WATER QUALITY, ABALONE GROWTH AND THE POTENTIAL FOR INTEGRATED MARICULTURE ON A SOUTH AFRICAN ABALONE *HALIOTIS MIDAE* L. FARM

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ABSTRACT

Abalone *Haliotis midae* farming in South Africa is highly intensive, employing pump-ashore, flow-through systems. Despite the known sensitivity of abalone to water quality, there is only a rudimentary understanding of water quality dynamics on South African abalone farms and its effects on abalone production. Furthermore, the potential for reusing the relatively dilute abalone farm effluent to culture other animal species has not been investigated. This study investigated the dynamics of water quality and growth on a South African abalone farm and assessed the suitability of the effluent for the culture of silver kob *Argyrosomus inodorus* and bloodworm *Arenicola loveni loveni*.

Monitoring of water quality and abalone growth in abalone tanks revealed that oxygen concentrations decreased, while H^+ ion and free-ammonia (NH_3) concentration increased in a gradient between the inflow and outflow. Abalone growth was positively correlated with oxygen concentration and negatively correlated with free-ammonia and H^+ ion concentration. The oxygen (*O*) concentration of the farm influent was dependent upon the influents' temperature (*T*) and was described by the relationship O (mg L^{-1}) = 11.244 – 0.208T (r^2=0.74). Linear regression analysis of data collected from abalone farm tanks revealed that the concentration of total ammonia at the outflow of abalone tanks (µg TAN L^{-1}) was dependant upon temperature (°C), flow-rate (L s^{-1} kg^{-1} *H. midae*), abalone size (g) and length of time since the tank was last cleaned (d) (n = 125, r^2 = 0.80). The production of total ammonia (µg TAN s^{-1} kg^{-1}) was related to temperature, abalone size and days that the tanks remained un-cleaned (n = 125; r^2 = 0.81). A diurnal cycle of respiration was evident in abalone tanks with higher oxygen consumption and H^+ ion production at night. The oxygen concentration of farm effluent was related to temperature,

farm biomass and flow rate by means of a linear regression equation (n = 40; $r^2 = 0.69$). The results demonstrated the importance of optimising the flow-rate per unit of biomass for various temperatures and sizes of abalone. As abalone size and temperature cannot be controlled under farm conditions, the flow-rate per unit of biomass which the abalone culture system receives will determine the quality of the culture water.

The specific growth rate (0.48 ± 0.01 % BW d^{-1}), mortality (1.8 ± 0.5 %), feed conversion ratio (3.0 ± 0.2) and protein efficiency ratio (1.0 ± 0.1) of silver kob kept in either abalone farm effluent or control seawater for 120 days did not differ significantly (*t*-test, $P>0.05$). A 90 day growth trial indicated that abalone farm effluent is a suitable culture medium for bloodworm. Bloodworm supplied with control seawater lost weight at 0.19 ± 0.04 % BW d^{-1}, while those given abalone effluent grew at 0.39 ± 0.07 % BW d^{-1}. Mortality was 6 ± 3 % in effluent and 11 ± 8 % in seawater. The bloodworm were efficient at processing solid waste. Abalone farm effluent initially contained 7.7 ± 13 mg L^{-1} more suspended solids than control seawater, which contained 3.5 ± 0.5 mg L^{-1}, but after passing through bloodworm systems the concentration in abalone effluent was reduced to only 1.4 ± 3.5 mg L^{-1} above that in control seawater. Therefore, abalone farm effluent could be reused as a culture medium for both silver kob and bloodworm. Future work is needed to investigate aspects of the feasibility of such systems such as growth rates at different sizes and stocking densities.

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CONTENTS

Chapter 1

General introduction

Abalone *Haliotis midae* L. farming is a relatively young sector of the South African aquaculture industry as farming only began in the 1990's (Sales & Britz, 2001). In spite of this abalone are now farmed on an industrial scale in intensive systems. The development of the industry has been fuelled by a great deal of research and development and good market demand for abalone products (Sales & Britz, 2001). In 2005 there were 13 farms in South Africa producing a total of approximately 508 tons of abalone with a value of ZAR 82 million (Botes et al., 2006). Globally, abalone production was 22 600 t in 2002, of which 8 600 t were farmed (Gordon & Cook, 2004). Most abalone are farmed in Asia (Gordon & Cook, 2004), while a minority of production occurs in South Africa, the USA, Australia, Mexico, New Zealand, Ireland, Iceland and recently, Chile. In the context of global abalone production, in 2004 South Africa was the largest producer outside of Asia (FAO, 2004). To date, research on the cultivation of *H. midae*, reviewed in Sales & Britz (2001), has investigated: spawning and seed production; the effect of temperature on abalone growth and nutritional indices; handling and transport; and the development of formulated diets. Furthermore, extensive research has investigated the potential for reuse of abalone farm effluent to culture seaweed (Robertson-Andersson, 2004; Troell et al., 2006). Very little research has investigated water quality on abalone farms or the potential for reuse of the abalone farm effluent to culture organisms other than seaweed.

Abalone farms in South Africa are all shore-based and most are flow-through systems, with a few farms using partial-recirculation. On farms which operate on the flow-through system, with which this thesis concerns itself, seawater is pumped ashore and makes a single-pass through abalone tanks before flowing by gravity back to the sea. In the grow-out phase of the production process the abalone are kept in baskets suspended in tanks through which seawater flows (Fig. 1.1). The abalone are typically fed either kelp *Ecklonia maxima* or a formulated diet, Abfeed® (26 - 34 % protein, 1.2 % lipid; Marifeed Pty Ltd, Hermanus) or a combination of the two. In 2004 the industry used approximately 5917 t wet weight of kelp and 180 – 200 t dry weight of Abfeed® (Troell et al., 2006). Since 1993 some *Gracilaria gracilis* and green seaweeds (*Ulva spp*.), grown

in abalone effluent, have also been used by certain farms as feed (Troell et al., 2006). While it is expected that the expansion of the abalone industry cannot be fuelled by increased harvesting of kelp, as this has now reached a maximum sustainable yield (Troell et al., 2006), it is not certain to what degree Abfeed® will be the future feed of choice or seaweeds grown in abalone effluent.

The suitability of the farm environment for the growth of abalone is, to a large degree, dependent upon the quality of the culture water. The water's quality is a relative measure because it is assessed with the optimum water quality requirements of abalone in mind. In well managed aquaculture systems, water quality is kept within the optimum requirements of the species being cultured. However, in poorly managed systems the quality of the water may become so altered that exposure to it is stressful for the organism (Schreck & Li, 1991). When this happens the growth of the organism may be reduced and in extreme cases it may die (Poxton & Allouse, 1982). In order to increase production, farmed abalone are kept at unnaturally high stocking densities. In the wild, population densities range from 82 – 133 g m^{-2} (Barkai & Griffiths, 1988) but on farms abalone are kept at higher densities of approximately 2.0 kg m^{-2} (G. Johnston, HIK Abalone Farm Pty Ltd, pers. comm.). The surface area per abalone basket is calculated as the surface area of the vertical plates and the underside of the feeder-plate (Fig. 1.1). Under these conditions the water in the culture environment may become altered by the abalone's metabolism. Furthermore, while some farms feed their abalone kelp (15 % protein; Troell et al., 2006), which forms the majority of their natural diet (Barkai & Griffiths, 1986), others feed a formulated diet which is relatively high in protein (26 - 34 %; Abfeed®, Marifeed Pty Ltd, Hermanus). The farmed abalone fed a formulated diet may consequently excrete more nitrogenous waste. Therefore, it is possible that on abalone farms water quality may become sub-optimal for the growth of abalone. While abalone farming is a lucrative sector of the South African aquaculture industry (Botes et al., 2006), few studies have investigated water quality on abalone farms.

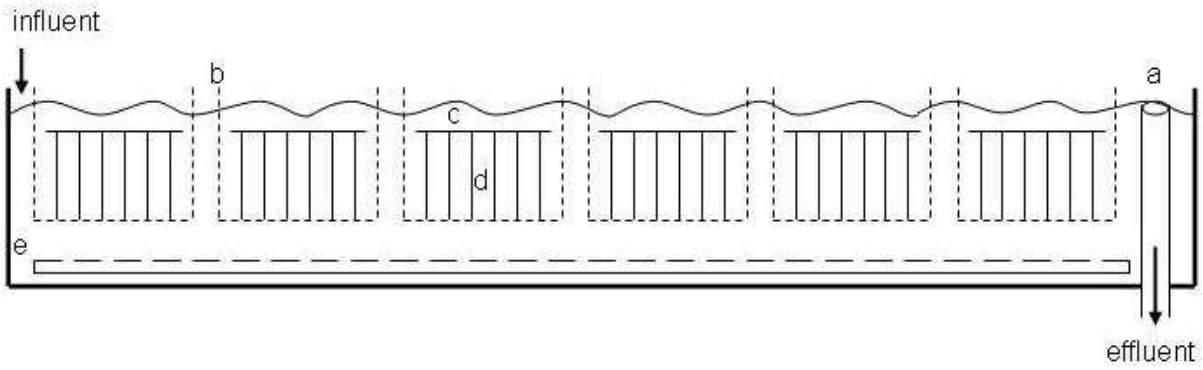


Fig. 1.1. A longitudinal section view of the tank design used on most South African abalone *Haliotis midae* farms. Influent enters the tank at the water-surface at one end and effluent leaves the tank via an outlet situated at the water-surface (a) at the opposite end. The tank shown contains six oyster-mesh baskets (b), although the number of baskets varies on different farms. On farms that feed a formulated diet the feed is placed on the feeder-plate (c). The vertical plates (d) provide a surface area for the abalone to attach themselves. Aeration is supplied beneath the baskets via a perforated pipe (e), improving water mixing.

Abalone farm effluent has been used successfully in integrated aquaculture systems for the culture of seaweeds, as reviewed by Troell et al. (2006), but its suitability as a culture medium for finfish and polychaete species has not been investigated. Integrated farming was defined by Edwards et al. (1998) as occurring when "an output from one subsystem in an integrated farming system, which otherwise may have been wasted, becomes an input into another subsystem resulting in greater efficiency of output of desired products from the land/water area under a farmers control". Typically these sorts of aquaculture systems offer economic and/or environmental benefits over conventional monoculture systems, in which only a single species is farmed. Furthermore, Neori et al. (2004) make the point that in some cases monoculture aquaculture systems are only successful because they have not been required to treat their waste-water. Therefore, if in future abalone farms were required to internalize the cost of waste-water treatment, integrated systems, which treat the abalone effluent while deriving an economic benefit may be the only strategy that remains profitable. Both environmental and economic benefits can be gained when the culture of a 'fed' organism is combined with that of an 'extractive' organism so that the waste from the 'fed' organism, that would otherwise pollute the

environment, is used by the 'extractive' organism to produce a secondary product (Chopin et al., 2001). Bolton (2002) argues that integrated aquaculture will be most successful when a fed organism is combined with an extractive species from low down the food-chain, as these are typically easy to grow and use resources efficiently. South Africa currently has a small, developing industry farming marine finfish. The preliminary investigations of Samsukal (2004) indicate that the effluent from South African abalone farms contains negligible dissolved total ammonia, nitrite and nitrate compared to levels considered toxic to finfish (Poxton & Allouse, 1982). In contrast, effluent from abalone tanks that are being cleaned may be rich in dissolved and particulate nutrients but this becomes diluted in the effluent from the farm as a whole. Therefore, utilising abalone farm effluent to supply flow-through or partial-recirculation finfish systems may be a viable alternative to pumping seawater ashore. This strategy would negate the need for the infrastructural and running costs associated with pumping seawater ashore for finfish aquaculture. Furthermore, the particulate matter produced in abalone tanks, and flushed out in the effluent, is rich in protein and energy (Chalmers, 2002) and may be a suitable feed for the detritivorous polychaete species *Arenicola loveni loveni*. This species, which is commonly known as bloodworm, has good market demand as it is a popular bait species (Britz et al., 2001; Mackenzie, 2005), which is time-consuming to collect. Furthermore, converting the particulate waste from abalone farms into bloodworm would reduce the amount of nutrients released by farms to the sea.

The aims of this study were to investigate (1) the spatial, diurnal and seasonal dynamics of water quality on a South African abalone farm and to develop tools that abalone farmers can use to improve water quality management on flow-through abalone farms that use formulated feeds and (2) to assess the suitability of abalone farm effluent for the culture of silver kob *Argyrosomus inodorus* Griffiths & Heemstra and bloodworm *Arenicola loveni loveni* Kinberg. In order to develop the research approach it was necessary to consider the effect that various water quality parameters may have in the culture environment.

Temperature is the primary environmental factor that determines the metabolic rate of poikilothermic animals (Fry, 1971). Hence, in the context of abalone farming, water temperature

is an important part of the abalones' environment. Studies by Britz et al. (1997), Gilroy and Edwards (1998), Kelly and Owen (2002) and Steinarsson and Imsland (2003) have documented the effects of temperature on *H. midae, Haliotis rubra, Haliotis laevigata, Haliotis tuberculata* and *Haliotis rufescens*. In *H. midae*, growth, feed conversion ratio and protein efficiency ratio increase from 12 ºC to 20 ºC and then decline sharply at temperatures higher than 20 ºC (Britz et al., 1997). The observations of Britz et al. (1997) were supported by Lyon (1995) who found that the ammonia excretion and oxygen consumption of *H. midae* were significantly higher above 20 ºC. Furthermore, the solubility of oxygen in water is reduced as temperature increases (Weiss, 1970) and at higher temperatures the toxicity of ammonia increases (Bower & Bidwell, 1978). The dynamics of temperature on abalone farms are relatively well understood as temperature is easily monitored. However, the effect of temperature on oxygen, ammonia and pH on abalone farms; and the indirect influence of temperature on water quality, through the metabolism of abalone, is not well understood.

Oxygen is needed for aerobic metabolism and hence can limit growth (Fry, 1971). Harris et al. (1999a) observed that juvenile *H. Laevigata* exposed to a dissolved oxygen concentration of 4.9 mg L^{-1} ate less, grew slower and had higher mortality compared to controls exposed to 8.9 mg L^{-1}. The oxygen consumption of *Haliotis discus hannai* varies diurnally with more oxygen being consumed during the period from dusk to midnight (Uki & Kikuchi, 1975). This diurnal variation in oxygen consumption can be attributed to their nocturnal feeding behaviour. Since *H. midae* also feeds nocturnally (Britz et al., 1996) it may have a similar pattern of oxygen consumption. Barkai and Griffiths (1987) and Lyon (1995) observed that the oxygen consumption of *H. midae* kept in small respirometer chambers did not vary diurnally. However, studies utilising respirometer chambers may not take the effect of movement during feeding into account since the abalones' movement is restricted (Barkai & Griffiths, 1987). Therefore, it is possible that oxygen consumption and hence concentration in abalone tanks changes on a diurnal cycle. The preliminary investigations of Lyon (1995) suggest that the oxygen consumption of *H. midae*, per gram of body weight, decreases as a linear function of abalone shell-length. In *H. discus hannai* the $\log_{10}$ oxygen consumption is linearly related to body weight (Uki & Kikuchi, 1975). Therefore, tanks containing different size-classes of abalone may have different oxygen

requirements. The oxygen consumption of abalone also varies according to temperature. Preliminary investigation suggests that the oxygen consumption of *H. midae* increases linearly with temperature (Lyon, 1995), while Uki and Kikuchi (1975) found a linear relationship between the $\log_{10}$ oxygen consumption of *H. discus hannai* and water temperature up to 20 ºC. Therefore, it is likely that oxygen concentrations in abalone tanks will vary according to the effect of temperature on oxygen consumption (Uki & Kikuchi, 1975; Lyon, 1995) and solubility (Weiss, 1970).

Ammonia is the main nitrogenous by-product of protein metabolism excreted by abalone (Bishop et al., 1983; Barkai & Griffiths, 1987). Numerous studies have documented the toxicity of ammonia to abalone (Harris et al., 1998a; Harris et al., 1998b; Basuyaux & Mathieu, 1999; Reddy-Lopata et al., 2006), other marine molluscs (Keppler, 2007, Widman et al., 2007) and marine finfish (Alderson, 1979; Wajsbrot et al., 1993; Rasmussen & Korsgaard, 1996; Person-Le Ruyet et al., 1997a; Person-Le Ruyet et al., 1997b; Lemarié et al., 2004). In aqueous solution ammonia exists in equilibrium between its un-ionized (NH_3) and ionized (NH_4) forms, which is controlled by pH, temperature and salinity (Bower & Bidwell, 1978). The sum of the two forms is known as total ammonia-nitrogen TAN ($NH_{3\text{-}4}$-N). The un-ionized form is more toxic because it is able to easily diffuse across cell-membranes (Thurston et al., 1981). Hence, for the purpose of toxicity testing, the concentration of the un-ionized form, expressed as free ammonia-nitrogen (FAN) is calculated from the total ammonia concentration, temperature and pH of the water. Reddy-Lopata et al. (2006) reported that 36 h exposure to a free ammonia concentration of 9.8 µg FAN L^{-1} causes 50 % mortality (i.e. 36 h LC_{50}) in juvenile *H. midae* of 10 – 25 mm shell length. Tolerance of free ammonia increases with body size and 10 – 15 cm abalone showed 50 % mortality at a higher concentration of 16.4 µg FAN L^{-1} (Reddy-Lopata et al., 2006). Chronic and acute exposure to free ammonia under laboratory conditions has also been shown to cause growth depression, increased oxygen consumption, reduced food consumption and changes in right-kidney histology in *H. laevigata* (Harris et al., 1998a; Harris et al., 1998b) and growth depression in *H. tuberculata* (Basuyaux & Mathieu, 1999). The 36 h LC_{50} FAN concentrations of 9.8 – 16.4 µg L^{-1} for *H. midae*, reported by Reddy-Lopata et al. (2006), are unusual in that they are lower than the threshold concentrations at which growth first becomes affected in other abalone and

mollusc species. *Haliotis laevigata* and *H. tuberculata* exposed to concentrations of 41 – 45 µg FAN L^{-1} for two months and two weeks respectively, showed only minor reductions in growth compared to controls and less than two percent mortality (Harris et al., 1998a; Basuyaux & Mathieu, 1999). Furthermore, the 72 h LC_{50} for juvenile (0.7 – 2.6 cm) bay scallops *Argopecten irradiana irradians* is 430 µg FAN L^{-1} (Widman et al., 2007), while in larger specimens (4.8 cm) of the oyster *Crassostrea viginica* cellular damage occurs only at concentrations between 250 – 520 µg FAN L^{-1} over 7 days exposure (Keppler, 2007). However, Vosloo (pers. comm., 2007) reports exposing 80 g *H. midae* to a free ammonia concentration of approximately 60 µg L^{-1} for 48 h without observing mortality. This result places the ammonia tolerance of *H. midae* closer to that of other molluscan species. Therefore, there is uncertainty surrounding the 36 h LC_{50} for *H. midae* reported by Reddy-Lopata et al. (2006) and the threshold, free ammonia concentration at which the growth of *H. midae* is reduced has yet to be established. Furthermore, the determinants of ammonia concentrations on abalone farms have not been documented. Elevated levels of total ammonia were recorded in abalone farm effluent in a preliminary study (Samsukal, 2004). However, while total ammonia levels were measured at a relatively large number of abalone farms (n = 7), it was not within the scope of the study to investigate seasonal or diurnal changes in ammonia concentration and the concentration of free ammonia was not reported. In laboratory-based studies the excretion of ammonia by *H. midae* has increased with increasing temperature between 16 – 20 °C (Lyon, 1995) and decreased exponentially with increasing body-weight (Barkai & Griffiths, 1978). Base-line data of free ammonia concentrations on abalone farms under different conditions have not been recorded and the influences of temperature and body-weight on ammonia excretion have not been verified.

In aquaculture systems the pH of the water may become altered due to the production of H^+ ions when carbon dioxide reacts with water to form an $HCO3^-$ molecule and a H^+ ion (Sanni & Forsberg, 1996). The concentration of the H^+ ion is commonly expressed as pH, which is the negative logarithm of the H^+ ion concentration (i.e. pH = - log [mol H^+ L^{-1}], Covington et al., 1985). The concentration of the H^+ ion has been shown to affect the growth rate and oxygen consumption of *H. laevigata* and *H. rubra* (Harris et al., 1999b). The mode of action by which growth and respiration are affected is unclear but exposure to seawater of reduced basicity, of

approximately 7.16 pH, causes the tubules and lumen of the kidney to become enlarged, and abnormalities in the structure of the gills (Harris et al., 1999b). Furthermore, the concentration of the H^+ ion controls the proportion of total ammonia in the toxic, free ammonia form (Bower & Bidwell, 1978) and hence can indirectly affect the health of animals in aquaculture systems by affecting the concentration of free ammonia. As basicity decreases, more of the total ammonia changes to the free ammonia form and therefore, in this context, high basicities are most dangerous. Base-line data of pH on abalone farms are not available and the determinants of pH have not been investigated.

In aquatic environments autotrophic bacteria oxidize ammonia to nitrite and nitrite to nitrate (Sharma & Ahlert, 1977). In abalone, nitrite has been shown to cause weakening of the immune system, damage to the gills, reduced oxygen consumption and growth depression (Harris et al., 1997; Harris et al., 1998b; Basuyaux & Mathieu, 1999; Cheng et al., 2004). In fresh-waters nitrite can move across the cell-membranes of aquatic animals and accumulate in the extra-cellular spaces in tissues, especially in the gills, liver, muscles and brain (Jensen, 1995) where it oxidizes other compounds (Colt & Armstrong, 1981). However, in marine finfish this occurs only at high concentrations that are unlikely to occur under aquaculture conditions (Wedemeyer & Yasutake, 1978). This is due to the increased concentration of chloride ions in saltwater, which competitively exclude nitrite ions from uptake via the gills' chloride cells (Wedemeyer & Yasutake, 1978). Whether the same is true for abalone has not been established (Harris et al., 1997). Samsukal (2004), documented elevated levels of nitrite in the effluent from abalone farms but investigations of nitrite dynamics have not proceeded beyond this preliminary work.

Nitrate is less toxic to aquatic organisms than ammonia or nitrite (Camargo et al., 2005). Basuyaux and Mathieu (1999) showed that exposure to a nitrate concentration of 250 mg NO_3-N L^{-1} for two weeks reduced the growth of *H. tuberculata*, while 100 mg NO_3-N L^{-1} had no affect. Since the highest nitrate concentration recorded during a preliminary survey of abalone farms was 0.3 mg NO_3-N L^{-1} (Samsukal, 2004), and the current study was concerned only with abalone health and growth, nitrate was not included in the water quality parameters investigated.

It is possible that high concentrations of suspended solids may promote the growth of the sabellid worm *Terebrasabella heterouncinata*, which infests the shells of abalone. Sabellid infestation can reduce the growth rate of abalone (Ruck & Cook, 1998). High concentrations of suspended solids may also damage the gills of abalone. A study by Cheung and Shin (2005) documenting damage to the gills of green-lipped mussels, *Perna viridis*, caused by acute exposure to suspended solids, found a linear relationship between suspended solids concentration and damage to the gill filaments. The same was found to be true for chronic exposure (Shin et al., 2002). Chalmers (2002), recorded the size-range and proximate composition of suspended solids in abalone farm tanks. However, the concentration of suspended solids in aquaculture systems can vary seasonally, with higher concentrations typically found when temperatures are warmer and consequently farmed animals are fed more (Tovar et al., 2000). On South African abalone farms the seasonal dynamics of the concentration and production of suspended solids have not been investigated.

In this thesis, the first experiments investigated water quality and growth dynamics on an abalone farm (Chapter 2). The first experiment of this chapter monitored the variation in water quality and growth at different positions within abalone tanks and correlated growth with water quality. Secondly, diurnal variations in oxygen, H^+ ion concentration, total ammonia and free ammonia were documented in abalone tanks. Thirdly, the quality of the farm influent and effluent were monitored over a period of twelve months. Variations in these water quality data over time were explained by changes in temperature, farm biomass and flow-rate. Models were developed for the prediction of the oxygen concentration of abalone farm effluent and the ammonia concentration in abalone farm tanks.

The third chapter used theoretical models to assess the effectiveness of various tank designs which have been employed by abalone farms in an attempt to reduce the variation in water quality and growth at different positions within abalone tanks. These variations in water quality and growth were documented in Chapter 2.

Anecdotal observations made while collecting the data presented in Chapter 2 suggested that the sludge which accumulated in the abalone tanks produced more ammonia, on a dry-weight basis, than the abalone themselves. Therefore, the fourth chapter investigated the effect that the accumulation of sludge in abalone tanks had on ammonia production.

In Chapter 5 the feasibility of reusing abalone farm effluent as a culture medium for silver kob and bloodworm was assessed. This involved recording the growth and mortality of these animals in abalone farm effluent and an unused seawater control, together with water quality in the culture systems.

Finally, the results of all four experimental chapters were discussed and the implications of the results for industry were assessed (Chapter 6).

Chapter 2

Water quality and growth dynamics on a South African abalone *Haliotis midae* farm that feeds a formulated diet

2.1 Introduction

South African abalone *Haliotis midae* L. farms almost exclusively operate on a pump-ashore, single-pass design. Anecdotal observations suggest that abalone growth on these farms changes seasonally and that abalone growth is poor at positions near the outflow of farm tanks. It was hypothesised that these apparent variations in growth are caused by changes in water quality within the abalone tanks.

Abalone farms in South Africa fall within the natural range of *H. midae* and therefore abalone are adapted to the environmental conditions of the coastal waters pumped ashore to supply the farms. However, within the relatively high-density farm environment it is expected that water quality will become altered by the abalones' metabolism. Abalone consume oxygen to fuel their metabolism and produce carbon dioxide (Hochachka et al., 1983) and ammonia (Bishop et al., 1983; Barkai & Griffiths, 1987) as the main metabolites. Therefore, the concentration of carbon dioxide and ammonia may become elevated while oxygen may become less abundant. In seawater ammonia exists in equilibrium between free ammonia (NH_3) and ammonium (NH_4^+). Total ammonia is the sum of the two. As the temperature or pH of seawater increases, ammonium changes to toxic, free ammonia (Bower & Bidwell, 1978). Carbon dioxide may also influence water quality by reducing the pH of the culture water (Sanni & Forsberg, 1996). Furthermore, nitrifying bacteria in the culture environment may convert ammonia to nitrite, increasing the concentration of nitrite in the abalones environment (Tal et al., 2003). These changes to the abalones' environment may have an affect on their health as oxygen (Harris et al., 1999a), free ammonia (Harris et al., 1998a; Basuyaux & Mathieu, 1999; Reddy-Lopata et al., 2006), pH (Harris et al., 1999b) and nitrite (Basuyaux & Mathieu, 1999; Harris et al., 1997) have been shown to affect the growth of abalone. Furthermore, particulate matter from feed and abalone

excretion, and the loss of nutrients from feed and solid waste into dissolved forms may alter water quality.

Thus, knowledge of water quality on abalone farms and the effect it has on the growth of *H. midae* is fundamental to good husbandry. Despite the industrial scale production of abalone in South Africa, which has been supported by a substantial research effort (Sales & Britz, 2001), few studies have investigated the metabolism of abalone or water quality on abalone farms. Barkai and Griffiths (1987) reported that the weight-specific oxygen consumption and ammonia production of wild *H. midae* decreases exponentially with increasing abalone size. The consumption of oxygen and production of ammonia appeared to increase linearly with increasing temperature (Lyon, 1995). Growth also increased linearly with increasing temperature between 12 – 20 °C (Britz et al., 1997). Samsukal (2004) conducted a preliminary investigation of nitrogenous waste in abalone farm effluent and identified elevated levels of total ammonia and nitrite. Reddy-Lopata et al. (2006) identified 9.8 – 16.4 µg FAN L^{-1} as the concentration range of free ammonia-nitrogen (NH_3-H) which caused 50 % mortality in 1 – 15 cm *H. midae*.

This work investigated (1) abalone growth and water quality at different positions within abalone tanks (2) diurnal changes in water quality (3) seasonal changes in the quality of abalone farm influent and effluent and (4) the variables which influenced the concentrations of ammonia and oxygen. The aims were to describe water quality dynamics under farm conditions and identify water quality variables that may affect abalone growth.

2.2 Materials and methods

Study site

Water quality and abalone growth were measured at HIK Abalone Farm (Pty) Ltd in Hermanus on the south-western coast of South Africa. The farm had a standing stock of approximately 110 – 126 tons. The abalone were grown to 20 – 120 g before sale although most were sold at sizes between 20 and 50 g (G. Johnston, HIK Abalone Farm Pty Ltd, pers. comm.). Abalone were weaned onto a formulated diet, Abfeed® (Marifeed Pty Ltd., South Africa; 34 % protein; 1.2 %

lipid), when less than 1 g in size. The abalone were grown in baskets, suspended within abalone tanks that received flow-through seawater at ambient temperature. The farm had a flow-rate per ton of standing stock (± standard deviation) of 6.9 ± 1.8 L s^{-1} t^{-1}. Water mixing within the tanks was improved by means of air-diffusers, positioned horizontally under the baskets. Sludge accumulated in the bottom of these tanks and was removed approximately every 10 days.

Statistical analyses

The significance level used in all statistical tests for this study was 5 %. When analysis of variance (ANOVA) was used the assumptions were verified using Levene's Test for homogeneity of variance and the Shapiro-Wilk *W*-test for normality. If the assumptions were not met a non-parametric test was used. Unless stated otherwise, the symbol ± indicates standard error.

Experiment 1: Abalone growth

The growth of marked abalone and water quality were monitored in baskets, placed sequentially between the inflows and outflows of four farm tanks (L 3.9 m, W 0.85 m, D 0.65m), over 104 days. Flow-through seawater entered the tanks at one end and flowed out the opposite end. Each tank held six oyster-mesh baskets, each of which was stocked with 7.4 ± 0.3 kg of 20 - 30 g abalone. The baskets in each tank were numbered 1 – 6, with 1 being at the inflow (control) and 6 nearest the outflow. Ten abalone (initial weight 23.7 ± 0.2 g) per basket were tagged on the shell with discs (two cm in diameter and two mm deep) of epoxy putty (Pratley's Putty®). The discs were uniquely marked with one to ten dots, to identify individual abalone. Maximum shell length (0.01 mm) was measured using digital vernier callipers and whole body weight (0.1 g) was measured, before and after tagging, using an electronic balance.

The temperature, oxygen concentration, total-ammonia concentration, free ammonia concentration and pH of the seawater in each basket was recorded approximately every 10 days. The concentration of nitrite was recorded in baskets 1, 4 and 6 at the same time as the other water quality parameters. The methods used to measure these variables remained the same in the other

experiments. The pH was measured using a pH meter (YSI Inc. Model # 60/10 FT; Yellow Springs, Ohio). Since the log-scale of pH did not meet the assumptions of many statistical tests and did not allow easily understandable reporting of changes in acidity or basicity, pH values were converted to the concentration of H^+ ions where relevant (nmol H^+ L^{-1} = [1 / 10^{pH}] X 10^9; Covington et al., 1985). Temperature and oxygen concentration were measured using an electronic meter (YSI Inc. Model # 55D; Yellow Springs, Ohio). Samples were analysed for total ammonia-nitrogen TAN ($NH_{3\text{-}4}$-N) and nitrite using a biochrom Novaspec II spectrophotometer and the phenolhypochlorite method of Solorzano (1969) and Merck nitrite test kits (Cat. no. 1.14776.0001) respectively. The samples were kept dark once the reagents were added. The absorbencies of colours obtained on the spectrophotometer were converted to concentrations of total ammonia or nitrite using relationships developed with standard solutions (n = 30, $r^2 > 0.96$). The percentage of TAN in the form of free ammonia-nitrogen (FAN) and consequently FAN concentration were calculated using the recorded pH, temperature and TAN values (Bower & Bidwell, 1978).

Abalone were fed Abfeed S34® to apparent satiation (monitored the following day) each afternoon. Every three days at mid-tide the flow-rate in each tank was set to an exchange rate of 100 % of the tank volume every 3.5 h^{-1}. The flow-rate of each tank was recorded as the time taken to fill a two L container and converted to L s^{-1}. Flow-rates fluctuated with changing tidal height. Tanks were cleaned approximately every 17 ± 7 days.

After 104 days, i.e. the average period that South African farmers wait before reducing the stocking density in a tank, the tagged abalone were again weighed and measured. Specific growth rate of marked abalone was calculated using the equation,

$$SGR = 100\ [\ln(Wf) - \ln(Wi)] / d \qquad (2.1)$$

where, *SGR* is specific growth rate (% BW d^{-1}), *Wf* is the final weight minus the weight of the tag, *Wi* is the weight prior to tagging and *d* is number of days.

Length gain ([final length – initial length]/experimental period in days) and condition factor (CF = 5575 X [weight (g)/length (mm)$^{2.99}$]; Britz, 1996) of marked abalone were calculated for all basket positions. Feed conversion ratios (FCR = dry weight feed consumed/wet weight gain) of individual baskets of abalone were calculated using the total weight of abalone (± 5 g) in the basket at the start and end of the experimental period.

The SGR, length gain and CF of abalone in individual baskets were each averaged before statistical analysis so that baskets, and not abalone, were used as replicates. The SGR of abalone in different positions were compared using analysis of covariance (ANCOVA), with initial weight as a co-variate. Length gain and FCR of abalone in different positions were compared using analysis of variance (ANOVA). CF at the start and end of the experiment were compared amongst different positions using repeated-measures ANOVA (R-M ANOVA). Regression analysis was used to test whether there was a change in temperature or the concentrations of H^+ ions, oxygen, free ammonia or nitrite in baskets between the tank inflow and outflow.

Experiment 2: Diurnal water quality dynamics

Temperature, pH, TAN and oxygen were recorded at the inflow and outflow of abalone tanks every three h over two 24 h periods (total n = 8 per sample time). Abalone biomass in the tanks averaged (± standard deviation S.D.) 109 ± 0.54 kg and the mean live abalone size was 34 ± 4.9 g, The flow-rate of the tanks averaged (± S.D.) 0.78 ± 0.26 L s^{-1}, while the mean temperature was 15.4 ± 0.7 °C. Although the flow-rate and temperature in the tanks at each sample time were adjusted for, they did not vary diurnally and therefore were not confounding factors (Kruskal-Wallis ANOVA; P = 0.96 and P = 0.66 respectively).

The production (net) of TAN and H^+ ions by tanks or the farm were calculated using the equation,

$$P = [(C_o - C_i) \text{ X } F] / B \qquad (2.2)$$

where: P is production (in weight per second per weight of biomass), C_o and C_i are concentration in the outflow and inflow water (weight L^{-1}), F is flow-rate ($L\ s^{-1}$) and B is live, whole weight of abalone biomass.

Oxygen consumption O_c ($mg\ s^{-1}\ kg^{-1}$) was calculated using the equation,

$$O_c = [(S_T - C_o)\ X\ F] / B \qquad (2.3)$$

where: S_T is solubility of oxygen in seawater of temperature T ($mg\ L^{-1}$; Weiss, 1970), T is temperature of the outflow water, C_o is oxygen concentration in the effluent water ($mg\ L^{-1}$), F is flow-rate ($L\ s^{-1}$) and B is abalone biomass (kg). The oxygen saturation of the influent seawater did not differ diurnally (108 ± 0.4 %, Kruskal-Wallis ANOVA, $P = 0.8$) and therefore was not a confounding factor.

Oxygen consumption in the tanks and TAN production at the various sample times were compared using analysis of covariance (ANCOVA) with temperature as a covariate. Kruskal-Wallis ANOVA was used to compare H^+ ion production within the tanks, and the H^+ ion concentration and oxygen concentration of the influent over the diurnal period. Total ammonia concentration of the influent over the diurnal period was compared using ANOVA.

Experiment 3: Abalone farm influent and effluent quality

The temperature, pH, oxygen saturation and concentration of oxygen, TAN, FAN, nitrite and suspended solids of the farm influent and effluent were sampled over a period of twelve months (June ’06 – May ’07). Ten samples, half collected at 09h00 and the remainder at 16h00, were taken each month. Oxygen saturation was calculated as a percentage of oxygen solubility at the observed temperature (Weiss, 1970). Suspended solids, larger than 10 µm, were filtered from five L of water and collected on pre-dried and weighed filter papers. The concentration of suspended solids ($mg\ L^{-1}$) was subsequently calculated as the dry weight gain of the filter paper divided by the volume of the water sample. The net production of TAN, nitrite, H^+ ions and suspended solids by the farm was calculated using equation 2.2. Total flow-rate of the farm was determined by

recording the height of effluent in the channel, which carries effluent to the sea, and converting this to volume of flow ($m^3\ s^{-1}$) using a height-flow curve ($n = 12$, $r^2 = 0.98$). The flow-height curve was developed using a flowmeter (Marsh-McBirney Inc. Model 2000; Frederick, Maryland) and the '0.2, 0.4, 0.8 of Depth Method',

$$F = [[((V_{0.2} + V_{0.8}) / 2) + V_{0.4}] / 2] \text{ X } d \text{ X } w \qquad (2.4)$$

where; F is flow-rate ($m^3\ s^{-1}$); $V_{0.2}$, $V_{0.4}$ and $V_{0.8}$ are flow velocity at 0.2, 0.4 and 0.8 of depth, from the channel bottom, ($m\ s^{-1}$); d is depth of effluent (m) and w is width of the effluent channel (m) (Marsh-McBirney Inc., 1990).

Regression analysis was used to determine whether the concentrations of oxygen, H^+ ions, suspended solids, nitrite or total ammonia in the influent were related to influent temperature. Influent quality in different months was compared using Kruskal-Wallis ANOVA. Farm biomass and flow-rate changed over time and therefore changes in effluent quality were not analysed according to season. Water quality variables were analysed for differences between the 09h00 and 16h00 sampling times.

Multiple-linear regression was used to relate effluent oxygen concentration ($mg\ L^{-1}$) to the farm's flow-rate ($L\ s^{-1}\ t^{-1}$) and the effluent's temperature (°C). The biomass of the farm was multiplied by the temperature of the farm's effluent since a linear relationship had been reported between oxygen consumption and temperature for *H. midae* within 16 – 20 °C (Lyon, 1995). Temperature was included separately from flow-rate in the equation as it influences the solubility of oxygen in seawater (Weiss, 1970).

Experiment 4: Predicting ammonia dynamics in tanks

Temperature, abalone size, the number of days since the tank was last cleaned and the flow-rate were recorded while monitoring ammonia concentrations at the inflow and outflow of abalone tanks (Table 2.1). These data were subsequently modelled using multiple-linear regression to develop two equations for the prediction of total ammonia concentration and production in tanks

under different conditions. Abalone size, W (g) was converted to weight-specific rate of ammonia excretion, E (µg TAN kg^{-1} h^{-1} = $0.856W^{-0.57}$), using the relationship between ammonia excretion and size at 14 °C determined by Barkai and Griffiths (1987). The values for E were multiplied by temperature since the relationship between ammonia excretion and temperature is linear within 16 – 20 °C (Lyon, 1995).

Table 2.1. Experimental conditions in *Haliotis midae* tanks from which data were collected for developing equations that predict total ammonia-nitrogen (TAN) concentration and production in abalone tanks (n = 125).

	Mean	min	max
Flow (L s^{-1} kg^{-1})	0.0070	0.0041	0.0108
Temperature (°C)	16.1	14.3	19.3
Size (g)	31	18	43
Uncleaned (d)	12	1	27
TAN (µg L^{-1})	65	23	146
TAN production (µg s^{-1} kg^{-1})	0.29	0.07	0.86

2.3 Results

Experiment 1: Abalone growth

The SGR of abalone in baskets 4 – 6 was slower than that of abalone in position 1 (ANCOVA, P = 0.0007, Fig. 2.1). Length gain in baskets 5 and 6 was also slower than that in the control (ANOVA, P = 0.04). Mean FCR (1.23 ± 0.03) of abalone in baskets 2 - 6 did not differ from that in basket 1 (ANOVA, P = 0.67). There was also no difference in initial (1.00 ± 0.01) or final (1.04 ± 0.01) condition factor of abalone in other baskets, relative to basket 1 (R-M ANOVA, P = 0.34).

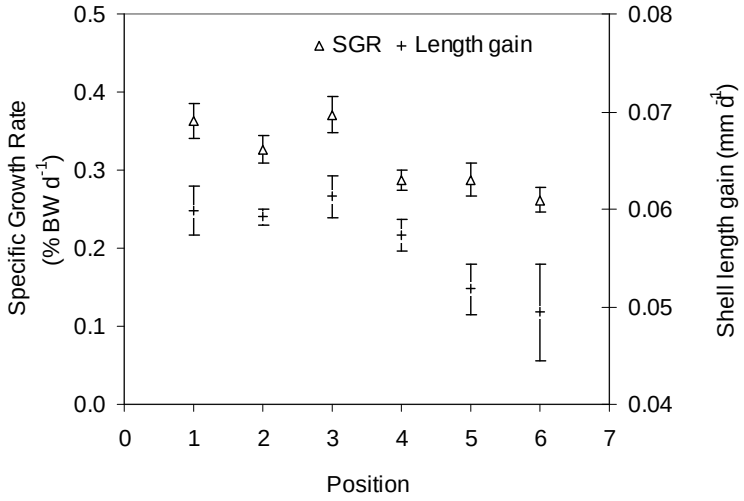


Fig. 2.1. Specific growth rate (SGR) and length gain of individually marked *Haliotis midae* (n = 10 basket^{-1}) in baskets positioned in a line between the inflow (position 1) and outflow (position 6) of four tanks. For each position the mean of 4 baskets (± standard error) is presented.

Flow-rates averaged (± standard deviation) 0.178 ± 0.042 L s^{-1} at the water quality sampling times (i.e. 14.5 and 9.7 L kg^{-1} h^{-1} at the initial and final stocking densities). The temperature was not significantly different in the various basket positions (Regression, $P = 0.22$). Oxygen concentration was highest in baskets nearest the inflow, decreasing steadily towards the outflow (Fig. 2.2; Regression, $P < 0.0001$). In basket 1 the lowest 50 % of recorded oxygen concentrations were between 6.1 – 7.1 mg L^{-1} while at the outflow the lowest 50 % were between 5.5 – 6.7 mg L^{-1}. H^+ concentrations increased from the inflow to the outflow (Regression, $P < 0.0001$). While the median free ammonia concentration increased from baskets situated at the inflow to those at the outflow, regression analysis revealed this to be non-significant at the 95 % confidence level (Fig. 2.3, $P = 0.07$). Similarly, the change in nitrite concentration between position 1, 4 and 6 was not significant at the 95 % confidence level ($P = 0.09$).

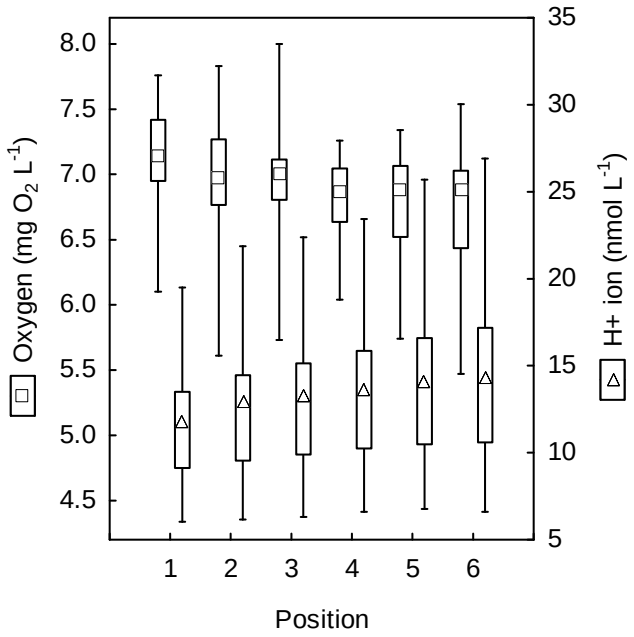


Fig. 2.2. Oxygen (n = 36) and H^+ ion (n = 56) concentrations measured, in baskets positioned in a line between the inflow (position 1) and outflow (position 6) of four tanks, approximately every ten days over 104 days. Indicated are the median, 25 - 75 percentiles and range. Regression analysis showed that oxygen concentration decreased from position 1 - 6 ($y = 7.17 – 0.082x$; $P < 0.0001$), while H^+ ion concentration increased ($y = 11.34 + 0.546x$; $P < 0.0001$).

Experiment 2: Diurnal water quality dynamics

There was no significant diurnal variation in the influent oxygen (8.69 ± 0.02 mg L^{-1}, ANCOVA, $P = 0.73$) and H^+ ion (11.75 ± 0.31 nmol H^+ L^{-1}, Kruskal-Wallis ANOVA, $P = 0.18$) concentration. Influent TAN concentration (19.59 ± 0.45 µg TAN L^{-1}) differed diurnally (ANOVA, $P = 0.002$), however, the difference between the minimum of 16.6 ± 0.8 µg L^{-1} at 16h00 and the maximum of 23.9 µg L^{-1} at 13h00 was negligible. TAN production did not differ diurnally with an overall mean of 0.178 ± 0.007 µg s^{-1} kg^{-1} (ANCOVA, $P = 0.17$). Oxygen consumption in tanks followed a diurnal pattern of greater consumption at night than during the day (Fig. 2.4, ANCOVA, $P = 0.03$).

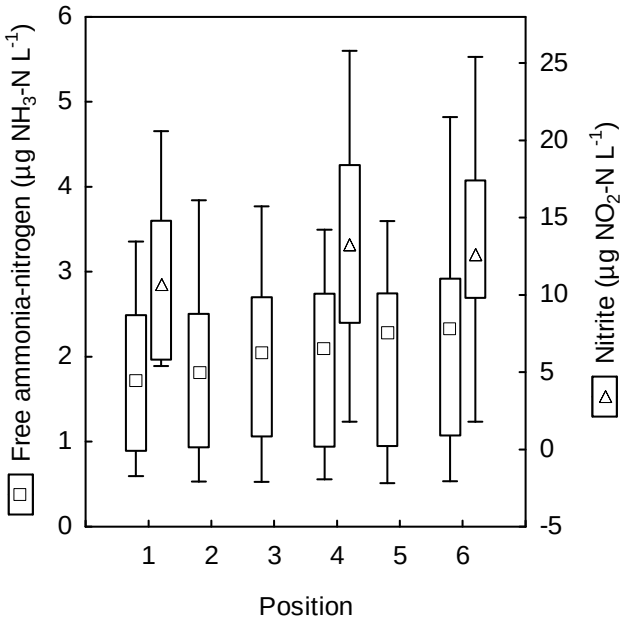


Fig. 2.3. Free ammonia-nitrogen (n = 46) and nitrite (n = 36) concentrations in baskets positioned in a line between the inflow (position 1) and outflow (position 6) of four tanks measured at ten day intervals over a 104 day period. Presented are the median, 25 - 75 percentiles and range. Change in free ammonia and nitrite concentration between position 1 – 6 was not statistically significant at the 95 % confidence level (Regression, $P = 0.07$ and $P = 0.09$ respectively).

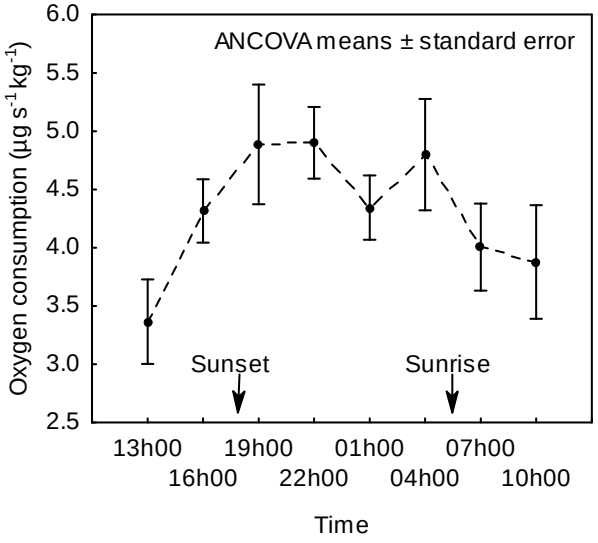


Fig. 2.4. Diurnal variation in net oxygen consumption in *Haliotis midae* tanks (n = 8).

Furthermore, the production of H^+ ions followed a similar diurnal pattern, increasing from 16h00 to a peak at 01h00 after which production decreased (Fig. 2.5). H^+ ion concentration at the outflow from tanks followed a similar pattern to production, increasing from 16h00 to a maximum at 04h00 after which concentrations were again lower at 07h00 and 10h00. Since pH is the negative logarithm of H^+ ion concentration (mol L^{-1}), pH at the outflow of the tanks was lower at 04h00 (pH 7.71) than at 16h00 (pH 7.88).

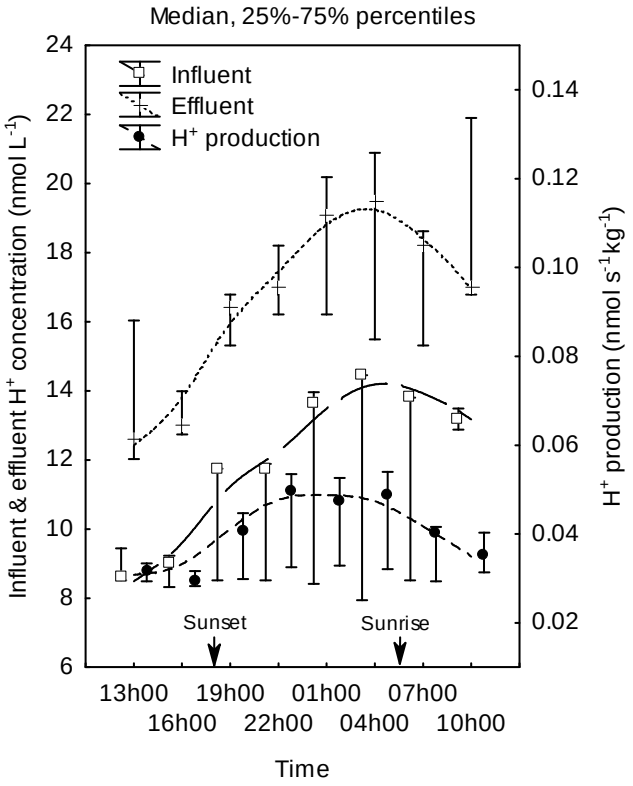


Fig. 2.5. Diurnal variation in influent and effluent H^+ ion concentration and H^+ ion production in *Haliotis midae* tanks (n = 8). Values are the median and 25 and 75 percentiles. H^+ ion production increased from 0.029 nmol s^{-1} kg^{-1} $tank^{-1}$ at 16h00 to 0.047 nmol s^{-1} kg^{-1} $tank^{-1}$ at 01h00 after which it decreased again, while H^+ concentration increased from 13.03 nmol L^{-1} (pH 7.88) at 16h00 to 19.50 nmol L^{-1} (pH 7.71) at 04h00.

Experiment 3: Abalone farm influent and effluent quality

Influent oxygen concentration was negatively correlated to influent temperature (Fig. 2.6; r^2 =

0.74) and thus monthly changes in temperature caused corresponding changes in oxygen concentration. The oxygen saturation of influent averaged 99.6 ± 0.67 %, with minimum and maximum values of 90.8 and 106 %. H^+ ion concentration of the influent was, to a small extent, negatively correlated to influent temperature ($r^2 = 0.13$, $P < 0.0001$).

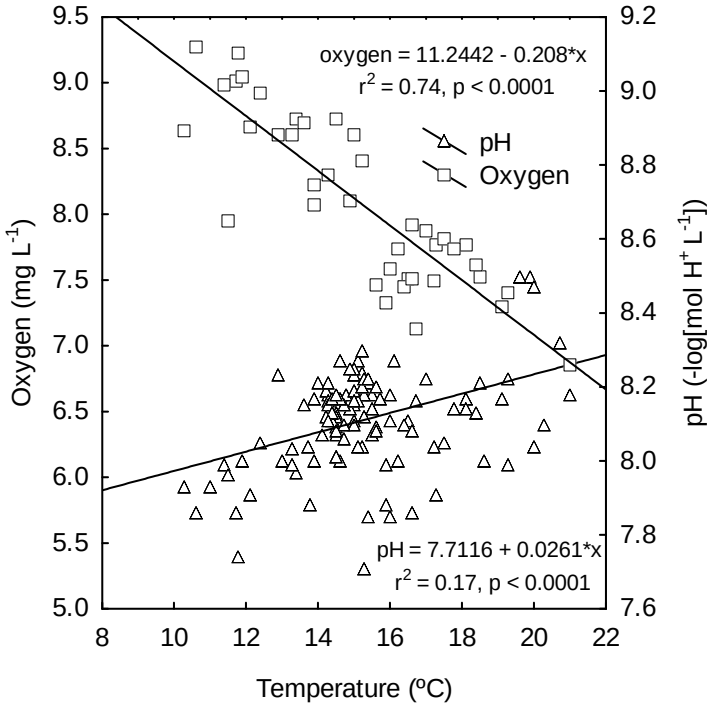


Fig. 2.6. Farm influent oxygen concentration (n = 40) and pH (n = 118) in relation to influent temperature.

The pH and free ammonia concentration of influent was lower at 09h00 than at 16h00 (Table 2.2). No differences in the temperature or concentration of oxygen, total ammonia or suspended solids in influent was detected between the morning and afternoon sample times ($P > 0.5$).

The pH, total ammonia concentration and concentration of suspended solids in influent all exhibited monthly variation (Table 2.3). Nitrite and free ammonia concentration did not vary monthly (Kruskal-Wallis ANOVA, $P > 0.05$). Concentrations of total ammonia, suspended solids and nitrite in influent were not correlated to influent temperature ($P > 0.05$).

In all cases the effluent oxygen concentration and pH were lower than that of the farm's influent while total ammonia and free ammonia concentrations were higher (Table 2.2). The pH, temperature and free ammonia concentration of the effluent were lower at 09h00 than at 16h00, while concentrations of suspended solids and oxygen were higher in the morning samples ($P < 0.05$; Table 2.2).

Production of H^+ ions by the farm as a unit averaged 26.71 ± 1.53 nmol s^{-1} t^{-1} $farm^{-1}$ (Table 2.2). Production of H^+ ions was positively correlated with the temperature of the effluent ($y = 4.69x + 4.70$; $P < 0.0001$, $r^2 = 0.35$). Neither production of suspended solids nor nitrite by the farm was correlated with temperature ($P > 0.05$). Production of suspended solids was greater at 09h00 than at 16h00.

The oxygen concentration of the farm effluent was modelled in relation to the farm biomass, flow rate and the effluent's temperature using the equation,

$$O_e = D - GT - KBT / F \qquad (n = 40;\ r^2 = 0.69)\ (2.5)$$

where O_e is predicted effluent oxygen concentration (mg L^{-1}), T is effluent temperature (°C), B is the farm biomass (t), F is the farm flow-rate (L s^{-1}) and D, G and K are the constants (± standard error) 10.672 ± 0.39, 0.157 ± 0.03 and 0.556 ± 0.17 respectively.

Table 2.2. Mean and standard error values for water quality parameters measured at 09h00 and 16h00 at a *Haliotis midae* farm over 12 months. pH data were converted to H^+ ion concentrations before the means and standard errors were calculated. Variables were compared between the two sample times using either Students *t*-test[t] or the Mann-Whitney *U* Test[u]. Differences were considered significant at $P < 0.05$.

	09h00		16h00			
	mean	st. err.	mean	st. err.	*P*-value	
Inflow						
Temperature	14.91	0.25	15.61	0.26	0.07	t
pH	8.06	8.04 - 8.08	8.13	8.12 - 8.15	0.02	u
Oxygen (mg O_2 L^{-1})	8.17	0.07	8.16	0.09	0.96	t
Total ammonia (µg TAN L^{-1})	18.23	0.75	18.26	1.14	0.98	u
Free Ammonia (µg FAN L^{-1})	0.46	0.03	0.57	0.04	0.04	t
Nitrite (µg NO_2-N L^{-1})	3.44	0.32	5.18	0.40	<0.001	t
Suspended solids (mg L^{-1})	3.93	0.47	3.39	0.45	0.43	t
Outflow						
Temperature	15.04	0.28	15.94	0.26	0.01	t
pH	7.90	7.89 - 7.92	7.95	7.94 - 7.96	0.01	u
Oxygen (mg O_2 L^{-1})	7.42	0.07	7.2	0.07	0.03	t
Total ammonia (µg TAN L^{-1})	55.62	2.65	60.98	3.36	0.21	t
Free Ammonia (µg FAN L^{-1})	0.98	0.07	1.32	0.11	0.01	u
Nitrite (µg NO_2-N L^{-1})	6.76	0.39	8.71	0.46	0.001	t
Suspended solids (mg L^{-1})	8.36	0.87	5.03	0.52	0.02	u
Production						
H^+ ion (nmol s^{-1} t^{-1} $farm^{-1}$)	27.18	2.18	26.19	2.15	0.75	t
Total ammonia (µg TAN s^{-1} kg^{-1})	0.27	0.02	0.29	0.03	0.50	t
Nitrite (µg NO_2-N s^{-1} kg^{-1})	0.023	0.002	0.024	0.003	0.74	t
Suspended solids (µg s^{-1} kg^{-1})	25.05	5.73	6.89	4.79	0.006	u

Table 2.3. Mean (± standard error) monthly pH, and concentration of total ammonia-nitrogen (TAN) and suspended solids (SS) measured in the influent of a *Haliotis midae* farm over 12 months (n = 10 month^{-1}). Data for November are not presented as only three samples were taken. The pH values were converted to H^+ ion concentrations before the means and standard errors were calculated and before analysis. Means, within each column, which do not share a common superscript were significantly different (Kruskal-Wallis ANOVA, $P < 0.05$).

Month	pH	TAN (µg L^{-1})	SS (mg L^{-1})
Jun	8.18 (8.17 - 8.19)^{a}	14.92 ± 1.42^{ab}	4.64 ± 1.04^{ab}
Jul	8.17 (8.15 - 8.18)^{a}	11.77 ± 0.65^{b}	5.14 ± 1.15^{a}
Aug	8.15 (8.14 - 8.17)^{ab}	15.13 ± 0.99^{abc}	5.20 ± 3.33^{ab}
Sep	8.10 (8.09 - 8.12)^{ab}	17.40 ± 2.46^{abc}	3.39 ± 0.51^{ab}
Oct	8.08 (8.03 - 8.12)^{ab}	18.04 ± 1.88^{abc}	3.01 ± 0.27^{ab}
Dec	8.07 (8.06 - 8.08)^{ab}	20.74 ± 1.84^{ac}	2.03 ± 0.57^{ab}
Jan	8.01 (7.98 - 8.04)^{b}	21.51 ± 2.43^{ac}	1.01 ± 0.25^{b}
Feb	8.15 (8.13 - 8.17)^{ab}	15.90 ± 1.34^{abc}	2.52 ± 0.98^{ab}
Mar	8.00 (7.95 - 8.06)^{ab}	21.60 ± 1.55^{ac}	3.76 ± 0.72^{ab}
Apr	8.09 (8.06 - 8.12)^{ab}	25.60 ± 1.38^{c}	4.95 ± 1.23^{a}
May	7.99 (7.96 - 8.03)^{b}	26.97 ± 3.06^{c}	3.81 ± 0.57^{ab}

Experiment 4: Predicting ammonia dynamics in tanks

Observed total ammonia concentration at the outflow of *H. midae* tanks was related to temperature, flow rate, body weight and the length of time since the tanks were last cleaned; and total ammonia production in *H*. midae tanks was related to temperature, body weight and length of time since last cleaned by the equations:

$$A_o = (0.216EBT + 0.368U) / F - 11.92 \quad (n = 125, r^2 = 0.80) \quad (2.6)$$

$$A_p = 0.323ET + 0.003U - 0.414 \quad (n = 125; r^2 = 0.81) \quad (2.7)$$

$$E = 0.856W^{-0.57} \quad \text{(after Barkai and Griffiths, 1987)} \quad (2.8)$$

where, A_o is outflow total ammonia concentration (µg TAN L^{-1}), E is total ammonia excretion rate by individual abalone (µg TAN kg^{-1} h^{-1}), B is abalone biomass (kg tank^{-1}), T is effluent temperature (°C), U is time since the tank was last cleaned (d), F is flow rate (L s^{-1}), A_p is total

ammonia production (µg TAN s^{-1} kg^{-1}) and *W* is mean, whole, body weight of *H. midae* (g). Observed values of A_p were calculated using equation 2.2.

2.4 Discussion

Experiment 1: Abalone growth

Reductions in abalone growth were observed within the range of concentrations of oxygen, H^+ ions and free ammonia recorded in the current study. The extent to which these growth reductions occurred was correlated to the extent to which oxygen concentration, H^+ ion concentration and FAN concentration were altered relative to levels in the influent. Oxygen concentrations in the present study often fell below the 7.36 mg L^{-1} level at which growth of the greenlip abalone *Haliotis laevigata* is reduced (Harris et al., 1999a). Furthermore, 75 % of observed H^+ ion values nearest the tank outflow were higher than the level that affects the growth of the blacklip abalone *Haliotis rubra* (pH 7.93; 11.7 nmol H^+ L^{-1}) and 25% where above the level that affects the growth of *H. laevigata* (pH 7.78; 16.6 nmol H^+ L^{-1}) (Harris et al., 1999b). The concentration of free ammonia which affects the growth of *H. midae* has not been established so it is uncertain whether free ammonia in the range recorded affects growth.

In order to obtain a measurable change in abalone growth within the tanks the flow rates in the current study (100 % exchange every 3.5 h) were purposely set slightly lower than the industry standard (100 % exchange every 3.0 h). Thus, exposure to altered oxygen, H^+ ion concentration and free ammonia, beyond the range measured in the current study, is unlikely on a South African abalone farm on which abalone are fed a formulated diet at temperatures within the range recorded in this study. Therefore, it is recommended that future studies testing the effect of oxygen, H^+ ion concentration and free ammonia on growth of *H. midae*, include the concentration ranges observed in the current study. In the interim, as a precautionary measure, flow-rates should be kept above the level of 9.7 – 14.5 L kg^{-1} h^{-1}, used in this study.

Experiment 2: Diurnal water quality dynamics

There was a net consumption of oxygen and production of H^+ ions and ammonia, within abalone tanks. This can be explained by the aerobic metabolism of abalone and their excretion of nitrogen as ammonia (Bishop et al., 1983; Barkai & Griffiths, 1987). In seawater, H^+ ions result from aerobic metabolism, as the carbon dioxide produced bonds with a hydrogen and oxygen atom from the water molecule, forming bicarbonate $HCO3^-$ and a H^+ ion (Sanni & Forsberg, 1996).

The diurnal pattern of greater oxygen consumption and H^+ ion production in tanks during hours of darkness indicates an increase in metabolic rate. Increased abalone metabolic activity at night is supported by Uki and Kikuchi (1975) who determined that the metabolic rate of *Haliotis discus hannai* is elevated between dusk and midnight. It is unlikely that changes in digestion or protein catabolism caused the fluctuation in metabolic rate since gut fullness decreases in a roughly linear fashion, over a period of 18 – 24 h, once satiation is reached (Britz et al., 1996) and a change in protein catabolism would be indicated by a change in ammonia production (Bishop et al., 1983). Therefore, it is hypothesised that the increase in metabolic rate during hours of darkness is predominantly due to an increase in metabolic processes fuelling the activity associated with finding and consuming food. In the current study the cycle of respiration correlates with the diurnal pattern of feeding activity. Anecdotal observations suggest abalone in tanks move very little unless feeding. Activity can cause a substantial increase in oxygen consumption in molluscs, as demonstrated by Donovan and Carefoot (1997) who found that mass-specific rate of oxygen consumption (Vo_2) in *Haliotis kamtschatkana* increased from 20.7 $\mu L\ O_2\ g^{-1}\ h^{-1}$ when resting to a minimum of 40.1 $\mu L\ O_2\ g^{-1}\ h^{-1}$ when moving – a minimum increase of 90 %. Barkai and Griffiths (1987) observed no diurnal pattern in respiration, in *H. midae* removed from the wild at various stages of the diurnal feeding cycle. However, they suggested that nocturnal rates may have been reduced because the respirometers used were too small to allow natural locomotory activity.

Unless there are fluctuations in temperature, which affect oxygen solubility (Weiss, 1970) the concentration of oxygen in tanks should be lowest at night, since this is when oxygen consumption is highest. H^+ ion concentration was lowest in the afternoon, due to reduced H^+ ion

production within tanks and the lower concentration of H^+ ions in the seawater pumped ashore. This diurnal change in the pH of seawater is similar to that documented by Park et al., (1958) in coastal waters. Since no significant diurnal variation in total ammonia concentration within tanks was found, diurnal variations in pH and temperature alone determined the observed fluctuations in free ammonia concentrations. As the pH and/or temperature of water increases, the percentage of total ammonia which is in the free ammonia form increases (Bower & Bidwell, 1978). Thus ammonia toxicity will be highest in the afternoon, as pH is highest at this time, unless mitigated by lower temperatures. However, mitigation by lower temperature was not observed as comparison of the water samples taken at 09h00 and 16h00 over the course of 12 months showed temperature of farm effluent was on average 0.9 °C higher in the afternoon than in the morning. Free-ammonia measured in the morning and afternoon over the 12 month period corresponded to the cycle of free ammonia concentration identified by *Experiment 2*, as concentrations were significantly higher (32 %) in the afternoon.

Experiment 3: Abalone farm influent and effluent quality

Concentrations of total ammonia and nitrite in the influent, monitored over 12 months, were similar to those recorded in other coastal waters (Cooper, 1933; Redfield & Keys, 1938; Horrigan et al., 1990). Seasonal variations in oxygen concentration conformed to the relationships between salinity, temperature and oxygen solubility reported by Weiss (1970). The influent was typically saturated with oxygen and therefore the oxygen concentration was lowest when temperatures where highest, i.e. 6.9 mg O_2 L^{-1} at 21 °C. The pH of influent was positively related to temperature and consistently fell within the normal range of 7.5 – 8.4 reported by Poxton and Allouse (1982). The relationship between influent pH and temperature can be explained by upwelling. Seawater with a low temperature is periodically brought to the surface off the southern cape coast from the deep-sea by upwelling (Shannon et al., 1983; Demarcq et al., 2003). Due to changes in biological processes, seawater in general becomes more acidic with increasing distance from the surface (Atkins, 1922; Park, 1968) and therefore, the cold upwelled seawater will have a lower pH (Park, 1968; Copin-Montégut & Raimbault, 1994).

The changes in the concentrations of oxygen, H^+ ions and ammonia between the farm inflow and outflow can be explained by the aerobic metabolism of abalone and the excretion of waste nitrogen as ammonia (Bishop et al., 1983; Barkai & Griffiths, 1987). The higher concentration of suspended solids in the farm effluent at 09h00, in comparison to 16h00, was caused by a higher production of solids by the farm. Solid waste, which accumulates at the bottom of tanks, was flushed out in the effluent when the tanks were cleaned and this may explain the discrepancy between the morning and afternoon samples. Tanks were cleaned between 08h00 – 15h30. Therefore, the effluent at 16h00 would have contained no solid waste released from the tanks during cleaning. The temperature, pH and free ammonia concentration of the afternoon samples were higher than those of the morning samples, which confirmed the conclusions drawn from the results of *Experiment 2*.

The oxygen concentrations of the farm effluent, predicted by equation 2.5, were highly correlated with the observed values as indicated by a high r^2-value (0.69). Higher temperatures resulted in lower oxygen concentrations, as expected based on the negative effect of temperature on the solubility of oxygen in seawater (Weiss, 1970) and its positive effect on the oxygen consumption of abalone (Uki & Kikuchi, 1975; Lyon, 1995).

Experiment 4: Predicting ammonia dynamics in tanks

The ammonia concentration and production in tanks, predicted by equations 2.6 and 2.7, were similar to the observed values ($r^2 \geq 0.8$). Thus, these equations appear to be valid, at least within the range of conditions under which the data were collected. These equations may require modification for accurate use on abalone farms in different locations or with different management methods. For example, farms with a higher input of solids through their influent may find that the ammonia equations under-estimate the accumulation of sludge over time and consequently the amount of ammonia produced. Differing levels of algal growth within tanks would affect the uptake of ammonia (Neori et al., 2004) thus affecting the accuracy of the equations. However, the basic relationships described by the equations should hold for other abalone farms feeding a formulated diet. For example, the oxygen concentration of the effluent will still increase with an increase in flow-rate and decrease with an increase in temperature,

although the strength of these relationships may differ. The equations should not be used to predict ammonia or oxygen dynamics on abalone farms which feed their abalone kelp *Ecklonia maxima* Osbeck. Kelp has a lower protein content than formulated diets (Troell et al., 2006) which may affect the abalones' rate of ammonia excretion. Furthermore, the metabolism of kelp will affect the levels of dissolved oxygen and nitrogenous waste in the tanks (Neori et al., 2004). However, the accuracy of the equations indicates that it is feasible for each farm to develop similar equations for the prediction of key water quality parameters. This would enable farms to make informed decisions about the effect of differing flow-rates, stocking densities, tank cleaning intervals and water temperatures on water quality.

In conclusion, ammonia and oxygen dynamics on abalone farms in South Africa that feed a formulated diet can be modelled successfully. This makes it possible to determine the appropriate flow-rate per unit of biomass to achieve desired threshold concentrations of oxygen or ammonia under different conditions of temperature and abalone size.

Chapter 3

The theoretical influence of three tank designs, used in abalone *Haliotis midae* culture, on water quality

3.1 Introduction

A few South African abalone *Haliotis midae* L. farms (e.g. HIK Abalone Farm Pty Ltd.; Roman Bay Sea Farm Pty Ltd.) have experimented with tank design in an attempt to improve water quality. On South African abalone farms seawater is pumped ashore and makes a single pass through abalone tanks before flowing by gravity back to sea. Typically, the seawater enters and leaves the tanks at opposite ends. In tanks of this design water quality becomes progressively altered between the inflow and outflow and abalone growth can be reduced nearer to the outflow (Chapter 2). Huchette et al. (2003) also reported that in serial-reuse raceways used to culture *Haliotis rubra* Leach, water quality and growth was negatively correlated with the number of abalone upstream. The tank designs that farmers have experimented with have divided the available water flow so that a portion of it enters the tank nearer to the outflow, thus 'improving water supply to the areas where growth is slowest'. One such design currently used in abalone culture has an inflow and outflow at opposing ends of the tank and another inflow in the middle of the tank (e.g. Roman Bay Sea Farm Pty Ltd.). Another design has two inflows situated at opposing ends of the tank and an outflow in the middle of the tank (e.g. HIK Abalone Farm Pty Ltd.). The additional plumbing these tanks require, in comparison to a tank with a single inlet, is a significant financial outlay (R. Krohn, HIK Abalone Farm Pty Ltd, pers. comm.). The carrying capacity of aquaculture systems is usually specified as the mass of fish per unit of water flow and the mass of fish per unit of water volume (Colt & Orwicz, 1991) However, tanks of these designs do not increase the flow-rate per unit of biomass (i.e. L s^{-1} kg^{-1} abalone) that the tank receives or decrease its stocking density, and the effect they have on water quality and abalone growth has not been investigated. Tanks, with a single inflow and outflow at opposing ends, are used to culture abalone (Hahn, 1989; Fallu, 1991) and other aquaculture species (Wheaton, 1977; Mayo, 1991). However, the use of tank's with an inflow and outflow at opposing ends and a second inflow in the middle, or two inflows situated at opposing ends and an outflow in the middle has not been documented.

This study investigated, in a theoretical manner, whether water quality in abalone tanks could be improved by dividing the available flow so that a portion of it enters the tank nearer to the outflow. This was done by modelling the oxygen concentration in three abalone tanks operated on three different scenarios of water flow: a) a single inflow and outflow, b) an inflow and outflow at opposing ends with an additional inflow in the middle of the tank and c) an inflow at each end and an outflow in the middle (Fig. 3.1). Oxygen was used as the water quality variable but this could be substituted by ammonia or another metabolite.

3.2 Materials and methods

For the purposes of the model it was assumed that the inflow water to the tanks contained 8.0 mg L^{-1} oxygen. The flow-rate per kg of abalone biomass was 0.2 L min^{-1} kg^{-1} for each tank and the abalone consumed oxygen at 0.4 mg min^{-1} kg^{-1}. All tanks contain six baskets, each stocked with 100 kg of abalone. These input parameters to the model have been rounded off to make the figures in the results easier to follow. However, the influent oxygen concentration used here is close to the mean of 8.16 ± 0.09 mg L^{-1} measured at a South African abalone farm (Table 2.2, Chapter 2). The oxygen consumption rate is taken from amongst the highest consumption rates recorded in *Experiment 2: Diurnal water quality dynamics* (Chapter 2). Abalone are commonly stocked at densities of 100 kg per basket. The flow-rate per kg of abalone biomass used (i.e. 0.2 L min^{-1} kg^{-1}) was approximately half that of the mean flow-rate per kg attained by HIK Abalone Farm (Pty) Ltd., a farm which has a relatively high flow-rate per kg in comparison to the rest of the abalone farming industry. It is unlikely that perfectly linear flow will occur between the inflow and outflow of tanks, while remaining homogenous in cross-section, but to simplify the model it was assumed that this perfect flow situation existed.

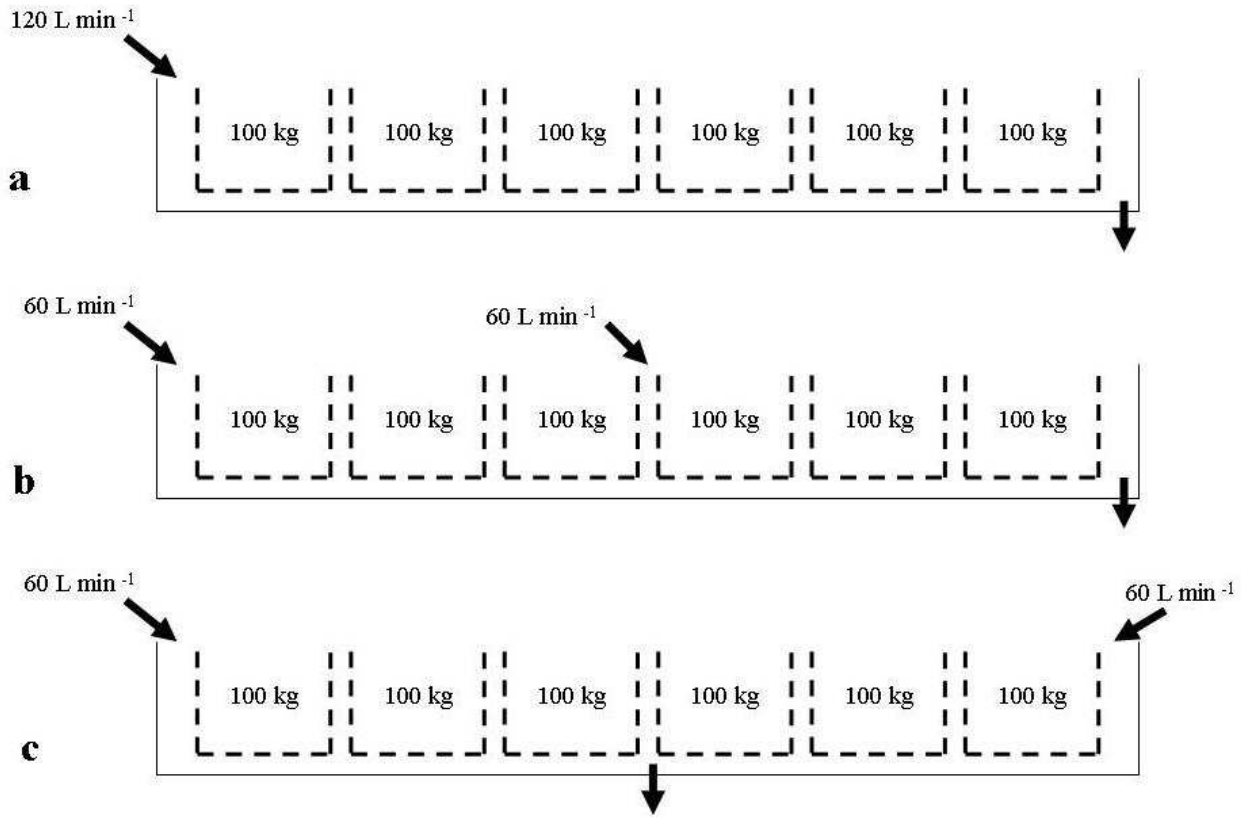


Fig. 3.1. Longitudinal section of three abalone tanks, each containing six oyster-mesh baskets with 100 kg of abalone per basket, and each with a flow-rate per kg of 0.2 L min^{-1} kg^{-1}, operated on three different scenarios: a) a single inflow and outflow, b) an inflow and outflow at opposing ends and an additional inflow in the middle and c) an inflow at either end and a central outflow

In each scenario the flow-rate per kg at the point of each basket in the tank, which can be used to assess the quality of the water at that point, was calculated as,

$$F\text{-}kg = F / Bp \tag{3.1}$$

where: *F-kg* is the flow-rate per kg of abalone biomass between the tank inflow and point *p* if the section between the tank inflow and the point *p* had to be considered as an individual unit, *F* is the flow-rate of the system (L min^{-1}) and B_p is the cumulative biomass (kg) between the inflow and point *p*.

The dissolved oxygen concentration (O_p) in mg L^{-1} at the point *p* of each basket within the tank was calculated as,

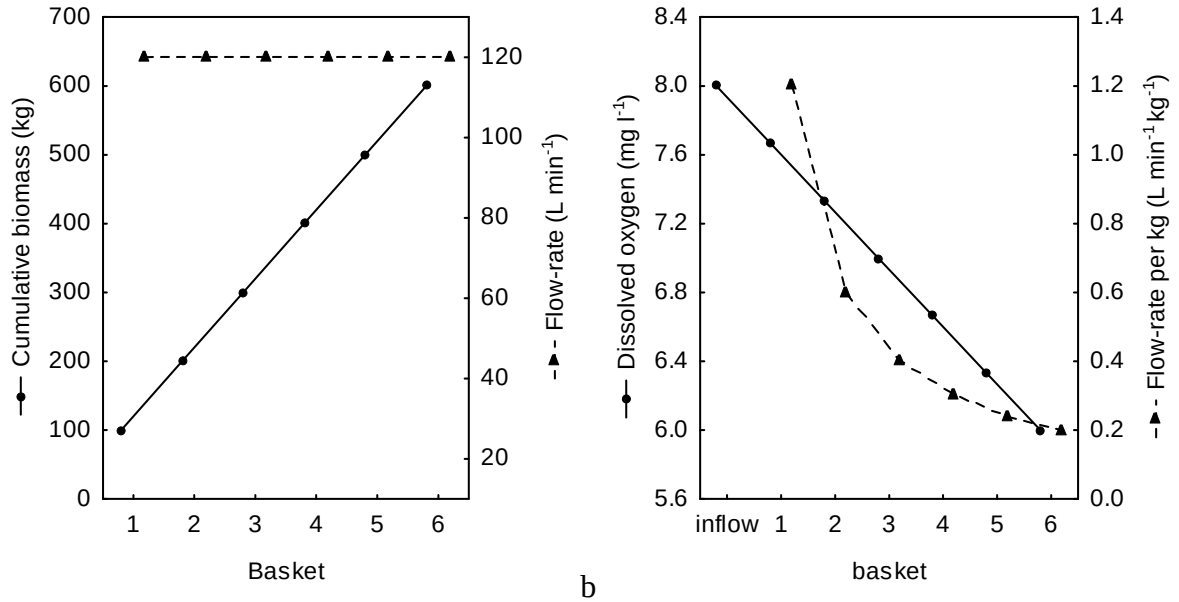
$$O_p = O_{inflow} - C/(F/Bp) \quad (3.2)$$

where: O_{inflow} is the oxygen concentration of the inflow water (8 mg L^{-1}), *C* is the oxygen consumption of the experimental abalone per kg (0.4 mg min^{-1} kg^{-1}), *F* is the flow-rate of the system (L min^{-1}) and B_p is the cumulative biomass (kg) between the inflow and point *p*.

3.3 Results and discussion

Scenario a) a single inflow and outflow

All available flow entered the tank at a single position so the flow-rate of 120 L min^{-1} flowed through all baskets. Therefore, the oxygen level at any point was affected only by the oxygen consumption of the cumulative biomass at that point. Since cumulative biomass increased linearly between the inflow and outflow a negative, linear gradient of oxygen concentration can be expected between the inflow and outflow (Fig. 3.2). Therefore, at any point within the tank dissolved oxygen was as high as possible given the flow-rate and cumulative biomass.

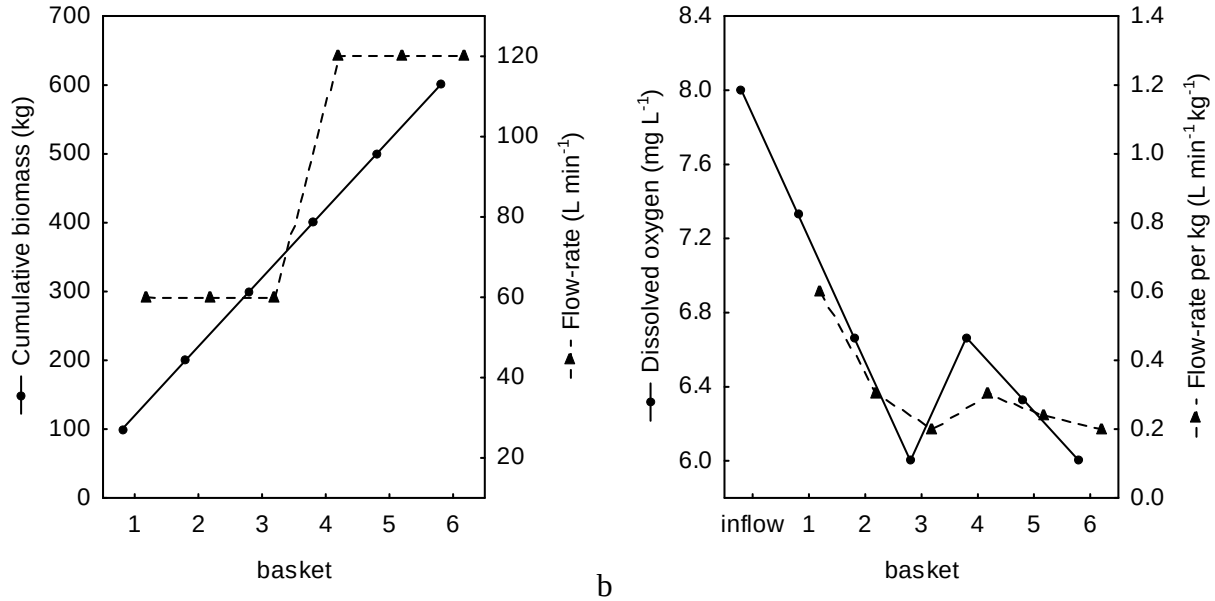


a b

Fig. 3.2. The cumulative biomass, flow-rate (a), flow-rate per kg and dissolved oxygen concentration (b) at different positions in a tank designed according to *scenario a*, i.e. a single inflow and outflow at opposite ends of the tank.

Scenario b) an inflow and outflow at opposing ends and an additional inflow in the middle

There was a linear increase in cumulative biomass down the length of the tank (Fig. 3.3). However, the flow-rate through baskets 1 - 3 was only 60 L min^{-1}. The remaining 60 L min^{-1} of available water flow entered the tank after basket 3 and thereafter the flow-rate through baskets 4 - 6 was the full 120 L min^{-1}. Consequently, the flow-rate per kg at baskets 1 - 3 (Fig. 3.3) was half that in baskets 1 - 3 in *scenario a* (Fig. 3.2). Therefore, relative to the inflow, oxygen concentration should decrease twice as fast. In *scenario a* dissolved oxygen concentration is reduced from 8.0 mg L^{-1} in the inflow water to 7.0 mg L^{-1} at basket 3, while in *scenario b* it is reduced to 6.0 mg L^{-1} at basket 3. Furthermore, *scenario b* had no advantage over *scenario a* at baskets 4 - 6, because at these points the flow-rate, cumulative biomass, flow-rate per kg, and hence oxygen concentration, were the same as in *scenario a*. Therefore, baskets 1 - 3 were unnecessarily exposed to low dissolved oxygen for no benefit at baskets 4 - 6. Furthermore, halving the flow-rate at baskets 1 – 3 may reduce water circulation. The modelled dissolved oxygen and flow-rate per kg showed that this approach would reduce water quality relative to a single inflow and outflow tank design.

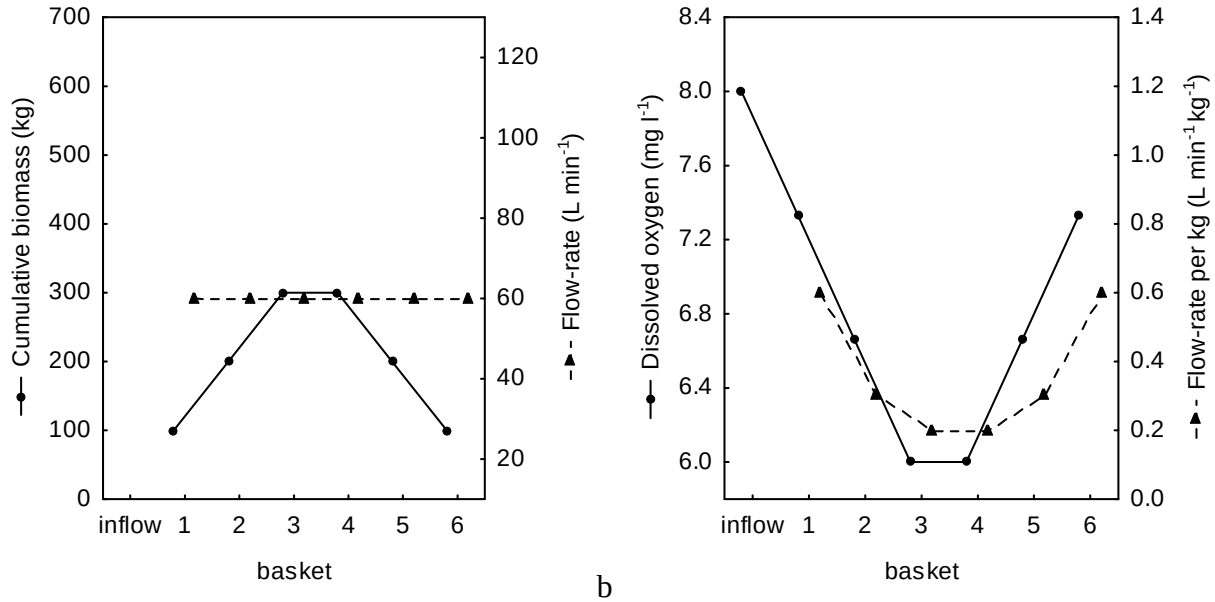


a b

Fig. 3.3. The cumulative biomass, flow-rate (a), flow-rate per kg and dissolved oxygen concentration (b) at different positions in a tank designed according to *scenario b*, i.e. an inflow and outflow at opposite ends of the tank and an additional inflow in the middle of the tank.

Scenario c) an inflow at either end and a central outflow

The cumulative biomass increased from 100 kg at basket 1 to 300 kg at basket 3 and increased in the same manner between basket 6 and 4 (Fig. 3.4). Thus, the maximum cumulative biomass was only half the maximum of 600 kg at basket 6 in *scenario a*. However, the flow-rate through each basket of 60 L min^{-1} was half that in *scenario a*. In *scenario c*, two gradients of oxygen concentration developed, one between each inflow and the central outflow (Fig. 3.4). The two gradients were twice as steep as the single gradient in *scenario a*. Therefore, the abalone in *scenario c* were exposed to the same water quality as those in *scenario a*. Thus, this design had no advantage over a single inflow and outflow design. Furthermore, dividing the available water-supply so that the flow-rate through each basket is halved may reduce water circulation, relative to *scenario a*.



a b

Fig. 3.4. The cumulative biomass, flow-rate (a), flow-rate per kg and dissolved oxygen concentration (b) at different positions in a tank designed according to *scenario c*, i.e. two inflows, each at opposite ends of the tank, and a central outflow.

Central to the tank designs shown in *scenario b* and *c* seems to be a belief that supplying some of the available seawater nearer to the outflow, where water quality is problematic, without increasing the flow-rate per unit of biomass that the tank receives, will improve water quality in

these areas. However, theoretical models show that this is unlikely and in the case of *scenario b* water quality could be worsened relative to a tank with a single inflow and outflow. Equations that are used to estimate the carrying capacity of aquaculture systems typically use inflow water quality, flow-rate, the amount of feed fed, and the desired outflow water quality as their independent variables (Haskell, 1955; Willoughby, 1968; Fivelstad, 1988; Colt & Orwicz, 1991). Therefore, as shown in this study, it is doubtful that shortening the distance between the inflow and outflow of tanks will improve carrying capacity. In support of this conclusion, long tanks 10 – 48 m in length, with a single inflow and outflow, are commonly used to culture abalone in other countries (Hahn, 1989; Fallu, 1991). In conclusion, the available evidence suggests that, in terms of water quality, the tank designs shown in *scenario b* and *c* do not offer any benefit compared to designs with a single inflow and outflow.

Chapter 4

Effect of settled sludge on dissolved ammonia concentration in tanks used to grow abalone *Haliotis midae* fed a formulated diet

4.1 Introduction

Abalone *Haliotis spp.* are sensitive to elevated ammonia in seawater, since it has been shown to negatively affect growth and survival of *Haliotis laevigata* Donovan, *Haliotis tuberculata* L. and *Haliotis midae* L. (Harris et al., 1998a; Basuyaux & Mathieu, 1999; Reddy-Lopata et al., 2006) and causes increased oxygen consumption, reduced food consumption and changes in right-kidney histology in *H. laevigata* (Harris et al., 1998a; Harris et al., 1998b). Farmed *H. midae* is subject to fluctuations in ammonia concentration in the pump-a-shore method of production in flow-through tanks used in South Africa (Fig. 4.1). Anecdotal observations suggest the sludge that accumulates in tanks contributes to ammonia production, which might negatively affect abalone health and growth. The aim of this study was to quantify the effect that sludge had on the level of dissolved ammonia in flow-through tanks in which the abalone were fed a formulated diet.

The sludge that accumulates at the bottom of *H. midae* tanks is made up of faeces, uneaten feed (in this case a formulated diet), and organic and inorganic particulate matter entering the tank with the incoming seawater. It begins as a brown layer that becomes deeper with time, and then develops a thin white layer on the surface of the sludge which is similar to the ‘white bacterial mat’ described by Hall et al. (1992) that covered the sludge below a marine trout cage farm. If the sludge layer in abalone tanks is disturbed, it becomes distributed through-out the water column by water movement, caused by heavy aeration. The sludge can be removed by cleaning, but this requires labour. Furthermore, certain cleaning strategies may disturb the abalone. It has not been established how often cleaning is necessary, since the effect that sludge has on water quality is not known.

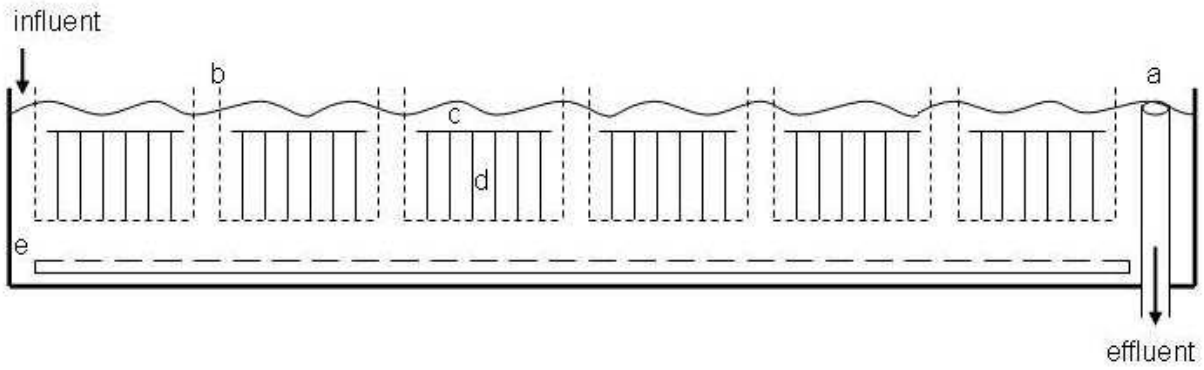


Fig. 4.1. A longitudinal section view of the tank design typically used on South African abalone *Haliotis midae* farms. Influent enters the tank at the water-surface at one end and effluent leaves the tank via an outlet situated at the water-surface (a) at the opposite end. The tank shown contains six oyster-mesh baskets (b), although the number of baskets varies on different farms. On farms that feed a formulated diet the feed is placed on the feeder-plate (c). The vertical plates (d) provide a surface area for the abalone to attach themselves. Aeration is supplied beneath the baskets via a perforated pipe (e), improving water mixing. Sludge accumulates on the bottom of the tank, below the pipe (e).

In seawater there is equilibrium between ammonia (NH_3) and ammonium (NH_4^+). The sum of the two is known as total ammonia, while ammonia alone is known as free ammonia. As the temperature or pH of seawater increases, ammonium converts to ammonia (Bower & Bidwell, 1978), which is toxic. Typically the concentration of total ammonia is measured and free ammonia concentration is subsequently determined by multiplying the concentration of total ammonia by the known proportion of free ammonia at the recorded temperature, pH and salinity (Bower & Bidwell, 1978). This study tested the hypothesis that the production of total ammonia, and consequently the abundance of free ammonia, is higher in *H. midae* farm tanks from which sludge was not removed compared to those from which it was.

4.2 Materials and methods

Net total ammonia-nitrogen TAN ($NH_{3\text{-}4}$-N) production, percentage of TAN in the form of free ammonia-nitrogen NH_3-N (% FAN) and FAN concentration were determined in tanks containing abalone from which sludge was removed, tanks containing abalone from which sludge was not removed, and tanks containing neither abalone nor sludge.

Fifteen tanks on a South African abalone farm (HIK Abalone Farm Pty Ltd., Hermanus), each with a length, width, depth and volume of 3.9 m, 0.85 m, 0.65 m and 2.155 m^3 respectively, were used. Five tanks contained no abalone while the other 10 each contained approximately 53 kg of 20-month-old abalone, with individual live, body weights of approximately 18 g, which had been acclimated to the tanks for two months. The abalone were divided equally among six oyster-mesh baskets per tank, suspended in a line between the inflow and outflow. Each basket contained vertical, plastic plates that increased the area for abalone attachment and a horizontal 'feeder-plate' positioned above the vertical ones onto which feed was placed. The abalone were fed a formulated diet (Abfeed®-S34: Marifeed Pty Ltd, South Africa; 34 % protein; 1.2 % lipid) to apparent satiation once a day. All 15 tanks were aerated using horizontal air-lines beneath the baskets, and were supplied with flow-through ambient-temperature seawater, filtered to 85 µm.

Of the 15 tanks, the 10 containing abalone were siphoned clean of sludge 24 days prior to first water quality data collection. Five of the 10 were randomly designated the 'sludge-remove' treatment and five the 'sludge-remain' treatment. The five empty tanks became the 'no abalone or sludge' treatment. The tanks that were stocked with abalone (i.e. the 'sludge-remove' and 'sludge-remain' treatments) were subsequently managed under farm conditions during the following 24 days to allow the accumulation of sludge in both treatments. Baseline temperature, flow rate, net TAN production, percent FAN and FAN concentration were determined in the 'sludge-remove' and 'sludge-remain' tanks after 24 days. On the following day (i.e. time = 0), sludge was siphoned from the 'sludge-remove' tanks, without disturbing the abalone. These same

water quality parameters were measured again in all 15 tanks 26 h, 44 h and 50 h after the sludge was removed from the 'sludge-remove' treatment. Sludge was not removed from any of the tanks from 0 h until the end of the experiment (i.e. 50 h).

At each sample time water quality variables were measured at the inflow of a single tank, since they all had a common water source, and at the outflow of each tank. TAN concentrations were determined using the phenolhypochlorite method of Solorzano (1969). Samples were kept in the dark once reagents were added and absorbencies were measured photometrically using a spectrophotometer (Biochrom Novaspec II; Cambridge). Absorbencies were converted to TAN concentrations using a calibration equation ($n = 30$, $R^2 = 0.95$) developed with stock solutions of ammonium-chloride. To correct for small differences in flow rate between tanks, net TAN production for each tank at each sample time was expressed as weight per second according to the equation:

$$A_p = F\,(A_o - A_i) \tag{4.1}$$

where, A_p is net TAN production ($\mu g\ s^{-1}$), F is flow-rate ($L\ s^{-1}$), A_o is outflow TAN concentration ($\mu g\ L^{-1}$), and A_i is inflow TAN concentration ($\mu g\ L^{-1}$).

Percentage FAN was calculated using the recorded pH and temperature values (Bower and Bidwell, 1978). FAN concentration was calculated by multiplying TAN concentration by percentage FAN.

The pH was measured using a pH meter (YSI Inc. Model # 60/10 FT; Yellow Springs, Ohio). Temperature was measured using an electronic thermometer (YSI Inc. Model # 55D; Yellow Springs, Ohio).

To verify that abalone biomass and individual abalone weight were equal in the 'sludge-remove' and 'sludge-remain' treatments the total weight of abalone in each basket was measured and summed to obtain the total weight per tank following the final water quality sample. Total weight

of abalone per tank averaged (± standard deviation, S.D.) 53.4 ± 1.1 kg in the 'sludge-remove' and 'sludge-remain' treatments and was not significantly different (Student's *t*-test: $P = 0.39$). This is equivalent to approximately 9.1 kg of soft abalone tissue per tank on a dry weight basis (Britz & Hecht, 1997; Sales & Britz, 2000). The mean individual weight of abalone in each tank was estimated from a sample of 30 randomly selected abalone per basket. Individual live weights averaged 18.4 ± 0.4 g and were also not significantly different (Student's *t*-test, $P = 0.75$).

The cumulative weight of feed, placed into each tank from the start of the trial up until each water quality sample, was recorded for the 'sludge-remove' and 'sludge-remain' treatments. These data were compared using multi-factorial analysis of variance (ANOVA) at $P < 0.05$. The 44 h and 50 h water quality samples fell between feeding times and so cumulative weight of feed fed did not change from 44 to 50 h. Feed was not placed into the 'no abalone or sludge' treatment.

Flow rate into tanks of the 'sludge-remain' and 'sludge-remove' treatments was recorded every 4 days at mid-tide during the sludge accumulation period, and in all tanks whenever water quality samples were taken. Although the flow of seawater into the tanks varied with tidal-height it was similar between the 'sludge-remove' and 'sludge-remain' treatments during the sludge accumulation period (ANOVA, $P = 0.96$) and amongst all treatments when water quality samples were taken (ANOVA, $P = 0.73$), with an overall mean of 0.37 ± 0.04 L s^{-1}. Flow rate into the treatments with abalone in the tanks was also calculated taking abalone biomass into account and remained similar between these treatments with a combined mean of 25.7 ± 3.0 L kg $abalone^{-1}$ h^{-1} (ANOVA, $P = 0.49$).

The dry weight of sludge in tanks of the 'sludge-remove' and 'sludge-remain' treatments at 0 h was estimated by collecting and drying the sludge siphoned from four tanks of the 'sludge-remove' treatment. The sludge was siphoned with seawater from these tanks into separate tanks and the volume recorded. The resulting sludge solution was stirred into a homogenous suspension and a 0.300 L sample was collected from each tank. The solids were filtered from these samples onto Whatman® 185 mm qualitative circles (Cat. No. 1001 185, porosity 11 µm) that had

previously been dried at 60 ºC until a constant weight (0.1 mg) was attained. The Whatman® circles with sludge were dried again and re-weighed, and the dry weight of sludge taken from each replicate of the 'sludge-remove' treatment was calculated using the equation:

$$S = (W_F - W_I)\, V_T / V_S \qquad (4.2)$$

where, S is dry weight (g) of sludge, W_F is final dry weight (g) of the Whatman circle, W_I is initial dry weight (g) of the Whatman circle, V_T is volume (l) of the sludge solution and V_S is volume (l) of the sample.

At the end of the trial, samples of the sludge was siphoned from each of the five 'sludge-remain' tanks and collected using a 10 μm sieve. The solids were centrifuged from these samples at 2000 rpm and nitrogen content was determined using the Dumas combustion method (Williams, 1984) and a LECO FP2000 Nitrogen Analyzer (St. Joseph, Michigan).

Net TAN production, percent FAN, FAN concentration and temperature data for the 'sludge-remove' and 'sludge-remain' treatments were compared using multi-factorial ANOVA and Fisher's LSD test ($P < 0.05$), over the experimental period, to test the null hypotheses that (1) production of total ammonia, (2) percentage of total ammonia in the free ammonia form and (3) concentration of free ammonia were similar in *H. midae* farm tanks from which sludge was removed to those from which it was not. Assumptions of normality and homogeneity of variance were checked using the Shapiro-Wilk W test and Levene's test.

4.3 Results

The cumulative weight of feed placed into the 'sludge-remove' and 'sludge-remain' treatments since the start of the trial (i.e. including the sampling period and the 24 days of sludge accumulation) did not differ significantly between treatments and averaged (± S.D.) 4.76 ± 0.59 kg tank^{-1} at -24 h, 5.26 ± 0.62 kg tank^{-1} at 26 h and 5.38 ± 0.61 kg tank^{-1} at 44 h and 50 h (ANOVA, $P = 0.94$). The dry weight of sludge removed from tanks of the 'sludge-remove' treatment after 24 days of sludge accumulation, averaged 735 ± 103 g tank^{-1}. The nitrogen

content of the sludge in the 'sludge-remain' treatment at the end of the trial averaged 3.6 ± 0.4 % of the dry weight.

Table 4.1. Total ammonia-nitrogen (TAN) concentration, net TAN production, percent FAN and free ammonia-nitrogen (FAN) concentrations in South African abalone (*Haliotis midae*) farm tanks (a) from which sludge that accumulated over 24 days was removed, (b) was not removed, or (c) that did not contain abalone or sludge (i.e. control), presented as the mean (± standard deviation) of five replicates. The data were analysed using a multifactor ANOVA ($P \leq 0.05$) with factors 'sludge-remove', 'sludge-remain' and sample time. Means with different superscripts within a row were significantly different and no superscript represents no significant difference between means within the row.

Sample time (h)	Inflow	(a) Sludge-remove ouflow	(b) Sludge-remain outflow	(c) Control outflow
TAN (µg L $^{-1}$)				
-24	35.6	106.0 ± 15.4	104.2 ± 26.0	-
26	14.6	104.3 ± 23.6	111.8 ± 8.1	20.9 ± 1.9
44	20.2	71.4 ± 6.4^{a}	96.0 ± 4.1^{b}	18.9 ± 1.5
50	24.4	108.0 ± 8.9^{a}	135.7 ± 8.4^{b}	24.8 ± 1.3
Net TAN production (µg L $^{-1}$)				
-24	-	25.4 ± 7.8	21.9 ± 5.7	-
26	-	29.5 ± 4.0	34.9 ± 3.3	2.4 ± 1.0
44	-	20.5 ± 1.8^{a}	30.0 ± 4.1^{b}	-0.4 ± 0.5
50	-	28.2 ± 4.5^{a}	40.0 ± 4.1^{b}	0.1 ± 0.5
FAN (%)				
-24	2.7	2.0 ± 0.1	2.1 ± 0.1	-
26	3.4	2.2 ± 0.1	2.1 ± 0.1	3.9 ± 0.2
44	2.7	1.7 ± 0.0	1.6 ± 0.1	2.6 ± 0.2
50	2.7	1.8 ± 0.1	1.7 ± 0.1	3.4 ± 0.2
FAN (µg L $^{-1}$)				
-24	1.0	2.1 ± 0.4	2.1 ± 0.5	-
26	0.5	2.2 ± 0.4	2.4 ± 0.1	0.8 ± 0.1
44	0.6	1.2 ± 0.1^{a}	1.6 ± 0.1^{b}	0.5 ± 0.0
50	0.7	1.9 ± 0.1^{a}	2.3 ± 0.3^{b}	0.8 ± 0.1

The mean temperature over all sample times was 17.6 ± 1.1 ºC. Temperature did not differ between the 'sludge-remove' and 'sludge-remain' treatments at any of the sample times

(ANOVA, $P = 1.0$). Temperature was higher in the 'no abalone or sludge' treatment when compared to the other treatments at 50 h (ANOVA, $P = 0.0002$), but was similar at all other sample times.

Net production of TAN at the 44 h and 50 h sample times was on average 44 % higher in tanks from which sludge was not removed, compared to those from which it was removed (Table 4.1; ANOVA, $P = 0.0006$). Tanks containing neither abalone nor sludge produced negligible TAN (Table 4.1). The percentage total ammonia in the free ammonia form did not differ between the 'sludge-remove' and 'sludge-remain' treatments at any of the sample times, with an overall mean of 1.9 ± 0.2 % (Table 4.1; ANOVA, $P = 0.54$). FAN concentrations were significantly higher in tanks from which no sludge was removed compared to those from which sludge was removed (Table 4.1; ANOVA, $P = 0.007$).

4.4 Discussion

The sludge which accumulated in *H. midae* tanks made a significant contribution to total dissolved ammonia. Tanks with sludge present had 44 % higher TAN production than those with sludge removed. This result is consistent with similar research on other aquaculture species. For example, the sludge below the cages of a marine trout (*Oncorhynchus mykiss* Walbaum) farm was found to release ammonium at a net rate of 4 mmol N m^{-2} d^{-1} (Hall et al., 1992); and sludge in shrimp (*Penaeus monodon* Fabricius and *Penaeus merguiensis* de Man) ponds produced ammonium at a net rate of 11.3 – 45.8 mmol NH_4^+ m^{-2} d^{-1}, which was found to be linearly related to sludge depth (Burford & Longmore, 2001). Further evidence that aquaculture sludge can produce total ammonia is given by Hopkins et al. (1994) who compared total ammonia concentrations for 148 days in two shrimp (*Penaeus setiferus* L.) ponds, one cleaned weekly and the other not at all, and found mean total ammonia concentration in the uncleaned pond was double that in the cleaned pond.

On a dry weight basis the sludge at the bottom of abalone farm tanks had a greater effect on net TAN production than the abalone themselves. Since farm tanks contain potential sinks of total

ammonia such as up-take by algae (Neori et al., 2004), conversion to nitrite by bacteria (Tal et al., 2003) and loss from water to air (Chiayvareesajja & Boyd, 1993), it was not possible to quantify total ammonia production per unit weight of sludge or abalone in this study. However, the removal of 0.735 kg of sludge (dry weight) from an abalone tank (which contained approximately 9.1 kg of abalone soft tissue on a dry weight basis, plus other potential ammonia sources and sinks in the system), resulted in a reduction in total ammonia production of 31 %. This indicates that the sludge is a potent source of dissolved ammonia, which is further supported by the presence of nitrogen (i.e. 3.6%) in the sludge.

The production of total ammonia by the sludge was not mitigated by a reduction in pH, which is reinforced by similar percentage FAN values in the 'sludge-remain' and 'sludge-remove' treatments. Consequently the concentration of toxic, free ammonia was higher in tanks with sludge present. It would thus be advisable to remove sludge from abalone tanks as often as practical considerations and disturbance of the abalone allow.

The mean flow rate of 25.7 ± 3.0 L kg^{-1} abalone h^{-1} (i.e. 62 ± 8 % exchange h^{-1}) resulted in a maximum FAN concentration of 2.4 µg L^{-1}. However, whether the observed FAN concentrations were below the level at which growth becomes affected is uncertain, since this level has yet to be established. Reddy-Lopata et al. (2006) reported that 36 h exposure to a FAN concentration of 9.8 µg L^{-1} caused 50 % mortality in 1 – 2.5 cm shell length *H. midae* kept under laboratory conditions; while exposure to 16.4 µg L^{-1} caused mortality in 10 – 15 cm animals. However, *H. midae* have been exposed to a FAN concentration of approximately 60 µg L^{-1} for 48 h under farm conditions without mortality (Vosloo, 2007; pers. comm.). The effect of free ammonia on the growth of *H. laevigata* is well documented (Harris et al., 1998a) but since the lowest concentrations found to affect growth of *H. laevigata* are higher than those reported by Reddy-Lopata et al. (2006) to cause mortality in *H. midae* they cannot be used as a comparison for the South African abalone. In light of the uncertainty surrounding the toxicity of free ammonia to *H. midae* we cannot discount the increase in free ammonia concentration in *H. midae* farm tanks, as a result of sludge, as having no negative effect on *H. midae*.

Future work should thus quantify the free ammonia EC_5 concentration for growth of farmed *H. midae* as well as the effects of handling stress associated with various strategies for sludge removal, in order to develop a management protocol that optimises the performance of farmed abalone, while minimising labour costs. In the interim, based on the significant effect that sludge has on FAN concentration in farm tanks, it is recommended that sludge removal be incorporated into management programmes on abalone farms.

Chapter 5

Growth and survival of silver kob *Argyrosomus inodorus* and bloodworm *Arenicola loveni loveni* in the effluent of a South African abalone *Haliotis midae* farm

5.1 Introduction

In South Africa abalone *Haliotis midae* L. farms are shore-based due to the exposed nature of the coastline. Large volumes of seawater (*ca*. 500 – 1500 L s^{-1} $farm^{-1}$) are pumped ashore to supply these farms, being fed through the abalone tanks in a single-pass system before flowing by gravity back to the sea. The abalone are usually fed a formulated feed (Abfeed®; Marifeed Pty Ltd, Hermanus) and/or kelp (*Ecklonia maxima* Osbeck). A preliminary investigation by Samsukal (2004) suggests that effluent from South African abalone farms contains levels of dissolved total ammonia, nitrite and nitrate within the limits required for culturing marine finfish species (Poxton & Allouse, 1982). Therefore, it may be possible to use abalone effluent as culture water for shore-based fish farms instead of bearing the infrastructural and running costs of pumping additional seawater ashore. Furthermore, the solid particulate matter produced in abalone tanks, and flushed out in the effluent, is high in protein (Chalmers, 2002) and could be used as feed for detritus feeders such as polychaete worms. Therefore, the suitability of abalone farm effluent for the culture of two candidate aquaculture species, the marine finfish silver kob (*Argyrosomus inodorus* Griffiths & Heemstra) and the polychaete, bloodworm (*Arenicola loveni loveni* Kinberg) was tested.

Bloodworm is a warm-temperate polychaete species which lives in sandy sediments (Wells, 1962). In South Africa, bloodworm have been recorded from Saldanha Bay on the west coast to Durban on the east coast (Wells, 1962). Although anecdotal observations suggest that, as in other arenicolids (Fauchald & Jumars, 1979), bloodworm feed by ingesting sediment, their feeding biology has not been investigated. Typically, arenicolids create an L-shaped burrow and ingest the sediment at the bottom end of this burrow (Fauchald & Jumars, 1979). Sediment from the sand-surface sinks downwards to replace the sediment ingested below the surface. In other *Arenicola* species, diatoms, meiofauna, organic matter and bacteria are ingested with the

sediment and digested (Hylleberg, 1975; Longbottom, 1970; Retraubun et al., 1996). Bloodworm are highly fecund (100 000 – 1000 000 oocytes female^{-1} y^{-1}) and spawning is governed by temperature (Lewis, 2005), both of which are desirable attributes in a candidate aquaculture species. The growth potential of bloodworm is not known although individuals as heavy as 100 g were collected by Barham (1979). Bloodworm potentially has good market demand as it is a popular bait species (Britz et al., 2001; Mackenzie, 2005), which is time-consuming to collect.

Silver kob is a popular sciaenid finfish which supports commercial and recreational fisheries in South African and Namibian waters (Griffiths & Heemstra, 1995). There is interest in the aquaculture potential of silver kob and the species has been successfully spawned and reared by commercial finfish farms. Silver kob were selected for the growth trial in abalone effluent as they have an entirely marine life-cycle (Griffiths, 1997) and may therefore be more sensitive to poor water quality than euryhaline finfish species. Consequently, their response to abalone effluent may be a better indication of its quality than that of a species like the dusky-kob (*Argyrosomus japonicus* Temminck & Schlegel), which is also an aquaculture candidate, but spends its early life history in the upper reaches of estuaries (Griffiths, 1996a).

To assess the suitability of abalone effluent as a culture medium for bloodworm and silver kob, the growth and mortality of these animals was recorded in abalone farm effluent and control seawater, which was unused.

5.2 Materials and methods

Control seawater and abalone effluent supply

Bloodworm and silver kob were supplied with either abalone effluent or control seawater. The abalone effluent was pumped from the underground effluent pipe of Roman Bay Sea Farm (Pty) Ltd. This farm operated on a flow-through design in which seawater was pumped ashore, filtered to 100 µm before entering a header-dam and then flowed by gravity through abalone tanks and back to sea. The farm had a biomass of 80 – 90 tons of abalone and a flow-rate of approximately 500 L s^{-1} (S. Ashlin, Roman Bay Sea Farm Pty Ltd., pers. comm., 2007). The abalone were fed a

formulated, pelleted feed (Abfeed®; 26 – 34 % protein, Marifeed Pty Ltd., Hermanus) and kelp (*E. maxima*). During the time in which the study was conducted abalone on the farm were fed approximately 8.92 t of Abfeed® S34 (34 % protein), 0.80 t of Abfeed® K26 (26 % protein) and 17.90 t of wet kelp per month (S. Ashlin, Roman Bay Sea Farm Pty Ltd., pers. comm., 2007). The abalone tanks on the farm were cleaned in rotation with the result that one was cleaned approximately every twenty minutes. The solid waste flushed from the tank during cleaning caused the effluent to become darker, anoxic-smelling and richer in suspended solids at these times. Control seawater for the experimental systems was gravity fed from the header-dam.

Experimental systems

The bloodworm were stocked into ten, cylindrical, plastic tanks (diameter 0.44 m, sand-height 0.51 m, surface area 0.15 m^2) filled with sea-sand. Water flowed into and out of each tank at the surface on opposing sides, with a water depth of 0.17 m above the sand. The flow-rate was sufficiently slow to allow suspended solids to settle onto the sand. The tanks were positioned outside and exposed to a natural photo-period. The top of each tank was covered in green, 80 % shade-cloth and the outside of each tank was painted white to reduce solar-heating. All tanks were supplied with flow-through seawater for four days prior to stocking. Thereafter, five were supplied with abalone effluent and five with control seawater. Flow rates averaged (± standard error) 0.052 ± 0.001 L s^{-1} (i.e. 0.34 L s^{-1} m^{-2}) over the experimental period.

The silver kob were stocked into eight cylindrical, green, plastic tanks with a water-depth of 0.68 m, diameter of 1.1 m and volume of 0.646 m^3 per tank. The tanks were aerated through a diffuser placed on the tank bottom. The tanks were situated outside, under a green, 80 % shade-cloth. Water flowed into each tank through an inlet at the side and out a central outlet. A ‘draw-pipe’ over the outlet meant water flowed out from the bottom of the tank. The flow rates over the experimental period averaged 0.152 ± 0.002 L s^{-1} (equivalent to 0.023 L kg^{-1} s^{-1} at the initial stocking density). For the sake of this preliminary trial the flow-rate was purposely kept at a level considered to be high. Future work may optimise the flow-rate to a lower value. The bottom of each tank was siphoned clean on Mondays, Tuesdays and Wednesdays. Algal growth was scrubbed from the tank surfaces once a week.

Experimental animals

Bloodworm were collected from Cape Town's Muizenberg beach (S34º 06N´ 54.61˝, E18º 27´ 50.95˝), at spring low tides, during December 2006 and January 2007, with a 'prawn-pump' and sieve. They were kept for a month in a holding tank, which was supplied with seawater and filled with sea-sand to a depth of 0.5 m. A formulated abalone feed (Abfeed®) and organic-rich sediment from an abalone farm hatchery was placed on the sand's surface as food. At the start of the growth experiment bloodworm were collected from the holding tank by hand and purged for 24 h. Excess water was then removed from their skin using absorbent paper and they were weighed to the nearest 0.01g. Seven bloodworm were placed in each tank (847.9 g m^{-2}). After 91 days the bloodworm were collected by hand, purged, dried and re-weighed. Mortality was calculated as initial minus final number per tank. The change in the body weight (BW) of bloodworm in each tank was expressed as mean specific growth rate using the equation:

$$SGR = 100\ [ln(Wf) - ln(Wi)] / d \qquad (5.1)$$

where *SGR* is specific growth rate (% BW d^{-1}), *Wf* is mean final weight (g), *Wi* is mean initial weight (g) and *d* is number of days.

Juvenile silver kob were obtained from I&J (Pty) Ltd., Marine Fish Hatchery. They had been kept at ambient temperature (11–19 °C) since the juvenile stage. They were acclimatized to the experimental system for 30 days at an initial stocking density of 10 kg m^{-3}. During this period all tanks were provided with flow-through control seawater. After acclimation the densities in the tanks were adjusted to achieve a mean stocking density of 10.0 ± 0.0 kg m^{-3}. The number of fish per tank ranged from 59 to 65. At the start of the trial (i.e. time 0) 25 randomly caught fish from each tank were anaesthetized with 0.25 ml L^{-1} 2-phenoxy-ethanol. These fish were measured for standard length (SL), to the nearest 1 mm, and weight, to the nearest 0.1 g. The water supply to four randomly chosen tanks was then gradually changed from seawater to abalone effluent over 24 h. A sample of 25 fish per tank was again randomly selected, anaesthetized and weighed and measured after 29, 59 and 89 days. All fish were counted, weighed and measured after 120 days. Mortality was monitored each day.

The silver kob were fed in excess in the morning and late afternoon with 4 mm trout pellets (Trout Grower; Aquanutro Pty Ltd., Malmesbury) and after 70 days with 6 mm pellets of the same brand. The feed contained 34 ± 2 % crude protein, 11 ± 1 % fat, 8 ± 2 % moisture, 10 ± 1 % ash and 19 ± 0.4 Mj kg^{-1} gross energy (n = 4).

SGR was calculated using equation 5.1. Feed conversion ratio (*FCR*) and protein efficiency ratio (*PER*) for each replicate, and condition factor (*CF*) for individual fish were calculated using the equations:

$$FCR = F_{consumed} / W_{gain} \quad (5.2)$$

$$PER = W_{gain} / (F_{consumed} \text{ X } F_{protein}) \quad (5.3)$$

$$CF = (W / TL^{3.0657}) \text{ X } 166667 \quad (5.4)$$

where, $F_{consumed}$ is the dry weight of feed consumed (g), W_{gain} is the wet weight gain of silver kob (g), $F_{protein}$ is the protein content of the feed (%), *W* is the wet weight (0.1 g) and *TL* is the total length (mm). The condition factor equation was developed from the mass-length relationship of wild silver kob (Griffiths, 1996b). SL was converted to total length according to Griffiths (1996b).

Quality of the abalone effluent and control seawater

The quality of the effluent and control seawater that flowed into the experimental tanks was recorded on 20 and 24 randomly-selected days during the bloodworm and silver kob growth trials respectively (Table 5.1). The pH, temperature, and concentrations of dissolved oxygen (DO), total ammonia-nitrogen TAN ($NH_{3\text{-}4}$-N) and suspended solids (SS) were recorded in the water flowing out of the tap, at the inflow of a tank in each treatment, before the water entered the tank. The pH was measured using a pH meter (YSI Inc. Model # 60/10 FT; Yellow Springs, Ohio). Temperature and DO concentration were measured using an electronic meter (YSI Inc. Model # 55D; Yellow Springs, Ohio). TAN concentration was determined using the phenolhypochlorite method of Solorzano (1969). Samples were not exposed to light once reagents were added. The

percentage of TAN in the free ammonia-nitrogen FAN (NH_3-N) form and FAN concentration were calculated using the pH, temperature and TAN values (Bower & Bidwell, 1978). Suspended solids, larger than 10 µm, filtered from five L of water were collected on pre-dried and weighed filter paper circles (Scleicher & Schüll®, diameter 70 mm; Dassel, W. Germany). The concentration of suspended solids (mg L^{-1}) was subsequently calculated as dry weight gain of the filter paper divided by the volume of the water sample. Temperature was recorded every weekday morning (08h30) and afternoon (16h30) using a mercury thermometer.

The recorded water quality parameters were compared between the two water sources using Student's *t*-test ($P < 0.05$).

Water quality in the culture tanks

The pH, temperature and concentrations of DO, TAN and FAN were recorded at the outflow of each silver kob tank. TAN, FAN and suspended solids concentrations were recorded at the outflow from each bloodworm tank and pH, temperature and DO concentration were recorded in the centre of the tank approximately 30 mm above the sand. Temperature was also recorded at the outflows of all tanks every weekday morning (08h30) and afternoon (16h30) using a mercury thermometer.

The organic content of sediment collected from the upper 10 cm in each bloodworm tank was determined at 0 and 91 days as the percentage weight (0.1 mg) lost when burnt in a muffle-furnace, at 460 ºC for 24 h, after pre-drying. The upper 10 cm was selected as particulate matter settled out on the sediment surface, from where it was moved downwards by a combination of the feeding of the bloodworm, which seemed to occur about 10 cm below the surface, and the excretion of sand by the bloodworm at the sand surface.

Statistical analyses

Length and weight data of silver kob in effluent and seawater at 0, 29, 59, 89 and 120 days were compared using repeated-measures analysis of variance (R-M ANOVA, $P < 0.05$). CF was

compared between treatments at 0 and 120 days using R-M ANOVA ($P < 0.05$). SGR, FCR, PER and mortality data were analyzed using Student's *t*-test ($P < 0.05$).

Initial and final weights of bloodworm in the effluent and seawater tanks were compared using R-M ANOVA ($P < 0.05$). SGR and mortality in effluent and seawater were compared using Student's *t*-test ($P < 0.05$). Initial and final organic content of the soil in the bloodworm tanks of the two treatments were compared using repeated-measures analysis of variance ($P < 0.05$).

Assumptions of normality and homogeneity of variance were checked using the Shapiro-Wilk *W* test and Levene's test.

5.3 Results

There were highly significant differences in pH, TAN and dissolved oxygen concentration between the control seawater and abalone effluent which supplied the experimental tanks ($P < 0.001$). The differences in FAN concentration between the two water sources averaged approximately 0.3 μg L^{-1} and were non-significant. Although the temperature of abalone effluent was consistently higher temperature by approximately 0.5 °C, the difference was not statistically significant. However, this non-significant result was likely caused by the high variability in the temperature data and should not be taken as evidence that the temperature of abalone effluent and control seawater is the same. The same was the case for the concentration of suspended solids in the two water sources. The abalone effluent had a mean suspended solids concentration of almost three times that of control seawater but due to large variability in the data-set this was not significant.

Weight-gain, length-gain, mortality and nutritional indices did not differ significantly in silver kob kept in either abalone effluent or control seawater ($P > 0.05$, Table 5.2, Fig. 5.1). SGR was 0.46 ± 0.01 % BW d^{-1} in seawater and 0.49 ± 0.01 % BW d^{-1} in abalone effluent. Condition factor

increased in both treatments from day 0 to day 120, and mortality was 2.0 ± 1.0 % in effluent and 1.6 ± 0.7 % in seawater.

Table 5.1. Mean (± standard error), minimum and maximum values for water quality parameters measured in the control seawater and abalone *Haliotis midae* effluent that supplied the silver kob *Argyrosomus inodorus* (n = 22) and bloodworm *Arenicola loveni loveni* tanks (n = 18).

	Control seawater			Abalone effluent			
	mean ± s.e.	min	max	mean ± s.e.	min	max	*P*-value
Silver kob							
Temp	15.9 ± 0.3	12.0	21.5	16.6 ± 0.3	12.1	22.1	0.53
pH	8.01	7.91	8.19	7.78	7.55	8.01	>0.001
TAN	39.5 ± 3.4	18.8	76.2	79.3 ± 5.8	27.2	153.2	>0.001
FAN	0.9 ± 0.1	0.3	1.9	1.2 ± 0.1	0.3	1.8	0.05
DO	7.83 ± 0.12	7.05	8.85	6.97 ± 0.13	6.04	7.97	>0.001
Bloodworm							
Temp	16.2 ± 0.3	12.0	21.5	16.9 ± 0.3	12.1	22.1	0.45
pH	7.99	7.78	8.19	7.79	7.55	8.01	>0.001
TAN	37.4 ± 3.8	18.8	75.5	69.9 ± 4.6	27.2	94.4	>0.001
FAN	0.9 ± 0.1	0.3	1.5	1.1 ± 0.1	0.3	1.7	0.06
DO	7.87 ± 0.14	7.05	8.85	7.06 ± 0.16	5.74	7.97	>0.001
SS	3.5 ± 0.5	0.0	5.8	11.3 ± 4.5	0.2	41.6	0.11

pH values were normalized ($1 / (10^{pH})$) before calculating the mean. TAN = total ammonia-nitrogen $NH_{3\text{-}4}$-N (µg L^{-1}), FAN = free ammonia-nitrogen NH_3-N (µg L^{-1}), DO = dissolved oxygen (mg L^{-1}), SS = dry weight of suspended solids (mg L^{-1}).

The mean temperature was 16.2 ± 0.3 ºC in silver kob tanks supplied with control seawater and 16.7 ± 0.1 ºC in those supplied with abalone effluent (Table 5.3). Dissolved oxygen concentration averaged 6.80 ± 0.09 mg L^{-1} in the tanks supplied with control seawater and 6.16 ± 0.09 mg L^{-1} in those supplied with effluent but dropped below 5 mg L^{-1} in both treatments on a few occasions in the morning when water temperatures were high. Maximum recorded FAN concentrations were 3.6 µg L^{-1} in seawater tanks and 3.4 µg L^{-1} in effluent tanks.

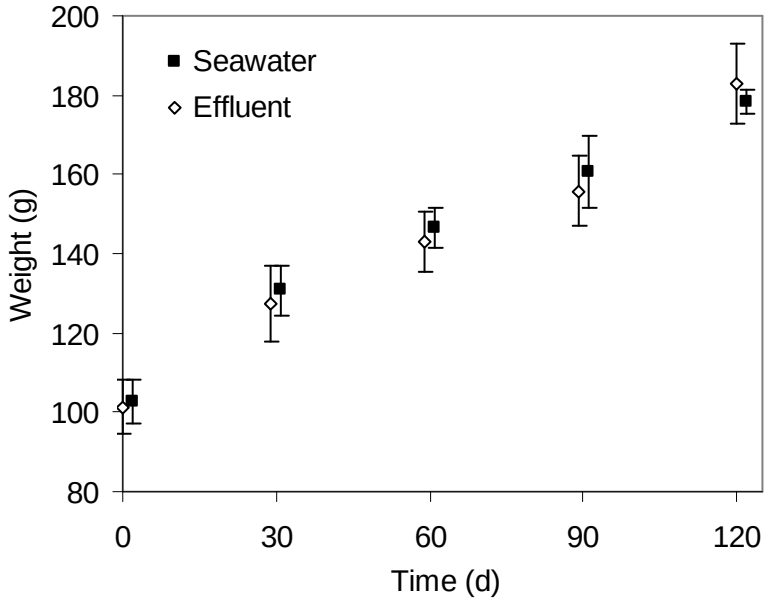


Fig 5.1. Mean body weight of silver kob *Argyrosomus inodorus* kept in circular tanks (n = 4 treatment^{-1}) supplied with either control seawater or abalone *Haliotis midae* effluent over 120 days. The repeated-measures analysis of variance mean and 95 % confidence intervals are indicated (P = 0.62).

Bloodworm kept in tanks supplied with control seawater lost weight at 0.19 ± 0.04 % BW d^{-1} over the 91 day trial period, while those kept in tanks supplied with abalone effluent gained weight at 0.39 ± 0.07 % BW d^{-1} (Table 5.2). Mortality was 5.7 ± 3.5 % in the effluent tanks and 11.4 ± 8.3 % in the seawater tanks and did not differ significantly between treatments (P = 0.54). The effluent water contained a greater concentration of suspended solids and 182 ± 56 g m^{-2} d^{-1} (28 g tank^{-1} d^{-1}) were deposited in tanks supplied with effluent compared to 46 ± 13 g m^{-2} d^{-1} (7 g tank^{-1} d^{-1}) in tanks supplied with seawater. The organic content of the sand averaged (± standard error) 1.6 ± 0.2 % in both treatments at day 0 and 1.9 ± 0.1 % in effluent tanks and 1.7 ± 0.1 % in control seawater tanks at day 91, however, this difference was not statistically significant (P = 0.22, Table 5.3).

Table 5.2. Growth and nutritional indices of silver kob *Argyrosomus inodorus* (n = 4 tanks treatment^{-1}) and bloodworm *Arenicola loveni loveni* (n = 5 tanks treatment^{-1}) over 120 and 91 days respectively when supplied with either control seawater or abalone *Haliotis midae* effluent.

	Control seawater	Abalone effluent	*P*-value	
Silver kob				
Weight$_i$ (g)	102.7 ± 1.7	101.3 ± 2.2	0.62	*
Weight$_f$ (g)	178.2 ± 1.0	182.9 ± 3.1	0.21	*
Length$_i$ (mm)	199 ± 2	198 ± 2	0.73	*
Length$_f$ (mm)	239 ± 1	239 ± 1	0.83	*
SGR	0.46 ± 0.01	0.49 ± 0.01	0.13	**
CF$_i$	0.79 ± 0.00	0.79 ± 0.00	0.47	*
CF$_f$	0.83 ± 0.00	0.85 ± 0.00	0.11	*
FCR	2.72 ± 0.20	3.21 ± 0.17	0.10	**
PER	1.09 ± 0.10	0.92 ± 0.05	0.11	**
Mortality (%)	1.6 ± 0.7	2.0 ± 1.0	0.77	**
Bloodworm				
Weight$_i$ (g)	18.4 ± 0.3	18.4 ± 0.7	0.99	*
Weight$_f$ (g)	15.5 ± 0.4^a	26.2 ± 0.9^b	< 0.0001	*
SGR (% BW d^{-1})	-0.19 ± 0.04^a	0.39 ± 0.07^b	< 0.0001	**
Mortality (%)	11.4 ± 8.3	5.7 ± 3.5	0.54	**

SGR = specific growth rate, CF = condition factor, FCR = feed conversion ratio, PER = protein efficiency ratio, Length = standard length, i = initial, f = final. All values are means ± standard errors. Treatment means were compared using repeated measures analysis of variance * or Student's *t*-test **. ^{a,b} Indicates significant differences between means within rows ($P < 0.05$).

Table 5.3. Mean (± standard error), minimum and maximum values (n = 90) for water quality parameters measured in the silver kob *Argyrosomus inodorus* and bloodworm *Arenicola loveni loveni* tanks supplied with either control seawater or abalone *Haliotis midae* effluent.

	Control seawater			Abalone effluent		
	mean ± s.e.	min	max	mean ± s.e.	min	max
Silver kob						
Temp	16.2 ± 0.1	11.5	22.1	16.7 ± 0.1	11.9	23.0
pH	7.88	7.62	8.08	7.65	7.39	7.90
TAN	88.6 ± 3.8	30.0	184.0	136.6 ± 6.1	37.7	248.4
FAN	1.6 ± 0.1	1.1	3.6	1.5 ± 0.1	0.4	3.4
DO	6.80 ± 0.09	4.84	8.83	6.16 ± 0.09	4.25	7.74
Bloodworm						
Temp	16.3 ± 0.1	11.2	22.1	16.8 ± 0.1	11.9	22.8
pH	7.98	7.67	8.16	7.78	7.40	8.00
TAN	36.7 ± 1.5	18.1	76.2	84.1 ± 3.4	42.6	154.6
FAN	0.9 ± 0.1	0.3	2.8	1.4 ± 0.1	0.6	3.6
DO	6.83 ± 0.08	4.90	8.31	5.99 ± 0.09	4.28	7.48
SS	2.5 ± 0.4	0.0	11.4	4.0 ± 0.6	0.0	14.2

pH values were normalized ($1 / (10^{pH})$) before calculating the mean. TAN = total ammonia-nitrogen $NH_{3\text{-}4}$-N (µg L^{-1}), FAN = free ammonia-nitrogen NH_3-N (µg L^{-1}), DO = dissolved oxygen (mg L^{-1}), SS = dry weight of suspended solids (mg L^{-1}).

5.4 Discussion

The positive weight gain of the bloodworm grown in abalone farm effluent indicates that this species could potentially be cultured downstream of abalone farms. The bloodworm in tanks supplied with control seawater lost weight indicating that the organically enriched farm effluent provided the bloodworm cultured in it with an adequate source of nutrition. This is reflected in the significantly higher level of suspended solids deposited in the tanks supplied with abalone farm effluent compared to those given control seawater. The importance of food availability has been demonstrated in populations of *Arenicola marina* L. where biomass density is positively correlated with the amount of organic matter in the sediment (Longbottom, 1970). Growth of the deposit-feeding polychaete *Capitella capitata* Fabricius increased with increasing nitrogen and

caloric ration (Tenore, 1983). Furthermore, food source had a significant effect on the growth of *C. capitata* as individuals lost weight when fed certain seaweed species, even at nitrogen and caloric rations that were adequate for growth (Tenore, 1983). Solids from abalone tanks are rich in protein (Chalmers, 2002). On a dry weight basis the solids from tanks in which abalone are fed kelp contain approximately 16.8 % protein, while those from tanks in which Abfeed® is fed are 30.2 % protein (Chalmers, 2002).

The bloodworm supplied with abalone farm effluent appeared to be quite efficient at processing the solids deposited in their tanks. Despite the significantly higher input of suspended solids into the tanks provided with effluent water, the organic content of the sediment was only slightly higher than the control, and this difference was not statistically significant. As with *A. marina*, which periodically move their feeding funnel to a new location, suggesting they deplete their local food-source over time (Rijken, 1979), the bloodworm in the tanks supplied with abalone farm effluent were efficient at removing the organic matter. Furthermore, a substantial proportion of the suspended solids in the abalone effluent was removed in the experimental bloodworm tanks as the concentration of suspended solids in the abalone effluent was 223 ± 89 % higher than that in the control seawater; whereas the suspended solid concentration in the effluent flowing from the bloodworm tanks supplied with abalone effluent was only 14 ± 2 % higher than the concentration in control seawater. Bloodworm culture could therefore be used to mitigate the production of solid waste by abalone farms.

The growth, mortality and nutritional indices of silver kob were similar in control seawater and abalone effluent, indicating that effluent from abalone farms can be used to culture this species without adverse effects on growth or health. The specific growth rate attained during the current study was approximately double that (0.26 % BW d^{-1}) of wild silver kob in the same size-range (i.e. 92–240 g) (calculated using data from Griffiths, 1996b). Furthermore, length gain of the silver kob in the abalone effluent treatment was approximately twice as fast as that achieved with silver kob of 34 g in a re-circulating system at a constant temperature of 18 °C (Ferreira, 2007). The mean feed conversion ratio (3.0 ± 0.2) was inefficient compared to the FCR's of 0.9 – 1.7 typically obtained with established finfish species in aquaculture (Austreng et al., 1987; Sveier et

al., 1999; Vergara et al., 1999; Daniel, 2004; Partridge et al., 2006), while silver kob has previously been cultured at a more efficient FCR of 2.3 (Ferreira, 2007). However, this was probably due to feeding in excess and the composition of the diet, which was not formulated for marine finfish. In this study feeding in excess could not be avoided as the fish fed predominantly at the bottom of the tanks, which were often not visible.

It is likely that the temperatures recorded in the current study (*ca.* 16.2 ºC in the control seawater tanks and 16.7 ºC in the effluent tanks) were below the optimal for growth of silver kob. Preliminary investigation suggests that the temperature preferendum, i.e. the temperature chosen in a choice experiment, of 50 g silver kob is between 16.9–18.1 °C (Dickens, 2007), and in the current study our observations suggested they only fed at the water surface above 20 °C and that feeding activity was minimal below 18 °C.

The concentrations of total ammonia in the abalone effluent were similar to those recorded at other South African abalone farms (Samsukal, 2004). It is unlikely that free-ammonia in either the tanks supplied with abalone effluent or control seawater affected the growth of the silver kob, as the highest recorded concentration was at least 18 times lower than concentrations shown to reduce growth in other marine finfish species over chronic periods of exposure (Alderson, 1979; Lemarié et al., 2004; Person-Le Ruyet et al., 1997a; Person-Le Ruyet et al., 1997b; Rasmussen & Korsgaard, 1996; Wajsbrot et al., 1993). In spite of the higher concentration of TAN in the silver kob tanks supplied with abalone effluent, compared to those given control seawater, the concentrations of FAN were similar due to the lower pH of the abalone effluent (Bower & Bidwell, 1978).

While the effect of oxygen concentration on growth of silver kob has not been investigated, the DO concentrations measured in this study were below those shown to affect the growth of other species of finfish (Davis, 1975). Davis's (1975) review of the metabolic rate of seven marine, nonanadromous species showed that metabolism becomes oxygen dependent below 4.20–7.12 mg L^{-1} and it was suggested that marine, nonanadromous species begin to show symptoms of

oxygen stress below 6.72 mg L^{-1}. Mean and minimum DO concentrations were 6.8 mg L^{-1} and 4.8 mg L^{-1} in the silver kob tanks supplied with seawater and 6.2 mg L^{-1} and 4.3 mg L^{-1} in those supplied with abalone effluent. In all cases the drop of oxygen concentration below 5 mg L^{-1} occurred in the morning. Although this was only recorded on a few occasions it is likely to have occurred more often as oxygen measurements were not taken every day. While no mortalities were recorded at, or in the days immediately after, these times the metabolism of fish in both treatments may have been limited by oxygen concentration which would have caused stress and a reduction in growth (Schrek & Li, (1991). This could be over-come by increased aeration or oxygenation.

The present results indicate that bloodworm and silver kob can be cultured in abalone farm effluent. Concentrations of free ammonia in effluent were low when compared to toxic levels for other finfish species but effluent may require oxygenation if used as a water source for commercial-scale finfish farming. The organic solid waste in the abalone farm effluent appeared to be an adequate source of nutrition for the bloodworm, which were relatively efficient at removing suspended solids from the effluent. It is uncertain how the growth rate of bloodworm and their tolerance of abalone effluent changes with size. Therefore, future work should investigate bloodworm growth and mortality in abalone effluent at the larval and early juvenile stages. Furthermore, the growth trials in this preliminary work were of a short duration and future work investigating the suitability of abalone effluent for the culture of finfish and bloodworm through a full cultivation cycle may be needed to detect disease or pathogenic bacteria.

Chapter 6

Concluding discussion

The research documented in this thesis was successful in its aims of (1) determining the dynamics of water quality on a South African abalone *Haliotis midae* L. farm and (2) assessing the suitability of abalone farm effluent for the culture of silver kob *Argyrosomus inodorus* Griffiths & Heemstra and bloodworm *Arenicola loveni loveni* Kinberg.

Diurnal water quality dynamics

The quality of the seawater within abalone tanks varied on a diurnal cycle. At night there was a greater consumption of oxygen and more H^+ ions were produced. This indicated an increase in metabolic-rate and was explained by the increased movement of the abalone when feeding. Due to the higher oxygen consumption at night, oxygen concentrations were lowest in abalone tanks during night-time. While the concentration of total ammonia in the influent changed diurnally, the variation was small enough to be discounted. Hence, the main determinants of free-ammonia concentration in tanks were pH and temperature. The pH was highest in tanks in the afternoon and therefore it was concluded that the concentration of free ammonia will be highest in tanks at this time unless mitigated by low temperatures. However, the large data-set of seawater temperatures, recorded in the morning and afternoon during *Experiment 3: Abalone farm influent and effluent quality* (chapter 2), showed that temperature was generally higher in the afternoon, which increased ammonia toxicity. The values for free ammonia recorded in the farm influent and effluent over a period of 12 months confirmed that free ammonia was higher in the afternoon, when compared to the morning, by an average of 32 %. For the purposes of water quality monitoring in abalone tanks it would thus be best to measure free ammonia concentrations in the afternoon and oxygen concentrations at night. Reducing or stopping the flow-rate of tanks at these times should be avoided in order to prevent low oxygen concentrations and high free ammonia concentrations. If water flow to the farm must be reduced or stopped this should preferably be done in the morning as this is when oxygen concentrations in tanks are highest and free ammonia concentrations are lowest.

The descriptions and explanations of diurnal water quality dynamics in abalone tanks, discussed here are the first for South African abalone farms which feed a formulated diet. The results of the experiment have been used to generate important management recommendations. A number of steps can now be taken to refine the findings of the current study. A sampling interval of less than three hours can be used to obtain a higher resolution in the measurements. Furthermore, a larger sample size than the eight tanks, in which water quality was recorded at each sample time over two periods of twenty four hours, would increase confidence in these important results.

Abalone growth and water quality at different positions within tanks

Free ammonia increased in a gradient between the inflow and outflow of abalone tanks, while oxygen and H^+ ion concentrations decreased (Chapter 2, *Experiment 1: Abalone growth*). Thus, under culture conditions abalone are exposed to elevated free ammonia and reduced oxygen and H^+ ion levels. The growth of abalone was correlated with changes in H^+ ion concentration, free ammonia and oxygen. Therefore, this experiment provided baseline data of the H^+ ion, oxygen and free ammonia levels in abalone tanks in which the growth of abalone were reduced. Thus, it is recommended that future studies investigating the effect of H^+ ions, oxygen or free ammonia on the growth of *H. midae* include the range of H^+ ions (6 – 27 nmol L^{-1} H^+), oxygen (5.5 – 8.0 mg L^{-1}) and free ammonia (0.6 – 4.8 µg L^{-1}), recorded in the current study. Furthermore, it is recommended that higher H^+ ion and lower oxygen concentrations are included as the range of values for these parameters recorded in the current study did not include the higher H^+ ion and reduced oxygen concentrations that occur in tanks at night.

In an effort to reduce the gradient of water quality and abalone growth which develops between the inflow and outflow of tanks (Chapter 2) farmers have experimented with different tank designs, which divide the available water-supply so that a portion of it enters the tank nearer to the outflow (Chapter 3, Fig. 3.1). However, models of oxygen concentrations in tanks of these designs showed that it is unlikely that they will improve water quality, in comparison to conventional tanks with a single inflow and outflow at opposing ends. Furthermore, these designs may actually adversely affect water quality. It was concluded that water quality is dependent upon the flow-rate per unit of abalone biomass which the tank receives rather than the distance

between the inflow and outflow. This conclusion is supported by equation 2.6, which showed that in abalone tanks, for a given temperature, abalone size and length of time since the tank was last cleaned, the ammonia concentration is dependent upon the tank's flow-rate per unit of biomass (Chapter 2). Therefore, the available evidence suggests that, in terms of water quality, these experimental tank designs do not offer any benefit compared to conventional designs with a single inflow and outflow.

Abalone farm influent and effluent quality

There was considerable variation in the quality of the abalone farm's influent and effluent over time. It was not assumed that these changes were a result of changes in 'season' (i.e. summer and winter). Rather, the changes were explained by correlation with particular environmental parameters like temperature, flow-rate and farm biomass. Changes in influent oxygen concentration were explained by a negative relationship with temperature, while variation in pH was, to a small extent, positively correlated with temperature.

The observed values for ammonia production and concentration within abalone tanks were highly correlated with the values predicted by equations 2.6 and 2.7 ($r^2 \geq 0.8$). Therefore, the results of this study supported the negative exponential relationship between abalone size and ammonia excretion, documented by Barkai and Griffiths (1987), and the positive linear relationship between temperature and ammonia excretion reported by Lyon (1995), under farm conditions within a temperature range of 14 – 19 °C and a size range of 18 – 43 g. In addition, the production of ammonia by the sludge that accumulated in abalone tanks (Chapter 4) was confirmed. As the growth of abalone is affected by free ammonia (Harris et al., 1998a; Harris et al., 1998b; Reddy-Lopata et al., 2006) these equations should be of great benefit to abalone farms as they can be used to determine the maximum biomass loading (kg L^{-1} s^{-1}) possible for a given set of environmental conditions and a desired ammonia concentration. However, there are some limitations to the application of these equations. The range of mean abalone sizes from which data were collected did not include sizes under 18 g or over 43 g. Furthermore, the tanks containing the smaller size classes were predominantly sampled when temperatures were high (± 18 °C), while tanks containing the larger size-classes were mainly sampled when temperatures

were lower (± 15 °C). Thus the equations would benefit from larger data sets, collected under a broader range of conditions. In addition, if farm management style and tank design influence ammonia production then the equations may need to be modified for use on other farms.

The fluctuation in the oxygen concentration of the farm's effluent was related to temperature, flow-rate and the farm biomass using equation 2.5. The values predicted by the equation were highly correlated with the observed values ($r^2 = 0.69$). The equation supported the finding of Lyon (1995) that the oxygen consumption of *H. midae* increases linearly with increasing temperature. Since the concentration of oxygen in the culture environment is known to affect abalone growth (Harris et al., 1999a) this equation could be used to improve the management of oxygen concentrations on abalone farms. For example, the equation can be used to derive estimates of the maximum abalone biomass which can be stocked by a farm per unit of flow-rate ($t\ L^{-1}\ s^{-1}$) at different temperatures, given a required effluent oxygen concentration (Table 6.1). However, it is acknowledged that the equation was developed using data collected only at 09h00 and 16h00 and therefore it will need further development to make it applicable to the full diurnal cycle. In its present form it is likely that the equation will over-estimate the oxygen concentration of abalone farm effluent at night, as it does not take into account the greater oxygen consumption in abalone tanks at this time. While equation 2.5 was developed for abalone farm effluent, its accuracy suggests that a similar equation could be developed for the culture water within abalone tanks. The equation would need to include abalone size as a variable as oxygen consumption declines exponentially with increasing abalone size (Barkai & Griffiths, 1987). This would enable farms to set the flow-rate of tanks with different abalone sizes, stocking densities and temperatures to achieve a required oxygen concentration.

Table 6.1. Maximum abalone *Haliotis midae* biomass loading (t L^{-1} s^{-1}) at different temperatures given a required effluent oxygen concentration.

Required oxygen:	5.0 mg l^{-1}	5.5 mg l^{-1}	6.0 mg l^{-1}	6.5 mg l^{-1}	7.0 mg l^{-1}	7.5 mg l^{-1}	8.0 mg l^{-1}
Temperature (℃):	Abalone biomass (t L^{-1} s^{-1})						
11	0.65	0.56	0.48	0.40	0.32	0.24	0.15
12	0.57	0.49	0.42	0.34	0.27	0.19	0.12
13	0.50	0.43	0.36	0.29	0.23	0.16	0.09
14	0.45	0.38	0.32	0.25	0.19	0.13	0.06
15	0.40	0.34	0.28	0.22	0.16	0.10	0.04
16	0.36	0.30	0.24	0.19	0.13	0.07	0.02
17	0.32	0.26	0.21	0.16	0.11	0.05	-
18	0.28	0.23	0.18	0.13	0.08	0.03	-
19	0.25	0.21	0.16	0.11	0.07	0.02	-
20	0.23	0.18	0.14	0.09	0.05	0.00	-
21	0.20	0.16	0.12	0.07	0.03	-	-

The production of dissolved ammonia by sludge

On a dry weight basis sludge was found to make a larger contribution to ammonia production in tanks than the abalone themselves (Chapter 4). In tanks containing approximately 9.1 kg of abalone soft tissue (dry weight), in addition to other potential sources and sinks of ammonia, the removal of 0.735 kg of sludge (dry weight) reduced total ammonia production by 31 %. The contribution of sludge to ammonia production in abalone tanks was also shown in equations 2.6 and 2.7. In these equations an increase in the length of time since an abalone tank was last cleaned resulted in an increase in the estimated ammonia concentration and ammonia production in the tank. The threshold free ammonia concentration at which the growth of *H. midae* becomes affected has not been established. Therefore, it is uncertain whether the presence of sludge elevates free ammonia concentrations to a level at which the growth of *H. midae* is affected. Thus, it is recommended that, as a precautionary measure, sludge removal be incorporated in management programmes. The rate at which sludge accumulates in tanks will vary under different conditions so a set time interval between sludge removal will not be the most efficient. In addition, excessive handling of the abalone for the purposes of cleaning abalone tanks may adversely affect their growth. Therefore, farms would benefit from research that could provide a

simple visual index for determining when tanks should be cleaned. In addition, it may be that filtration of the farm's influent water will reduce the accumulation of sludge in tanks, thereby lengthening the possible time intervals between cleaning.

The potential for reuse of abalone effluent as a culture medium for finfish and bloodworm

The growth and survival of silver kob and bloodworm in abalone effluent and control seawater indicated that abalone farm effluent could be used to culture these species. Since approximately five to six percent of the total cost of abalone farming is spent pumping seawater ashore, reuse of the abalone farm effluent to culture other species would significantly improve the economic efficiency of abalone farming (du Plessis, 2005; pers. comm.). Integrated systems with abalone and finfish or abalone and bloodworm would also be able to produce a greater diversity of products. The effectiveness of bloodworm at removing solid waste from abalone farm effluent indicated that the culture of this species in an integrated system with abalone would reduce the release of solid waste by abalone farms into the marine environment. Since bloodworm did not require feeding the production of bloodworm could be achieved without feed costs. While the growth of silver kob compared well with that of wild individuals (Griffiths, 1996b), there is evidence to suggest that the mean temperature of the abalone effluent (*ca.* 16 .6 °C) was below the optimal for silver kob (Dickens, 2007). In addition, this study was conducted during months with relatively warm water temperatures and the annual mean will likely be lower than 16 .6 °C. For example, the annual mean documented by *Experiment 3: Abalone farm influent and effluent quality* was approximately 15.5 °C (Chapter 2). Growth of the silver kob was relatively slow compared to that achieved by commercially successful finfish species at their optimal temperatures (Austreng et al., 1987; Sveier et al., 1999; Vergara et al., 1999; Daniel, 2004; Partridge et al., 2006). However, this study was preliminary and optimisation of the diet and culture techniques should improve growth. Since the concentration of oxygen affects the growth of finfish (Davis, 1975), one of the most important factors which will determine the biomass of finfish that can be stocked per unit of flow-rate of abalone effluent is the oxygen concentration of the effluent (Haskell, 1955; Willoughby, 1968; Fivelstad, 1988; Colt & Orwicz, 1991). In this case, equation 2.5, which can be used to predict the oxygen concentration of the abalone effluent will be useful in determining the amount of finfish that can be stocked under different conditions.

At a flow-rate (0.34 l s^{-1} m^{-2}), stocking density (847.9 g m^{-2}) and growth rate (0.39 % BW d^{-1}) equal to that of the current study, abalone farms with effluent flow-rates of 500 - 1000 l s^{-1} would be able to sustain bloodworm farms of approximately 1500 - 3000 m^2, capable of producing 4.8 - 9.6 kg bloodworm d^{-1}. This production could be improved by approximately 54 %, to 7.4 - 14.8 kg bloodworm d^{-1}, as in the current study only 65 % of the suspended solids in the abalone farm effluent were deposited in the bloodworm tanks. The preliminary investigation of Samsukal (2004) indicates that the concentrations of suspended solids recorded in the current study were similar to those in the effluent from other abalone farms. However, the production (µg s^{-1}) of suspended solids by an abalone farm, and hence the capacity for bloodworm production in its effluent, will depend more upon the biomass of abalone on the farm than the farm's flow-rate. In this case the values for the production of suspended solids per unit of abalone biomass (µg s^{-1} t^{-1} abalone) recorded in *Experiment 3: Abalone farm influent and effluent quality* (Chapter 2), will be useful in estimating the production of solids (µg s^{-1}) by a particular farm based on its biomass. However, it must be noted that these values were measured on a farm with a size range of abalone from 1 – 120 g and that the production of solids may vary on farms with a different size-range of abalone.

Conclusions

Water quality in the abalone farm environment was primarily influenced by the flow-rate per unit of abalone biomass, and temperature. Changes in pH, oxygen and free ammonia in the farm environment were explained by the metabolism of abalone in which oxygen is consumed and carbon-dioxide (Hochachka et al., 1983) and ammonia are produced. Carbon dioxide reacts with water to form H^+ ions, which decrease the waters' pH (Sanni & Forsberg, 1996). Furthermore, the linear relationship between temperature and metabolism documented by Lyon (1995) and the negative, exponential relationship between body-size and metabolism reported by Barkai and Griffiths (1987) were verified under farm conditions. The effect of the flow-rate per unit of biomass in culture systems, on water quality, was indicated by the inverse relationship between the ammonia concentration and the flow-rate per unit of biomass in abalone tanks. The oxygen

concentration in abalone farm effluent was also inversely related to the flow-rate per unit of biomass of the farm.

Therefore, water quality in the abalone farm environment was not random but was determined by the flow-rate per unit of biomass, abalone size and temperature. This demonstrated the importance of optimising the flow-rate per unit of biomass for various temperatures and abalone sizes. As abalone size and temperature cannot be controlled, the only way in which farmers will be able to control water quality is by varying the flow-rate per unit of biomass which the abalone culture system receives.

Abalone and bloodworm or abalone and finfish could be farmed in integrated systems. This would improve the economic efficiency of pumping seawater ashore and increase the diversity of products produced by abalone farms. Furthermore, integrated systems of abalone and bloodworm would release less solid waste to the marine environment than abalone farms. The investigations of water quality dynamics on abalone farms, presented in this thesis, can contribute to estimating the biomass of finfish and bloodworm that could be stocked in abalone farm effluent.

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