to four, no samples exceeded the estimated EC_5 of 4 µg L^{-1} for *H. rubra*. However, in tank positions five to seven, values between 4–6 µg L^{-1} FAN were recorded 38 times (15 % of samples), occurring at least three times in each tank. Reddy-Lopata *et al*. (2006) showed that the 36 h LC_{50} for 50–80 mm *H. midae* was 12.9 µg FAN L^{-1}. The authors also recorded a 59 % decrease in specific weight gain in 10–25 mm *H. midae* after chronic exposure to 7.4 µg FAN L^{-1} at pH 7.8 and stated that the EC_5 for *H. midae* is likely to be lower than that estimated for *H. rubra* (EC_5, 4–6 µg L^{-1}). Percentage weight gain was 52 % lower in tank position seven than in tank position one, where FAN concentrations averaged 2.7 µg FAN L^{-1}. Although the concentrations of FAN experienced by abalone in the lower tank positions are slightly lower than those presented by Harris *et al*. 1998, Huchette *et al*. 2003 and Reddy-Lopata *et al*. 2006, these tanks had lower pH values (pH 7.63–7.60) than the top tanks. The 96-h LC_{50} concentrations for FAN in rainbow trout (*Oncorhynchus mykiss* Walbaum), fathead minnows (*Pimephales promelas* Rafinesque) (Thurston *et al*. 1981) and silver catfish (*Rhamdia quelen* Quoy & Gaimard) (Miron *et al*. 2008) were lower at lower environmental pH. It is hypothesised that EC_5 and EC_{50} concentrations would be affected similarly and that the presence of H^+ ions may increase the toxicity or bioavailability of the NH_3 molecule.

Peaks in FAN concentration in weeks 4, 8 and 11 are likely to have been caused by high ambient temperatures resulting in increased TAN production by the abalone and during the breakdown of feed and faecal wastes. As tanks were cleaned on the Wednesday of all even numbered weeks, the peaks in FAN during week 4 and 8 would have coincided with a period when accumulated feed and faecal wastes were at their maximum.

Yearsley *et al*. (2009) showed that the presence of accumulated sludge on the tank bottom resulted in TAN concentrations 44 % higher then tanks containing no sludge.

Although the nitrite concentration increased throughout the experimental period, being highest in tank positions five to seven, nitrite was the least likely cause for reduction in abalone growth. Nitrifying bacteria are slow growing, autotrophic bacteria (Colt *et al*. 2009) and require ammonia as an energy source. Nitrifying bacteria were not counted or identified in this study. However, it is hypothesised that the availability of ammonia initiated and maintained bacterial growth leading to ammonia being oxidised to nitrite. Nitrifying bacteria are sessile and may have been attached to the abalone shells, the basket, rack and feeder plate, which were merely moved during tank cleaning and never scrubbed or sprayed clean. Establishing nitrifying bacteria within the tank before the introduction of abalone may help reduce ammonia concentrations in the lower tank positions of the serial-use raceway, in particular when operating at low flow indices. In freshwater fish species, transport of nitrite occurs via the chloride cells and can result in blood plasma nitrite concentrations above the concentrations in the water (Lewis & Morris 1986; Russo & Thurston 1991; Tomasso & Grosell 2005). Due to the high chloride concentration in seawater, marine fishes developed an opposite osmotic gradient to freshwater fishes thereby preventing nitrite uptake via this pathway (Jensen 2003). However, the high concentration of chloride ions in marine systems may not protect all invertebrates (Tomasso 1994). Nitrite may enter marine organisms through the drinking of seawater and subsequent uptake across the intestinal epithelium, or via diffusion of nitrous acid across epithelial cells (Jensen 2003).

Nitrite is generally less toxic in seawater, especially at the naturally occurring high pH levels (Wedemeyer & Yasutake 1978). There is a paucity of data on the chronic toxicity of nitrite to abalone. Nitrite oxidises the respiratory pigment haemocyanin to form methaemocyanin, thereby reducing oxygen transport. In *H. laevigata*, Harris *et al*. (1997) recorded significant and uniform reductions in growth regardless of concentration in the range of 0.56 – 7.8 mg NO_2-N L^{-1} relative to a control treatment (0.024 mg NO_2^--N L^{-1}). Mean nitrite concentrations in the serial-use raceways were similar to those of the control used by Harris *et al*. (1997). The variations in influent

dissolved oxygen and pH over time were probably caused by weather patterns such as up-welling, discharge of acidic river waters, rough seas and high temperatures.

Assuming a farm with a standing stock of 100 t, costs of R 0.29 m^{-3} of water pumped and a feed cost of R 17 kg^{-1}, operating at the minimum prescribed flow index would reduce operating costs by 30 – 32 % when compared to the current flow indices used on commercial abalone farms in South Africa. Due to the high value nature of abalone, and the slightly better production at high flow indices, the minimum flow index suggested here is not the most profitable. However, in South Africa, the electricity price is expected to rise by 25 % $year^{-1}$ for the next three years. By the end of the third year, a flow index of 7.2 – 9.0 L h^{-1} kg^{-1} is expected to generate more profit due to lower pumping costs than currently used flow indices. Thus, based on these estimates there is a justification to design future studies to conduct a comprehensive economic analysis of serial-use systems.

Conclusions

Water flow was set low in order to determine the minimum required flow indices, and to understand how the concentrations of dissolved oxygen, pH, ammonia and nitrite affect growth in a commercially operated system. Deterioration of water quality due to serial-use negatively affected the wet weight gain and FCR of 60–70 mm *H. midae*, but not their survival. The minimum flow per unit biomass was estimated to range from 7.2 to 9.0 L h^{-1} kg^{-1} to prevent significant growth reduction. The model equations for weight gain and FCR can be used by farmers to calculate the most economic flow indices within the safe range suggested here, depending on the cost of pumping and the stocking strategy employed. By comparing the results of this experiment with those by other researchers, it is suggested that low pH was the first growth-limiting variable in the serial-use raceways through its interaction with FAN which becomes more toxic as pH decreases.

CHAPTER 3

The effect of dietary protein level on total ammonia nitrogen and free ammonia nitrogen concentration in an abalone, *Haliotis midae*, serial-use raceway

Introduction

The protein content and protein source of formulated abalone diets have been optimised according to their impact on feed cost and their importance for muscle growth (Britz 1996a; Britz 1996b; Fleming *et al*. 1996; Sales *et al*. 2003; Guzman & Viana 1998; Coote *et al*. 2000; Bautista-Teruel *et al*. 2003; Montano-Vargas *et al*. 2005; Cho *et al*. 2008; Green *et al*. 2011a). However, the effect of dietary protein content on ammonia concentrations under intensive culture conditions such as those occurring in recirculating systems and serial water use systems requires further research (Naylor *et al*. 2011). Ammonia is the principle nitrogenous metabolite of protein catabolism in abalone (Bishop *et al*. 1983, Barkai & Griffiths 1987). Furthermore, mineralization of uneaten feed and faeces increased the concentration of ammonia in flow-through abalone culture tanks (Yearsley *et al*. 2009). Ammonia occurs either in its ionized form (NH_4^+) or un-ionized form (NH_3, free ammonia nitrogen, FAN), the latter concentration being a function of the concentration of NH_4^+, water pH, temperature and salinity (Bower & Bidwell 1978, Thurston *et al*. 1981). The sum of these two forms is the total ammonia nitrogen concentration (TAN).

Under intensive culture conditions FAN concentrations as low as 4 – 41 μg L^{-1} reduced growth and condition of abalone (Harris *et al*. 1998; Huchette *et al*. 2003; Reddy-Lopata *et al*. 2006; Naylor *et al*. 2011). South African abalone farmers use pump-ashore flow-through systems with constant dilution of metabolites by the inflowing water. With an increasing interest in water reuse systems designed to reduce production cost, and as a result of growing concerns over the effects of farm effluent on the environment, formulated diets that result in lower TAN and FAN concentrations in the water will be beneficial for both the health of the cultured abalone and the environment. Using a

serial-use system, Naylor *et al.* (2011) described the changes in water quality as a function of the intensity of water use. FAN concentrations > 4 μg L^{-1} in combination with reduced water pH reduced abalone growth.

It is hypothesised that water quality in intensive systems could be improved by using formulated diets with improved protein conversion efficiency. In abalone, *Haliotis midae*, the protein content of the diet could be reduced from the commercially used 34 %, to as low as 22 % while maintaining good growth (Jones & Britz 2006; Green *et al*. 2011b). The effect of low protein diets on water quality has not been tested in intensive serial-use systems. The aim of this experiment was to quantify the effect of dietary protein levels of 33, 26 and 22 % of iso-energetic diets on TAN and FAN concentrations in a serial-use raceway operated under farm conditions. The results are expected to benefit diet development for intensive abalone systems.

Methods and Materials

The experimental system comprised six serial-use raceways each with three 300 L tanks in series, set-up at different heights to allow for gravitational water flow. The tanks in series were labelled as position one, two and three, with position one being first in series. The water flow between the second and third tank in series was halved to simulate conditions of flow indices (L h^{-1} kg^{-1} biomass) at which reductions in growth due to sub-optimal water quality have occurred (Naylor *et al*. 2011). To achieve this, water flow between the second and third tank in series was controlled by a valve with excess water being returned to the ocean (Figure 3.1).

Water flow into each raceway was measured daily using a 2 L graduated container and stopwatch and adjusted when necessary. Water flow into the first tank of a series averaged 181.7 ± 1.6 L h^{-1}. Due to variations in ambient temperature and water temperature and resultant changes in farm management, flow rate to the experimental system varied between days. Thus, water quality variables were expressed as a function of flow index (L h^{-1} kg^{-1}), percentage protein level, and time (days since the beginning of the experiment).

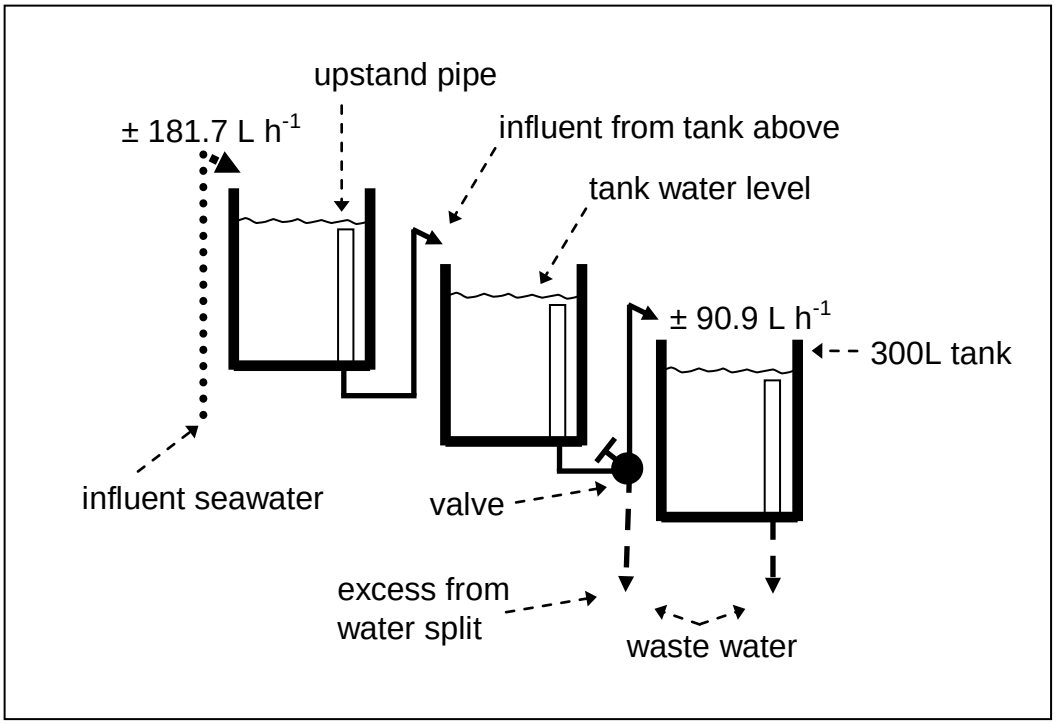


Figure 3.1: Diagram of the serial-use raceway set-up. Each basket also contained a rack and feeder plate providing additional surface area for the abalone. Tanks were aerated via individual airlines placed horizontally beneath the abalone basket.

The flow index accounts for the accumulated abalone biomass which increased with serial water use. Each tank contained one abalone basket stocked with 7.6 ± 0.1 kg of *H. midae* with a mass of 45 – 55 g.

The iso-energetic experimental diets contained 22 (P22), 26 (P26) or 33.2 % (P33) protein (Table 3.1). The protein levels were chosen to resemble the most commonly used commercial diet in South Africa, Abfeed® S34 (Marifeed Pty Ltd., South Africa), and two diets under commercial development, Abfeed S26 and Abfeed S22. Fishmeal and soya were the protein source. Abalone were fed every second day according to commercial feeding methods. Each tank was fed one cup of food containing 57.8 ± 2.2 g which amounted to a feeding rate of 0.4 % body mass day^{-1}.

Table 3.1: Protein, lipid, energy and protein to energy ratio of the three diets used a serial-use abalone raceway system.

	P33	P26	P22
Protein (%)	33.2	26	22
Lipid (%)	3.03	3.03	3.03
Energy (MJ kg^{-1})	15.6	15.6	15.6
Protein: Energy ratio	2.13	1.67	1.41

Temperature, pH, total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) concentrations were recorded for 17 days. Although the experimental design accounted for daily measurements, there were a few days when the pH meter did not function. These data were excluded from the data set.

The concentration of dissolved oxygen (O_2, mg L^{-1}) and percentage oxygen saturation were recorded every third day. Temperature and pH were measured using a pH meter (YSI Inc. Model # 60/10 FT; Yellow Springs, Ohio, U.S.A.). Dissolved oxygen concentration and oxygen saturation were measured using an oxygen meter (YSI Inc. Model # 55D; Yellow Springs, Ohio, U.S.A.). TAN concentrations were determined according to Solorzano (1969) using a spectrophotometer (Prim Light, Secomam, 30319 Ales, France). Absorbance was converted into the concentration of TAN using the coefficients derived from least-square linear regression calibration curves (NH_4CL, n = 12, $r^2 = 0.996$, Appendix B). The concentration of FAN was calculated using the values for NH_4^+, temperature, pH and salinity of the water sample (Bower & Bidwell 1978).

Statistical analysis

Data were analysed using multiple regression modelling with stepwise inclusion of variables. Water quality variables, i.e., the concentrations of TAN, FAN and dissolved oxygen, percentage oxygen saturation and pH were used as dependent variables and flow index, time, and dietary protein levels were the independent variables. The decision for the model of best fit was made according to the distribution of residuals,

the t-statistics to test for inclusion of each variable and the respective p-value. In some cases, the least-square fit could be improved by coding the data and using the logarithmic value of the flow index. An α error level of 5 % ($p < 0.05$) was used for inclusion of all coefficients.

Results

Changes in water quality over time (Figures 2.2 - 2.5) were related to changes in flow index which were a result of management, water temperature, and fluctuations related to setting the daily flow rate.

The concentrations of free ammonia (FAN) and total ammonia (TAN) (μg L^{-1}) were a function of both percentage protein (P) ($t = 6.41$, $p < 0.0001$ (FAN) and $t = 6.63$, $p < 0.0001$ (TAN)) and flow index (FI) [$t = 3.9$, $p < 0.0002$ (FAN) and $t = 5.42$, $p < 0.0001$ (TAN)], and could be estimated as FAN = 0.132 P – 1.10 log (FI), and TAN = 9.73 P – 110.3 log (FI). When using percentage protein, time (T = days), and FI to estimate pH values, percentage protein did not significantly contribute to the model ($t = 1.05$, $p = 0.295$), while FI and T contributed significantly ($t = 11.3$, $p < 0.00001$ (FI) and $t = 4.72$, $p < 0.00001$ (T)). The suggested model was pH = 7.73 – 0.004 T + 0.17 log (FI).

There was no correlation between FI or P and the concentration of dissolved oxygen, while there was a small significant increase in dissolved oxygen level of 0.017 mg L^{-1} d^{-1} ($t = 3.81$, $p < 0.0002$). The percentage oxygen saturation was not correlated to any of the independent variables and averaged 95.5 % ranging from 92.3 – 99.5 %. Over all treatments, dissolved oxygen concentration averaged 7.69 mg L^{-1}, ranging from 7.28 – 8.17 mg L^{-1} O_2.

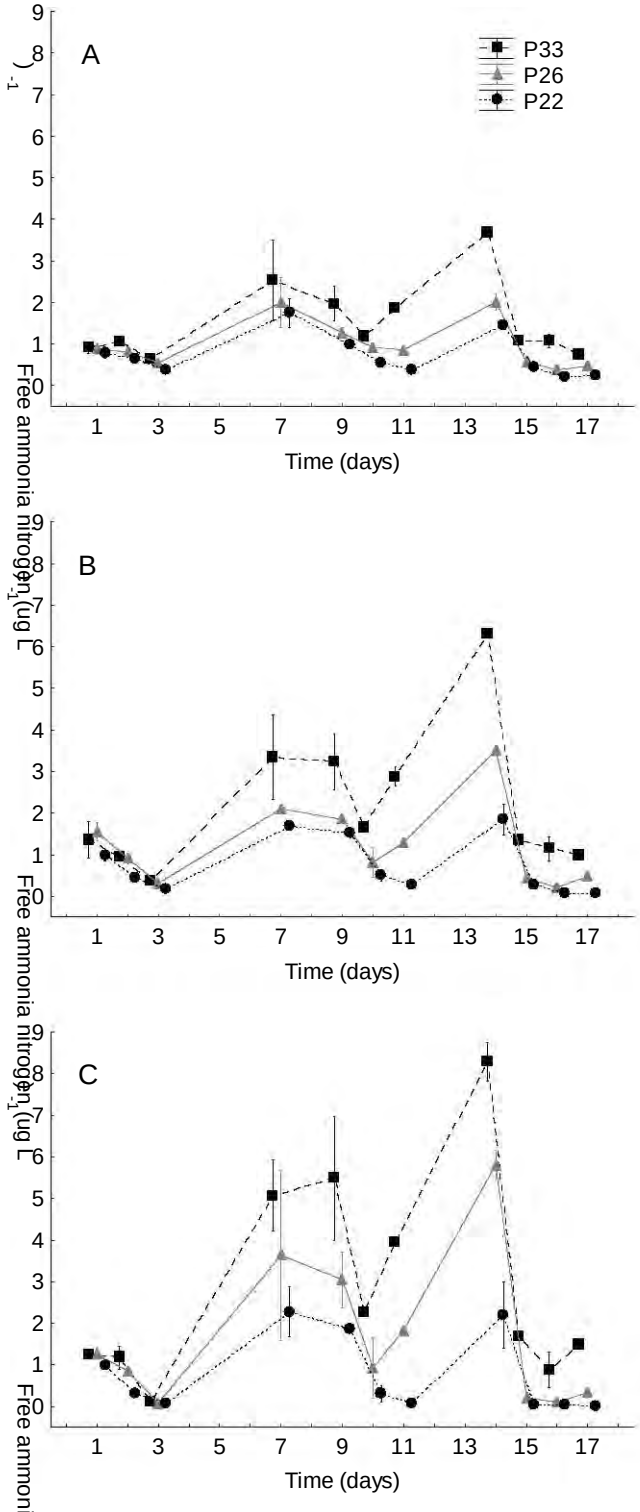


Figure 3.2: Free ammonia nitrogen concentration (FAN) (µg L^{-1}) in an abalone culture system with 3 serial-passes: first tank (A), second tank (B), and third tank (C) in series. Three abalone diets with varying protein levels were fed to satiation: 33 (P33), 26 (P26), and 22 % (P22). Values are mean ± SD.

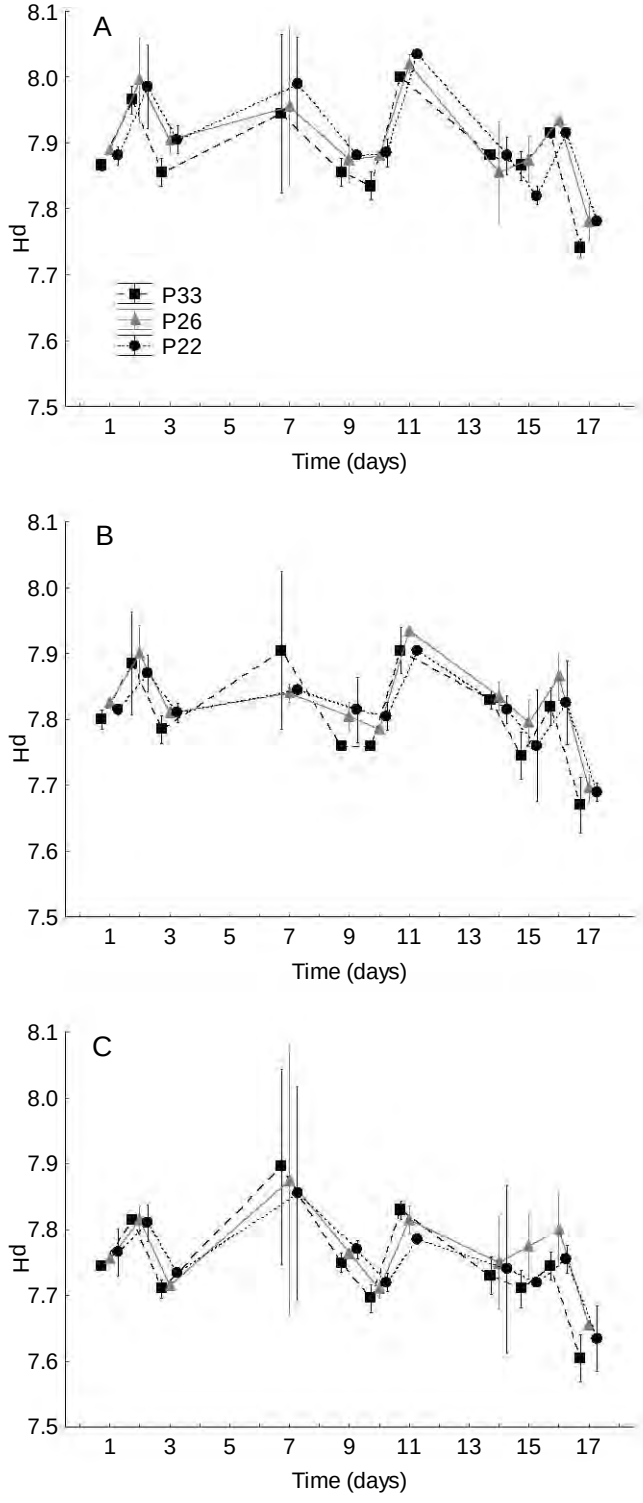


Figure 3.3: Water pH in an abalone culture system with 3 serial-passes: first tank (A), second tank (B), and third tank (C) in series. Three abalone diets with varying protein levels were fed to satiation: 33 (P33), 26 (P26), and 22 % (P22). Values are mean ± SD.

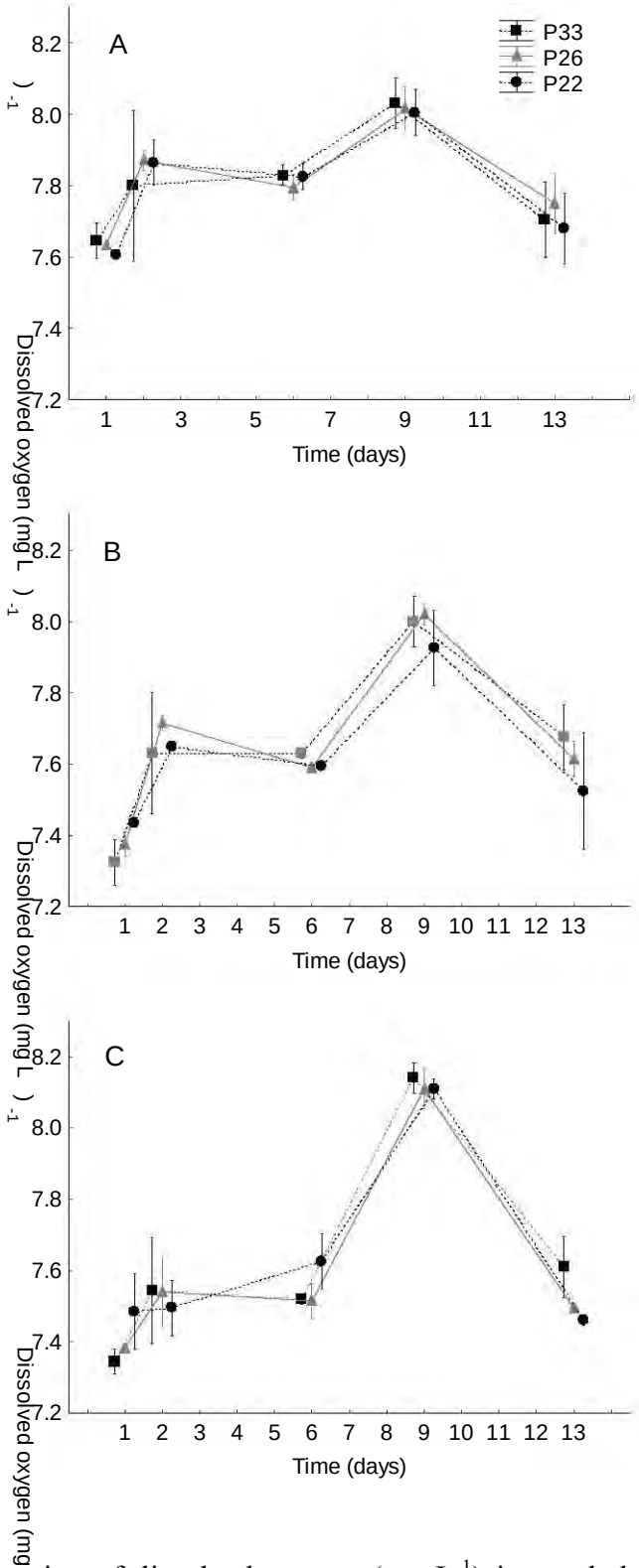


Figure 3.4: Concentration of dissolved oxygen (mg L^{-1}) in an abalone culture system with 3 serial-passes: first tank (A), second tank (B), and third tank (C) in series. Three abalone diets with varying protein concentrations were fed to satiation: 33 (P33), 26 (P26), and 22 % (P22). Values are mean ± SD.

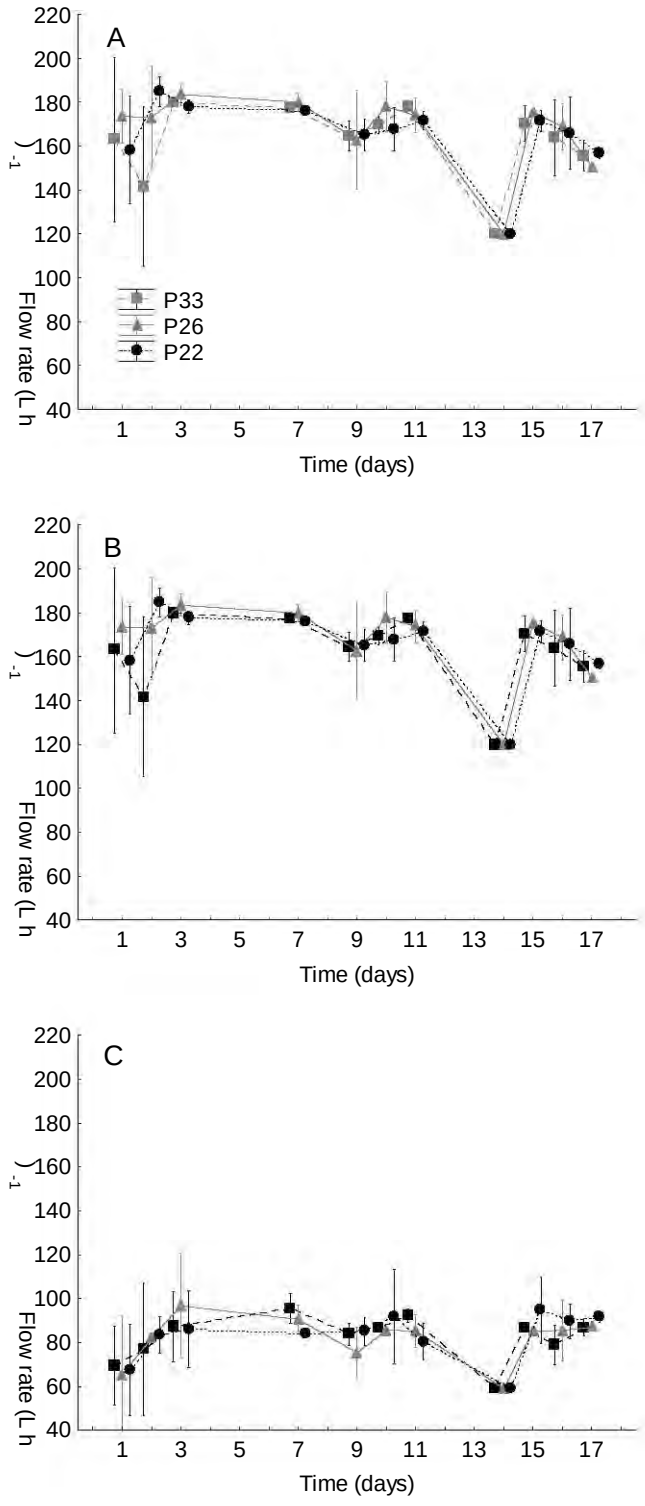


Figure 3.5: Flow rate through an abalone culture system with 3 serial-passes: first tank (A), second tank (B), and third tank (C) in series. Three abalone diets with varying protein concentrations were fed to satiation: 33 (P33), 26 (P26), and 22 % (P22). Values are mean ± SD.

On average, TAN concentrations could be reduced by 54 % (position one), 67 % (position two), and 75 % (position three) by lowering the dietary protein level from 33 to 22 % (Table 3.2).

Table 3.2: Mean concentrations ± standard error of total ammonia nitrogen (TAN) and free ammonia nitrogen (FAN) and mean flow index for three dietary protein levels and three levels of water reuse, i.e., positions one - three.

Dietary protein level (%)	**Total ammonia nitrogen (µg L^{-1})**	**Free ammonia nitrogen (µg L^{-1})**	**Mean flow index (L h^{-1} kg^{-1})**
Position one			
33	88.8 ± 10.9	1.52 ± 0.20	21.6 ± 0.6
26	54.8 ± 6.8	0.97 ± 0.12	22.2 ± 0.56
22	39.0 ± 5.3	0.70 ± 0.10	21.9 ± 0.52
Position two			
33	147.3 ± 23.1	2.14 ± 0.36	10.8 ± 0.30
26	80.9 ± 13.4	1.22 ± 0.20	11.1 ± 0.27
22	47.4 ± 9.3	0.71 ± 0.39	11.0 ± 0.26
Position three			
33	227.2 ± 41.7	2.87 ± 0.53	3.6 ± 0.13
26	121.6 ± 28.9	1.64 ± 0.39	3.6 ± 0.13
22	53.3 ± 13.6	0.73 ± 0.20	3.7 ± 0.13

Discussion

The percentage protein in the formulated diets had a significant effect on the concentration of TAN and FAN in the serial-use raceway. It is hypothesised that both the metabolic activity of the abalone with the resultant excretion of ammonia (Barkai & Griffiths 1987) and the sludge, i.e., the accumulated uneaten feed and faeces in the tanks contributed to the rise in ammonia levels. As an increase in dietary protein did not affect feed consumption in *H. midae* under similar conditions (Britz 1996a), it is suggested that with ingestion of increasing amounts of dietary protein, ammonia production by the abalone will increase. A positive relationship between dietary protein content and

ammonia excretion has been shown for red drum, *Sciaenops ocellatus* (Webb & Gatlin 2003), Australian short-finned eel, *Anguilla australis australis* (Engin & Carter 2001) and silver perch, *Bidyanus bidyanus* (Yang *et al*. 2002). Yearsley *et al*. (2009) showed that the accumulated sludge on the bottom of commercial flow-through tanks for *H. midae* was a significant contributor to the concentration of TAN. In shrimp ponds, total ammonia nitrogen concentrations were significantly lower in ponds where sludge was removed weekly, than in un-cleaned ponds and in ponds where sludge was re-suspended (Hopkins *et al*. 1994).

The reduction in water pH as a function of flow index was significant but small. FAN and TAN concentrations followed similar changes over time. The magnitude of the differences in TAN and FAN concentrations as a function of percentage dietary protein has implications for water use efficiency and management.

Haliotis midae is sensitive to ammonia with an estimated growth-reducing EC_5 for FAN of < 4 µg L^{-1} (Reddy-Lopata *et al*. 2006). Using all data from this experiment, this value was exceeded in 16 and 1.5 % of the measurements for treatments P33 and P26, respectively (normal distribution, z-test).

The results suggest that water flow and energy costs for pumping could be reduced by lowering the protein concentration in the diet. TAN and FAN concentrations were reduced by 67 % when lowering the dietary protein concentration from 33 to 22 %. Thus, it should be tested whether a reduction in flow index to 8 L h^{-1} kg^{-1} (Position one), 4 L h^{-1} kg^{-1} (Position two) and 1.3 L h^{-1} kg^{-1} (Position three) in a raceway fed P22, would allow the same production of abalone biomass as a raceway with abalone fed P33 operated at the flow indices used in the current study. Protein content did not influence the changes in water pH which has been reported to drop with increasing reuse (Naylor *et al*. 2011). Thus, assuming a growth reducing EC_5 for pH of 7.78 (Harris *et al*. 1999a) water could be used more efficiently by either testing lower flow indices or increasing abalone biomass. The concentrations of FAN and pH recorded in this experiment should be tested in growth trials to identify the first limiting variable. Diets containing > 33 % protein are likely to result in FAN concentrations that greatly exceed the EC_5 for other

abalone species, and are therefore not suitable for serial-use raceways or perhaps even recirculating systems.

The use of diets containing lower percentages of protein may also help farmers comply with regulations pertaining to the nitrogen concentration of effluent discharge while achieving good abalone growth.

Although increased dietary protein levels have been associated with an increase in *H. midae* growth (Britz 1996a; Britz & Hecht 1997; Sales *et al*. 2003), Green *et al*. (2011b) showed that dietary protein content could be lowered without reducing growth in *H. midae*, provided that dietary energy levels were > 13.5 MJ kg^{-1}, supplied through carbohydrates. Research into essential amino acid requirement and protein sparing effects of lipids and carbohydrates should aim at designing formulated abalone diets that contain less fishmeal protein while achieving good abalone growth. The models proposed from this study can contribute to estimating the environmental effects of abalone diets as a function of their protein level within the ranges tested and experimental conditions.

Conclusion

This study showed that a reduction of dietary protein can reduce the concentrations of TAN and FAN in a serial-use raceway, as FAN concentrations were on average 67 and 41% lower in raceways with abalone fed P22 and P26 than in those fed P33.

CHAPTER 4

The effect of free ammonia nitrogen, pH and supplementation with oxygen on the growth of South African abalone, *Haliotis midae* in an abalone serial-use raceway with three passes

Introduction

Due to South Africa's high-energy coastline, abalone are farmed intensively in land-based flow-through systems at high water exchange rates. Such systems require efficient utilisation of pumped water in order to remain profitable (Siikavuopio *et al*. 2004). Thus, under such conditions, identifying potentially limiting water quality variables and species-specific tolerance limits is essential (Siikavuopio *et al*. 2004; Colt 2006). This important aspect is not well documented in the culture of *H. midae*.

In flow-through and serial-use raceways, oxygen is generally considered to be the first limiting variable (Colt & Orwicz 1991a; Sanni & Forsberg 1996). However, in systems emplying a degree of water reuse an increase in the concentration of the metabolic waste products carbon dioxide (CO_2) and ammonia may occur (Sanni & Forsberg 1996; Huchette *et al*. 2003; Piedrahita 2003; Colt *et al*. 2009; Naylor *et al*. 2011). In water, total CO_2 and ammonia exist in both the ionized form, i.e., as bicarbonate, HCO_3^- or carbonate, CO_3^{2-} and ammonium, NH_4^+, and in the un-ionized form, i.e., as aqueous carbon dioxide, CO_2^{aq}, and free ammonia nitrogen, FAN or NH_3. The equilibrium reactions are controlled by pH, temperature and ionic strength of the water (Bower & Bidwell 1978; Smith 1988; Randall & Tsui 2002). The sum of the ionized and un-ionized forms of ammonia is total ammonia nitrogen, TAN. When CO_2 hydrates in water, hydrogen ions (H^+) are released which results in changes in water pH (Covington *et al*. 1985).

The toxic effects of CO_2 and ammonia are mostly related to the concentration of the un-ionized forms (Thurston *et al*. 1981; Sanni & Forsberg 1996). In *H. rubra* Leach, and *H. laevigata* Donovan, FAN concentrations of 4 μg L^{-1} and 41 μg L^{-1}, respectively, were estimated to cause growth reductions and an increase in oxygen consumption (Harris *et al*. 1998; Huchette *et al*. 2003). In *H. midae* of 10 – 20 mm, 50 – 80 mm and 100 – 150 mm shell length, the 36-h LC_{50} for FAN was estimated at 9.3, 12.7 and 16.2 μg L^{-1}, respectively (Reddy-Lopata *et al*. 2006). The authors suggested that exposure to concentrations as low as 4 μg L^{-1} may reduce growth in *H. midae*. However, in addition to an increase in FAN concentrations, intensive water use can also reduce water pH as a result of the accumulation of CO_2^{aq} (Harris *et al*. 1999a; Colt *et al*. 2009). The effect that water pH has on growth and health has only been studied in *H. laevigata* and *H. rubra*. Harris *et al*. (1999a) estimated that in these species a 50 % reduction in growth (EC_{50}) could be expected at pH values of 7.39 and 7.37. A decrease in oxygen consumption was also noted at low pH. Exposure of the marine mussel, *Mytilus galloprovincialis* Lamarck, to pH 7.3, using CO_2-gassed water, resulted in a reduction in growth, haemolymph pH, and metabolic rate and an increase in haemolymph CO_2 concentration (Michaelidis *et al*. 2005). A decrease in pH can also lower the saturation state of essential carbonate minerals, calcite and aragonite, which are deposited during shell formation in calcifying organisms (Kleypas *et al*. 2006; Lopez *et al*. 2011).

In this study, medium-size abalone (Shell length; 65.9 ± 2.6 mm) were exposed to a range of FAN concentrations and variations in water quality at three positions in a serial-use raceway on an abalone farm. As the dosing of ammonia can be costly and technically demanding under farm conditions, the percentage protein in the formulated pelleted diet fed to the abalone was changed to create a range of FAN concentrations. As ammonia is the primary metabolite of nitrogen catabolism in *H. midae* (Barkai & Griffiths 1987), it was hypothesized that increasing the amount of dietary protein will result in increased ammonia excretion. This has been observed in both the red drum, *Sciaenops ocellatus* L. (Webb & Gatlin 2003) and silver perch, *Bidyanus bidyanus* Mitchell, (Yang *et al*. 2002). Growth in *H. midae* had been positively correlated to percentage dietary protein (Britz 1996a; Britz & Hecht 1997). However, recent studies showed that reducing the dietary protein content to ≤ 26 % did not reduce growth of *H.*

midae of > 50 mm shell length (Jones & Britz 2006; Green *et al*. 2011b) and has been attributed to the maintenance of dietary energy levels above 13.5 MJ kg^{-1} (Green *et al*. 2011b). This study tested the extent to which FAN and changes in water quality influenced abalone growth in a serial-use raceway. In order to elimate dissolved oxygen as the first limiting water quality variable in an abalone serial-use raceway, the effect that supplementing dissolved oxygen at high FAN and low pH had on abalone growth was tested.

Methods and Materials

The study was conducted at HIK Abalone Farm (Pty) Ltd in Hermanus, on the south-west coast of South Africa (34°26'04.35"S; 19°13'12.51"E) from July to October 2009. The abalone, *H. midae*, had a mean shell length of 65.9 ± 2.6 mm and a mass of 49.3 ± 3.3 g (n = 3080) at the beginning of the experiment and formed part of the same age cohort. They were spawned in the farm hatchery and reared in well aerated, flow-through tanks at ambient water temperatures (12–20 ºC). They were weaned from diatom coated plates onto a formulated diet (Abfeed®, Marifeed (Pty) Ltd., South Africa) at a young age.

Experimental design

Experiment 1 – Effect of serial reuse intensity on abalone growth

To test the effect of FAN concentration on abalone growth, a diet containing 26 % protein (P26) and a diet containing 33 % protein (P33) were used to create a low FAN and a high FAN treatment in triplicate serial-use raceways. Dietary protein level was used to manipulate FAN concentrations in the raceways without confounding growth rates, since dietary protein level, in the range used here, does not alter the growth of *H. midae* in the size class used in this experiment as long as the dietary energy levels are above 13.5 MJ kg^{-1} (Green *et al.* 2011b). Each diet was fed to all tanks in three raceways thereby creating a range of water quality conditions.

Experiment 2 – Effect of oxygen supplementation at high FAN and low pH

Supplementation with oxygen allowed for a comparison of abalone growth at high FAN (2.4 ± 1.1 μg L^{-1}), low pH (7.6 ± 0.1) and high dissolved oxygen concentrations (103 ± 8 %) (P33 with O_2 added) with a treatment in which the mean FAN concentration was high, and where pH and dissolved oxygen concentration was low (92 ± 6 %). Treatments were done in triplicate.

Experimental system

The experiment was conducted in six serial-use raceways each with three tanks in series. Each tank (0.9 × 0.6 × 0.6 m; 300 L water volume) contained one abalone basket, with a surface area of 3.2 m^2 provided by a plastic rack and feeder plate. Each tank was aerated by polyvinylchloride (PVC) airlines placed horizontally beneath the abalone basket, 100 mm above the tank bottom. Filtered seawater (100 μm) entered the first tank in series (position one) and flowed by gravity to tank positions two and three. Water flow between the second and third tanks in series was halved by means of a valve to simulate conditions of low flow indices (L h^{-1} kg^{-1}) (Figure 4.1). These flow rates were checked and maintained each time water samples were taken. Where abalone were fed the low-protein diet, half of the water from tank position two entered tank position three, with the other half going to waste, i.e., being returned to the ocean. To create the conditions for an additional experimental design, effluent water from abalone fed the high-protein diet was also split equally to flow into two tanks with one tank supplemented with oxygen (P33-O_2) while the other tank remained part of the experiment designed to test the effect of FAN on abalone growth (Figure 4.1). Oxygen supplementation allowed oxygen saturation in these tanks to be maintained at approximately 100 %, and thus, a comparison between two treatments was made possible, i.e., a treatment with high FAN-concentrations, reduced pH and reduced oxygen levels, and a treatment with high FAN-concentrations, reduced pH and oxygen supplementation. All tanks were cleaned every 14 days.

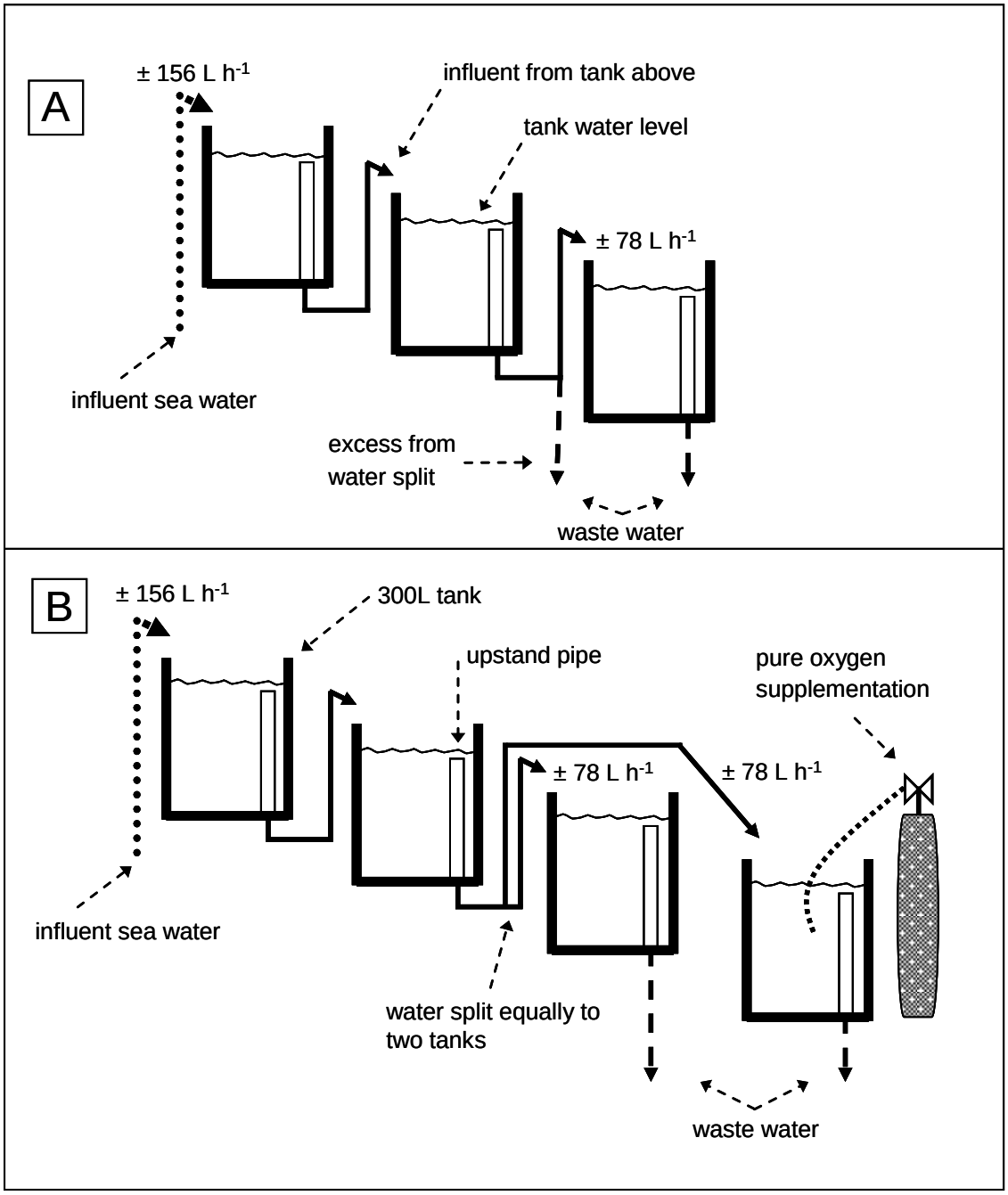


Figure 4.1: Diagram of the serial-use raceway set-up. (A) Tank arrangement for serial-use raceways in which the low protein diet (P26) was fed. (B) Tank arrangement for serial-use raceways in which the high protein diet (P33) was fed, showing the inclusion of an extra tank in to which pure oxygen was added to maintain ± 100 % oxygen saturation. Each tank contained one abalone basket, and each basket contained a rack and feeder plate providing additional surface area for the abalone. Tanks were aerated via individual airlines placed horizontally beneath the abalone basket.

Experimental diets and feeding

The diets were iso-energetic and contained a low percentage of lipid (Table 4.1). Fishmeal and soya-meal were used as the protein source. The abalone were fed to apparent satiation at 16:00 daily with feed from a bucket assigned to each tank. They were fed according to farm protocol to obtain results representative for these conditions. The buckets were weighed and refilled at two-week intervals, with the amounts fed added up to calculate the total mass of feed given per basket.

Table 4.1: Protein, lipid, energy and protein to energy ratio of the two diets used to create high and low FAN concentrations.

	P33	P26
Protein (%)	33.2	26
Lipid (%)	3.03	3.03
Energy (MJ kg^{-1})	15.6	15.6
Protein: Energy ratio	2.13	1.67

Growth analysis

At the start of the trial, each tank (n = 21) was stocked to 7.5 ± 0.01 kg of size graded *H. midae* (45 – 55 g $abalone^{-1}$). Fifty abalone per tank were weighed individually to the nearest 0.01 g using an electronic balance (Snowrex BBA-600, Snowrex International, Taipei, Taiwan) and measured to the nearest 0.1 mm from photographs using the software, SigmaScan® Pro 5 (Systat Software Inc., San Jose, California, U.S.A.). Photographs were taken from directly above the abalone using a digital camera on a tripod, and calibrated using length measurements taken with Vernier callipers. After 105 days, i.e., the duration of the farm management's quarterly grading schedule, the abalone were weighed and measured again to determine mean wet weight gain (g $abalone^{-1}$), shell length gain (mm $abalone^{-1}$) and condition factor (CF) of abalone in each tank. CF was determined using the equation: $CF = 5575 (\text{weight} \times \text{length}^{-2.99})$ (Britz 1996b), where weight is in g $abalone^{-1}$ and shell length in mm $abalone^{-1}$. Feed

conversion ratio (FCR) was calculated as FCR = dry weight feed given / biomass gain, where biomass gain is the difference in total wet weight of abalone within a tank between the start and end of the growth period.

Water quality analysis

The concentration of total ammonia nitrogen (TAN), nitrite (NO_2^- - N), dissolved oxygen, temperature, pH, and oxygen saturation were monitored twice a week. Measurements or samples were taken at the outflow of each tank during mid-morning. This sampling time was chosen as suggested by Yearsley (2008) for diurnal water quality patterns in *H. midae* tanks. Hand-held meters were used to measure temperature, pH (YSI Inc. Model # 60/10 FT; Yellow Springs, Ohio, U.S.A.), dissolved oxygen and oxygen saturation (YSI Inc. Model # 55D; Yellow Springs, Ohio, U.S.A.). The pH and oxygen meters were calibrated weekly using a three-point calibration with buffer solutions of pH 4, 7 and 10, and air-saturated seawater. The oxygen meter membrane was replaced at intervals prescribed by the manufacturer. TAN concentrations were measured using the method described by Solorzano (1969), while Merck Nitrite Test Kits (Cat. no. 1.14776.0001, Merck, Modderfontein, South Africa) were used to measure the concentration of nitrite-N. Colour absorbance for TAN and nitrite were read using a spectrophotometer (Prim Light, Secomam, 30319 Ales, France) and converted to concentration (μg L^{-1}) using the coefficients derived from least-square linear regression standard curves (TAN, ammonium chloride, n = 15, r^2 = 0.990, Appendix B; nitrite-N, sodium nitrite, n = 10, r^2 = 0.975, Appendix C).

The concentration of FAN was calculated using the values for TAN, temperature, pH and salinity of the water sample (Bower & Bidwell 1978). The flow rate of clean seawater entering the first tank in series of each serial-use raceway was measured in duplicate at each sampling time using a 2-L graduated container and a stop watch. Adjustments to the flow rate were made when necessary to maintain flow rates at 155 L h^{-1}.

Statistical analysis

As there were two diets and three serial-use positions per diet, data were analysed using multi-factorial analysis of variance including a test for interactions between the two main effects, diet and serial-use. Differences between means of the dependent variables where compared among tank positions using Tukey's HSD test. A student's t-test for independent data was used to compare means of the dependent variables for experiment 2 which was designed to test the effect of oxygen supplementation on growth. The assumptions of equality of variance and normality of residuals were checked with Levene's test (Levene 1960) and the Shapiro-Wilk test (Shapiro & Wilk 1965), respectively. An α-error level of 5 % was used for all tests. Values presented in the text are mean ± standard deviation (SD), unless otherwise stated. Repeated Measures Analysis of Variance was used to estimate the within-subject variance of repeated measurements of water quality to avoid the bias of pseudoreplication. Least-square regression analysis and correlation tests were used to model weight gain and shell length gain as a function of water pH, and the concentrations of dissolved oxygen and FAN. Step-wise forward multiple regression analysis ($F_{stop} = 1$) was used to estimate which of the water quality variables was the best predictor of weight and shell length gain. For this weight and length gain were regressed to pH, dissolved oxygen and FAN-concentration, and the product of these independent variables.

Results

Effect of diet and serial-use on abalone growth

At the start of the experiment mean shell length (66.04 ± 0.14 mm) and weight (49.4 ± 0.21 g abalone^{-1}) did not differ between dietary treatments ($p = 0.99$; $p = 0.39$) and the three serial-use positions ($p = 0.64$; $p = 0.83$). For weight gain (g abalone^{-1}), there was a significant interaction between diet and serial-use position ($F_{2, 12} = 10.4$; $p = 0.0024$), and mean values for all serial-use positions differed significantly from each other ($F_{2, 12} = 200.5$; $p < 0.0001$; Figure 4.2). Diet did not have a significant effect on weight gain ($F_{1, 12} = 1.09$; $p = 0.31$). Thus, the significant drop in weight gain in both dietary treatments with increasing water reuse was more pronounced in abalone fed P33 (Figure

4.2). Serial-use reduced weight gain by 60.2 % from 12.3 g abalone^{-1} in position one to 4.9 g abalone^{-1} in position three.

Shell length gain (mm abalone^{-1}) differed significantly among tank positions ($F_{2, 12}$ = 53.0; $p < 0.0001$), but not between diets ($F_{1, 12}$ = 1.08; $p = 0.31$) with no significant interaction between these two main effects ($F_{2, 12}$ = 3.05; $p = 0.09$). The mean shell length gain of both dietary treatments was 49 % lower in position three than in position one. While there were no significant differences in CF between dietary treatment and serial-use position ($p = 0.47$; $p = 0.31$), there was a significant interaction between these two effects at the end of the experiment ($F_{2, 12}$ = 5.5; $p = 0.02$) as CF had dropped between position two and three in abalone fed P33 (Figure 4.2).
Although there were no differences in the total amount of feed fed during the 105-day experimental period (2446 g ± 18 g tank^{-1}) between dietary treatments and serial-use ($p = 0.86$ and $p = 0.99$, respectively), mean FCR differed among all tank positions ($F_{2, 12}$ = 41.4; $p < 0.0001$), but not between diets ($F_{1, 12}$ = 1.77; $p = 0.21$). There was a significant interaction between the two main effects ($F_{2, 12}$ = 4.64; $p = 0.03$; Figure 4.2) showing a relatively higher increase, i.e., reduced feed conversion rate, from position two to three in abalone fed the high-protein diet. A 100 % survival rate was recorded in tank positions for both dietary treatments.

The difference in the dietary protein level of the feed resulted in significantly different TAN (within-subjects analysis of variance, $F_{42, 252}$ = 4.01; $p < 0.0001$) and FAN (within-subjects analysis of variance, $F_{42, 252}$ = 2.79; $p < 0.0001$) concentrations between treatments at each position within the raceways (Figure 4.3 & 4.4). FAN concentrations in tanks fed P26 were on average 51 % lower than in tanks fed P33 (Table 4.2). Variations in water quality were due to fluctuations in environmental conditions and management (Table 4.2).

Weight gain, Wg, (g abalone^{-1}) and shell length gain, Lg, (mm abalone $^{-1}$) could be predicted from water pH using least-square regression analysis (Wg: $F_{1, 19}$ = 64.5; $p < 0.0001$; $r^2 = 0.76$; Lg: $F_{1, 19}$ = 41.9; $p < 0.0001$; $r^2 = 0.67$) (Figure 4.5). The relationship between weight gain and shell length gain and dissolved oxygen concentration was not

significant (Wg: $F_{1, 19} = 0.86$; $p = 0.36$; Lg : $F_{1, 19} = 0.004$; $p = 0.95$). Weight gain was significantly correlated to FAN concentration (Wg: $F_{1, 19} = 7.4$; $p = 0.01$), while there was no significant relationship between this variable and shell length gain ($F_{1, 19} = 3.5$; $p = 0.07$) (Figure 4.5).

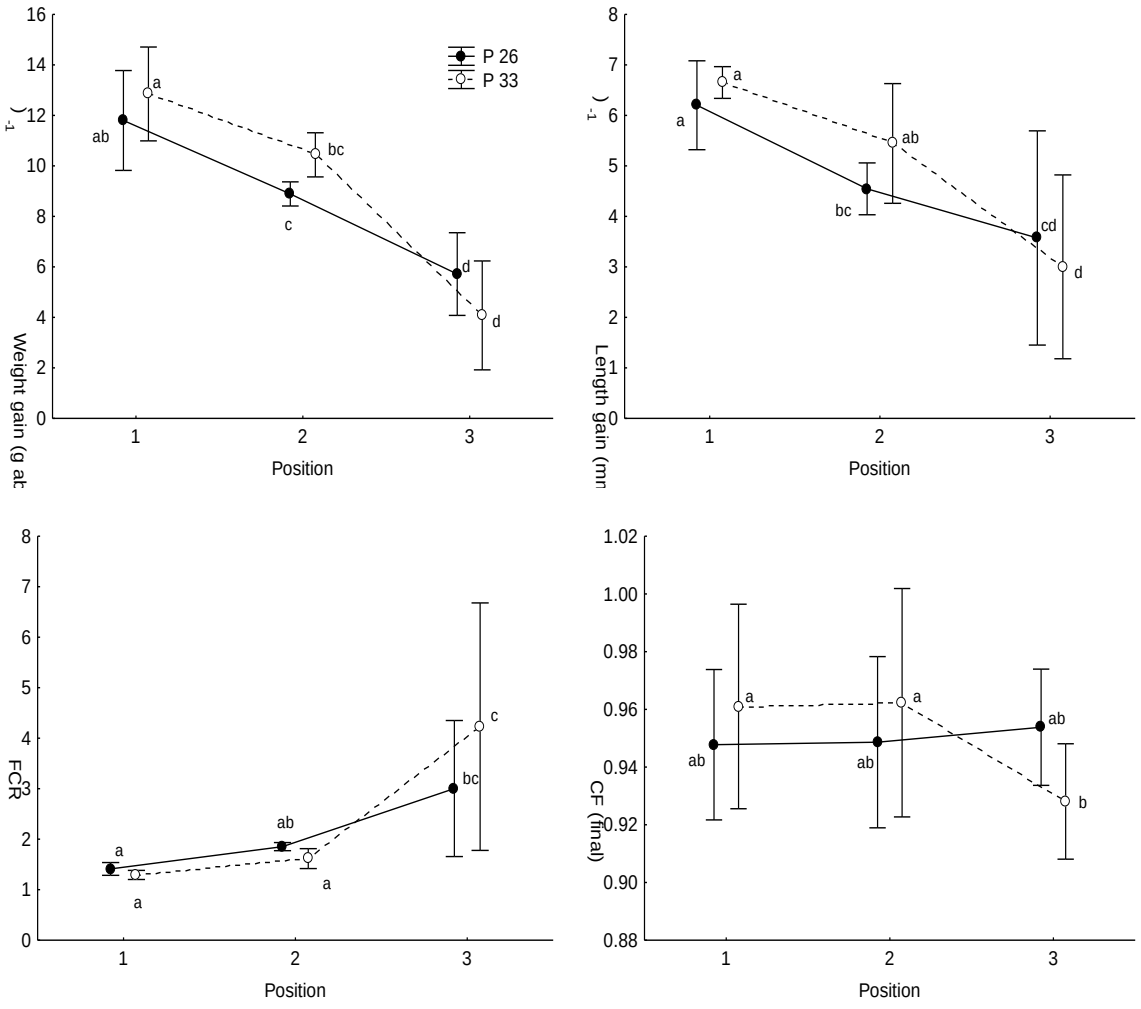


Figure 4.2: Weight gain (g abalone^{-1}), shell length gain (mm abalone^{-1}), final condition factor (CF) and food conversion ratio (FCR) of *H. midae* grown in a serial-use raceway with three tanks in series. Abalone were fed a diet containing either 26 or 33 % protein for 105 days. Values are means and 95 % confidence intervals. Different superscripts represent significant differences.

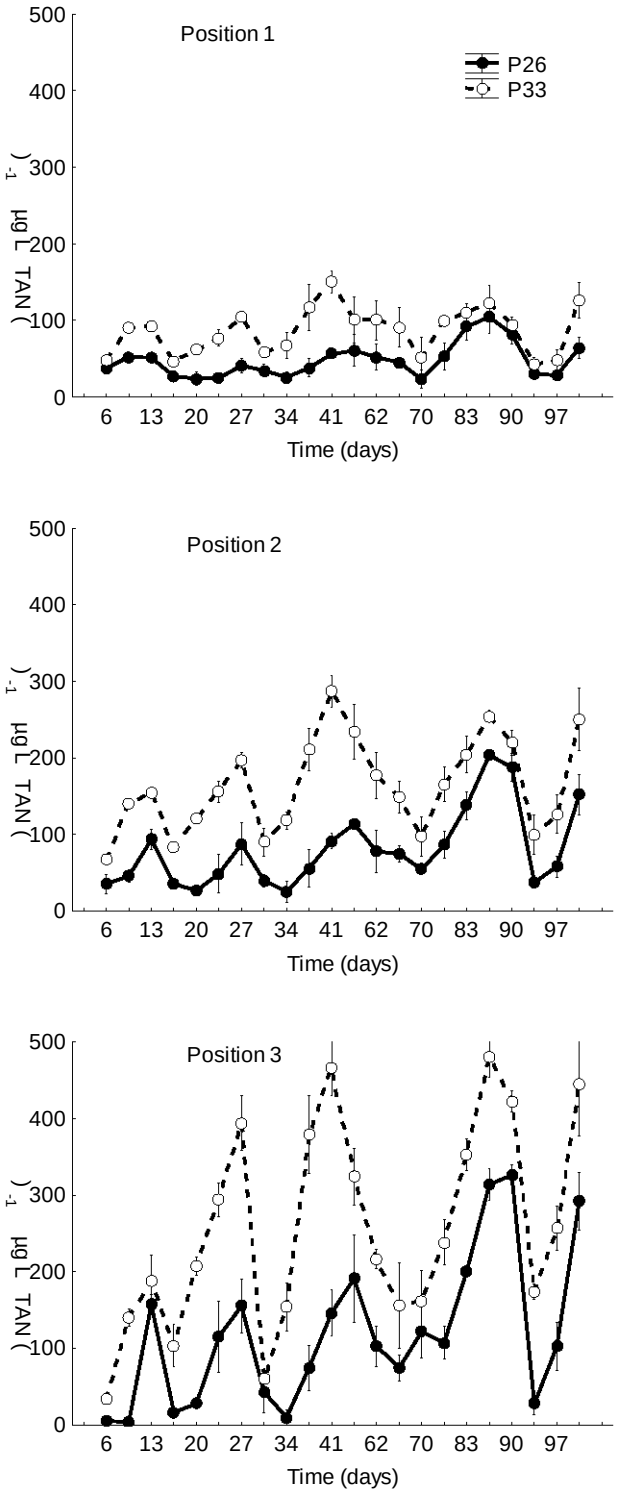


Figure 4.3: Mean (± SD) total ammonia nitrogen (TAN) concentration at three positions in an abalone serial-use raceway for two diets differing in percentage protein.

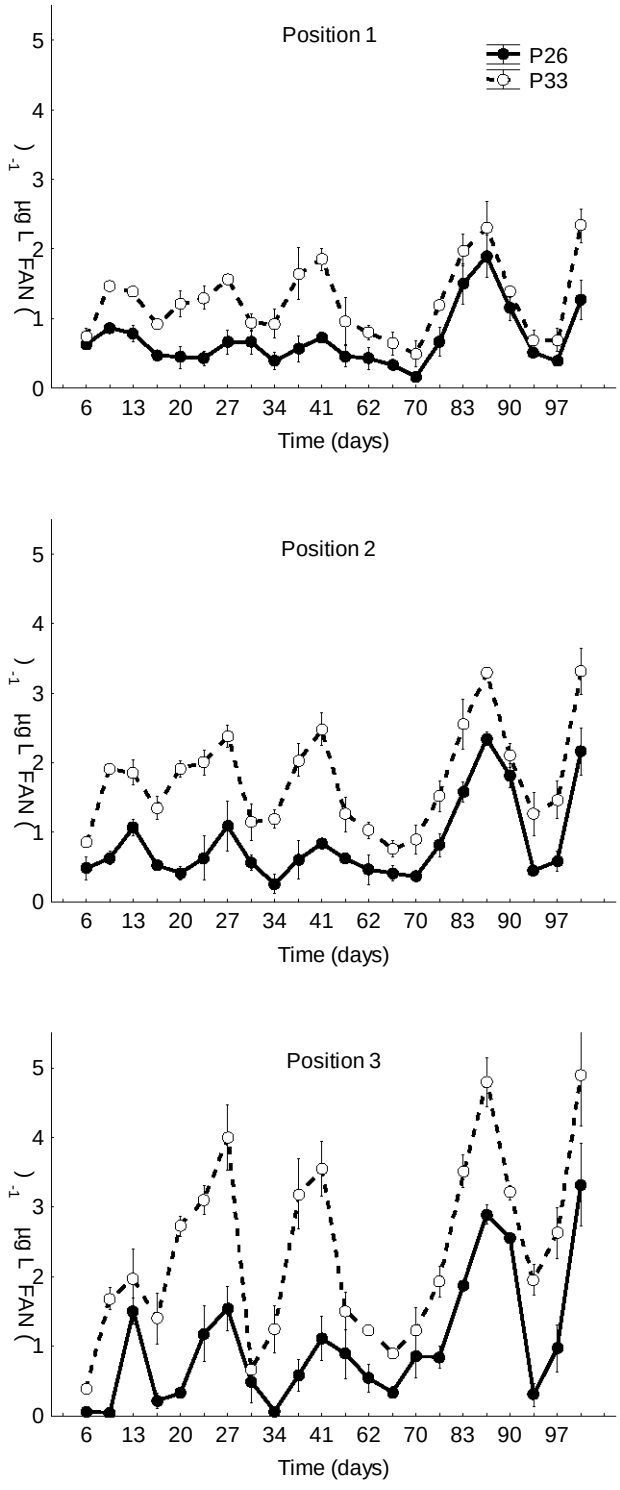


Figure 4.4: Mean (± SD) free ammonia nitrogen (FAN) concentration at three positions in an abalone serial-use raceway for two diets differing in percentage protein.

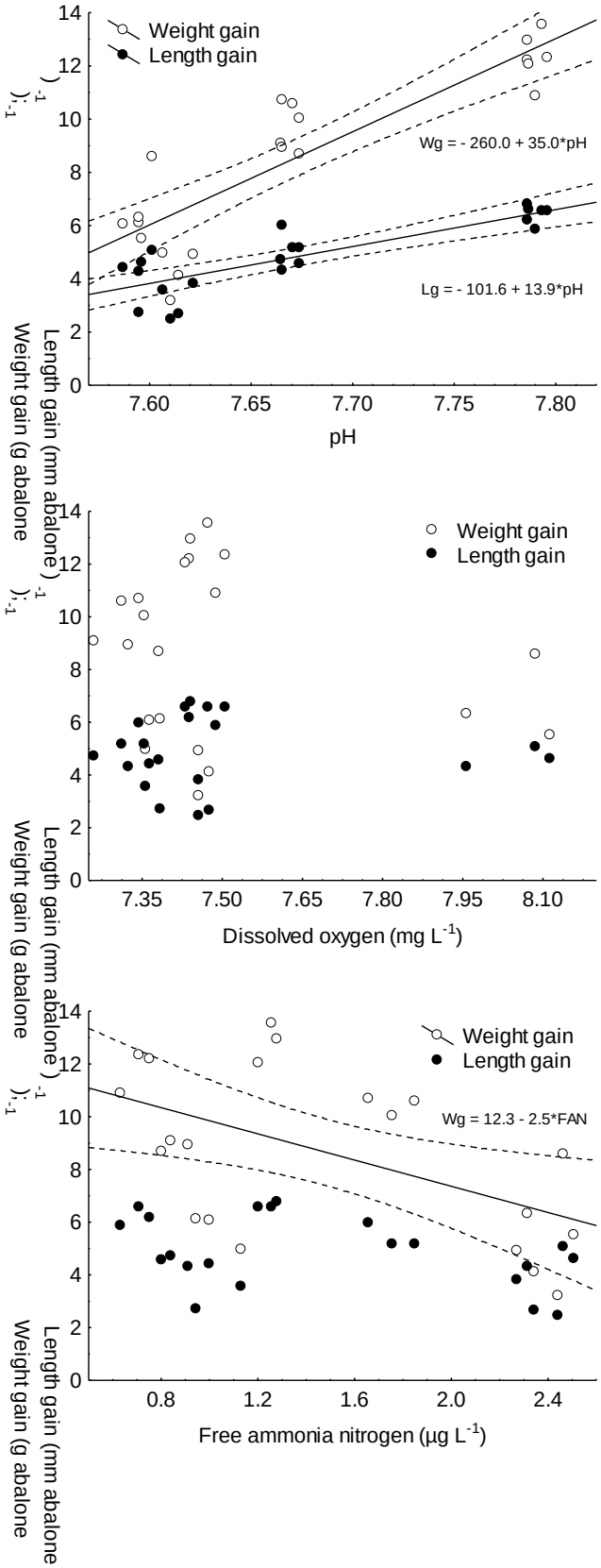


Figure 4.5: Correlation plots between weight gain (g abalone^{-1}) and shell length gain (mm abalone^{-1}) with least-square regression lines and 95 % confidence intervals for pH, dissolved oxygen (mg L^{-1}) and free ammonia nitrogen (FAN) (µg L^{-1}). There was a significant correlation between weight and shell length gain and pH. Dissolved oxygen did not have an effect on weight and shell length gain, while weight gain but not shell length gain was significantly correlated to the concentration of FAN (significant regression equations included).

Stepwise forward regression analysis with pH, FAN, pH x FAN, DO x FAN, pH x DO as independent variables and weight gain or shell length gain as the dependent variables suggested pH as the best predictor as the other variables did not contribute significantly to the multiple regression model.

Effect of oxygen supplementation at low flow indices

The comparison of production variables (Table 4.3) in tanks in the third position of the serial-use system in which one treatment received additional oxygen showed that there were no significant differences in mean wet weight gain ($t = 2.61$; $p = 0.059$) of abalone between the control and the tanks supplemented with oxygen. Shell length gain differed significantly ($t = 3.44$; $p = 0.026$) between treatments. Total amount of feed given was significantly lower ($t = 3.97$; $p = 0.017$) in the oxygen supplemented tanks (2262 ± 68 g) than in the control tanks (2436 ± 35 g). Abalone in tanks supplemented with oxygen had a significantly better FCR ($t = 3.04$; $p = 0.04$) than those from the control tanks (Table 4.3). There were no differences in condition factor between treatments. Due to oxygen supplementation, the P33-O_2 treatment had significantly higher (within-subjects analysis of variance: O_2 (mg L^{-1}), $F_{14, 56} = 15.8$, $p < 0.0001$; Oxygen saturation, $F_{14, 56} = 35.4$, $p < 0.0001$) dissolved oxygen concentrations and saturation percentages (8.3 ± 0.7 mg L^{-1}; 103 ± 8 %), when compared to control tanks (7.5 ± 0.5 mg L^{-1} O_2; 92 ± 6 %). Mean FAN concentrations in the P33 and P33-O_2 treatments were 2.4 ± 1.3 µg L^{-1} and 2.4 ± 1.1 µg L^{-1}, respectively. Mean pH for both treatments ranged between 7.60 and 7.62. Mean flow indices in the P33-O_2 and P33 treatments were 3.34 ± 0.13 L h^{-1} kg^{-1} and 3.35 ± 0.04 L h^{-1} kg^{-1} respectively.

Table 4.2: Mean values (± SD) of temperature, total ammonia nitrogen (TAN), free ammonia nitrogen (FAN), water pH, nitrite - N (NO_2^- - N), dissolved oxygen and oxygen saturation for abalone fed either a 26 (P26) or 33. 2 % (P33) protein diet, in each of three tanks in series.

	Dietary treatment	**Position one**	**Position two**	**Position three**
Temperature (°C)	P26	15.5 ± 0.6	15.3 ± 0.7	15.1 ± 0.9
	P33	15.5 ± 0.6	15.3 ± 0.7	15.0 ± 0.9
TAN (µg L^{-1})	P26	47.6 ± 24.1	80.3 ± 51.7	118.9 ± 99.1
	P33	86.3 ± 32.7	163.7 ± 63.2	256.8 ± 134.5
FAN (µg L^{-1})	P26	0.7 ± 0.4	0.9 ± 0.6	1.0 ± 0.9
	P33	1.2 ± 0.5	1.8 ± 0.7	2.4 ± 1.3
pH	P26	7.79 ± 0.14	7.67 ± 0.15	7.60 ± 0.14
	P33	7.79 ± 0.13	7.67 ± 0.15	7.62 ± 0.14
Nitrite – N (µg L^{-1})	P26	7.9 ± 1.3	10.3 ± 2.1	17.4 ± 9.0
	P33	9.1 ± 1.8	13.5 ± 4.8	31.5 ± 20.4
Dissolved oxygen (mg L^{-1})	P26	7.5 ± 0.6	7.3 ± 0.6	7.4 ± 0.6
	P33	7.4 ± 0.6	7.3 ± 0.6	7.5 ± 0.5
Oxygen saturation (%)	P26	92.8 ± 8.0	90.5 ± 7.4	90.7 ± 6.9
	P33	92.3 ± 7.5	90.6 ± 7.5	91.8 ± 6.0

Table 4.3: Mean (± SD) for production variables of abalone in the last tanks of a serial-use system using three passes without (control) and with supplementation of oxygen (treatment). Abalone were reared for 105 days on a 33 % protein diet. T-statistics and p-values compare means between control and treatment. The column on the right shows values from the first tank in series of abalone fed the same diet. W_0 and W_e are mean initial and final weight (g abalone^{-1}), L_0 and L_e are mean initial and final shell length (mm abalone^{-1}), Wg and Lg are mean weight gain and shell length gain, CF_0 and CF_e are mean initial and final condition factor, Feed is total amount of feed fed (g), and FCR is feed conversion ratio (Wg feed fed^{-1}).

	Control	**Treatment**	**t-statistics / p-value**	**Position one**
W_0	49.5 ± 1.33	48.7 ± 0.55	t = 0.93; p = 0.40	50.2 ± 0.49
W_e	53.6 ± 0.76	55.6 ± 2.06	t = 1.54; p = 0.19	63.1 ± 1.27
Wg	4.1 ± 0.87	6.8 ± 1.59	t = 2.61; p = 0.06	12.9 ± 0.80
L0	66.5 ± 1.19	65.3 ± 1.20	t = 1.23; p = 0.29	65.9 ± 0.20
L_e	69.5 ± 0.52	70.0 ± 1.29	t = 0.56; p = 0.60	72.5 ± 0.25
Lg	3.00 ± 0.73	4.66 ± 0.39	t = 3.44; p = 0.026	6.65 ± 0.35
CF_0	0.97 ± 0.03	1.02 ± 0.05	t = 1.29; p = 0.27	1.02 ± 0.02
CF_e	0.93 ± 0.01	0.94 ± 0.02	t = 1.08; p = 0.34	0.96 ± 0.01
Feed	2436 ± 34.83	2261 ± 67.79	t = 3.97; p = 0.017	2427 ± 122.98
FCR	4.23 ± 0.99	2.32 ± 0.45	t = 3.05; p = 0.04	1.29 ± 0.05

Discussion

FAN concentration in a serial-use raceway

The percentage dietary protein had a significant effect on the concentration of FAN at each tank position. Thus, within the range of protein levels tested an increase in dietary protein level of 21 % resulted in 50 % higher FAN concentrations. The large peaks and troughs in TAN and FAN concentration (Figure 4.3 & 4.4) were the result of an accumulation of uneaten feed and faeces and changes in temperature.

In *H. midae*, growth has been shown to be independent of dietary protein, provided that dietary energy is maintained above 13.5 MJ kg^{-1} and that it is provided mostly in the form of carbohydrates (Green *et al*. 2011b). The diets used here conformed with these requirements, in that energy levels were 15.6 MJ kg^{-1} and lipid content was low (3 %). There was also no difference in growth between the two diets for position one where the flow indices were above those shown to cause reductions in growth as a function of water quality (Naylor *et al*. 2011). Thus, it is hypothesised that differences in growth were the result of water quality or environmental conditions.

Despite being exposed to lower FAN concentrations, abalone in the P26 treatment did not gain weight or length faster at any of the positions within the serial-use raceways. However, the reduction in growth as a result of increasing water use for both dietary treatments was more pronounced in abalone fed the high-protein diet from the second to the third tank in the series. It is hypothesised that a low-protein diet may become more beneficial as the intensity of water use increases. Abalone are sensitive to FAN, and have demonstrated a large reduction in growth rate (Harris *et al*. 1998; Basuyaux & Mathieu 1999; Huchette *et al*. 2003; Reddy-Lopata *et al*. 2006) well below the 50 – 200 µg L^{-1} safe limit described for many marine finfish species (Russo & Thurston 1991; Person-Le Ruyet *et al*. 1997; Lemarie *et al*. 2004). Although no information is available regarding the EC_5 for FAN in *H. midae*, Reddy-Lopata *et al*. (2006) determined that the EC_{50} for FAN in juvenile *H. midae* was < 7 µg L^{-1} and speculated that the EC_5 would be lower than the 4 µg L^{-1} as was reported by Huchette *et al*. (2003) for *H. rubra*. Since there was no difference in growth between the P26 and P33 treatments at position three, where FAN concentrations were 1.0 ± 0.9 and 2.4 ± 1.3 µg L^{-1}, respectively, suggests

that FAN concentration in the P33 treatment was below the level that can significantly reduce growth. This may be due to the larger size class used (69.5 ± 2.5 mm versus 10 – 25 mm used by Reddy-Lopata *et al*. 2006), as larger abalone are less sensitive to high concentrations of FAN (Fallu 1991; Reddy-Lopata *et al*. 2006). Other environmental conditions, especially pH (Thurston *et al*. 1981) may also influence the estimation of EC_5. Since the mean FAN concentration could not explain differences in growth between tank positions, other water quality variables probably influenced growth.

Using predictions by Britz *et al*. (1997), the decrease in temperature between position one and position three accounts for only 11 – 12 % of the difference in weight gain observed and therefore may have influenced growth, but this was not likely the most limiting variable. Similarly, mean dissolved oxygen concentration (7.3 – 7.5 mg L^{-1} O_2) and percentage oxygen saturation (90.5 to 92.8 %) were similar among positions and treatments and are unlikely to have caused the differences in abalone growth. This was supported by the low correlation between oxygen concentration and growth, and since oxygen concentration, within the range tested here, did not contribute to a predictive model. The EC_{50} for dissolved oxygen in *H. laevigata* has been estimated at 5.91 mg L^{-1} and 77 % saturation (Harris *et al*. 1999b).

Although nitrite–N concentrations increased with increasing serial-use, reaching a maximum of 34.1 µg L^{-1}, these values were below those shown to affect the growth in other abalone species. Harris *et al*. (1997) recorded growth reductions in *H. laevigata* at nitrite–N concentrations above 540 µg L^{-1}, while Basuyaux & Mathieu (1999) estimated safe concentrations at < 5000 µg L^{-1} in *H. tuberculata* Linnaeus.

pH

There was, however, a significant correlation between the reduction in growth and the decrease in water pH. In addition, water pH contributed the most to a multiple regression model to predict growth as a function of water quality. The hypothesis that low pH caused a reduction in growth, is supported by the negative effects of chronic hypercapnia on growth rate and shell dissolution shown in echinoderms (Shirayama 2002), gastropods (Crim *et al*. 2011) and bivalves (Michaelidis *et al*. 2005). In this study, the decrease in pH was most likely caused by the respiration of the abalone and

decomposition of organic matter and subsequent release of CO_2. As CO_2 hydrates in water, it dissociates into aqueous carbon dioxide (CO_2^{aq}), carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), carbonate (CO_3^{-2}) and hydrogen ions (H^+), in a pH-equilibrated reaction (Smith 1988). The lower the pH, the greater the proportion of CO_2^{aq}, which could lead to reduced CO_2 diffusion at the gills and subsequent blood acidosis and altered oxygen-haemocyanin affinity (Sanni & Forsberg 1996; Harris *et al*. 1999a). In *H. corrugata* Wood, Burnett *et al*. (1988) showed a decrease in haemocyanin oxygen affinity with a decrease in pH. In the marine mussel, *Mytilus galloprovincialis*, exposure to pH 7.3 caused a significant reduction in haemolymph pH and metabolic rate and increased haemolymph CO_2-concentrations, when compared to controls held at pH 8.05 (Michaelidis *et al*. 2005). A decrease in pH also reduces the availability of essential CO_3^{-2} ions (Kleypas *et al*. 2006; Lopez *et al*. 2011) and it lowers the saturation state of calcite and aragonite, which are calcium carbonate minerals essential for the formation of skeletal structures and shell in many marine organisms (Feely *et al*. 2004). In the greenlip abalone, *H. laevigata* the EC_5 and EC_{50} values for pH have been estimated as 7.78 and 7.39, while in the blacklip abalone, *H. rubra*, EC_5 and EC_{50} values were 7.93 and 7.37 (Harris *et al*. 1999a). The pH values recorded in this study were therefore within the range known to affect other abalone species. They were also well below the mean pH recorded by Yearsley (2008) for morning (pH 8.06) and afternoon (pH 8.13) samples of influent water quality for this specific farm during 2007, representing the pH levels of this species' natural environment.

Effect of oxygen supplementation at low flow indices

Supplementation of pure oxygen to saturation levels of 103 ± 8.3 % resulted in a significantly higher shell length gain in abalone exposed to high FAN concentrations and low pH. It is hypothesised that a significant statistical difference in wet weight gain could have emerged as these conditions continue over a longer growth period. The growth was, however, not as good as that observed in position one, where oxygen saturation percentages averaged only 92 ± 8. It is suggested that the oxygen available in the supplemented tanks alleviated some of the negative effects of either low pH or high FAN concentrations, or a combination of both, but was not sufficient to overcome the negative effects of reduced pH and increased FAN concentrations. As abalone reared

under conditions of low dissolved oxygen levels and low pH were less efficient at converting feed into biomass, it is suggested that the water quality conditions reduced growth by influencing FCR.

However, a low water pH as a result of a high CO_2 concentration in the water can lead to reduced CO_2 excretion at the gills and subsequent blood acidosis. In *H. diversicolor supertexta* Reeve, Cheng *et al*. (2004b) reported that the partial pressure of haemolymph CO_2 was inversely related to environmental dissolved oxygen concentrations. As a result of this metabolic imbalance, the increased CO_2 concentration of the haemolymph may affect the oxygen carrying capacity of haemocyanin (Burnett *et al*. 1988; Harris *et al*. 1999a). Under natural sea conditions, the energetic cost of respiration is estimated at 32 % of absorbed energy (Barkai & Griffiths 1988). The increased dissolved oxygen concentration in the supplemented tanks may have reduced the energetic cost of respiration when compared to non-supplemented tanks, thereby allowing a greater proportion of energy to be used for somatic growth, and is supported by the lower FCR values recorded in this treatment.

Conclusion

Dietary protein level had a significant effect on mean FAN concentrations within the serial-use raceways. The resultant high FAN and low FAN conditions did not cause significant differences in growth and indicates that the mean FAN concentrations ($\leq$ 2.4 $\mu g\ L^{-1}$) were within a range that did not affect growth. However, significant differences in growth were observed between positions, and thus under the conditions prevalent in this serial-use system, the concentration of FAN was not the most limiting water quality variable. Weight gain and shell length gain were significantly correlated to water pH. Supplemental oxygen improved the length gain of abalone held at low pH and high FAN concentrations. Future studies should examine the effect of pH on abalone growth through its effect on the carbonate system in seawater.

CHAPTER 5

The effect of dosing with sodium hydroxide ($NaOH^-$) on water pH and growth of *Haliotis midae* Linnaeus, in an abalone serial-use raceway

Introduction

The majority of abalone farms in South Africa use pump-ashore flow-through systems for the commercial production of the South African abalone, *Haliotis midae* Linnaeus. To promote maximum growth and health, water quality is maintained through water exchange rates of 800 - 1000 % of system volume per day (personal observation). The infrastructure and electricity cost of pumping such large volumes of seawater places a major burden on the economic success of abalone farming (Wassnig *et al*. 2010; Vivanco-Aranda *et al*. 2011). In many parts of the world, and especially in South Africa, electricity cost is increasing faster than annual inflation and the increase in the price of the farmed product. This has prompted research into testing alternate farming methods to increase production efficiency (Evans & Langdon 2000; Robertson-Andersson *et al*. 2008; Wassnig *et al*. 2010; Naylor *et al*. 2011; Vivanco-Aranda *et al*. 2011).

Before the farming system can be improved or redesigned, a thorough understanding of the water quality requirements of *H. midae* is needed. At a flow index of 7.2 – 9.0 L $h^{-1}kg^{-1}$, growth was significantly reduced by water quality changes, and it has been suggested that a drop in pH may be the first growth-limiting variable (Naylor *et al*. 2011). Marine calcifying organisms are sensitive to changes in dissolved carbon dioxide (Gattuso *et al*. 1998; Siikavuopio *et al*. 2007; Ellis *et al*. 2009; Talmage & Gobler 2009; Crim *et al*. 2011; Yu *et al*. 2011) and the resultant changes in pH and carbonate ion concentrations (Fivelstad *et al*. 1998; Feely *et al*. 2004; Orr *et al*. 2005).

Carbon dioxide (CO_2) is a highly soluble gas that hydrolyzes and dissociates in seawater in a pH-equilibrated reaction (Figure 5.1) (Manahan 1984; Smith 1988; Summerfelt

2000) forming aqueous carbon dioxide (CO_2^{aq}), carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), carbonate (CO_3^{-2}) and hydrogen ions (H^+):

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{-2} + 2H^+$$

Thus, in high-intensity culture systems changes in pH are likely to occur from the accumulation of CO_2 released by the cultured animal (Sanni & Forsberg 1996; Fivelstad *et al*. 1998, Colt *et al*. 2009) and CO_2 produced during nitrification (Harris *et al*. 1999a; Colt *et al*. 2009).

Alkalinity is defined as the quantity of bases, usually bicarbonates, carbonates and hydroxides (OH^-), and it represents the capacity of water to neutralize acids (Wurts & Durborow 1992; Wedemeyer 2000a). Seawater generally has a high alkalinity and is therefore regarded as having a high buffering capacity, thus pH is often not considered a factor of concern (Wedemeyer 2000b; Ringwood & Keppler 2002). However, Naylor *et al*. (2011) showed that pH can decline rapidly in a serial-use abalone raceway system. As pH is measured on a logarithmic scale, small changes may have significant physiological effects (Ringwood & Keppler 2002).

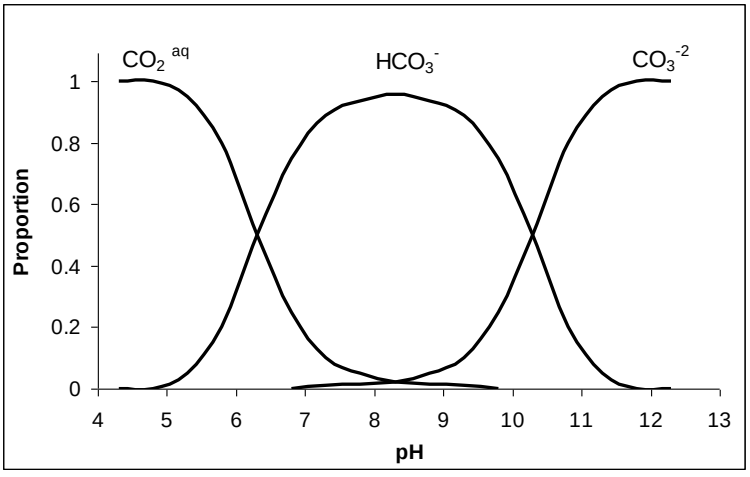


Figure 5.1: The typical proportion of carbonate species, CO_2, HCO_3^- and CO_3^{-2} in seawater as a function of pH (redrawn from Manahan 1984). The proportion of carbonate species is also temperature dependent.

In general, the toxic effects of increased CO_2^{aq} and reduced pH relate to a diminished CO_2 gradient at the gills, which leads to blood acidification (Sanni & Forsberg 1996; Michaelidis *et al*. 2005). Low blood pH reduces the ability of blood pigments to bind and carry oxygen (Sanni & Forsberg 1996), ultimately leading to a reduction in metabolic rate. Burnett *et al*. (1988) showed that at reduced pH the haemocyanin in *H. corrugata* Wood, had decreased affinity to oxygen and a reduced co-operativity of the hem groups. In larval northern abalone, *H. kamtschatkana* Jonas, exposure to elevated CO_2 resulted in significant reductions in survival and growth and an increase in the number of abalone with abnormal shell development (Crim *et al*. 2011). In juvenile greenlip, *H. laevigata* Donovan, and blacklip, *H. rubra* Leach, abalone, EC_5 values (5 % reduction in specific growth rate) for pH were estimated to be 7.78 and 7.93, respectively, when compared to specific growth rates at pH 8.25 (Harris *et al*. 1999a). A reduction in oxygen consumption was also shown in *H. laevigata* after exposure to low pH (Harris *et al*. 1999a). In intensive aquaculture systems, aeration to allow degassing of CO_2^{aq} and addition of alkaline chemicals are suitable options for the control of pH (Summerfelt 2000). In a recirculating system Vivanco-Aranda *et al*. (2011) improved growth of juvenile red abalone, *H. rufescens* Swainson, by adding sodium bicarbonate to increase both pH and alkalinity. Degassers are inexpensive to build and to operate, however, efficiency may vary. The addition of alkaline chemicals requires a constant running cost, even though they can be sourced relatively cheaply. By raising the pH of the inflowing water in an experimental serial-use raceway with sodium hydroxide ($NaOH^-$), this study was designed to determine the effect of pH on *H. midae* growth and estimate its importance as a limiting variable in an abalone serial-use system.

Methods and Materials

The study was conducted at HIK Abalone Farm (Pty) Ltd. in Hermanus, South Africa (34°26'04.35"S; 19°13'12.51"E) from July to October 2010. The abalone were approximately 36 months old and originated from a single spawning in the HIK hatchery. The abalone had been weaned onto a formulated diet, Abfeed® (Marifeed Pty Ltd., South Africa) at < 6 months of age. They had been reared in flow-through tanks at

temperatures between 12 – 20 ºC in accordance with farm stocking density and feeding procedures.

Experimental system

Six serial-use raceways each with three 300-L tanks (0.9 × 0.6 × 0.6 m) in series were used. The tanks were set-up at different heights to allow gravitational water flow (161 ± 4 L h^{-1}) between tank position one, two, and three. These flow rates were maintained and checked at each water quality sampling time, using a 2-L graduated container and a stopwatch. All tanks were cleaned on the same day at intervals of 12 – 14 days.

Each tank contained one abalone basket stocked with 8.0 ± 0.02 kg *H. midae*. Each basket therefore contained 138 – 139 abalone. There was no significant difference between treatments in the mean initial weight (58.0 ± 0.33 g; $F_{2, 12} = 1.8$; $p = 0.20$) or shell length (69.7 ± 0.55 mm; $F_{2, 12} = 1.0$; $p = 0.38$). Following commercially used methods each basket contained a plastic rack providing surface area and a feeder plate covering the basket (Yearsley *et al*. 2009). Each tank was individually aerated by airlines placed 100 mm off the tank bottom, and supplied with air from the farm's blower system. Three of the serial-use raceways were connected to a common header tank (300 L), into which a solution of $NaOH^-$ (Associated Chemical Enterprises, CAS No. 1310-73-2, Johannesburg, South Africa) was dosed. The $NaOH^-$ solution was replenished when necessary at a concentration of 2 g L^{-1} $NaOH^-$. A small aquarium pump and an airline were used to mix the water in the dosing tank.

To minimise the effect of ambient air temperature on water temperature in the common header tank, the retention time of water in the header tank was kept low, with a large volume of water being returned to the ocean. Thus, large quantities of $NaOH^-$ were required to achieve the desired pH levels. Influent seawater was filtered through a100-µm drum filter before use.

The abalone were fed Abfeed S34® (proximate composition in g kg^{-1}: 340 g protein, 530 g carbohydrates, 1.2 g lipids) to apparent satiation daily, using a feeder cup and in accordance with a method developed by the farm to be both practical and to minimise

feed waste. The number of cups of feed given was recorded for each tank and multiplied by the average weight of feed in a cup (64 ± 2.8 g; n = 30) to determine the dry mass of feed given.

Growth analysis

All abalone were of the same age and grow-out history. Mean wet weight (g abalone^{-1}) for each tank was determined from a sample of 50 abalone weighed individually to the nearest 0.01 g using an electronic balance (Snowrex BBA-600, Snowrex International, Taipei, Taiwan). At the same time, digital photographs were taken from directly above the abalone. The software program, SigmaScan® Pro 5 (Systat Software Inc., San Jose, California), was used to determine the length of each of the weighed abalone. Each photograph was calibrated by the inclusion of Vernier callipers (set at 70 mm) in the image. The experiment lasted 95 days, after which the weighing and measuring process was repeated to determine mean wet weight gain (g abalone^{-1}), shell length gain (mm abalone $^{-1}$) and condition factor (CF) of abalone in each tank. CF was determined using the equation: CF = 5575 (weight x length$^{-2.99}$) (Britz 1996b). Initial CF was 1.00 ± 0.04. Feed conversion ratio (FCR) was calculated as FCR = dry weight feed given / biomass gain, where biomass gain is the difference in total wet weight of abalone in the tank between the start and end of the experiment.

Water quality analysis

Temperature, pH and dissolved oxygen concentration and percentage saturation were measured at the outflow of each tank every 5 days, using hand-held meters (YSI Inc. Model # 60/10 FT and YSI Inc. Model # 55D, respectively, Yellow Springs, Ohio, USA). The pH meter was calibrated using a three-point calibration with buffer solutions of pH 4, 7 and 10 while the oxygen meter was calibrated using air-saturated seawater.

The accumulation of sludge in the bottom of abalone tanks has been shown to affect the total ammonia nitrogen (TAN) concentration, with TAN being positively correlated to the length of time since the tanks were last cleaned (Yearsley 2008). TAN concentrations were measured at the mid-point of each tank cleaning cycle, as consistently measuring TAN before or after this time would result in under or over

estimation of mean TAN concentrations. The phenol-hypochlorite method (Solorzano 1969) was used to measure TAN concentration. Water samples were taken in acid-washed glass bottles and processed within 30 minutes of sampling. Colour absorbance was read through a spectrophotometer (Prim Light, Secomam, 30319 Ales, France) at 640 Å, and the concentration of TAN calculated from the coefficient of the ammonia chloride standard curve (Appendix B). The concentration of free ammonia nitrogen (FAN) was calculated using the measured concentrations of TAN, pH, temperature and salinity, according to the method by Bower & Bidwell (1978).

Statistical analysis

Values are presented as mean ± standard deviation (SD), unless otherwise stated. Abalone growth data were analysed using multi-factorial analysis of variance (ANOVA) including a test for interaction between the two main effects, i.e., dosing with $NaOH^-$ and position in the serial-use raceway. Tukey's HSD test was used to compare differences among means of dependent variables. With each tank being an experimental unit, the mean weight gain, shell length gain and CF-values of abalone in a tank were used for the analysis. The assumptions of equality of variance and normality of residuals were checked with Levene's test (Levene 1960) and the Shapiro-Wilk test (Shapiro & Wilk 1965), respectively. Water quality changes over time were analysed using repeated measures analysis of variance.

Weight gain and shell length gain were modelled as a function of water pH, dissolved oxygen and FAN, using least-square regression analysis. The mean water pH, dissolved oxygen and FAN concentrations for each tank were used in a step-wise forward multiple regression analysis ($F_{stop} = 1$) to determine the contribution of each of the three variables to a model predicting weight and length gain as a function of water quality.

Results

There was no significant interaction between serial-use and $NaOH^-$ dosing for weight gain ($F_{2, 12} = 0.62$; $p = 0.55$), shell length gain ($F_{2, 12} = 0.42$; $p = 0.67$), FCR ($F_{2, 12} = 0.87$; $p = 0.44$) and final CF ($F_{2, 12} = 0.60$; $p = 0.54$).

Serial-use position had a significant effect on weight gain (g $abalone^{-1}$) ($F_{2, 12} = 14.1$; $p = 0.0007$), shell length gain (mm $abalone^{-1}$) ($F_{2, 12} = 29.3$; $p = 0.0002$) and FCR ($F_{2, 12} = 16.07$; $p = 0.0004$) (Figure 5.2). In the $NaOH^-$ dosed treatment, weight gain (Wg) and shell length gain (Lg) were reduced by 35 and 25 % respectively, between position one (Wg: 14.4 g $abalone^{-1}$; Lg: 5.3 mm $abalone^{-1}$) and position three (Wg: 9.3 g $abalone^{-1}$; Lg: 3.8 mm $abalone^{-1}$). Similarly, in the control treatment, Wg and Lg were reduced by 28 and 24 % respectively, between position one (Wg: 12.0 g $abalone^{-1}$; Lg: 4.9 mm $abalone^{-1}$) and position three (Wg: 8.6 g $abalone^{-1}$; Lg: 3.7 mm $abalone^{-1}$). A 100 % survival rate was achieved in all tanks.

Dosing with $NaOH^-$ resulted in significant differences in pH between treatments at each position within the serial-use raceways (repeated measures analysis of variance, $F_{36, 216} = 12.00$; $p < 0.0001$) (Figures 5.3 & 5.4). At an error level 5.5 % ($p = 0.055$) both weight gain and shell length gain were higher in the tanks dosed with $NaOH^-$ ($F_{1, 12} = 4.51$; $p = 0.055$; $F_{1, 12} = 4.56$; $p = 0.054$), while mean FCR and CF respectively, did not differ between these two treatments ($F_{1, 12} = 2.93$; $p = 0.11$; $F_{1, 12} = 1.98$; $p = 0.18$) (Figure 5.2). The largest differences in weight and length gain occurred in position one, decreasing to position three. The differences in mean pH between dosed and control treatments were not constant between positions. Differences were 0.30, 0.27 and 0.21 pH units at positions one, two and three, respectively.

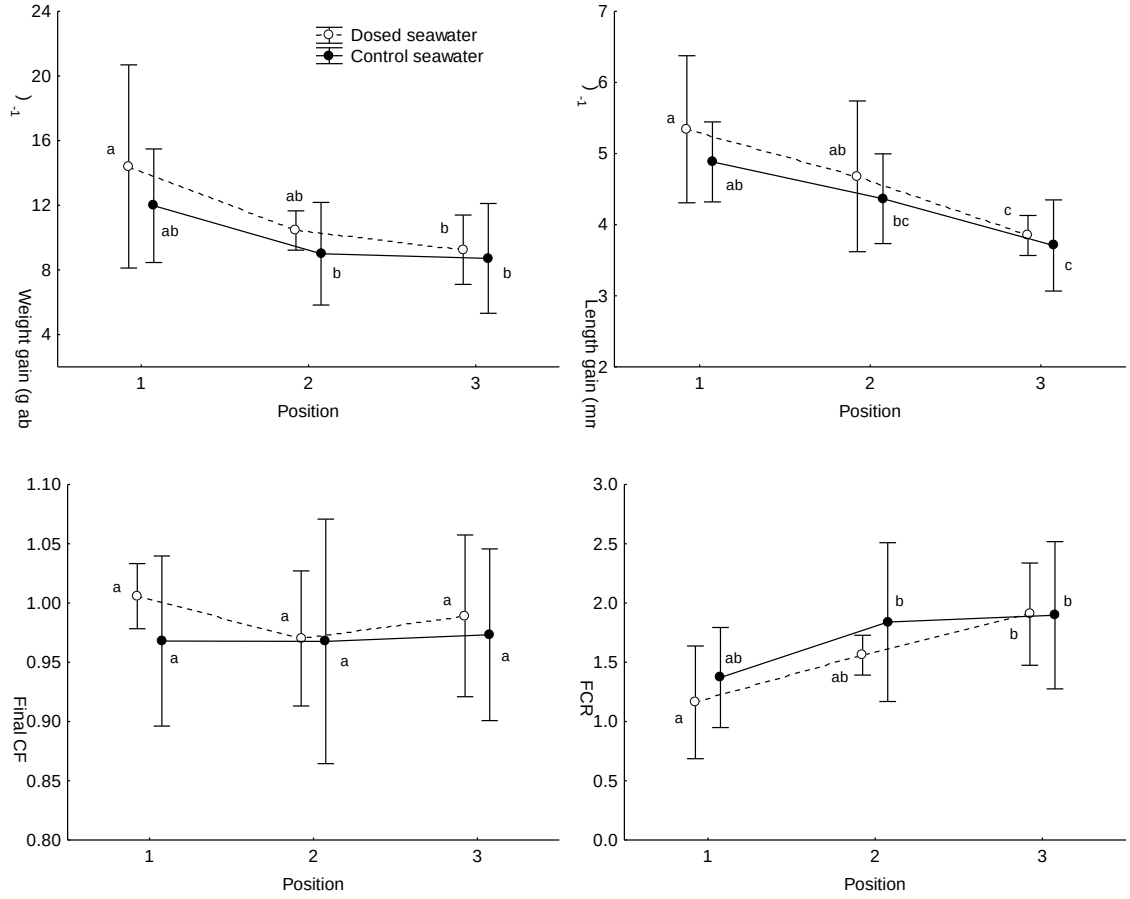


Figure 5.2: Weight gain (g abalone^{-1}), shell length gain (mm abalone^{-1}), final condition factor (CF) and feed conversion ratio (FCR) of *H. midae* grown in serial-use raceways with three tanks in series. A sodium hydroxide ($NaOH^-$) solution (2 g L^{-1}) was dosed into the treatment raceways to raise water pH above that of control raceways. Values presented are mean ± 95 % confidence intervals. Different superscripts represent significant differences.

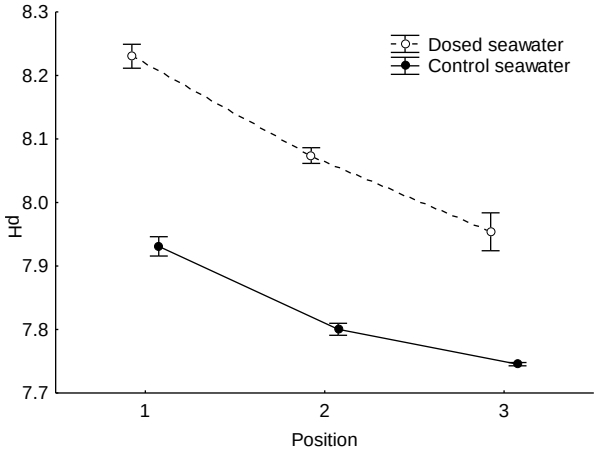


Figure 5.3: Mean (± 95 % confidence intervals) water pH at three positions in replicate abalone serial-use raceways receiving ambient seawater (control) or seawater dosed with a $NaOH^-$ solution (dosed) over the 95 day experimental period.

There was a significant difference in the amount of feed given between treatments ($F_{2, 12}$ = 25.7; $p < 0.0001$). Abalone in tank position three of the $NaOH^-$ dosed treatment required significantly more feed (2426 ± 11 g) than other tanks, while the total amount of feed given to all other tanks was similar, and ranged from 2240 – 2285 g.

Significant differences in FAN were recorded between treatments at each position (repeated measures ANOVA, $F_{16, 96}$ = 1.82; $p = 0.04$), with abalone in the treatment tanks being exposed to 155, 106 and 53 % higher FAN concentrations at position one, two and three respectively (Figure 5.5). Mean values of all water quality data are presented in Table 5.1.

Weight gain and shell length gain were positively correlated to water pH (Wg: $F_{1, 16}$ = 16.35, $p = 0.0009$, $r^2 = 0.51$; Lg: $F_{1, 16}$ = 16.16, $p = 0.0009$, $r^2 = 0.50$), but showed no relationship to FAN (Wg: $F_{1, 16} = 0.01$, $p = 0.97$; Lg: $F_{1, 16} = 0.23$, $p = 0.64$) or dissolved oxygen concentrations (Wg: $F_{1, 16} = 0.04$, $p = 0.84$; Lg: $F_{1, 16} = 2.04$; $p = 0.17$) (Figure 5.6). pH was the best predictor of weight gain and shell length gain (Table 5.2). Dissolved oxygen concentration did not contribute significantly to a model predicting shell length gain as a function of water quality.

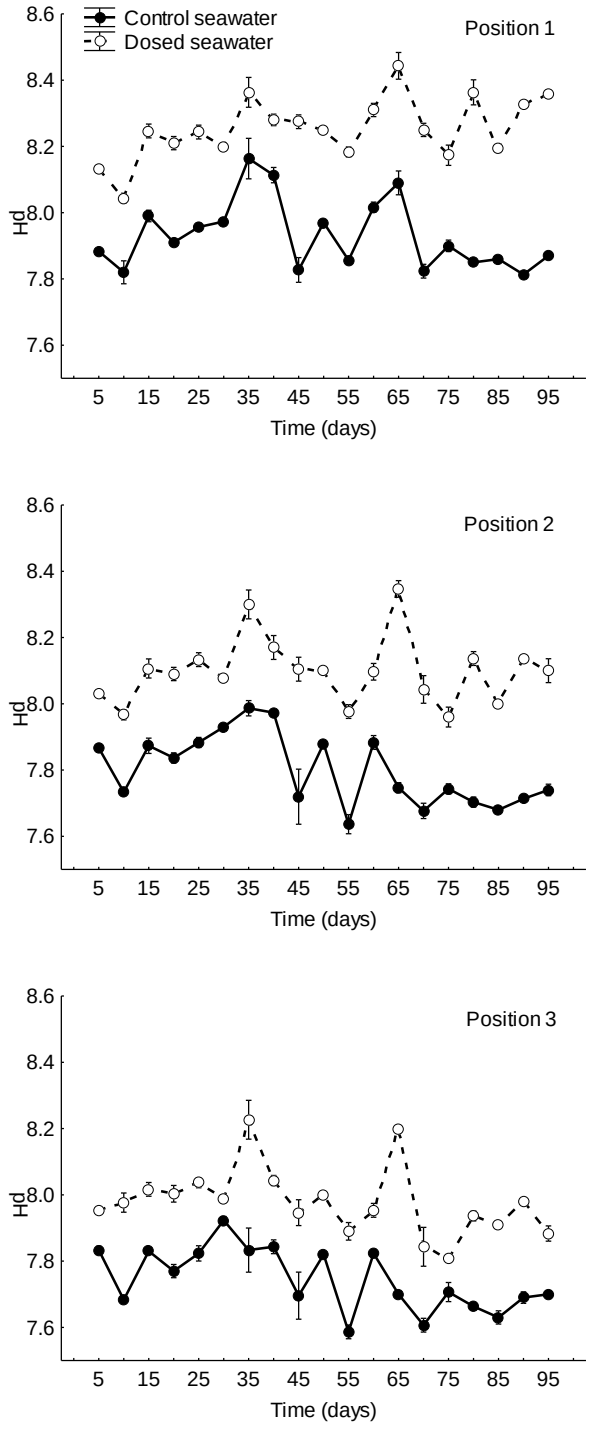


Figure 5.4: Mean (± SD) water pH in positions one, two and three at each sampling time in replicate abalone serial-use raceways, where treatment raceways were dosed with a solution of sodium hydroxide ($NaOH^-$) to raise pH above control levels. Variation in pH between days was due to the variation in influent seawater pH, flow rates and dosing volumes.

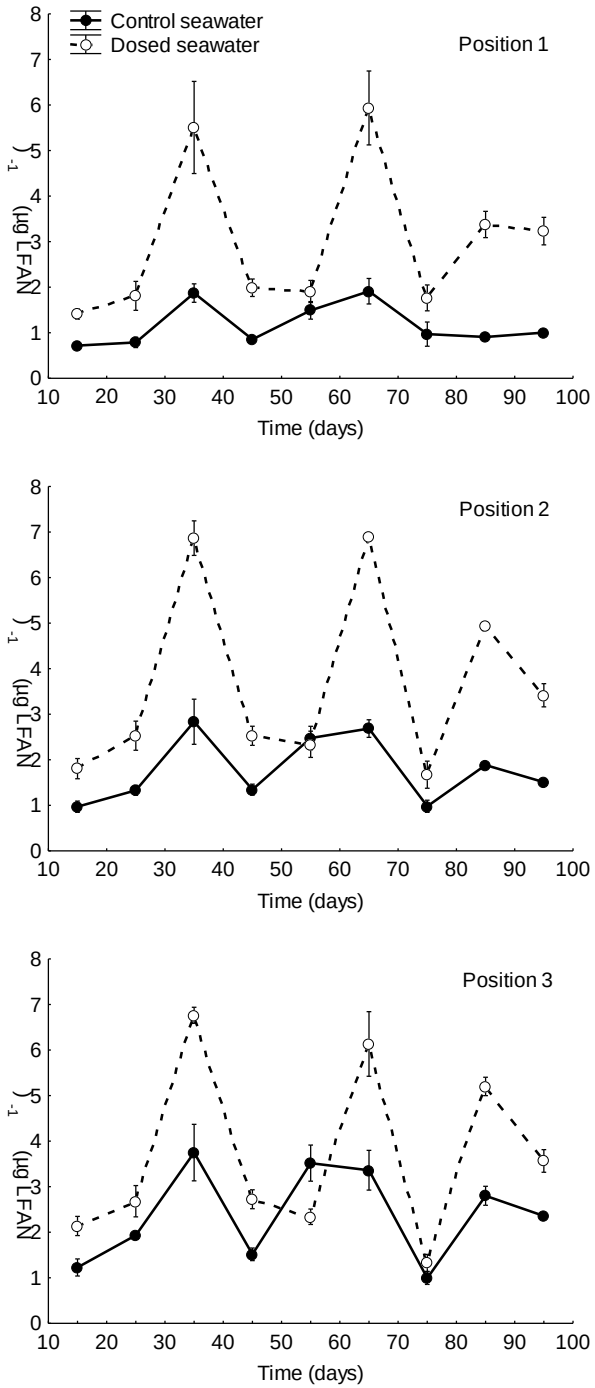


Figure 5.5: Mean (± SD) free ammonia nitrogen (FAN) concentration in positions one, two and three at each sampling time in replicate abalone serial-use raceways, where treatment raceways were dosed with a solution of sodium hydroxide ($NaOH^-$) to raise pH above control levels. Variation in FAN concentration between days was due to the variations in pH, temperature and flow rates.

Table 5.1: Mean values (± SD) of water temperature, water pH, free ammonia nitrogen (FAN), total ammonia nitrogen (TAN), dissolved oxygen, percentage oxygen saturation and initial and final flow index for abalone reared in raceways with three tanks in series. Treatment raceways were dosed with a $NaOH^-$ solution to raise the water pH above that of seawater.

	Treatment	**Position one**	**Position two**	**Position three**
Temperature	Dosed	14.6 ± 0.9	14.5 ± 1.1	14.4 ± 1.2
(°C)	Control	14.6 ± 0.9	14.5 ± 1.0	14.4 ± 1.2
pH	Dosed	8.23 ± 0.14	8.07 ± 0.14	7.95 ± 0.13
	Control	7.93 ± 0.11	7.80 ± 0.11	7.74 ± 0.10
FAN	Dosed	3.0 ± 1.8	3.7 ± 2.1	3.7 ± 2.0
($\mu g\ L^{-1}$)	Control	1.2 ± 0.5	1.8 ± 0.8	2.4 ± 1.1
TAN	Dosed	80.1 ± 38.0	148.6 ± 72.0	203.9 ± 107.9
($\mu g\ L^{-1}$)	Control	74.6 ± 36.6	148.0 ± 75.2	211.8 ± 109.3
Dissolved oxygen	Dosed	7.74 ± 0.3	7.70 ± 0.4	7.76 ± 0.5
($mg\ L^{-1}\ O_2$)	Control	7.78 ± 0.4	7.66 ± 0.4	7.85 ± 0.5
Oxygen saturation	Dosed	94.9 ± 3.2	94.1 ± 3.6	94.5 ± 4.0
(%)	Control	95.1 ± 3.5	93.6 ± 3.7	95.7 ± 3.9
Initial flow index	Dosed	20.1 ± 0.7	10.0 ± 0.4	6.7 ± 0.2
($L\ h^{-1}kg^{-1}$)	Control	20.0 ± 0.5	10.0 ± 0.2	6.7 ± 0.1
Final flow index	Dosed	16.2 ± 1.2	8.3 ± 0.4	5.6 ± 0.3
($L\ h^{-1}kg^{-1}$)	Control	16.6 ± 0.6	8.5 ± 0.2	5.7 ± 0.1

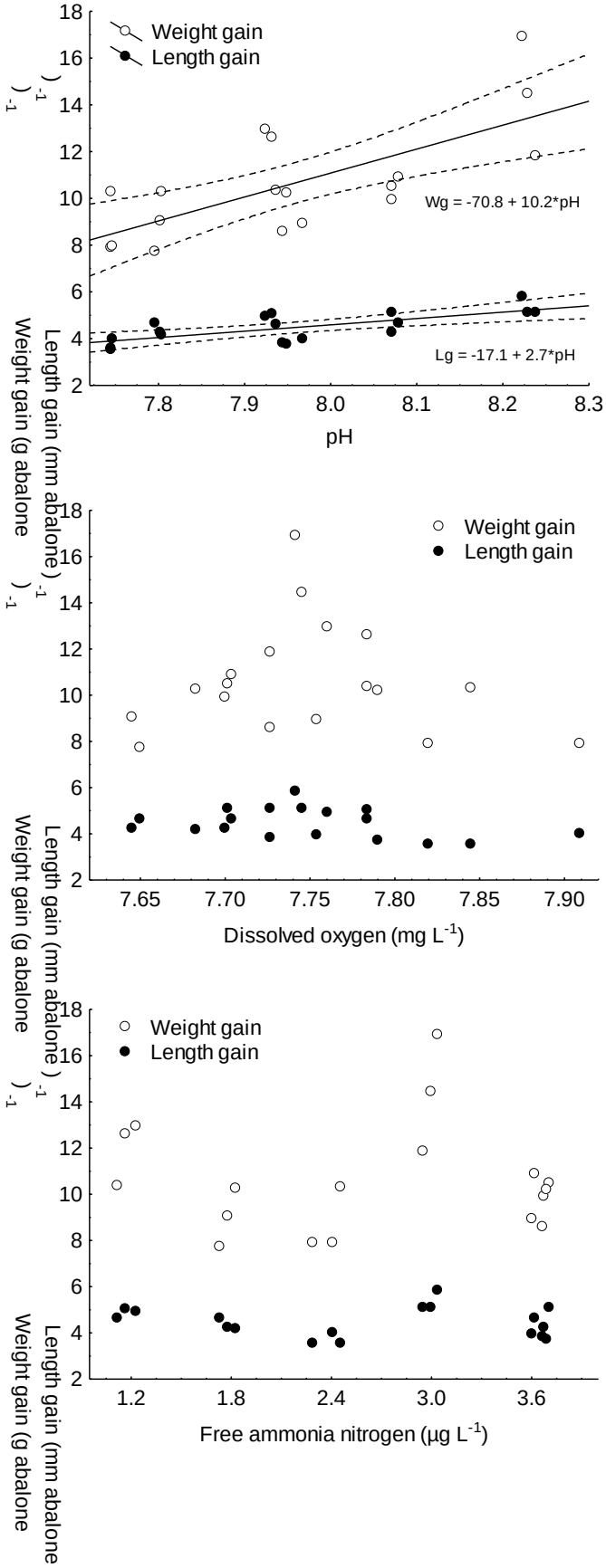


Figure 5.6: Correlation plots of weight (g abalone^{-1}) and shell length (mm abalone^{-1}) gain as a function of pH, dissolved oxygen (mg L^{-1}) and free ammonia nitrogen (FAN) (µg L^{-1}). Plots are least-square regression lines and 95 % confidence intervals. There was a significant correlation between weight and shell length gain and pH (significant regression equations included). Neither weight gain nor shell length gain were significantly correlated to dissolved oxygen or FAN concentration.

The total abalone biomass produced from each raceway was on average 12.5 % higher in the treatment raceways with increased pH. Total production from the three replicate raceways was 14.2 kg and 12.3 kg in the treatment and control raceways, respectively.

Table 5.2: Step-wise multiple regression summary of weight gain (g abalone^{-1}) and shell length gain (mm abalone $^{-1}$) of *H. midae* as a function of mean water pH, free ammonia nitrogen (FAN) and dissolved oxygen concentration.

Variable	Multiple r	Multiple r^2	F	p	variables included
Weight gain (g abalone^{-1})					
pH	0.711	0.505	16.35	0.0009	1
FAN	0.818	0.670	7.45	0.0155	2
Dissolved oxygen	0.839	0.704	1.64	0.2209	3
Shell length gain (mm abalone^{-1})					
pH	0.709	0.502	16.16	0.0009	1
FAN	0.885	0.784	19.50	0.0005	2

Discussion

Dosing with $NaOH^-$ resulted in significant differences in pH between treatments at each position. The addition of $NaOH^-$ resulted in an increase in pH above the levels recorded in the control treatments by increasing the concentration of OH^- and therefore the buffering capacity. The increased pH also indicates a greater proportion of HCO_3^- and CO_3^{-2} in the water (Manahan 1984; Smith 1988; Summerfelt 2000). However, in both treatments pH decreased with increasing serial-use. In an experiment using a similar system under similar conditions, Naylor *et al*. (2011) reported a decrease in mean pH from 7.96 to 7.62 between the influent and effluent of a seven-tier abalone serial-use raceway, and modelled pH as a function of flow rate and accumulated abalone biomass.

It is hypothesised that in both studies the decrease in pH was caused by the excretion of CO_2 by the abalone.

Abalone in the $NaOH^-$-dosed treatment were probably exposed to higher concentrations of HCO_3^- and CO_3^{-2} relative to the control and may have benefited from the increased saturation state of calcite and aragonite at higher pH (Gattuso *et al*. 1998). For example, there were significant positive correlations between calcification rate and aragonite saturation levels in the zooxanthellate coral, *Stylophora pistillata* Esper, (Gattuso *et al*. 1998). Animals use bicarbonate in extra-cellular fluids, such as blood plasma or haemolymph, to maintain the physiologically required acid-base balance and to limit potential blood acidosis caused by exposure to increased CO_2^{aq} concentration in the water (Fivelstad *et al*. 1998; Pörtner *et al*. 2004). Bicarbonate accumulation can be detected through changes in gill epithelium histology. Harris *et al*. (1999a) noted such changes in *H. laevigata* and *H. rubra* exposed to low and high pH. Future studies to should include gill histopathology to test this hypothesis.

Few authors have studied the effect of pH on abalone growth. Although it was hypothesised that increasing pH would improve growth, a pH value above a presumed optimal range may also have an adverse effect on abalone growth when compared to the effects caused by pH vales below the optimal range. In the greenlip abalone, *H. laevigata* and blacklip abalone, *H. rubra*, upper EC_5 values for pH were 8.77 and 8.46, respectively (Harris *et al*. 1999a). Regression models presented by Harris *et al*. (1999a) suggested that pH 8.25 was optimal for growth in *H. laevigata* and *H. rubra*. $NaOH^-$ dosing in the current experiment thus aimed to achieve a pH of approximately 8.25 in position one.

During the study period (July – October 2010), the growth rates of the farmed stock from which the experimental animals were selected, may have been affected by gonad production and spawning (Riddin *et al*. 2011) although no spawning event was noticed in the experimental animals. Wild *H. midae* spawn in spring and late summer (Newman 1967). This may have increased the variability in weight and length gains and added to the within-treatment variance.

The choice of alkaline dosing solution should be studied further, as Vivanco-Aranda *et al*. (2011) reported significantly better growth and survival in juvenile red abalone, *H. rufescens*, grown in a recirculating system compared to flow-through tanks, where the only measured differences were the pH and alkalinity of the respective systems. These authors used sodium bicarbonate to maintain alkalinity at 139.0 ± 8.0 mg L^{-1} $CaCO_3$ and pH at 8.13 ± 0.04 in the recirculating system, while in the flow-through tanks, alkalinity and pH averaged 115.1 ± 4.8 mg L^{-1} $CaCO_3$ and 8.01 ± 0.03 (mean ± standard error).

A significant decrease in growth and increase in FCR-values between positions was observed for both treatments as a similar drop in pH occurred between positions for both treatments. However, assuming that the $NaOH^-$ treatment tanks had higher alkalinity, a greater amount of CO_2^{aq} would have been needed in order for this to occur. It is therefore hypothesised that total inorganic carbon may have been higher in the $NaOH^-$ treatment tanks, and thus even though a greater proportion may have been in the form of HCO_3^- and CO_3^{-2}, the concentration of CO_2^{aq} may have been relatively similar at each position. The continuous aeration of the concentrated dosing solution may have encouraged uptake of atmospheric CO_2 which would be converted into HCO_3^- and CO_3^{-2}. Upon mixing with the system water, the equilibrium would shift to the left resulting in a greater proportion of CO_2^{aq}. Hypercapnic conditions can result in reduced diffusion of CO_2 at the gills and lead to blood acidosis, with subsequent long term effects on the ability of the organism to maintain homeostasis (Hargreaves & Brunson1996; Sanni & Forsberg 1996; Pörtner *et al*. 2004; Langenbuch *et al*. 2006). Significant decreases in metabolic rate and haemolymph pH have been reported in the marine mussel, *Mytilus galloprovincialis* Lamarck, exposed to CO_2-gassed seawater at pH 7.3 compared to control specimens held at pH 8.05 (Michaelidis *et al*. 2005). Similarly, Atlantic salmon (*Salmo salar* Linnaeus) post-smolts consumed less oxygen and showed decreased weight and length gain after exposure to elevated CO_2^{aq} concentrations (44 mg L^{-1}; pH 6.37) than controls maintained at 1.3 mg L^{-1} CO_2^{aq}, and pH 7.88 (Fivelstad *et al*. 1998). In the non-calcifying marine sipunculid, *Sipunculus nudus* Linnaeus, exposure to increased CO_2 concentrations resulted in metabolic disturbance, increased mortality and reduced rates of protein synthesis essential for somatic growth (Langenbuch & Pörtner

2004; Langenbuch *et al*. 2006). Ringwood & Keppler (2002) reported a significant positive correlation between environmental pH and growth in juvenile clams, *Mercenaria mercenaria* Shumacher. Growth rates were reduced by > 50 % at pH values below 7.5.

In larval northern abalone, *H. kamtschatkana*, Crim *et al*. (2011) showed that a CO_2 concentration of 400 ppm (pH 8.3) which represented ambient conditions resulted in 65 % survival and 98 % shell normality. However, when CO_2 concentrations were raised to 800 ppm (pH 8.07) and 1800 ppm (pH 7.81), survival rate dropped to 40 %. In the 800 ppm CO_2 treatment, only 60 % of larvae developed normal shells, while in the 1800 ppm CO_2 treatment 99 % of larvae developed either highly abnormal shells or no shell at all. Similar effects of CO_2 on larval survival and shell development have been shown in the gastropod *Littorina obtussata* Linnaeus, (Ellis *et al*. 2009) and the bivalves *Crassostrea gigas* Thunberg, (Kurihara *et al*. 2007), *Mytilus galloprovincialis* (Kurihara *et al*. 2009), *Mercenaria mercenaria* and *Argopecten irradians* Lamarck, (Talmage & Gobler 2009). In the sea urchin, *Paracentrotus lividus* Lamarck, skeletal growth was completely inhibited when CO_2^{aq} concentrations reached five to nine times that of seawater (Grosjean *et al*. 1998). Harris *et al*. (1999a) showed that pH influences weight gain and length gain differently in juvenile *H. laevigata* and *H. rubra*. Elevated CO_2^{aq} concentrations may therefore be the reason for both the reduction in *H. midae* weight gain and length gain with increasing serial-use, as no differences in condition factor were noted between positions.

The significant correlation between pH and abalone growth not only highlights the importance of monitoring and controlling this variable in water reuse systems where water quality changes are likely to occur, but also addresses the potential effects of ocean acidification on marine calcifying organisms and on industries that produce such animals. As the oceans continue to accumulate atmospheric CO_2, and pH starts declining at a rapid rate (Feely *et al*. 2004; Orr *et al*. 2005), abalone farmers employing flow-through systems will start noticing a decline in growth rate and health of their stock irrespective of the volumes of water they pump through the tanks.

The indirect consequence of altering pH in this study was its effect on the proportion of FAN, which is recognized as the toxic form of ammonia due to its permeability across cell membranes (Thurston *et al*. 1981; Randall & Tsui 2002). Previous research suggested that haliotids may be particularly sensitive to FAN concentrations with an estimated EC_5 concentration of < 4 μg L^{-1} (Huchette *et al*. 2003; Reddy-Lopata *et al*. 2006; Naylor *et al*. 2011). However in the current study, FAN concentrations were significantly higher in the $NaOH^-$ treatment tanks where mean weight gains and shell length gains were significantly higher. FAN contributed toward multiple regression analysis to predict weight and shell length gain, indicating that it may have a small influence on growth at low flow indices.

From an economic point of view, dosing with alkaline chemicals may be a profitable scenario for serial-use or recirculating abalone culture systems. In this study, the extra abalone biomass produced in the $NaOH^-$ dosed serial-use raceways would translate into a substantial increase in farm productivity. Alternative methods of increasing pH, such as the use of agricultural grade $NaOH^-$ or investment into CO_2 degassing towers may be economically viable on a commercial scale. The method of increasing pH on a large-scale should be optimised in future research.

Conclusion

Dosing with a $NaOH^-$ solution, and the resultant increase in pH, improved the growth of abalone. The position in the series had a significant effect on weight gain, shell length gain and FCR, and was most likely caused by an accumulation of CO_2^{aq}. However, the mechanisms affecting growth under moderately increased CO_2^{aq} concentrations need to be investigated for *H. midae*. Studies could include measurements of alkalinity, total inorganic carbon and pH in order to better explain differences in abalone growth at low flow indices. Addition of CO_2 gas to treatment tanks, rather than increasing buffering capacity through addition of alkaline substances may be a better approach to understanding the effects of pH and CO_2^{aq} on abalone growth. Future studies should establish to what extent ocean acidification may pose a threat to the economic viability of abalone farming. Since the best weight and length gains were achieved in *H. midae*

exposed to the highest pH, we suggest increasing pH to 8.4 in future studies and determining the cost-benefit of such an approach.

CHAPTER 6

General Discussion

The aim of this study was to produce data on the effects of water quality on abalone growth in a serial-use raceway and to use this information to provide water quality management suggestions to the abalone industry. Instead of performing toxicity experiments, where only one variable is altered while the remaining variables are maintained at optimal levels, the serial-use raceway was used as a tool to create various water quality conditions under which weight gain, shell length gain, FCR and condition factor of abalone could be monitored. This method is novel in that it simulates the conditions that are likely to occur in commercial serial-use raceways, recirculating systems and intensive flow-through systems. Such a study provides a degree of production forecasting based on which economic decisions can be made. System design factors such as tank size, basket size and design, abalone handling procedures, stocking densities and feeding methods closely matched those used on commercial flow through abalone farms in the Overberg region of South Africa, making the results of this thesis directly applicable to these farmers.

The results (chapters 2 – 5) can be used to (1) describe the changes in water quality between tanks in an abalone serial-use raceway in relation to cumulative abalone biomass, flow-rate and flow index, (2) correlate temperature, pH, dissolved oxygen, TAN, FAN and nitrite to *H. midae* growth and (3) suggest the first limiting water quality variable for abalone serial-use systems based on a combination of data analysis and review of the literature.

For all experiments, abalone in the size range of 45 – 60 g abalone^{-1} were used. This size class represents the largest proportion of stock on most South African abalone farms (G. Johnston – HIK Abalone Farm, pers. comm.), and thus the results of this study are applicable to the majority of farms. There is evidence that size may play a role in an abalone's tolerance to certain water quality variables (Fallu 1991; Reddy-Lopata *et*

al. 2006), and future experimental work should apply these results to both smaller and larger *H. midae* so that farming methods can be further refined.

Temperature

H. midae growth was positively correlated with temperature in the range of 12–20 °C, as an increase in temperature results in increased metabolic rate and feed consumption (Britz *et al*. 1997). To avoid large differences in temperature between treatments, shading was provided to minimise direct sun light on the serial-use raceways, and retention time of water in the header tank was kept low. Ambient air temperature did, however, have a small effect on the water temperature as retention time increased with water reuse. In chapter 1, mean water temperature increased by 0.3 °C between position one and position seven. In chapter 4 and 5, water temperature decreased between positions one and three by 0.5 °C and 0.3 °C, respectively. Using a model by Britz *et al*. (1997), describing the relationship between temperature and growth of *H. midae*, the maximum potential error in weight gain induced by the differences in temperature among tank positions would be between 8 and 12 %. The model by Britz *et al*. (1997) was based on juvenile *H. midae* (17.5 ± 1.25 mm, 1.1 ± 0.25 g) where an increase in temperature elicited a much stronger response on feed consumption rates (Britz *et al*. 1997) than in larger abalone. Steinarsson & Imsland (2003) showed that red abalone, *H. rufescens*, exhibited size-dependent differences with respect to the temperature for optimal growth, with mature abalone performing best at slightly lower temperatures. Deviations from the temperature for optimum growth had a lesser effect on the growth rate of *H. rufescens* > 66 mm, when compared to juvenile animals < 33 mm (Steinarsson & Imsland 2003). The mean temperature of all tanks from all experimental chapters was in the range of 14.4 – 15.5 °C. For the size range of abalone used in the experimental chapters (60 – 70 mm), it is suggested that the differences in mean temperature among tank positions had a negligible effect on growth as no significant effects on feed consumption were noted.

Yearsley (2008) described the annual mean (± standard error) influent water temperature at 09:00 and 16:00 for this particular farm (HIK Abalone Farm) as 14.91 ±

0.25 °C and 15.61 ± 0.26 °C respectively. The mean (± standard error) temperatures of influent water in this study were 15.07 ± 0.25 (chapter 2), 15.65 ± 0.10 (chapter 3), 15.47 ± 0.10 (chapter 4) and 14.64 ± 0.17 (chapter 5) respectively. The models describing changes in water quality and production parameters as a function of flow-index were therefore developed at temperatures close to the mean annual temperature, and may describe representative conditions for commercial operations in this region.

Dissolved oxygen

Dissolved oxygen is a primary limiting factor for aquatic organisms, as it affects the capacity for aerobic metabolism (Fry 1971; Harris *et al*. 1999b). In the serial-use raceways, dissolved oxygen concentration decreased in some cases with increasing serial-use (chapter 1), while in other cases it remained constant or increased slightly (chapter 4 and 5). The most likely explanation for this is the effect of temperature on the solubility of oxygen (Weiss 1970). A decrease in dissolved oxygen concentration with increasing serial-use was apparent only in raceways where temperature had increased. Lyon (1995) showed that oxygen consumption in *H. midae* increases linearly with temperature.

Abalone are oxygen regulators, which means that they consume oxygen independent of environmental concentrations down to a specific critical partial pressure (P_c), at which point they become oxygen conformers and consume oxygen in relation to environmental concentrations (Harris *et al*. 1999b). Below P_c, aerobic metabolism is reduced. In *H. laevigata* and *H. diversicolor supertexta*, P_c has been estimated at 4.9 mg L^{-1} O_2 and 5.5 mg L^{-1} O_2, respectively (Jan & Chang 1983; Harris *et al*. 1999b). In the present experiments, dissolved oxygen concentration rarely fell below 7.3 mg L^{-1}, with the majority of measurements in the range 7.5 – 8 mg L^{-1} O_2. These values are above the estimated EC_5 of 7.36 mg L^{-1} O_2 for *H. laevigata* (Harris *et al*. 1999b). Least-square regression analysis revealed no significant correlation between weight or length gain and dissolved oxygen concentration. Dissolved oxygen also did not contribute significantly to multiple regression models predicting weight gain and shell length gain as a function of water quality. It is thus suggested that dissolved oxygen was present in

sufficient quantities to maintain acceptable levels of aerobic metabolism, and that it was not the first limiting factor for growth. In abalone exposed to low pH (7.6 ± 0.1) and increased FAN concentrations (2.4 ± 1.1 μg L^{-1}), addition of pure oxygen resulted in higher weight gain and significantly higher shell length gain, relative to tanks with no supplemental oxygen, but failed to produce growth rates equivalent to those observed in position one where flow indices were well above the estimated minimum level (chapter 4). It is hypothesised that low pH or high FAN inhibited the abalone's ability to efficiently use the available oxygen.

TAN and FAN

Ammonia is a toxic metabolite produced by aquatic organisms during the breakdown of digested protein (Barkai & Griffiths 1987; Basuyaux & Mathieu 1999; Randall & Tsui 2002). Formulated diets are extensively used for commercial production and contain 20 - 50 % crude protein (Fleming *et al*. 1996). Depending on the combination of flow rate and stocking density (flow index), ammonia concentrations in the culture water may reach toxic or growth-limiting levels. Discharge of nitrogen rich effluent water is also considered one of the main pollutants of intensive aquaculture (Lemarie *et al*. 1998).

TAN and FAN were negatively correlated with flow index (chapter 2). TAN concentrations increased linearly with increasing water reuse, while the concentration of FAN increased slower due to the decreasing pH in the lower tank positions. In chapters 3 – 5 TAN and FAN were highest in tanks with the lowest flow index. These results are in agreement with Huchette *et al*. (2003), where TAN concentrations in a serial-use raceway containing juvenile *H. rubra*, were positively correlated with cumulative biomass. Yearsley *et al*. (2009) determined that as much as 45 % of TAN was generated from the "sludge" of uneaten feed and faeces that accumulated at the bottom of flow-through abalone tanks. In marine sediments, the rate of ammonia production is directly related to the nitrogen and carbon content of recently settled organic matter, and may be enhanced through bioturbation by benthic macrofauna or sediment re-suspension (Caffery 1995; Hargreaves 1998). In the present study, the raceways were cleaned every 12-14 days, and sludge was removed on all occasions. It is therefore assumed that

sludge contributed to an increase in TAN concentration, however, this contribution was not quantified. Future experiments should examine the combined effects of the tank cleaning regime on water quality and abalone growth to find a balance between the negative effects of altered water quality and stress induced from excessive handling.

Management protocols for reducing the concentration of TAN and FAN should include operating at appropriate flow-indices (Naylor *et al*. 2011), strict control of feeding rates in relation to temperature, stocking density and abalone size (Rasmussen & Korsgaard 1996; Britz *et al*. 1997) while regularly removing sludge (Yearsley *et al*. 2009). As the rate of ammonia production by the abalone (Lyon 1995) and mineralization of sludge by aerobic bacteria (Hargreaves 1998) is primarily linked to temperature, increases in temperature are likely to lead to spikes in FAN concentrations. Such spikes were evident at week 4, 8 and 11 (chapter 2, Figure 2.4), and were correlated to an increase in water temperature and the number of days since the tanks were last cleaned.

Reducing the dietary protein content of the feed had a significant effect on the concentration of TAN and FAN in the serial-use raceways and should also be considered as a management tool for improving water quality. TAN and FAN concentrations were on average 67 % lower in raceways fed a diet containing 22 % protein, when compared to raceways fed abalone feed containing 33 % protein. The nitrification components of a recirculating system are dependent on the excretion rate of nitrogenous waste products and the removal efficiency of the specific component (Qian *et al*. 2001). The differences in TAN recorded at the various dietary protein levels has implications for reuse systems that require biological filtration, as the size and complexity of the bio-filter unit could be reduced for a given biomass of abalone. The nitrogen concentration of effluent discharge from flow-through systems will be equally affected, and using diets with a lower percentage protein may provide a mechanism for farmers to comply with effluent quality regulations and sustainability standards. There was no significant difference in growth between the 26 and 33 % protein diets (Chapter 4), which supports findings by Green *et al*. (2011b). In order to achieve environmental sustainability certification, the recently released WWF "Abalone Dialogue Standards" requires farmers to produce abalone at a forage fish efficiency ratio (FFER) ≤ 1. Only

the 26 % protein diet satisfied these requirements. Depending on abalone size and FCR, the 26 % protein diet achieved FFER's of 0.60 – 0.95, while the standard commercial diet containing 34 % protein achieved FFER's in the range of 1.10 – 1.80 (Yearsley & Jones 2011). At elevated temperatures, such as those likely to occur in recirculating systems, lower protein diets may reduce the susceptibility of *H. midae* to "bloat", which results from uncontrollable fermentation of gut contents and the formation of gas bubbles in the digestive tract (Green *et al*. 2011b).

The concentration of FAN below the minimum flow index of 7.2 – 9.0 L h^{-1} kg^{-1} was within the range of 2.0 – 4.8 µg L^{-1}, suggested by Huchette *et al*. (2003), Reddy-Lopata *et al*. (2006) and Yearsley (2008) to cause reductions in growth in *H. rubra* and *H. midae*. According to these authors, the range suggested would induce a 5 % reduction in growth. The weight gain of abalone held at flow indices below 7.2 – 9.0 L h^{-1} kg^{-1} was, however, reduced by 35 – 70 %. The observed reductions in growth did not agree with that predicted by other authors, suggesting that either the abalone used in this study were more sensitive to elevated FAN concentrations than predicted or that at low flow indices another water quality variable, i.e., pH, had a more pronounced or synergistic effect on growth. In chapter 4, the raceways fed a diet containing 26 % protein had a mean FAN concentration of 1.0 ± 0.9 µg L^{-1} in position three, yet growth was still significantly reduced relative to abalone in position one where mean FAN concentration was 0.7 ± 0.4 µg L^{-1}. It is highly unlikely that such a small difference in FAN concentration could elicit such a response.

Raising the pH (chapter 5) resulted in significantly higher FAN concentrations (Figure 5.5), however, weight gain, shell length gain and FCR were better in this treatment when compared to controls. There was also no significant correlation between FAN concentration and weight gain or shell length gain (Chapter 4 and 5).

pH

In all experiments serial-use of water resulted in a decrease in water pH. Tanks with the lowest flow index displayed the lowest pH. The changes in pH were most likely related to the excretion of CO_2 by the abalone, which hydrolyzes in water to form a weak acid (H_2CO_3) and subsequent increase in the concentration of hydrogen ions. Even in seawater, which generally has a high alkalinity and therefore a good buffering capacity, respiration by the cultured animal can lead to significant reductions in water pH (Sanni & Forsberg 1996; Colt *et al*. 2009). In a simulated systems failure experiment, designed to test the effect of pulses of sub-optimal water quality on growth of *H. laevigata* and *H. rubra*, a decrease in the pH of treatment tanks was attributed to respiration and metabolic excretions (Hindrum *et al*. 2001). Nitrification may have also contributed to the decrease in pH with increasing serial-use (Harris *et al*. 1999a), however, the relatively low concentrations of nitrite suggest that its contribution was most likely small.

Few studies have examined the effect of pH on abalone growth and health. Harris *et al*. (1999a) reported the EC_5 and EC_{50} for pH in *H. laevigata* as 7.78 and 7.39, and modelled specific growth rate (SGR) as a function of pH (x); $SGR = 10.4x - 0.63x^2 - 42.16$. In chapter 1, a significant decrease in weight gain was observed in abalone held at pH of 7.63 ± 0.07. When converted into SGR, the reduced weight gain equates to a 28 % reduction in SGR relative to that of abalone held in position one, where mean pH was above the EC_5 predicted in *H. laevigata*, and therefore in a "no effect" range. The model developed by Harris et al (1999a) suggests that at the same mean pH as experienced in chapter 1, SGR in *H. laevigata* would be reduced by 27 %. The growth response of *H. midae* and *H. laevigata* to low pH is therefore relatively similar.

More recently, however, studies focussing on ocean acidification have shown that gastropods, bivalves and echinoderms are sensitive to elevated CO_2 concentrations and the changes in carbonate chemistry and pH which accompany it (Grosjean *et al*. 1998; Ringwood & Keppler 2002; Kurihara *et al*. 2007; Ellis *et al*. 2009; Talmage & Gobler 2009; Crim *et al*. 2011). In the serial-use raceway a process similar to ocean acidification may be occurring. Here, however, the source of CO_2 was the respiration by

the abalone in the serial-use raceway rather than the elevated atmospheric CO_2 in acidification studies. In larval northern abalone, *H. kamtschatkana*, reducing pH to 7.81 ± 0.02 with CO_2-enriched air, resulted in a 25 % decrease in survival and 99 % prevalence of abnormal or no shell development relative to control treatments maintained at pH 8.3 ± 0.02 (Crim *et al*. 2011).

In chapter 4 and 5, pH was the best predictor of weight and shell length gain. These results lead to the conclusion that pH is the first limiting variable in abalone serial-use raceways. The correlation between water pH and abalone growth displayed in this study not only has potential impacts for the design of reuse systems, but also for flow-through systems where the effects of acidification may result in lower production irrespective of the flow indices used. The effects of CO_2 alone on *H. midae* metabolic rate, energy expenditure and body mass growth, calcification rate and shell formation and health should be tested in future research. We hypothesize that increases in dissolved CO_2, measured in this case as a result of the decrease in pH, resulted in blood acidosis and an inability to efficiently use oxygen for aerobic metabolism. Also, the low pH may have altered the saturation states of the calcium carbonate polymorphs deposited during shell formation (Feely *et al*. 2004; Kleypas *et al*. 2006; Lopez *et al*. 2011). In the current study, the occurrence of "shiny shell", which is the exposure of shiny nacre as a result of the dissolution of the outer calcite layer of the shell was observed in many instances, but could not be quantified. Future studies could determine methods to easily quantify this observation and use it as an independent variable in studies focussed on low pH and accumulated CO_2.

Management practises such as dosing with alkaline chemicals or system components such as CO_2 degassing towers for preventing a decrease in pH as a result of water reuse should be investigated further. A solution of $NaOH^-$ effectively raised the water pH of treatment raceways above those of control raceways (chapter 5). Depending on the control and efficiency of the dosing apparatus, and the price for agricultural grade $NaOH^-$, addition of sodium hydroxide may result in increased profits.

Nitrite

Nitrite concentrations increased between tanks in series, similar to the increase in the concentration of TAN (Chapter 3 and 4). Nitrite concentrations were lower in raceways fed the low protein P26 diet, corresponding to the lower TAN concentrations in these raceways. Nitrifying bacteria, such as *Nitrosomonas* spp. oxidise ammonia into nitrite (Lewis & Morris 1986, Chen *et al*. 2006). The concentration of nitrite increased with time, especially in tank positions at the lower end of the raceways. It is hypothesized that the consistently higher TAN concentrations recorded in these tanks promoted the growth of *Nitrosomonas* bacteria, leading to elevated nitrite concentrations, a reaction also known from studies on the conditioning of biological filters in recirculating aquaculture systems.

However, mean nitrite concentrations recorded at the lower end of the raceways were orders of magnitude lower than the concentrations shown to affect the growth of other abalone species. For *H. laevigata* and *H. tuberculata* safe levels of nitrite were < 540 $\mu g\ L^{-1}$ and < 5000 $\mu g\ L^{-1}$, respectively (Harris *et al*. 1997; Basuyaux & Mathieu 1999). The most common method of nitrite uptake in freshwater organisms occurs actively at the gills in the chloride cells (Lewis & Morris 1986; Jensen 2003). Due to the high chloride concentration in seawater, marine organisms developed an opposite osmotic gradient to freshwater fishes thereby preventing nitrite uptake via this pathway (Jensen 2003). It is hypothesized that the nitrite concentrations observed in the serial-use raceways exerted no negative effects on abalone growth, and that the concentrations required to reduce abalone growth are not likely to occur under farming conditions.

In chapter 2 it was suggested that allowing nitrifying bacteria to colonise baskets before being used in the production system, may represent a method of reducing the sometimes high concentrations of TAN experienced when operating under low flow conditions. However, the process of nitrification results in the release of H^+ ions which decreases pH, and consumes alkalinity (Chen *et al*. 2006), the latter controlling the acid buffering capacity of the water. As the results of this study suggest that changes in pH may exert more negative effects on abalone growth than TAN, this strategy should be approached with caution.

Suspended solids

In the serial-use raceway with seven passes (chapter 1), mean suspended solid concentration increased marginally from 4.2 ± 2.0 mg L^{-1} in position one to 4.8 ± 2.5 mg L^{-1} in position seven. In contrast, the mean influent water suspended solid concentration was 6.06 ± 2.9 mg L^{-1}. Thus, although some changes occurred between tanks in series, the lower concentration in position one compared to the influent water suggested settlement of particles. The suspended basket system, and the airlines raised 50 mm off the tank bottom may have provided a quiescent zone where settlement of particulate matter could occur. Removal of settled particulates on a regular basis will be important for reducing TAN concentrations and the biological oxygen demand (Losordo *et al*. 1998; Cripps & Bergheim 2000).

Flow indices

The future profitability of abalone farming in South Africa will depend on the producer's ability to reduce production costs and improve production efficiency and biomass (Troell *et al*. 2006; Vosloo & Vosloo 2006). The simplest method of increasing operational efficiency and production volume is to optimise stocking density and flow rate. However, these factors are not independent of each other. Thus, there is a need to consider the interactive effects of these variables on water quality (Huchette *et al*. 2003).

Unlike fish, abalone require two-dimensional space. Under commercial conditions, the plastic rack and feeder plate provide the necessary surface area, with stocking density expressed as the percentage of surface area covered by the area of the shells of the abalone within a basket. When reporting flow rate, the time taken to complete 100 % exchange of water within a tank is most commonly used. For management purposes water flow to all tanks on an abalone farm may be set to achieve the same water exchange rate, even if the biomass of abalone differs among tanks. The use of a flow index that takes into account the biomass of abalone may be more appropriate for water

flow management, in particular under the conditions prevalent in serial-use systems where biomass accumulates with each pass. The minimum flow index range to avoid a significant reduction in growth as a consequence of altered water quality was estimated at 7.2 – 9.0 L h^{-1} kg^{-1} for 50 – 60 g *H. midae* (Chapter 2). For 20 – 30 g *H. midae*, Yearsley (2008) recommended flow indices of 9.7 – 14.5 h^{-1} kg^{-1} for commercial flow-through system. Although not directly comparable, these results potentially indicate size-specific tolerances to water quality, and support the suggestion by Fallu (1991) and Reddy-Lopata *et al*. (2006) that small abalone are particularly sensitive to changes in water quality. Thus, for the design of stocking density trials flow rates should be chosen such that flow-indices are equal among treatments reducing the potential of water quality becoming a confounding factor. Due to the sometimes large head height which farmers need to pump water, the time of day at which flow rates are checked and set can have an influence on the flow-indices. Flow rates should be set at low tide, ensuring the flow-indices do not drop below a minimum level of 7.2 – 9.0 L h^{-1} kg^{-1}. Pumps fitted with variable speed drives would ensure a constant water supply at a chosen flow-index, and result in electricity savings during high tide.

Depending on the cost of electricity for pumping water, farmers should consider the modelled relationship between weight gain (Wg; g abalone $^{-1}$) and flow-index (FI; L h^{-1} kg^{-1}) over a quarterly grading schedule: Wg = (FI / 0.26 + 0.07FI) (Chapter 2). The profitability of a medium sized (130 ton standing stock) commercial flow-through abalone farm under various scenarios of stocking density or standing stock, electricity usage, electricity price and product sales price where modelled in Table 6.1 & 6.2. The assumptions of the models were: 1) the farm has existing water pumping infrastructure that cannot be increased due to permit regulations, coastline characteristics or electricity supply; 2) total abalone production during the month is sold at the end of the month; 3) stocking density, and therefore farm standing stock, can be increased without causing negative effects on growth through competition for space or feed; and 4) multiplying the weight gain of the average size abalone for a specific farm by the total number of abalone on the farm gives a reasonable estimate of abalone production. Model calculations and input values are presented in Table 6.3.

If electricity can be sourced cheaply, then the increased production when operating at high flow-indices represents a more profitable scenario, when compared to simply lowering pumping volumes in an effort to save costs (Farm A vs Farm B, Table 6.1). However, if the electricity price rises dramatically, the nominally increased production at high flow-indices can no longer be justified, and opting to save on pumping costs becomes more economical (Farm W vs Farm Y, Table 6.2).

Instead of reducing water supply, farmers may choose a more aggressive stocking density thereby increasing the standing stock of the farm and reducing average flow indices to more efficient levels. At an expected electricity price of R 0.65 kWh^{-1}, increasing the farms standing stock by 10, 25 or 50 %, will result in a profit increase of 10, 24 and 44 % respectively (Farm A vs Farm C, D & E) (Table 6.1).

If the electricity price was to rise to R 1.30 kWh^{-1} (expected Winter tariff for 2013), in order for a 130 ton abalone farm to make the same profit levels as at R 0.65 kWh^{-1}, the standing stock would need to be increased by 32 % (Farm X), or the product sales price would need to be increased by 8.3 % (Farm Z).

In Table 6.1, a serial-use farm with two grow-out units in series compares positively to a standard flow-through farm (Farm A vs Serial-use farm). However, the benefits of developing a serial-use farm would most likely only be realised in a situation where space for grow-out tanks is available, but where the supply of sufficient electricity to operate a flow-through system at high flow indices cannot be guaranteed.

Table 6.1: Modelled scenarios of abalone farm profitability as a function of increased farm standing stock (Farm C, D & E), average flow index, reduced electricity consumption (Farm B), FCR and weight gain. Farm A represents a current commercial flow-through abalone farm. Serial-use farm indicates the potential profitability of a farm employing a serial-use system with two units in series.

Production parameters	**Farm A**	**Farm B**	**Farm C**	**Farm D**	**Farm E**	**Serial -use farm (first tier)**	**Serial –use farm (second tier)**
Farm standing stock (t)	130	130	143	162.5	195	130	130
Average animal size (g)	50	50	50	50	50	50	50
Total number of animals on farm	2600000	2600000	2860000	3250000	3900000	2600000	2600000
Average flow index (L h^{-1} kg^{-1})	20	10	18.18	16.00	13.33	20	10
Total water used $month^{-1}$ (m^3)	1872000	936000	1872000	1872000	1872000	1872000	***
Electricity usage (kWh $month^{-1}$)	300000	150000	300000	300000	300000	300000	
Price kWh^{-1}	R 0.65	R 0.65	R 0.65	R 0.65	R 0.65	0.65	
Total water pumping cost $month^{-1}$	R 195 000	R 97 500	R 195 000	R 195 000	R 195 000	195000	
FCR *(chapter 2)*	1.09	1.32	1.12	1.16	1.23	1.09	1.32
Feed required (kg)	11388	12030	12679	14614	17818	11388	12030
Feed cost kg^{-1}	R 18	R 18	R 18	R 18	R 18	R 18	R 18
Total feed cost	R 204 985	R 216 543	R 228 228	R 263 060	R 320 726	R 204 985	R 216 543
Other expenses							
(R ton^{-1} $month^{-1}$) ~ 0 - 130 t	R 10 000	R 9 000	R 10 000	R 10 000	R 10 000	R 10 000	
(R ton^{-1} $month^{-1}$) ~ 130 - 260 t			R 8 000	R 8 000	R 8 000		R 8 000
Total other expenses ~ 0 - 130 t	R 1 300 000	R 1 170 000	R 1 300 000	R 1 300 000	R 1 300 000	R 1 300 000	
Total other expenses ~ 130 - 260 t			R 104 000	R 260 000	R 520 000		R 1 040 000
Total costs	R 1 699 985	R 1 484 043	R 1 827 228	R 2 018 060	R 2 335 726	R 2 956 527	
Wg $animal^{-1}$ $month^{-1}$ *(chapter 2)*	4.02	3.50	3.95	3.86	3.72	4.02	3.50
Total production $month^{-1}$ (kg)	10442	9100	11309	12560	14525	10442	9100
Price Kg^{-1} ($)	30	30	30	30	30	30	30
R / $ exchange rate	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Turnover	R 2 349 398	R 2 047 401	R 2 544 484	R 2 826 087	R 3 268 156	R 4 396 798	
Profit	R 649 413	R 563 358	R 717 256	R 808 027	R 932 431	R 1 440 271	
% profit relative to Farm A		-13.25	10	24	44	122	

Table 6.2: Modelled scenarios of abalone farm profitability as a function of a rise in electricity price (Farm W, Y, X, Z), farm standing stock (Farm X), reduced electricity consumption (Farm W), sales price (Farm Z), average flow index, FCR and weight gain. Farm A represents a current commercial flow-through abalone farm.

Production parameters	**Farm A**	**Farm W**	**Farm Y**	**Farm X**	**Farm Z**
Farm standing stock (t)	130	130	130	171.2	130
Average animal size (g)	50	50	50	50	50
Total number of animals on farm	2600000	2600000	2600000	3423600	2600000
Average flow index (L h^{-1} kg^{-1})	20	20	10	15.19	20
Total water used $month^{-1}$ (m^3)	1872000	1872000	964080	1872000	1872000
Electricity usage (kWh $month^{-1}$)	300000	300000	150000	300000	300000
Price kWh^{-1}	R 0.65	R 1.30	R 1.30	R 1.30	R 1.30
Total water pumping cost $month^{-1}$	R 195 000	R 390 000	R 195 000	R 390 000	R 390 000
FCR *(chapter 2)*	1.09	1.09	1.32	1.18	1.09
Feed required (kg)	11388	11388	12030	15473	11388
Feed cost kg^{-1}	R 18	R 18	R 18	R 18	R 18
Total feed cost	R 204 985	R 204 985	R 216 543	R 278 522	R 204 985
Other expenses (Rand ton^{-1} $month^{-1}$) ~ 0 - 130 t (Rand ton^{-1} $month^{-1}$) ~ 130 - 260 t	R 10 000	R 10 000	R 9 000	R 10 000 R 8 000	R 10 000
Total other expenses ~ 0 - 130 t	R 1 300 000	R 1 300 000	R 1 170 000	R 1 300 000	R 1 300 000
Total other expenses ~ 130 - 260 t				R 329 440	
Total costs	R 1 699 985	R 1 894 985	R 1 581 543	R 2 297 962	R 1 894 985
Wg $animal^{-1}$ $month^{-1}$ *(chapter 2)*	4.02	4.02	3.50	3.83	4.02
Total production $month^{-1}$ (kg)	10442	10442	9100	13099	10442
Price Kg^{-1} ($)	30	30	30	30	32.5
R / $ exchange rate	7.5	7.5	7.5	7.5	7.5
Turnover	R 2 349 398	R 2 349 398	R 2 047 401	R 2 947 382	R 2 544 398
Profit	R 649 413	R 454 413	R 465 858	R 649 420	R 649 413
% profit relative to Farm A		-30	-28	0	0

Table 6.3: Abalone farm profitability model calculations and initial input values.

Farm standing stock	The total tonnage (t) of abalone on the farm
Average animal size	The average size in grams (g) of abalone on a farm at any one time.
Total animals on Farm	= ((farm standing stock * 1000) * 1000) / average size The total number of abalone on the farm is used to calculate monthly production
Average flow index	= Total water used month^{-1} / (Farm standing stock * 1000) The average flow index (FI) figure is used to calculate weight gain animal^{-1} month^{-1} and FCR
Total water used month^{-1}	The average volume of water pumped to supply a 130 ton commercial flow-through abalone farm.
Electricity usage	The average kilo watt hours (kWh) of electricity used to pump approximately 1872000 m^3 of water per month. This data was provided by a commercial flow-through abalone farm of approximately 130 tons standing stock.
Price	The expected Summer (0.65 R kWh^{-1}) and Winter (1.3 R kWh^{-1}) price for electricity in 2013
Total water pumping cost	= Electricity usage * Price
FCR	= $2.6*FI^{-0.29}$ Calculated from the Average Flow index, Chapter 2, Figure 2.2
Feed required	= Total production month^{-1} * FCR
Feed cost	The current price of Abfeed S34 (Marifeed Pty Ltd). The most common formulated abalone diet used in South Africa
Total feed cost	= Feed required * Feed cost
Other expenses	= R 10 000 t^{-1} month^{-1} or R 9 000 t^{-1} month^{-1} or R 10 000 t^{-1} month^{-1} Other expenses include all other running and overhead costs such as salaries, wages, chemicals, export costs etc. These costs were related to Farm standing stock. R 10 000 t^{-1} month^{-1}: Based on average values for an existing abalone farm R 9000 t^{-1} month^{-1}: For Farm B and Farm Y, the pumping requirements are halved and therefore the "other expenses" figure is reduced by R 1000 t^{-1} month^{-1} to reflect the decreased amount of maintenance and technical staff required R 8000 t^{-1} month^{-1}: An assumption was made that the cost t^{-1} month^{-1} would decrease by 20 % for the total tonnage above a standing stock of 130 tons due to economy of scale
Total costs	= Total water pumping cost + Total feed cost + Total other expenses
Weight gain animal^{-1} month^{-1}	= (FI / (0.26 + 0.07 * FI)) / 3.36 Calculated from the relationship between average flow index and weight gain (Wg), Chapter 2, Figure 2.2 The weight gain in grams (g) from the model equation in Chapter 2 was divided by 3.36 to obtain a Wg month^{-1} at the given flow index
Total production month^{-1}	= (Wg animal^{-1} month^{-1} * Total number of animals) / 1000
Price	= $ 30 kg^{-1} The average price for abalone product across the size range 0 – 100 g
R / $ exchange rate	$ 1 = R 7.5
Turnover	= Total production * Price ($) * R / $ exchange rate
Profit	= Turnover – Total costs
***	Indicates where water is reused and therefore no pumping costs apply

Conclusions

Under conditions tested in this study, the reuse of water in a serial-use raceway resulted in the deterioration of water quality. The changes in water quality could be accurately modelled as a function of tank position or flow index. At flow-indices between 7.2 – 9.0 L h^{-1} kg^{-1}, the changes in water quality were severe enough to cause significant reductions in weight gain and shell length gain, and significantly increase FCR values. For efficient utilization of available water, farmers should consider the flow-index as an indicator of tank loading density, rather than using flow-rate and stocking density as separate variables. Based on regression analyses and the study of the relevant literature, the changes in pH, and therefore an assumed accumulation of CO_2^{aq}, are hypothesized to be the first limiting water quality variable for abalone serial-use raceways. To achieve good growth of *H. midae*, reuse systems should incorporate apparatus such as CO_2 degassing columns or addition of suitable alkaline chemicals. Reducing the protein content of formulated diets may provide farmers with an environmentally friendly mechanism of improving tank water quality, limiting the negative effects of nitrogen-rich effluent discharge and reducing the need for large bio-filtration units that may require management efforts. Increasing standing stock to reduce flow indices to more efficient levels may greatly increase farm profitability.

CHAPTER 7

References

Au D.W.T., Pollino C.A.,Wu R.S.S., Shin P.K.S., Lau S.T.F. & Tang J.Y.M. (2004) Chronic effects of suspended solids on gill structure, osmoregulation, growth, and triiodothyronine in juvenile green grouper *Epinephelus coioides*. *Marine Ecology Progress Series* **266**, 255-264.

Badillo L., Segovia M. & Searcy-Bernal R. (2007) Effect of two stocking densities on the growth and mortality of the pink abalone Haliotis corrugata in recirculating and flow-through systems. *Journal of Shellfish Research* **26**, 801-807.

Barkai R. & Griffiths C.L. (1987) Consumption, absorption efficiency, respiration and excretion in the South African abalone *Haliotis midae*. *South African Journal of Marine Science* **5**, 523-529.

Barkai R. & Griffiths C.L. (1988) An energy budget for the South African abalone *Haliotis midae* Linnaeus. *Journal of Molluscan Studies* **54**, 43-51.

Basuyaux O. & Mathieu M. (1999) Inorganic nitrogen and its effect on growth of the abalone *Haliotis tuberculata* Linnaeus and the sea urchin *Paracentrotus lividus* Lamarck. *Aquaculture* **174**, 95-107.

Bautista-Teruel M.N., Fermin A.C. & Koshio S.S. (2003) Diet development and evaluation for juvenile abalone, *Haliotis asinina*: animal and plant protein sources. *Aquaculture* **219**, 645-653.

Bishop S.H., Ellis L.L. & Burcham J.M. (1983) Amino acid metabolism in Molluscs. In: *The Mollusca* **1**. Metabolic biochemistry and molecular biomechanics (ed. by Hochachka, P.W.), pp. 244-327. Academy Press; New York.

Branch G.M., Griffiths C.L., Branch M.L. & Beckley L.E. (2010) Lamp shells & molluscs. In: *Two Oceans – A guide to the marine life of Southern Africa*, pp. 144-218. Struik Nature, Cape Town, South Africa.

Brinker A., Schroder H.G. & Rosch R. (2005) A high-resolution technique to size suspended solids in flow-through fish farms. *Aquacultural Engineering* **32**, 325-341.

Britz P.J. (1996a) Effect of dietary protein level on growth performance of South African abalone, *Haliotis midae*, fed fishmeal-based semi-purified diets. *Aquaculture* **140**, 55-61.

Britz P.J. (1996b) The suitability of selected protein sources for inclusion in formulated diets for the South African abalone, *Haliotis midae. Aquaculture* **140**, 63-73.

Britz P.J. & Hecht T. (1997) Effect of dietary protein and energy level on growth and body composition of South African abalone, *Haliotis midae*. *Aquaculture* **156**, 195-210.

Britz P.J., Hecht T. & Mangold S. (1997) Effect of temperature on growth, feed consumption and nutritional indices of *Haliotis midae* fed a formulated diet. *Aquaculture* **152**, 191-203.

Botes L., Smit A.J. & Cook P.A. (2003) The potential threat of algal blooms to the abalone (*Haliotis midae*) mariculture industry situated around the South African coast. *Harmful Algae News 2* **4**, 247–259.

Bower C.E. & Bidwell J.P. (1978) Ionization of ammonia in seawater: effects of temperature, pH and salinity. *Journal of Fisheries Research Board Canada* **35**, 1012-1016.

Burnett L.E., Scholnick D.A. & Mangum P. (1988) Temperature sensitivity of molluscan and arthropod hemocyanins. *Biological Bulletin* **174**, 153-162.

Caffrey J.M. (1995) Spatial and seasonal patterns in sediment nitrogen remineralization and ammonium concentrations in San Francisco Bay, California. *Estuaries* **18**, 219-233.

Carter C.G., Houlihan D.F. & Owen S.F. (1998) Protein synthesis, nitrogen excretion and long-term growth of juvenile *Pleuronectes flesus*. *Journal of Fish Biology* **5**, 272-284.

Chen S., Ling J. & Blancheton J. (2006) Nitrification kinetics of biofilm as affected by water quality factors. *Aquacultural Engineering* **34**, 179-197.

Cheng W., Li C. & Chen J. (2004a) Effect of dissolved oxygen on the immune response of *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemolyticus*. *Aquaculture* **232**, 103-115.

Cheng W., Liu C., Cheng S. & Chen J. (2004b) Effect of dissolved oxygen on acid-base balance and ion concentration of Taiwan abalone *Haliotis diversicolor supertexta*. *Aquaculture* **231**, 573-586.

Cho S.H., Park J., Kim C. & Yoo J.H. (2008) Effect of casein substitution with fishmeal, soybean meal and crustacean meal in the diet of the abalone *Haliotis discus hannai*. *Aquaculture Nutrition* **14**, 61-66.

Colt J. (2006) Water quality requirements for reuse systems. *Aquacultural Engineering* **34**, 143-156.

Colt J. & Orwicz K. (1991a) Modeling production capacity of aquatic culture systems under freshwater conditions. *Aquacultural Engineering* **10**, 1-29.

Colt J. & Orwicz K. (1991b) Aeration in intensive aquaculture. In: *Water Quality and Aquaculture, Advances in Aquaculture*, vol. 3. (ed. by Brune D.E. & Tomasso J.R.), pp. 198-271.World Aquaculture Society, Baton Rouge.

Colt J.,Watten B. & Rust M. (2009) Modeling carbon dioxide, pH, and un-ionized ammonia relationships in serial reuse systems. *Aquacultural Engineering* **40**, 28-44.

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

Covington A.K., Bates R.G. & Durst R.A. (1985) Definition of pH scales, standard reference values, measurement of pH and related terminology. *Pure and Applied Chemistry* **57**, 531-542.

Crim R.N., Sunday J.M. & Harley C.D.G. (2011) Elevated seawater CO_2 concentrations impair larval development and reduce larval survival in endangered northern abalone (*Haliotis kamtschatkana*). *Journal of Experimental Marine Biology and Ecology* **400**, 272-277.

Cripps S.J. & Bergheim A. (2000) Solids management and removal for intensive land-based aquaculture production systems. *Aquacultural Engineering* **22**, 33-56.

Eikebrokk B (1990) Design and performance of the BIOFISH water recirculation system. *Aquacultural Engineering* **9**, 285-294.

Ellis R.P., Bersey J., Rundle S.D., Hall-Spencer J.M. & Spicer J.I. (2009) Subtle but significant effects of CO_2 acidified seawater on embryos of the intertidal snail, *Littorina obtusata*. *Aquatic Biology* **5**, 41-48.

Engin K. & Carter C.G. (2001) Ammonia and urea excretion rates of juvenile Australian short-finned eel (*Anguilla australis australis*) as influenced by dietary protein level. *Aquaculture* **194**, 123-136.

Eshchar M., Mozes N. & Fediuk M. (2003) Carbon dioxide removal rate by aeration devices in marine fish tanks. *The Israeli Journal of Aquaculture* **55**, 79-85.

Evans F. & Langdon C.J. (2000) Co-culture of dulse *Palmaria mollis* and red abalone *Haliotis rufescens* under limited flow conditions. *Aquaculture* **185**, 137-158.

Fallu R. (1991) *Abalone Farming*. Fishing News Books, Oxford, England. 195 pp.

Feely R.A., Sabine C.L., Lee K., Berelson W., Kleypas J., Fabry V.J. & Millero F. J. (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science* **305**, 362-366.

Fivelstad S. (1988) Waterflow requirements for salmonids in single-pass and semi-closed land-based seawater and freshwater systems. *Aquacultural Engineering* **7**, 183-200.

Fivelstad S., Bergheim A. & Tyvold T. (1991) Studies of limiting factors governing the waterflow requirement for Atlantic salmon (*Salmo salar* L.) in land-based seawater systems. *Aquacultural Engineering* **10**, 237-249.

Fivelstad S., Haavik H., Lovik G & Olsen A.B. (1998) Sublethal effects and safe levels of carbon dioxide in sea water for Atlantic salmon postsmolts (*Salmo salar* L.): ion regulation and growth. *Aquaculture* **160**, 305-316.

Fleming A.E., Van Barneveld R.J. & Hone P.W. (1996) The development of artificial diets for abalone: A review and future directions. *Aquaculture* **140**, 5-53.

Fry F.E.J. (1971) The effect of environmental factors on the physiology of fish. In: *Fish Physiology* **9**. (ed. by Hoar W.S. & Randall D.J.). pp. l-98. Academic Press, New York.

Gattuso J.P., Frankignoulle M., Bourge I., Romaine S. & Buddemeier R.W. (1998) Effect of calcium carbonate saturation of seawater on coral calcification. *Global and Planetary Change* **18**, 37-46.

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

Gordon H.R. & Cook P.A. (2001) World abalone supply, markets and pricing: historical, current and future. *Journal of Shellfish Research* **20**, 567-570.

Green A.J., Jones C.L.W. & Britz P.J. **(**2011a**)** Effect of dietary lipid level on growth and feed utilization in cultured South African abalone *Haliotis midae* L. fed diets with a constant protein-to-energy ratio. *Aquaculture Research* **42**, 1501-1508.

Green A.J., Jones C.L.W. & Britz P.J. **(**2011b**)** The protein and energy requirements of farmed South African abalone *Haliotis midae* L. cultured at optimal and elevated water temperatures. *Aquaculture Research* **42**, 1653-1663.

Grosjean P., Spirlet C., Gosselin D., Vaittilingon D. & Jangoux A. (1998) Land based, closed-cycle echiniculture of *Paracentrotus lividus* (Lamarck) (Echinoidea: Echinodermata): a long term experiment at pilot scale. *Journal of Shellfish Research* **17**, 1523-1531.

Guzman J.M. & Viana M.T. (1998) Growth of abalone *Haliotis fulgens* fed diets with and without fish meal, compared to a commercial diet. *Aquaculture* **165**, 321-331.

Hargreaves J.A. & Brunson M. (1996) Carbon Dioxide in Fish Ponds. *SRAC Publication* **468**, 1-6.

Hargreaves J.A. (1998) Nitrogen biogeochemistry of aquaculture ponds. *Aquaculture* **166**, 181-212.

Harris J.O., Maguire G.B., Edwards S.J. & Hindrum S.M. (1997) Effect of nitrite on growth and oxygen consumption for juvenile greenlip abalone *Haliotis laevigata* Donovan. *Journal of Shellfish Research* **16**, 395-401.

Harris J.O., Maguire G.B. & Hindrum S.M. (1998) Effect of ammonia on the growth rate and oxygen consumption of juvenile greenlip abalone, *Haliotis laevigata* Donovan. *Aquaculture* **160**, 259-272.

Harris J.O., Maguire G.B., Edwards S.J. & Hindrum S.M. (1999a) Effect of pH on growth rate, oxygen consumption rate, and histopathology of gill and kidney tissue for juvenile greenlip abalone, *Haliotis laevigata* Donovan and blacklip abalone, *Haliotis rubra* Leach. *Journal of Shellfish Research* **18**, 611-619.

Harris J.O., Maguire G.B., Edwards S.J. & Johns D.R. (1999b) Low dissolved oxygen reduces growth rate and oxygen consumption rate of juvenile greenlip abalone, *Haliotis laevigata* Donovan. *Aquaculture* **174**, 265-278.

Hecht T. (1994) Behavioural thermoregulation of the abalone, *Haliotis midae*, and the implications for intensive culture. *Aquaculture* **126**, 171-181.

Hindrum S.M., Burke C.M., Edwards S.J. & Johns D.R. (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**, 679-684.

Hopkins J.S., Sandifer P.A. & Browdy C.L. (1994) Sludge management in intensive pond culture of shrimp: effect of management regime on water quality, sludge characteristics, nitrogen extinction, and shrimp production. *Aquacultural Engineering* **13**, 11-30.

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

Jan R. & Chang K. (1983) The oxygen consumption by formosan abalone, *Haliotis diversicolor supertexta* Lishke, during decline of ambient oxygen. *Bulletin of Institutional Zoology, Academia Sinica* **22**, 43-48.

Jensen F.B. (2003) Nitrite disrupts multiple physiological functions in aquatic animals. *Comparative Biochemistry and Physiology Part A* **135**, 9-24.

Jones C.L.W. & Britz P.J. (2006) Development of a low-protein, water stable kelp diet for the South African abalone culture industry. *The Sixth International Abalone Symposium, Puerto Varas, Chile*, 19-24 February, pp 152.

Kelly M.S. & Owen P.V. (2002) Growth of the abalone *Haliotis tuberculata* L. at Scottish sea temperatures. *Aquaculture Research.* **33**, 729-733.

Kleypas J.A., Feely R.A., Fabry V.J., Langdon C., Sabine C.L. & Robbins L.L. (2006) Impacts of ocean acidification on coral reefs and other marine calcifiers: A guide for future research. *Report of a workshop held 18–20 April 2005, St. Petersburg, FL, sponsored by NSF, NOAA, and the U.S. Geological Survey*, 88 pp.

Kurihara H., Kato S. & Ishimatsu A. (2007) Effects of increased seawater pCO_2 on early development of the oyster *Crassostrea gigas*. *Aquatic Biology* **1**, 91-98.

Kurihara H., Asai T., Kato S. & Ishimatsu A. (2009) Effects of increased seawater pCO_2 on early development in the mussel *Mytilus galloprovincialis*. *Aquatic Biology* **4**, 225-233.

Langenbuch M & Pörtner H.O. (2004) High sensitivity to chronically elevated CO_2 levels in a eurybathic marine sipunculid. *Aquatic Toxicology* **70**, 55-61.

Langenbuch M., Bock C., Leibfritz D. & Pörtner H.O. (2006) Effects of environmental hypercapnia on animal physiology: A 13C NMR study of protein synthesis rates in the marine invertebrate *Sipunculus nudus*. *Comparative Biochemistry and Physiology Part A* **144**, 479-484.

Lemarie G., Dosdat A., Coves D., Dutto G., Gasset E. & Person-Le Ruyet J. (2004) Effect of chronic ammonia exposure on growth of European seabass (*Dicentrarchus labrax*) juveniles. *Aquaculture* **229**, 479-491.

Lemarie G., Martin J.L.M., Dutto G. & Garidou C. (1998) Nitrogenous and phosphorous waste production in a flow-through land-based farm of european seabass (*Dicentrarchus labrax*). *Aquatic Living Resources* **11**, 247-254.

Levene H. (1960) Robust tests for equality of variances. In: *Contributions to probability and statistics* (ed. by Olkin I. & Hotelling H.), pp. 278-292. Stanford University Press, Stanford, CA, United States.

Lewis W.M. & Morris D.P. (1986) Toxicity of nitrite to fish: a review. *Transactions of the American Fisheries Society* **115**, 183-195.

Lopez M.I., Chen P.Y., McKittrick J. & Meyers M.A. (2011) Growth of nacre in abalone: Seasonal and feeding effects. *Materials Science and Engineering* C **31**, 238-245.

Losordo T.M., Masser M.P. & Rakocy J. (1998) Recirculating aquaculture tank production systems: an overview of critical considerations. *SRAC Publication* **451**, 1-6.

Loubser, N.C. (2005) Abalone farming in South Africa. *The seventh conference of the Aquaculture Association of South Africa, Grahamstown, South Africa*, 12-16 September, pp 150.

Lyon R.G. (1995) *Aspects of the physiology of the South African abalone, Haliotis midae L., and implications for intensive abalone culture.* M.Sc. Thesis, Rhodes University, Grahamstown, pp 85.

Manahan S.E. (1984) *Environmental Chemistry*, pp. 1-612. Willard Grant Press, Boston.

Michaelidis B., Ouzounis C., Paleras A. & Pörtner H.O. (2005) Effects of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Marine Ecology Progress Series* **293**, 109-118.

Miron D.S., Moraes B., Becker A.G., Crestani M., Spanevello R., Loro V.L. & Baldisserotto B. (2008) Ammonia and pH effects on some metabolic parameters and gill histology of silver catfish, *Rhamdia quelen* (Heptapteridae). *Aquaculture* **277**, 192-196.

Montano-Vargas J., Viana M.T., D'Abramo L.R., Shimada, A. & Vasquez-Pelaez, C. (2005) Growth and energy utilization of juvenile pink abalone *Haliotis corrugata* fed diets containing different levels of protein and two starch:lipid ratios. *Journal of Shellfish Research* **24**, 1179-1185.

Morrison J.K. & Piper R.G. (1988) Effect of reused water on Atlantic salmon. *The Progressive Fish-Culturist* **50**, 110-112.

Muir J.F. (1982) Recirculated water systems in aquaculture. In: *Recent Advances in Aquaculture* (ed. by Muir J.F. & Roberts R.J.). pp. 357- 447. Westview Press, Boulder, Colorado, USA.

Naylor M., Kaiser H. & Jones C.L.W. (2011) Water quality in a serial-use raceway and its effect on growth of South African abalone, *Haliotis midae* Linnaeus, 1758. *Aquaculture Research* **42**, 918-930.

Newman G.G. (1967) Reporduction of the South African abalone, *Haliotis midae*. *Investigational Report Division of Sea Fisheries South Africa* **64**, 1-21.

Orr J.C., Fabry V.J., Aumont O., Bopp L., Doney S.C., Feely R.A., Gnanadesikan A., Gruber N., Ishida A., Joos F., Key R.M., Lindsay K., Maier-Reimer E., Matear R., Monfray P., Mouchet A., Najjar R.G., Plattner G., Rodgers K.B., Sabine C.L., Sarmiento J.L., Schlitzer R., Slater R.D., Totterdell I.J., Weirig M., Yamanaka Y. & Yool A. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**, 681-686.

Park J., Kim H., Kim P. & Jo J. (2008) The growth of disk abalone, *Haliotis discus hannai* at different culture densities in a pilot-scale recirculating aquaculture system with a baffled culture tank. *Aquacultural Engineering* **38**, 161-170.

Person-Le Ruyet J., Galland R., Le Roux A. & Chartois H. (1997) Chronic ammonia toxicity in juvenile turbot (*Scophthalmus maximus*). *Aquaculture* **154**, 153–169.

Piedrahita R.H. (2003) Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. *Aquaculture* **226**, 35-44.

Pörtner H.O., Langenbuch M. & Reipschläger A. (2004) Biological impact of elevated ocean CO_2 concentrations: Lessons from animal physiology and earth history. *Journal of Oceanography* **60**, 705-718.

Poxton M.G. & Allouse S.B. (1982) Water quality criteria for marine fisheries. *Aquacultural Engineering* **1**, 153-191.

Qian P., Wu M. & Ni I. (2001) Comparison of nutrients release among some maricultured animals. *Aquaculture* **200**, 305-316.

Rasmussen R.S. & Korsgaard B. (1996) The effect of external ammonia on growth and food utilization of juvenile turbot (*Scophthalamus maximus* L.). *Journal of Experimental Marine Biology and Ecology* **205**, 35-48.

Randall D.J. & Tsui T.K.N. (2002) Ammonia toxicity in fish. *Marine Pollution Bulletin* **45**, 17-23.

Reddy-Lopata K., Auerswald L. & Cook P. (2006) Ammonia toxicity and its effect on the growth of the South African abalone *Haliotis midae* Linnaeus. *Aquaculture* **261**, 678-687.

Riddin N., Jones C.L.W. & Kaiser H. (2011) Gonad development and growth in farmed South African abalone *Haliotis midae*. *14th South African Marine Science Symposium and 49th Estuarine and Coastal Sciences Association International Conference*, *Grahamstown, South Africa*, 4 -7 April, pp 88.

Ringwood A.H. & Keppler C.J. (2002) Water quality variation and clam growth: is pH really a non-issue in estuaries? *Estuaries* **25**, 901-907.

Robertson-Andersson D.V., Potgieter M., Hansen J., Bolton J.J., Troell M., Anderson R.J., Halling C. & Probyn T. (2008) Integrated seaweed cultivation on an abalone farm in South Africa. *Journal of Applied Phycology* **20**, 579-595.

Russo R.C. & Thurston R.V. (1991) Toxicity of ammonia, nitrite and nitrate to fishes. In: *Water Quality and Aquaculture, Advances in Aquaculture*, vol. 3. (ed. by Brune D.E. & Tomasso J.R.) pp. 58–89. World Aquaculture Society, Baton Rouge, USA.

Sales J. & Britz P.J. (2001) Research on abalone (*Haliotis midae* L.) cultivation in South Africa. *Aquaculture Research* **32**, 863-874.

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

Sanni S. & Forsberg O.I. (1996) Modelling pH and carbon dioxide in single-pass sea water aquaculture systems. *Aquacultural Engineering* **15**, 91-110.

Schuenhoff A., Shpigel M., Lupatsch I., Ashkenazi A., Msuya F.E. & Neori A. (2003). A semi-recirculating, integrated system for the culture of fish and seaweed. *Aquaculture* **221**, 167-181.

Shapiro S.S. & Wilk M.B. (1965). An analysis of variance test for normality (complete samples), *Biometrika* **52**, 591-611.

Shin P.K.S, Yau F.N., Chow S.H., Tai K.K. & Cheung S.G. (2002) Responses of the green-lipped mussel *Perna viridis* (L.) to suspended solids. *Marine Pollution Bulletin* **45**, 157-162.

Shirayama Y. (2002) Towards comprehensive understanding of impacts on marine organisms due to raised CO_2 concentration. *The fifth International Symposium on CO_2 fixation and efficient utilization of energy, Tokyo, Japan*. pp 177-181.

Siikavuopio S.I., Dale T., Foss A. & Mortensen A. (2004) Effects of chronic ammonia exposure on gonad growth and survival in green sea urchin *Strongylocentrotus droebachiensis*. *Aquaculture* **242**, 313-320.

Siikavuopio S.I., Mortensen A., Dale T. & Foss A. (2007) Effects of carbon dioxide exposure on feed intake and gonad growth in green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture* **266**, 97-101.

Smith R.G. (1988) Inorganic carbon transport in biological systems. *Comparative Biochemistry and Physiology Part B* **90**, 639-654.

Solorzano L. (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. *Limnology and Oceanography* **14**, 799-801.

Steinarsson A. & Imsland A.K. (2003) Size dependent variation in optimum growth temperature of red abalone (*Haliotis rufescens*). *Aquaculture* **224**, 353-362.

Summerfelt R.C. & Penne C.R. (2005) Solids removal in a recirculating aquaculture system where the majority of flow bypasses the microscreen filter. *Aquacultural Engineering* **33**, 214-224.

Summerfelt S.T. (2000) Carbon dioxide. In: *Encyclopedia of Aquaculture* (ed. by Stickney R.R.), pp. 141-147. John Wiley and Sons Inc., 605 Third Avenue, New York.

Summerfelt S.T., Davidson J.W., Waldrop T.B., Tsukuda S.M. & Bebak-Williams J. (2004) A partial-reuse system for coldwater aquaculture. *Aquacultural Engineering* **31**, 157-181.

Talmage S.C. & Gobler C.J. (2009) The effects of elevated carbon dioxide concentrations on metamorphosis, size and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and Eastern oysters (*Crassostrea virginica*). *Limnology and Oceanography* **54**, 2072-2080.

Thurston R.V., Russo R.C. & Vinogradov G.A. (1981) Ammonia toxicity to fish: effect of pH on the toxicity of the un-ionized ammonia species. *Environmental Science and Technology* **15**, 837-840.

Tomasso J.R. (1994) Toxicity of nitrogenous wastes to aquaculture animals, *Reviews in Fisheries Science* **2**, 291-314.

Tomasso J.R. & Grosell M. (2005) Physiological basis for large differences in resistance to nitrite among freshwater and freshwater-acclimated euryhaline fishes. *Environmental Science and Technology* **39**, 98-102.

Troell M., Robertson-Anderson D., Anderson R.J., Bolton J.J., Maneveldt G., Halling C. & Probyn T. (2006) Abalone farming in South Africa: An overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. *Aquaculture*. **257**, 266-281.

Vergara J.M., Lopez-Calero G., Robaina L., Caballero M.J., Montero D., Izquierdo M.S. & Aksnes A. (1999). Growth, feed utilization and body lipid content of gilthead seabream *Sparus aurata* fed increasing lipid levels and fish meals of different quality. *Aquaculture* **179**, 35-44.

Vivanco-Aranda M., Gallardo-Escarate C.J. & Del Rio-Portilla M.A. (2011). Low-density culture of red abalone juveniles, *Haliotis rufescens* Swainson 1822, recirculating aquaculture system and flow-through system. *Aquaculture Research* **42**, 161-168.

Vosloo A. & Vosloo D. (2006) Routes of water loss in South African abalone (*Haliotis midae*) during aerial exposure. *Aquaculture* **261**, 670–677.

Wassnig M., Roberts R.D., Krsinich A. & Day R.W. (2010) Effects of water flow rate on growth rate, mortality and biomass return of abalone in slab tanks. *Aquaculture Research* **41**, 839-846.

Webb K.A. Jr. & Gatlin D.M. III. (2003) Effects of dietary protein level and form on production characteristics and ammonia excretion of red drum *Sciaenops ocellatus*. *Aquaculture* **225**, 17-26.

Wedemeyer G.A. & Yasutake W.T. (1978) Prevention and treatment of nitrite toxicity in juvenile steelhead trout (*Salmo gairdneri*). *Journal of Fisheries Research Board Canada* **35**, 822-827.

Wedemeyer G.A. (2000a) Alkalinity. In: *Encyclopedia of Aquaculture* (ed. by Stickney R.R.), pp. 25-26. John Wiley and Sons Inc., 605 Third Avenue, New York.

Wedemeyer G.A. (2000b) pH. In: *Encyclopedia of Aquaculture* (ed. by Stickney R.R.), pp. 646-647. John Wiley and Sons Inc., 605 Third Avenue, New York.

Weiss R.F. (1970) The solubility of nitrogen, oxygen and argon in water and seawater. *Deep-Sea Research* **17**, 721-735.

Wurts W.A. & Durborow R.M. (1992). Interactions of pH, carbon dioxide, alkalinity and hardness in fish ponds. *SRAC Publication* **464**, 1-4.

Yang S., Liou C. & Liu F. (2002) Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). *Aquaculture* **213**, 363-372.

Yearsley R.D. (2008) *Water quality, abalone growth and the potential for integrated mariculture on a South African abalone Haliotis midae L. farm*. M.Sc. thesis, Rhodes University, Grahamstown, South Africa. 77 pp.

Yearsley R.D., Jones C.L.W. & Britz P.J. (2009) Effect of settled sludge on dissolved ammonia concentration in tanks used to grow abalone (*Haliotis midae* L.) fed a formulated diet. *Aquaculture Research* **40**, 166-171.

Yearsley R.D. & Jones C.L.W. (2011) New Marifeed abalone diet reduces fishmeal use and satisfies WWF standards. *AFMA Martix* **20**, 24-26.

Yu P.C., Matson P.G., Martz T.R. & Hofmann G.E. (2011) The ocean acidification seascape and its relationship to the performance of calcifying marine invertebrates: Laboratory experiments on the development of the urchin larvae framed by environmentally-relevant pCO_2/pH. *Journal of Experimental Marine Biology and Ecology* **400**, 288-295.

APPENDIX A

Peer reviewed journal articles emanating from this thesis:

Naylor M.A., Kaiser H. & Jones C.L.W. (2011) Water quality in a serial-use raceway and its effect on growth of South African abalone, *Haliotis midae* Linnaeus, 1758. *Aquaculture Research* **42**, 918-930.

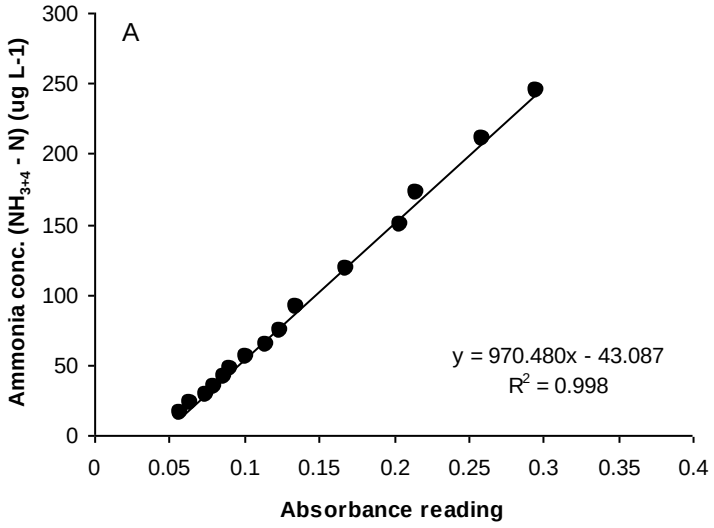
Naylor M.A., Kaiser H. & Jones C.L.W. (2011) The effect of dietary protein level on total ammonia nitrogen and free ammonia nitrogen concentration in an abalone, *Haliotis midae*, serial-use raceway. *Journal of Shellfish Research* **30**, 1-5.

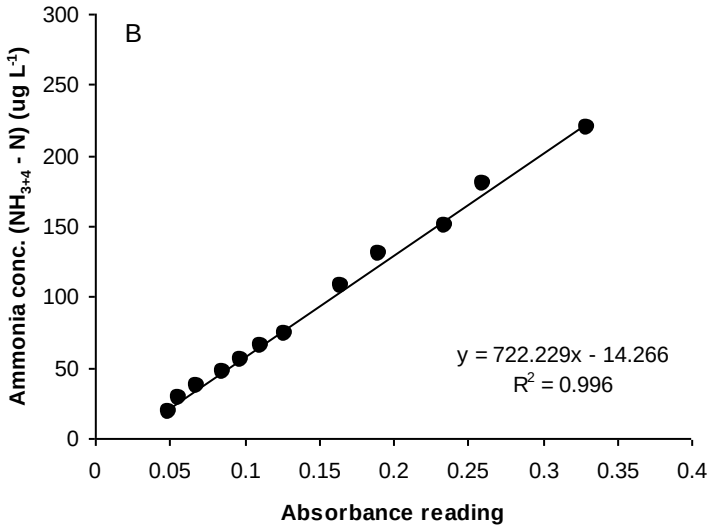
Naylor M.A., Kaiser H. & Jones C.L.W. (2012) The effect of free ammonia nitrogen, pH and supplementation with oxygen on the growth of South African abalone, *Haliotis midae* L. in an abalone serial-use raceway with three passes. *Aquaculture Research* (in review).

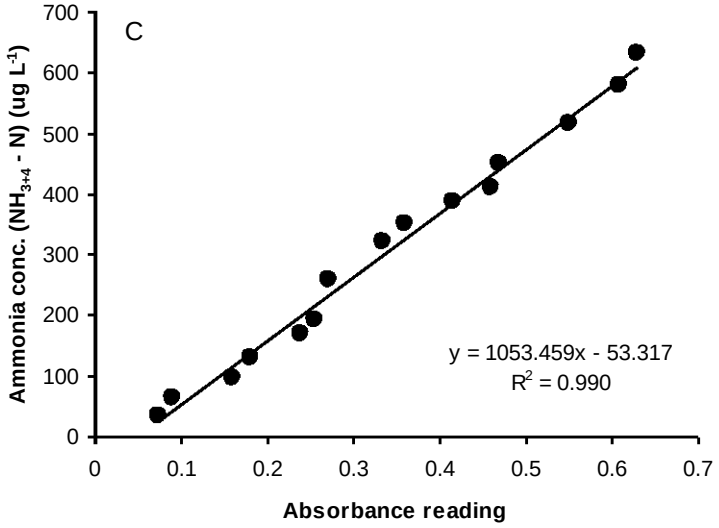
Naylor M.A., Kaiser H. & Jones C.L.W. (2012) The effect of dosing with sodium hydroxide ($NaOH^-$) on water pH and growth of *Haliotis midae* in an abalone serial-use raceway. *Journal of Shellfish Research* (in review).

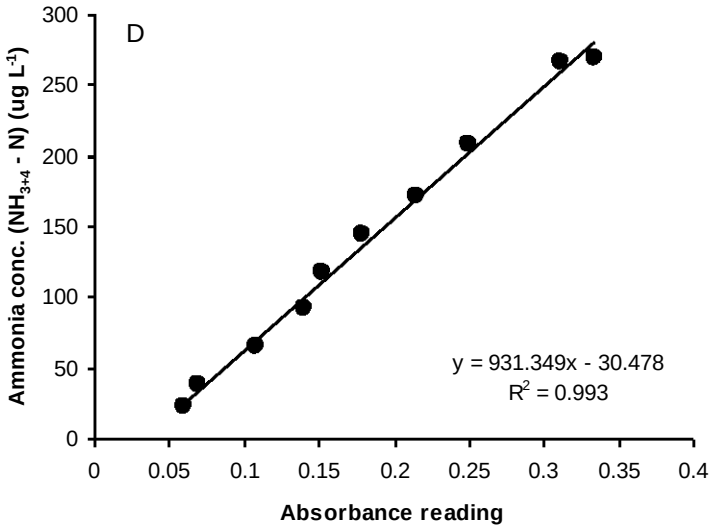
APPENDIX B

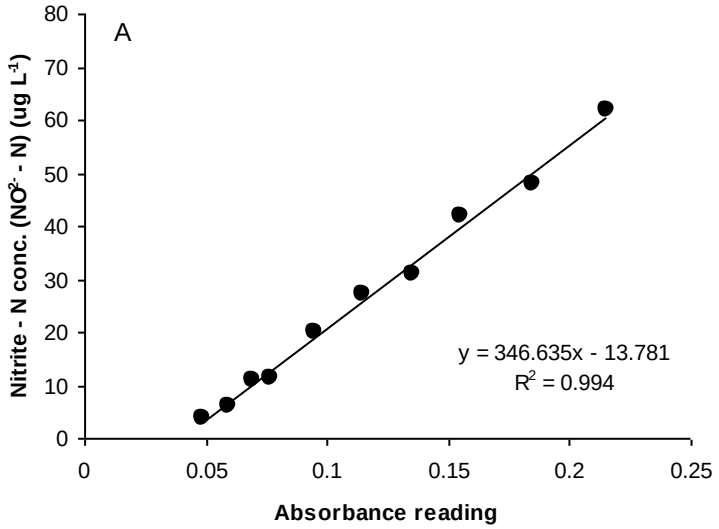
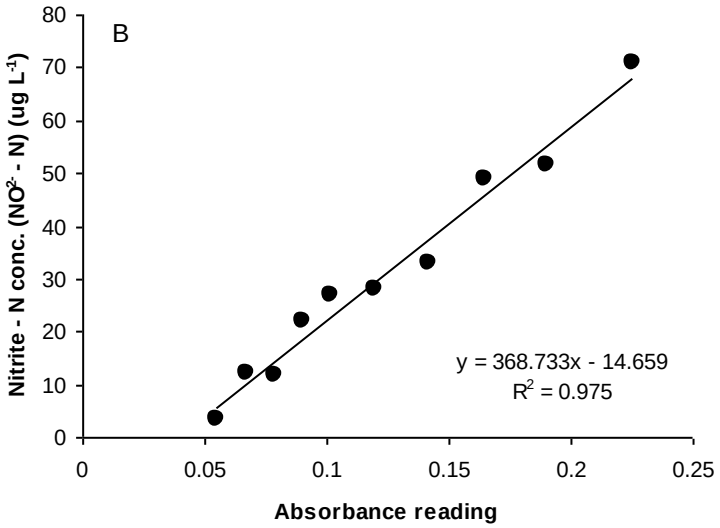
Ammonium chloride standard curves, showing least-square linear regression coefficients for calculation of total ammonia nitrogen (TAN) in Chapter 2 (A), Chapter 3 (B), Chapter 4 (C) and Chapter 5 (D).






C
Ammonia conc. (NH_{3+4} - N) (ug L^{-1})
700
600
500
400
300
200
100
0
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Absorbance reading
y = 1053.459x - 53.317
R^2 = 0.990


D
Ammonia conc. (NH_{3+4} - N) (ug L^{-1})
300
250
200
150
100
50
0
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
Absorbance reading
y = 931.349x - 30.478
R^2 = 0.993

APPENDIX C

Sodium nitrite standard curves, showing least-square linear regression coefficients for calculation of nitrite - N (NO^{2-} - N) in Chapter 2 (A) and Chapter 4 (B).