ASPECTS OF THE PHYSIOLOGY OF THE SOUTH AFRICAN ABALONE, HALIOTIS MIDAE L., AND IMPLICATIONS FOR INTENSIVE ABALONE CULTURE.

THESIS

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CONTENTS

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ABSTRACT:

A physiological study was carried out to determine the effects of temperature, quantity of food fed, feeding activity and size class on the oxygen consumption and ammonia production of the South African abalone *Haliotis midae* L.. A set of intermittent flow respirometer chambers were used in a recirculating system to measure the oxygen consumption rates of fed (postprandial) and unfed (postabsorptive) abalone for three different size classes (15, 30, and 50mm) at three different temperatures (16°, 20° and 23°C) over a 24 hour period. Ammonia production rates and food consumption rates were simultaneously determined.

Oxygen consumption and Ammonia production rates per gram abalone were linear functions of abalone size, temperature, and mass of food consumed. Oxygen consumption and ammonia production rates were found to increase proportionally to temperature and in inverse proportion to abalone size. These rates were significantly higher for fed as opposed to unfed abalone. Equations were derived to predict oxygen consumption and ammonia production for fed and unfed animals for a range of temperatures and size classes of abalone.

A 96h LC50 lethal toxicity test for exposure to ammonia established 1.08mg.l^{-1} of unionised ammonia as the lethal limit. A test on the long term effects on growth of acute exposure (12h) to various sublethal concentrations of ammonia showed no significant effects on growth for concentrations below 0.88 mg.l^{-1}. A safe chronic exposure level of 0.02 mg.l^{-1} NH_3-N was used to predict optimal flow rates required per kilogram of abalone in a rearing tank over a range of size classes and temperatures. The physiological and biological requirements of *H. midae* and the implications of this knowledge for rearing tank management and optimal design are discussed.

INTRODUCTION

Abalone has become one of the most popular marine products, particularly in Asia where it is considered a delicacy and is believed to be an aphrodisiac. The demand for abalone, combined with its undersupply, has resulted in abalone products having an extremely high market value ranging between US$ 20-40/kg live weight. Presently abalone are harvested in France, the Mediterranean, South Africa, Japan, Korea, Taiwan, China, Australia and in New Zealand and Mexico, U.S.A. and Canada (Olley and Thrower 1977, Lindberg 1992). There are more than 100 species of these marine gastropods worldwide, all of which belong to the genus *Haliotis* (Hahn 1989). The small size of some of the species means that not all of them are utilised commercially. Depletion of abalone stocks by overfishing and the high value of abalone on overseas markets has led to increased research to develop abalone aquaculture. The first effective experimental spawning of abalone was achieved by Murayama (1935), followed by Ino (1952). This was followed by the experimental culture of abalone in Nagi in Japan in 1959 (Shepherd 1976). Abalone are presently cultured in Japan (*H. gigantea* and *H. discus hannai*), Taiwan (*H. diversicolor supertexta*), California predominantly in Morro bay (*H. rufescens*, *H. fulgens*, and *H. corrugata*), Australia (*H. ruber*), New Zealand (*H. iris* or the paui), South Africa (*H. midae*), and in China *H. discus hannai* (Bardach et al. 1972, Chen 1989, Ebert 1992, Nie 1992).

Six species of *Haliotis* have been identified on the South African coast (Muller 1986). *Haliotis midae* is the largest of the South African abalone species and is the only one which is commercially exploited. The small size and low biomass of the other species makes them commercially unattractive. *H. midae* is distributed from St. Helena Bay in the western Cape

to Port St. Johns on the Transkei coast (Newman 1969, Muller 1986). The harvesting of *H. midae* is most productive in those areas of the south western Cape with an average yearly temperature variation from 15 to 17°C. The greater proportion of the population is concentrated between the low tide mark and a depth of approximately 25 metres (Newman 1969) with most animals occurring at depths of 2-10m in beds of the kelp *Ecklonia maxima* (Tarr 1992). Juvenile *H. midae* are found in the subtidal zone under small rocks in the shallower waters.

Fisheries and aquaculture Trends

The South African abalone has a present export price in excess of R110 per kg live weight with a total export value in excess of R16 million per annum (Tarr 1992). This makes the South African abalone a highly sought after resource, which has placed tremendous pressure on the abalone stocks whose restricted distribution makes them very vulnerable to exploitation by commercial and recreational divers as well as poachers. Records of the catch per unit effort for *H. midae* have shown a decrease from ca. 1000 kg/diver/month in 1960 to between 300 and 400 kg/diver/month in 1990 (Tarr 1992). Concern about the decline in the catch of the South African abalone following the exploitation of accumulated abalone beds, particularly in the Hermanus area, lead to the imposition of catch quotas in an attempt to facilitate a recovery of the stocks. This has led to an improvement in yields from the early 1980's low of 200 kg/diver/month to ca. 350 kg/diver/month in 1990. A serious decline in abalone fisheries all over the world has occurred as a result of the high market demand for this product and has provided an incentive for the culture of abalone. Abalone have in many cases been cultured for reseeding following the drastic reduction of natural populations

(Garza and Bernal 1992, Tong and Moss 1992). Following the successes of such programs the emphasis has moved towards the intensive culture of abalone for growout in on shore facilities.

Intensive culture of marine organisms requires considerable research to develop appropriate technology. The current status of the abalone aquaculture has been reviewed by Hahn (1992), Shepherd et al. (1992) and Fallu (1972). Research has been done on various aspects of the taxonomy (Shepherd 1976), physiology (Barkai and Griffiths 1987, 1988, Dixon 1992), ecology and distribution (Stephenson 1944, Newman 1969), growth (Newman 1968), reproduction (Newman 1967, Genade et al. 1988) and diet (Barkai and Griffiths 1986, Britz et al. 1994, Knauer et al. 1993, 1994) of *H. midae*. This information has provided a foundation upon which to develop technology for the commercial culture of the South African abalone.

The development of an artificial feed for *H. midae* (Britz et al. 1994), was an important breakthrough for the intensive culture of this organism. This is because an artificial diet has several advantages over the natural seaweed diet. Firstly, it is a reliable source of food which provides a balanced (complete) diet and which is easily produced by the animal feed industry. Formulated diets are a more nutritious and concentrated diet than seaweed and produce animals with consistently higher growth rates and food conversion efficiencies. Britz (1993) found that artificial feeds produced a feed conversion ratio (dry feed fed: wet weight gain) close to 1:1 while dried kelp produced the worst feed conversion ratio at 5. Artificial feed is also more digestible. Artificial feed is also easily stored and transported, takes up less space, is easily accessible to the abalone and can be evenly distributed over the tank floor.

It also has a high water stability (Britz 1993) which ensures that the abalone have a chance to derive as much nutrient as possible from the diet before leaching occurs. Artificial feeds thus eliminate the risks associated with the harvest of seaweed and make commercial scale culture possible even where no exploitable seaweed resources exist.

Intensive aquaculture strives to maximise production while reducing the associated capital and operating costs. The most important consideration in tank design for shore based production systems is to ensure that the equipment is designed for efficient use and that the profitability per unit volume of water pumped ashore is maximised (Klapsis and Burley 1984). In physical terms, the productivity of an abalone rearing system is thus determined by the mass of abalone produced per unit of tank rearing space and per litre of flow. The mass rearing of animals on an industrial scale requires relatively high stocking densities within each tank. This can have an adverse effect on the water quality within the tank which in turn affects the animals. Several important factors need to be considered in tank design: Good water quality conditions need to be maintained; the tank needs to be self cleaning with minimal disturbance of the cultured animal; stocking densities of the animals concerned should be optimal and conditions which promote maximum growth, such as access to feed, need to be promoted. In addition, harvesting needs to be cost effective, simple and efficient. Stress during harvesting should also be minimised especially if the animals are to be transported to the market live and in good condition. The advantages of optimal tank design are improved survival, fitness and performance, reduced pumping costs or conservation of water, reduced feed wastage and reduced labour costs.

The water quality and flow requirements within a raceway are dictated by the environmental

requirements of the organisms being cultured and the rate of water quality change as a result of their metabolic processes (Muir 1982). Adequate water circulation and rapid mixing of influent and tank water is therefore important in tank design (Westers and Pratt 1977). Improper mixing and short circuiting can result in local depletion of oxygen in certain areas, as well as the simultaneous build-up of ammonia and the accumulation of detritus (Klapsis and Burley 1984). A number of studies have been conducted to determine how flow dynamics are affected by tank design (Burrows and Chenowith 1955, Klapsis and Burley 1984, Cripps and Poxton 1992, Cripps and Poxton 1993). These studies have focused exclusively on flow dynamics in fish tanks for the maintenance of optimal water quality conditions through even flow distribution. The main concern in tank design has been removal of wastes and good exchange of water.

Oxygen consumption by intensively cultured abalone determines the oxygen concentration in the water and is the prime limiting factor in determining the carrying capacity of a rearing tank. The main limiting metabolite after oxygen is unionised ammonia (Willoughby 1968). Ammonia-nitrogen is also the main end product of protein catabolism in most aquatic poikilotherms. High animal stocking densities can lead to a build-up of this metabolite to potentially toxic levels. Ammonia-nitrogen occurs in two forms, an unionised form (NH_3-N) which is toxic, and an ionised form (NH_4^{+}-N) which is non-toxic (Thurston et al. 1981). Unionised ammonia, because of its lack of charge and low solubility is more toxic, as it can readily diffuse across gill membranes. The ionised form of ammonia occurs as a larger, charged, hydrated molecule that cannot pass easily through the hydrophobic micropores of the gill membrane, rendering it less toxic (Fromme and Gillette 1968). The equilibrium between the two forms of ammonia-nitrogen, is determined primarily by the pH of the water.

Consequently ammonia-nitrogen is more toxic in seawater, which is a better buffer and has a higher pH, than freshwater. The proportion of unionised ammonia-nitrogen present in solution also increases as the temperature increases and decreases as the ionic strength of the water increases (Alabaster and Lloyd 1980). The temperature effect is due to increased hydrolysis of ammonium ions (NH_4) ions at higher temperatures (Hampson 1976). Ammonia toxicity is caused by high ammonia concentrations in the blood resulting from an inability of the animal to excrete ammonia, or from the uptake of ammonia from the water at the surface membranes, particularly at the gills (Hampson 1976). Symptoms of ammonia toxicity include hyperplasia, proliferation, clubbing, and eventual fusion and consolidation of the gill lamellae which leads to asphyxiation (Burrows 1964). There may also be damage to the liver, kidneys and nervous system of fish (Jeney et al. 1991). In an intensive aquaculture system the primary factor limiting a build up of ammonia is the rate of supply of the water. Knowledge of the ammonia production levels and the tolerance of the aquatic animal to different concentrations of ammonia-nitrogen facilitates the management of flow rates so that optimal water quality conditions can be maintained at maximum stocking densities. While extensive studies have been performed on fish, no work has yet been published on the effects of chronic and acute exposure of abalone to ammonia.

The levels of oxygen consumed and ammonia produced by an ammonotelic animal is determined to a large degree by the food consumption rate of the animal. Haskell (1955) stated that the amount of oxygen consumed and the quantity of metabolic products excreted is proportional to the amount of food consumed. Liao (1970) also found that dissolved oxygen uptake and the ammonia and phosphate production rates of fish are proportional to their feeding rates and inversely proportional to the fish size, loading densities and water

supply rates (oxygen concentration in the water). Uneaten food also fouls the water through leaching and decay which increases ammonia levels and decreases oxygen levels because of the bacterial and chemical oxygen demand. Speece (1973) found that if the carrying capacity of trout in a pond ($kg.m^3$) for a given flow rate is known for any particular size of fish at a particular temperature, then the safe carrying capacity for other sizes and temperatures is that quantity of fish which will require the same weight of feed daily. Thus the quantity of feed consumed can be used to determine the carrying capacity of a tank (i.e. the mass and number of animals that can be cultured in that tank) irrespective of the size of the fish and the ambient temperature. Abalone, which are also poikilotherms, fed on formulated diets could theoretically be expected to show a similar relationship between the quantity of feed consumed and the carrying capacity. It may be hypothesized that regardless of size and temperature, abalone which are fed the same quantity of feed should consume the same quantity of oxygen and excrete the same quantity of ammonia. This knowledge could make the management of water flow rates within abalone tanks possible using the quantity of food consumed as a criterion to predict ammonia excretion rates and hence flow rates required to maintain suitable ammonia levels.

The physiology of poikilotherms is affected by several environmental factors which have been classified by Fry (1971) as limiting, directive, controlling, masking and lethal factors. These factors may affect their physiology by influencing metabolic rate or the effect on the physiology may be independent of the metabolic rate of the animal. Lethal temperatures, for example, are regarded as lethal factors that lead to the death of the animal without affecting the metabolic rate. The two main factors that affect the rate of metabolism of poikilotherms are controlling factors and limiting factors. These factors usually work in combination to

influence the metabolic rate. Controlling factors act by influencing the state of molecular activation of the metabolic chain and include factors such as temperature. Limiting factors affect the metabolic rate by restricting the supply or removal of the materials in the metabolic chain. Limiting factors include metabolites, food, water, and the respiratory gases. The quantity, rate and time at which feed is consumed is determined by controlling factors such as temperature, limiting factors, such as oxygen levels and quality of feed consumed, and directive factors, such as photoperiod. Directive factors are responsible for all behavioural regulation and for anticipatory adjustments in physiological regulation like hormonal responses. Buss and Miller (1971) have shown that the response of trout to feed is determined by the availability of oxygen and the water temperature. In general the metabolic rate of a poikilotherm increases with temperature and activity, and decreases with body size (Liao and Mayo 1972, Bayne and Newell 1983). Like all poikilotherms, abalone respiration rates have been found to be dependant both on temperature and on body size (Uki and Kikutchi 1982).

Some work has been done on respiration (oxygen consumption) and metabolic rate in abalone (Tamura 1939, Peck et al. 1987, Nimura and Yamakawa 1989, Uki and Kikuchi 1975, 1982, Segawa 1991) but very little information exists on how it is affected by water temperature, body size, time of day, or the quantity of food consumed. Energy budgets have been developed for *H. tuberculata* and *H. midae* based on seaweed diets (Peck et al. 1987, Barkai and Griffiths 1987, 1988). Barkai and Griffiths (1987) found that the oxygen consumption rate of abalone increases with increasing mass, and feed consumption per unit weight decreases. Uki (1981) working on *H. discus hannai* found that the hourly feeding rate, and the duration of intensive feeding and consequently the quantity of feed consumed increases

with higher temperature.

Considerable work has been done on the influence of directive factors such as photoperiod and its affect on feeding activity and respiration rates of poikilotherms. Most organisms show either diurnal or nocturnal peaks in activity with metabolic responses that show a clear correlation with photoperiod (Newell 1979). Some poikilothermic animals such as Sockeye Salmon (Brett and Zala 1975) and Fiddler Crabs Brown et al. (1954) have distinct diurnal, lunar, and semilunar fluctuations for both oxygen consumption and ammonia production. Abalone have been found to have a diel activity rhythm with a nocturnal pattern of feeding (Barkai and Griffiths 1986, Knauer et al. 1994). Consequently their respiration rate would be expected to be greatest at night when their activity is greatest. Uki (1981) working on *H. discus hannai* found that feeding shows clear diel periodicity and takes place from dusk to midnight at a constant rate. Uki and Kikutchi (1982) found that oxygen consumption in *H. discus hannai* showed a circadian rhythm with the rate increasing from dusk to midnight and decreasing from midnight to midday. This indicates that the most active feeding period for *H. discus hannai* is mainly during the initial portion of the dark period and is not constant throughout the night. Knauer et al. (1994) in their study on the feeding behaviour of juvenile *H. midae*, found that there was a clear nocturnal feeding activity with no feeding taking place between 07h00 and 18h00. They also found that at any one time less than 7% of the animals were actively feeding but most animals were active. Previous work by Barkai and Griffiths (1987) on *H. midae* and Peck et al. (1987) on *H. tuberculata*, has also shown clear diel patterns in the feeding periodicity of these animals. These authors however found that, despite observed peaks in the nocturnal activity of abalone, oxygen consumption rates over a 24 hour period did not differ significantly.

Environmental parameters such as controlling, limiting, and directive factors affect the metabolic rate and hence the physiology of abalone. The response of abalone to these parameters has important implications for the management and design of rearing tank units. A change in the metabolic rate of abalone brought on by these environmental parameters, ultimately affects the water quality and hence carrying capacity of the rearing tank. The present study was thus undertaken in order to understand the dynamics of the production of these limiting metabolites and recommend management protocols for water quality maintenance in intensive abalone culture. It was decided to quantify the nitrogenous waste production and oxygen consumption of *H. midae* for various temperatures, size classes, feeding regimes (fed and unfed animals) and time of day. This data would facilitate the testing of the hypothesis derived from Haskell (1955), namely that the relationship between oxygen consumption, ammonia production and the quantity of food fed should be a constant for all size classes. To define effective management guidelines for abalone the water quality requirements of the abalone had to be established i.e. safe levels of unionised ammonia for acute exposure (96h LC50). The effect on growth of short term exposure to sublethal levels of ammonia also needed to be determined in order to establish the long term effects of acute exposure to ammonia following an accidental increase in levels of ammonia due to a system failure. This data would facilitate the determination of flow rates required to maintain optimum water quality conditions for different size classes, at different stocking densities and temperatures, and for different quantities of food consumed.

CHAPTER 2 An overview of respirometry techniques

Several experimental techniques for measurement of respiration rates have been developed, which are generally referred to as methods of respirometry. Respirometry is a procedure in which an organism's rate of oxygen consumption is measured in order to calculate its metabolic rate. There are many problems associated with the measurement of metabolic rate. For example the metabolic rate of an organism can be influenced by environmental conditions such as temperature, feeding and nutritional state, day length and water quality (Schmidt-Nielson 1984). Furthermore poor water quality conditions in the respirometer apparatus may stress the experimental animals and affect their metabolic rate. Experimental protocols can also have an adverse effect on the metabolic rate of an organism, for example, the time of measurement relative to circadian cycles, time of measurement after introduction of fish into the experimental apparatus, size of the experimental chamber, degree of suppression of external stimuli, and the presence of other animals in the chamber can all influence metabolic rate (Schmidt-Nielson 1984). An understanding of the various types of respirometer apparatus and their applications is necessary in order to select the most appropriate method for a particular experimental situation.

Respirometry has been used in the past to determine energy budgets based on measurements of oxygen, carbon dioxide, and ammonia. Measurements are made either directly using electrodes or probes (Springer and Neill 1987, Mitz and Newman 1988), and manometers (Ghosh et al. 1985, Mitz and Newman 1988), or indirectly by means of Winklers titrations followed by a spectrophotometric analysis (Peck et al. 1987). Measurements may be made either in an air or a water medium within which the experimental animal is confined during

the investigation. The animal subject for the investigation is retained in a sealed chamber, usually glass or perspex, which forms part of the respirometer apparatus. A series of measurements are taken over a period of time to derive a rate of oxygen consumption or carbon dioxide production. Types of respirometry include closed or static respirometry (Ghosh et al. 1986, Springer and Neill 1988, James and Probyn 1989), flow-through or open respirometry (Cho et al. 1982, Gehrke et al. 1990), and intermittent-flow (Livingston 1968, Steffenson 1989), semi-closed (Oikawa et al. 1991) or Gilson differential flow respirometry (Dixon 1951, Mitz and Newman 1989).

Closed respirometers

Closed respirometry involves measurement of the change in gas content of the water over a period of time in a closed or chamber. It was first used by Humboldt and Provencal in 1809 (Steffenson 1989). The closed method is based therefore on the depletion of oxygen in water in a sealed container (Oikawa et al. 1991). Oxygen concentrations are measured in closed systems by titrations (e.g. Winklers), manometric or volumetric methods (Scholander et al. 1943), and oxygen electrodes (Springer and Neill 1988).

Advantages and disadvantages

The advantage of this technique is that readings can be taken over short time spans making it easier to distinguish between standard oxygen consumption (oxygen consumption of resting, postabsorptive (unfed) animals and routine oxygen consumption (oxygen consumption arising from spontaneous activity) (Fry and Hart 1948, Fry 1957, 1971). Disadvantages of this

technique are that measurements over short time intervals reduce the ability to measure changes in the concentration of oxygen accurately and stratification of gas content in the water may affect readings as a result of improper mixing of gases in the water.

Accumulation of production of carbon dioxide and other metabolites as well as nitrogenous excretory products may affect the respiration rates of the animal (Springer and Neill 1988). In addition bacterial oxygen consumption may also influence results particularly in a closed system with a relatively large volume where the decline in concentration of oxygen will be relatively slow. In a situation such as this, the consumption of oxygen by bacterial oxygen demand is often not considered. This may cause a significant error in the calculation of the oxygen consumption of the animal concerned (Springer and Neill 1988, Steffenson 1989). Oikawa et al. (1991) in a study on *Pagrus major* has overcome this problem by determining the respiration of bacteria in water to cancel the effects of bacterial decomposition of organic matter in water.

Open respirometry

Open respirometry involves measurement of the difference in gas content or loss of oxygen relative to the water flow rate through the test chamber (Ege and Krogh 1914, Oikawa et al. 1991). Open respirometry was introduced by Ege and Krogh (1914). Usually the oxygen consumption is determined in open respirometry by using the Fick principle (Fick 1870; cited in Dixon 1951). This is an equation used to calculate oxygen consumption based on determinations of oxygen content of the water running in and going out, and the water flow through the respirometer. The water flow rate in the respirometer is reduced to allow a 20%

drop in the oxygen content in the water running through the chamber. Once the flow rate has been adjusted a period of time is allowed for the oxygen levels to stabilise at a new steady state which usually takes from 20-60 minutes and which is referred to as a transformation.

Advantages and disadvantages

The advantage of an open respirometer system is that there is no build up of metabolites such as carbon dioxide or nitrogenous wastes that can influence readings. The disadvantage of open respirometry is that it doesn't make allowance for the reservoir or washout effects within the chamber. The washout effect is a delay or lag in the change of oxygen consumption following a change in the activity of the animal (Spoor 1946). It is a function of the dilution factor of the chamber, that is, the ratio of water flow through the respirometer chamber, to the volume of water in the respirometer.

Intermittent-flow respirometry

Intermittent flow respirometry is a semi-closed respirometry technique which allows for periodic flushing between readings to avoid the build up of excretory products such as carbon dioxide and ammonia (Forstner 1983) and a drop in the oxygen levels beyond the critical oxygen concentration (Spoor 1946), below which the animals respiration rate becomes dependant on the oxygen concentration of the water (oxygen conformer). Intermittent flow respirometers are an effective means of taking measurements over short time spans. Repeated experiments can also be performed within narrow limits of time and partial pressure changes by flushing of respirometers using automatic or manual control of valves. A flow-through

of water in the respirometer occurs during the period of equilibration of the respirometer and manometer. Livingston (1968) used a modified volumetric or intermittent flow respirometer. He found that such a system combined the advantages of a volumetric technique with those of a running water system to allow the continuous measurement of oxygen consumption under controlled conditions. With this method oxygen consumption can be determined during three to ten minute intervals with a decrease in partial pressure of oxygen of only 2 to 5 mm Hg (Steffenson et al. 1984). Dixon (1951) refers to intermittent flow respirometers as differential type respirometers.

Advantages and Disadvantages

The advantage of these respirometers is that they allow for readings to be taken over short periods of time while reducing the problems of accumulation of carbon dioxide and other excretory products by allowing for periodic flushing of the chambers using valves.
Other advantages of intermittent-flow respirometers is that they make use of volumetric or manometric methods which determine instantaneously changes in oxygen consumption associated with a change in activity thus eliminating the problems associated with the reservoir or washout effect (Steffenson 1989). These respirometers may also be used with oxygen meters. A disadvantage of this system is that periodic flushing might have an effect on the activity of the animal thereby influencing results for respiratory rates. The level of water in the chamber changes following flushing which may affect readings particularly if the level is not carefully adjusted to its original marked level.

Oikawa et al. (1991) used several respirometer systems in a study on *Pagrus major*. These

systems included a closed system, an open or continuous flow system and two semi-closed respirometer systems, one of which was equipped with an oxygen electrode the other not. His results demonstrated that all techniques were reliable for the determination of oxygen consumption rates, however there were differences in size ranges between the fish used for each technique making it impossible to compare these techniques directly.

Measuring Techniques

Several techniques are used for measuring oxygen consumption in respirometry.

Probes

Oxygen probes are used for work on flow through or open systems and can also be used in closed or intermittent flow systems. It certainly is the quickest method of determining levels of certain substances like oxygen. These probes are usually connected to computer logging devices so that data is automatically recorded. The advantage of this technique is that the oxygen levels can be constantly monitored and recorded. Computerised data acquisition using oxygen probes has been used in several studies using all the types of respirometry (Gehrke et al. 1990, Cho et al. 1982, and Springer and Neill 1988).

Analytical titrations (Winklers)

This technique involves the titration of water samples taken from respirometers at various time intervals. The disadvantage of this method is that readings can not be taken

immediately. The oxygen levels of sample water often changes with time or exposure, with the result that readings become unreliable with time. The number of samples that can be taken during an experiment is also limited using this technique (APHA 1971).

Volumetric determinations

These techniques involve the use of manometers which are calibrated glass tubes filled with a viscous liquid like Gilsen's Respirometer Fluid which is moved by a change in pressure in the tube following respiration of the animal within a chamber to which the manometer is connected. Manometric techniques can only be used in a closed or semi-closed (intermittent-flow) system. The manometric respirometry technique has several operational advantages over the oxygen electrode respirometry method. Oxygen consumption measurements are logistically easier to make and more quickly obtained using the manometric method. This technique also provides very precise and accurate measurements by producing consistent and replicated measurements of the data. Also an oxygen electrode requires frequent, careful calibration which is not required for the manometric method. Umbreit et al. (1972) have described the three types of manometric respirometry, namely constant volume manometry, constant pressure manometry, and differential manometry.

In constant volume manometry the volume is held constant and the change in pressure measured in order to derive the amount of gas consumed or released. It may also be referred to as the Warburg Method (Umbreit et al. 1972). Constant pressure manometry involves maintaining a constant pressure in the reaction chamber and measurement of the change in volume of gas in order to derive how much oxygen is consumed and released. There are a

variety of these methods so that no specific name can be associated with these respirometers though the most popular is Gilsens respirometer (Umbreit et al. 1972). In differential manometry both pressure and volume change, and the amount of gas consumed or released is determined by measuring both these changes. It is referred to as the Barcroft or Differential respirometer. A differential respirometer is essentially a closed system formed of two flasks connected by a manometer. The volumes of liquid and gas are the same on both sides. One chamber contains the respiring animal and is known as the reaction chamber and the other chamber is known as the compensation chamber or vessel and compensates for changes in the temperature and barometric pressure during the course of an experiment.

The Warburg constant volume respirometer has been the most widely used unit for respirometry. It's popularity is derived primarily from its greater simplicity in callibration and use and its greater compactness than the Barcroft differential respirometer (Umbreit et al. 1972). The differential respirometer is superior for precise work as it is not affected by barometric changes and is less sensitive to temperature changes.

Having considered all the options it was determined that the ideal respirometry technique for the purposes of this study would be an intermittent-flow respirometer incorporating an oxygen meter. The reason for selecting this method was that it eliminated many of the problems associated with most other respirometry techniques such as accumulation of metabolites, reservoir or washout effects, and the stratification of gases. It also reduced handling stress between runs since it allowed animals to be resident in the chambers, frequent measurements could be taken, and experiments could be run over a long period i.e. 24 hours. The disadvantages of this system were that one oxygen probe had to be shared between

respirometers, and water samples could only be taken after oxygen readings as the chambers had to be opened for siphoning. An oxygen meter was chosen as the preferred method for measuring oxygen consumption as the manometric technique was not considered practical for our purposes.

Chapter 3 Oxygen consumption and ammonia production of *H. midae* and its implications for the management of flow rates in commercial rearing tank units.

INTRODUCTION:

Aquatic organisms have a profound effect on the water quality of their environment at high stocking densities. The major constraints on carrying capacity (Haskell 1955) and hence aquacultural production are the depletion of dissolved oxygen, accumulation of toxic metabolites (Soderberg et al. 1983, Westers and Pratt 1977) and extreme temperature fluctuations (Brockway 1950, Redner and Stickney 1979). Oxygen stress has been found to affect growth to a greater degree than does ammonia (Speece 1973), however in flowing water fish culture systems this can be ameliorated by additional aeration (Soderberg 1982), and it is the accumulation of un-ionised ammonia which is the primary limiting factor in production (Westers and Pratt, 1977).

Nitrogen pollution as NH_3-N may arise either from the production of metabolites or from the breakdown and leaching of feed. Nitrogen pollution from feeding can be attributed to food wastage from overfeeding, poor feed water stability, high solubility of foods in water and limited retention and absorption of ingested nitrogen by the cultured animal (Burrows and Combs 1968). Nitrogen pollution from feed leachate is particularly relevant to abalone which have a slow feeding rate. Haskell (1955) found that the amount of oxygen consumed by trout and the quantity of metabolic products are proportional to the amount of food fed. He reasoned that if the carrying capacity in terms of a given quantity of feed fed is known for any particular size of fish at a particular temperature, then the safe carrying capacity for

other sizes and temperatures is that quantity of fish which will require the same weight of feed daily. It can be hypothesised that ammonia excretion and oxygen consumption rates for aquatic poikilotherms such as abalone at various temperatures for a given quantity of food fed should be a constant. If this hypothesis is valid it would be possible to predict the minimum flow rates required to maintain oxygen and ammonia concentrations at safe levels in rearing containers according to the temperature and amount of feed fed.

The main aim of this project was to establish water quality guidelines for abalone culture. The objective of this experiment was to quantify the ammonia production and oxygen consumption rates for *H. midae* as functions of animal size, temperature, feed consumption and time of day.

METHODS AND MATERIALS:

Experimental System

A recirculating seawater system incorporating 12 intermittent-flow respirometers (Fig. 1 and 2) was designed for the measurement of the oxygen consumption and ammonia production of three size classes of *H. midae*. The chambers were adapted from a Gilsens respirometer design to incorporate a Schotte-geratte oxygen meter probe which was inserted through the lid of the chamber (Fig. 3.). Each respirometer consisted of a 1.5l perspex respiration chamber which contained the experimental animals. The chambers were closed with perspex lids and sealed with dry silicon rubber seals over which petroleum jelly was smeared to make an airtight fit. The lids were screwed onto the chambers by four brass screws fitted with wing nuts. Access to the chambers between experimental runs was through a 25mm hole

through which the airstone was inserted. This hole was also used to take readings with the oxygen probe and to siphon off samples of water for the ammonia analyses. The probe was inserted through a rubber bunge to make an airtight seal. The respirometer chambers were placed into glass waterbaths so that the temperature of the entire system could be kept constant during each experiment (Fig. 4). The temperature of the experimental system was regulated by means of thermosatatically controlled heater and chiller units. The water level within each of these water baths was kept just below the lids of the respirometer chambers. The glass waterbaths were connected to each other by 50mm PVC piping which kept the water level constant. Photoperiod was controlled by a timer switch and was set at a 12 hours light:dark cycle.

Experimental protocol

Animals used in this experiment were acclimated in the recirculating system for at least one week at each temperature. All animals were weighed and measured before they were placed into the experimental chambers. A further period of 4-5 days was allowed for the animals to acclimate to conditions within the respirometer chambers. During this time the animals were fed a percentage of their body weight using a table of feeding levels by Mangold (pers. comm.). Animals were fed in their chambers during each experimental run. Any excess or uneaten food pellets were removed daily from each of the chambers by siphoning. In addition to the supply of fresh seawater, the oxygen levels in the water were maintained at saturation level by airstones placed within the chambers between experimental runs. When readings were not being taken the chambers were sealed using rubber bunges. Readings were taken once every 6 hours over a 24 hour period. An initial oxygen reading was taken before the

chambers were sealed. In addition a sample of water was siphoned out of each tank for ammonia analysis. The chambers were sealed and the animals were allowed to respire for 20-30 minutes before the chambers were reopened. A final oxygen reading was then taken as well as a sample of water for ammonia analysis. Between runs the chambers were opened, aerated and flushed with fresh seawater to replace the oxygen used.

The water samples were tested for ammonia-nitrogen using the Indophenol spectrophotometric method (APHA 1971). Fractions of unionised ammonia at each of the temperatures and pH's were calculated using formulae from Rogers and Klemetson (1985). Initial and final oxygen and ammonia readings were then subtracted and the difference was divided by the mass of the animals within each chamber, the chamber volume, and the time period between readings and samples. Oxygen consumption and ammonia production for each size class was then expressed as mg NH_3/g abalone/hour. Readings of oxygen consumption and ammonia production per gram body weight were taken for fed and for unfed animals, at three different temperatures (16,20,23°C) for each of three size classes (10-20mm, 20-40mm and 40-60mm). The animals were acclimated over a period of weeks to the different temperatures and used in all the experiments. Two separate trials were run at each temperature, one for fed and another for unfed abalone. Three empty chambers were used as controls. Food consumption was determined by weighing the oven dried mass (100°C) of food fed and subtracting the dry mass from the uneaten food after it had been dried at 100°C in an oven. The masses of food fed to the abalone were corrected for the 10% moisture content in air dried pellets. All readings taken during the dark period were read under a red lamp so that the animals were disturbed as little as possible.

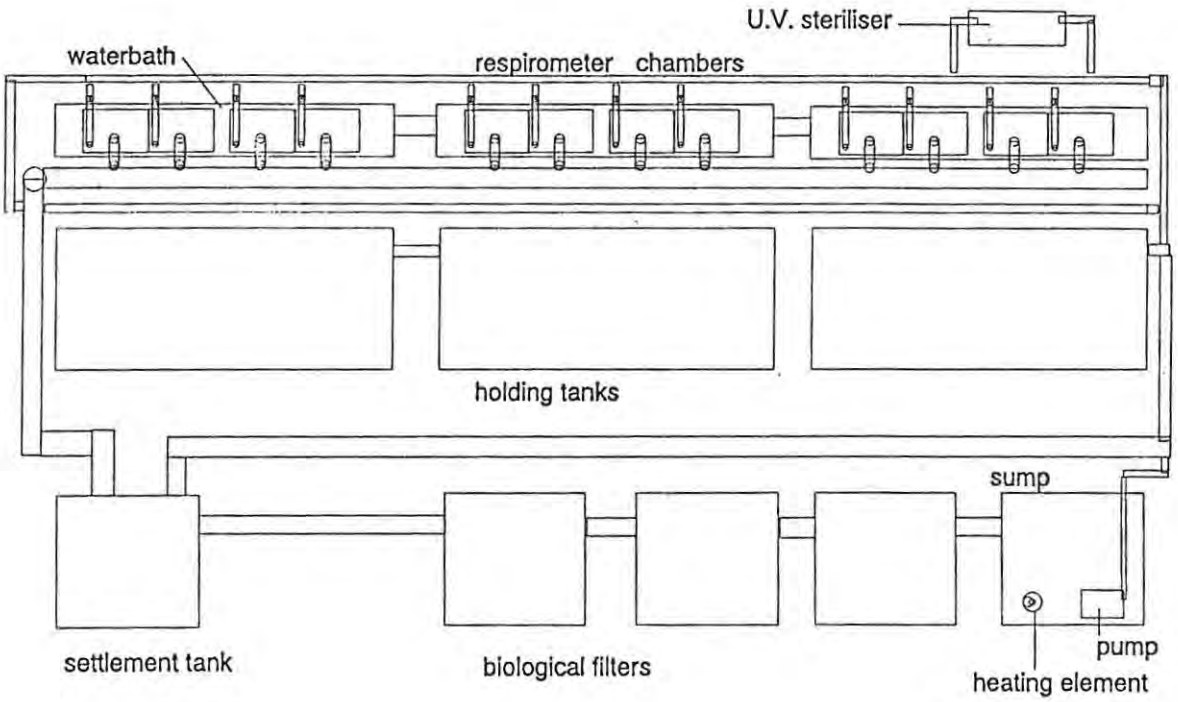


Figure. 1. Diagram to show the recirculating seawater system and respirometer chambers used for the respirometry experiments.

Statistical methods

Analysis of variance was run on the data to determine whether there were any significant differences in oxygen consumption rates and ammonia production per gram of abalone as functions of time, temperature, size class and feeding regime (fed or unfed animals). Regressions of oxygen consumption and ammonia production were plotted for each size class against temperature for both fed and unfed animals. A multiple regression was performed on the size class and temperature data in order to formulate a model of oxygen consumption and ammonia production.

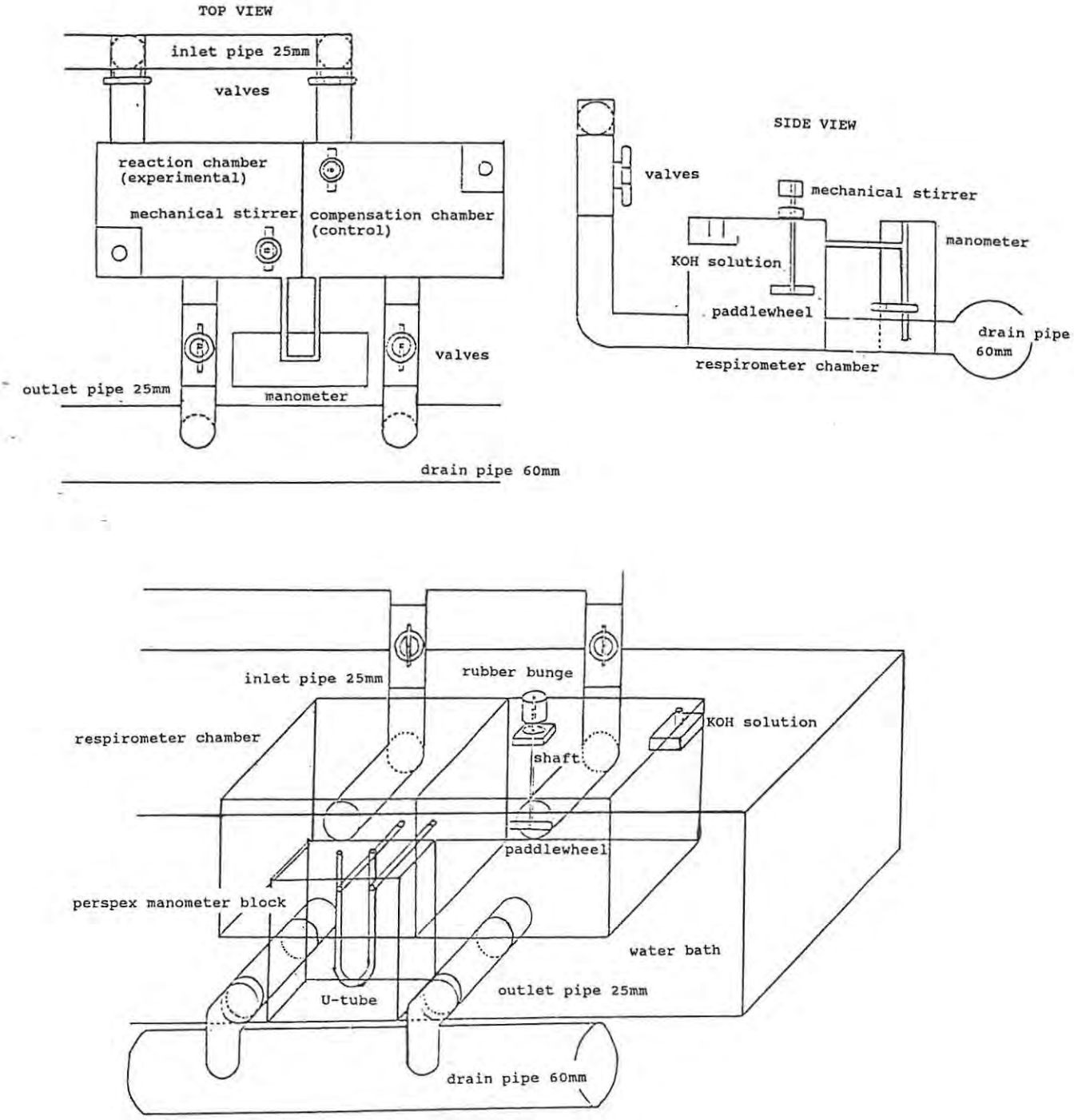


Figure 2. Plan diagram of the respirometer design based on the Gilsens intermittent-flow respirometer system in top, side and 3-D front view.

Figure 3. Photograph of the author taking an oxygen reading from one of the respirometer chambers. Note rubber bunge on probe used to seal the chamber (Photograph by Jentz Knauer).

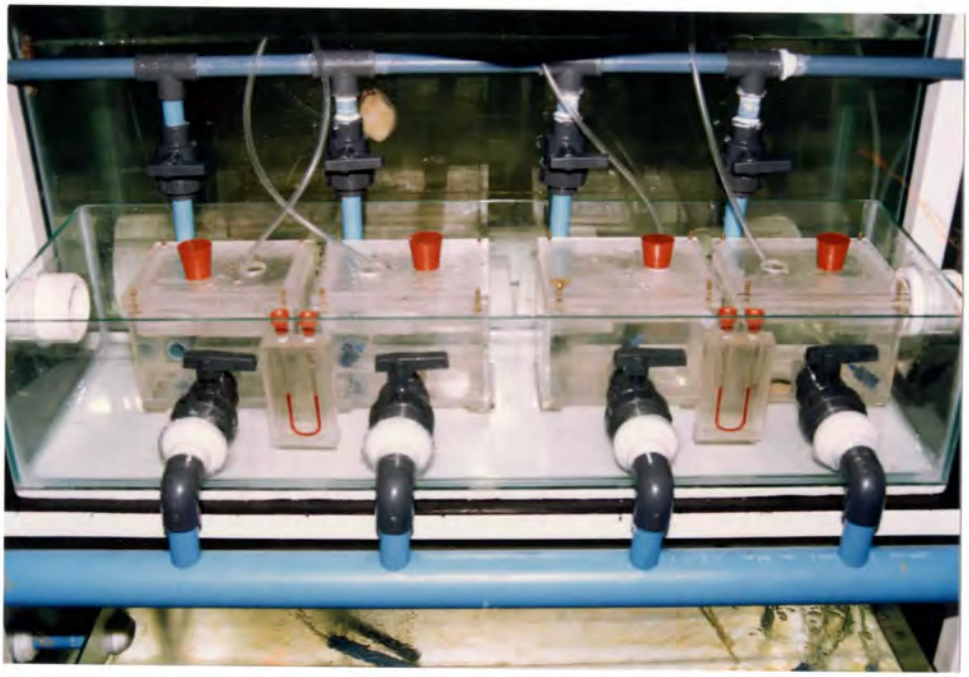


Figure 4. Photograph of experimental respirometers sitting within glass waterbaths (Photograph by Jentz Knauer).

RESULTS:

A mean plot of oxygen consumption and ammonia production rates against time revealed that there is no significant change in oxygen consumption and ammonia production over a 24 hour period ($P<0.05$, Fig. 5 and 6). The data for each 24h experimental run was therefore pooled. A comparison of the oxygen consumption and ammonia production rates of fed and unfed animals (Table 1.) revealed that fed animals had higher oxygen consumption and ammonia production rates than unfed animals. These differences were however not all significant. Regressions of oxygen consumption and ammonia production for fed (Fig. 7 and 8) and unfed animals (Fig. 9 and 10) and the variables of temperature and size class yielded linear relationships. Oxygen consumption and ammonia production per gram of abalone increased as the temperature increased for both fed and unfed abalone. Rates of oxygen consumption and ammonia production however decreased with increasing abalone size. The food consumption levels (% body weight) of *H. midae* showed similar trends, with a decrease in consumption levels as the size of the abalone increased and an increase in food consumption as temperature increased (Table 2). The food consumption data and the regressions suggest that the amount of food consumed is dependant on the abalone size and the ambient temperature of the water and is a rate limiting factor determining the oxygen consumption and ammonia production levels.

Using the data from Table 1, a multiple regression analysis was generated to produce a predictive model of oxygen consumption and ammonia production levels as functions of temperature and abalone size. The equations are for oxygen consumption and ammonia

Regressions of oxygen consumption and ammonia production per kilogram of food consumed (Fig. 11 and 12) both indicated an increasing trend as abalone size increases. Oxygen consumption however showed no significant change ($p < 0.05$) with increasing temperature (Fig. 11, Table 3). Ammonia production, however, increased with increasing abalone size and with an increase in the ambient water temperature (Fig. 12, Table 4). The trends in the data for oxygen consumption and ammonia production per kilogram of food consumed are not as consistant as when they are expressed as a function of abalone mass. This data has therefore not been used to generate multiple regressions.

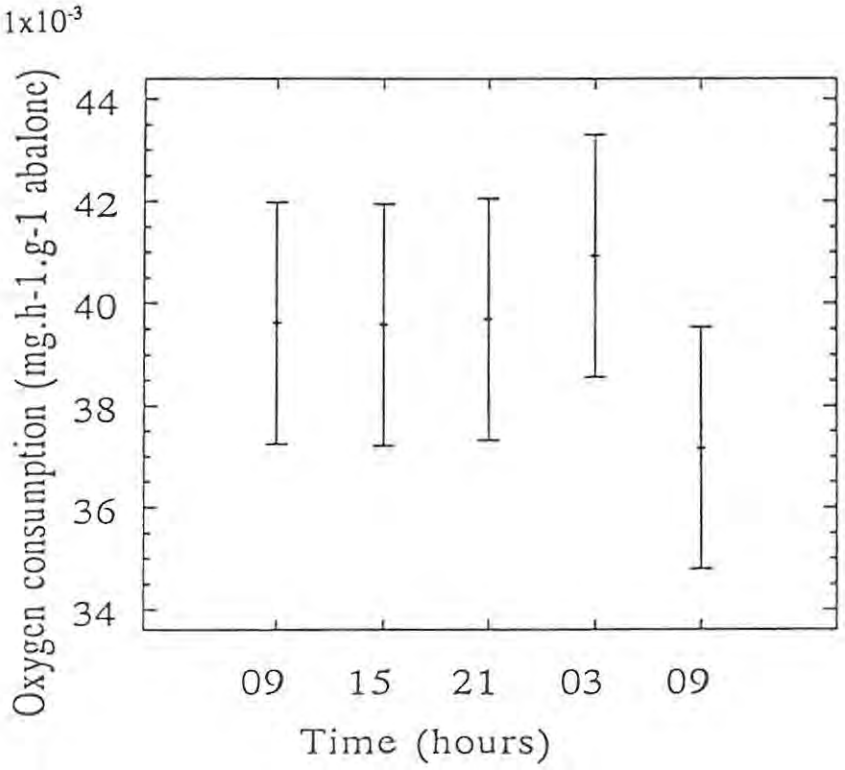


Figure 5. Mean plot of oxygen consumption in milligrams per hour per gram of abalone as a function of time in hours.

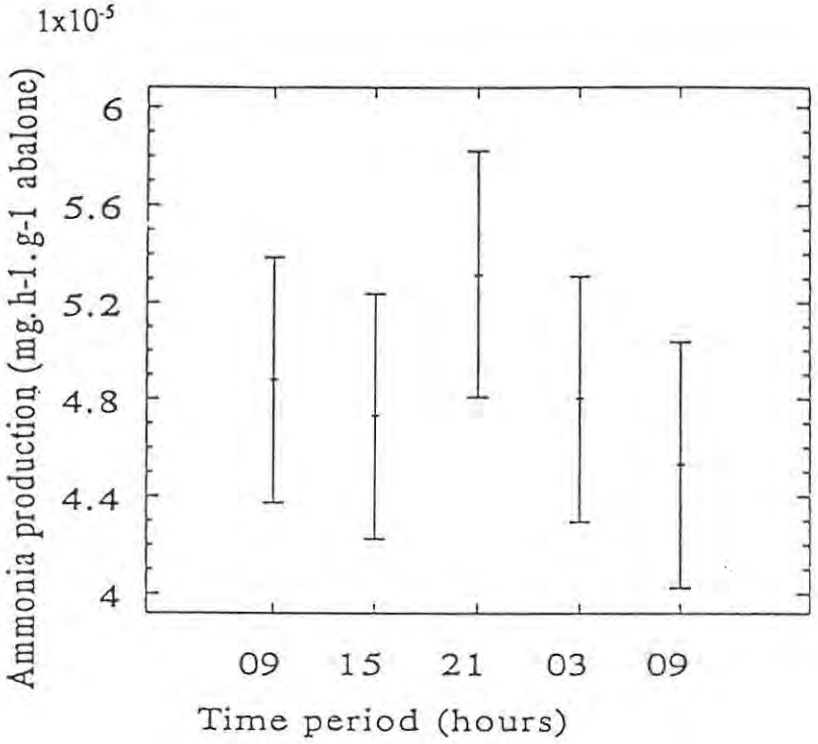
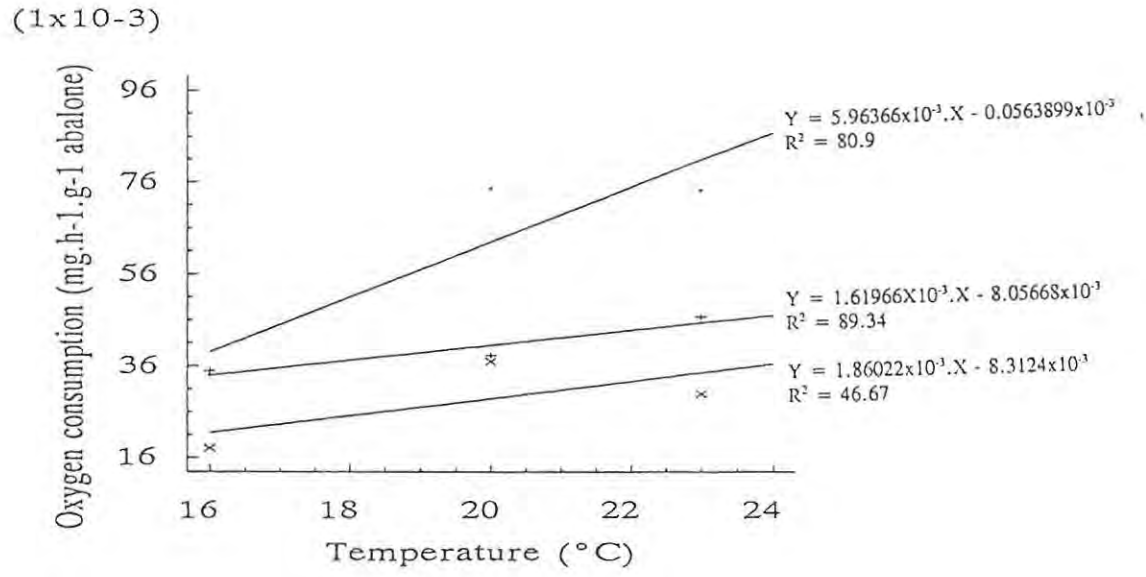


Figure 6. Mean plot of ammonia production in milligrams per hour per gram of abalone as a function of time in hours.



. 15mm + 30mm x 50mm

Figure 7. Plot of regressions of oxygen consumption in milligrams per hour per gram of abalone for fed abalone for each size class against temperature.

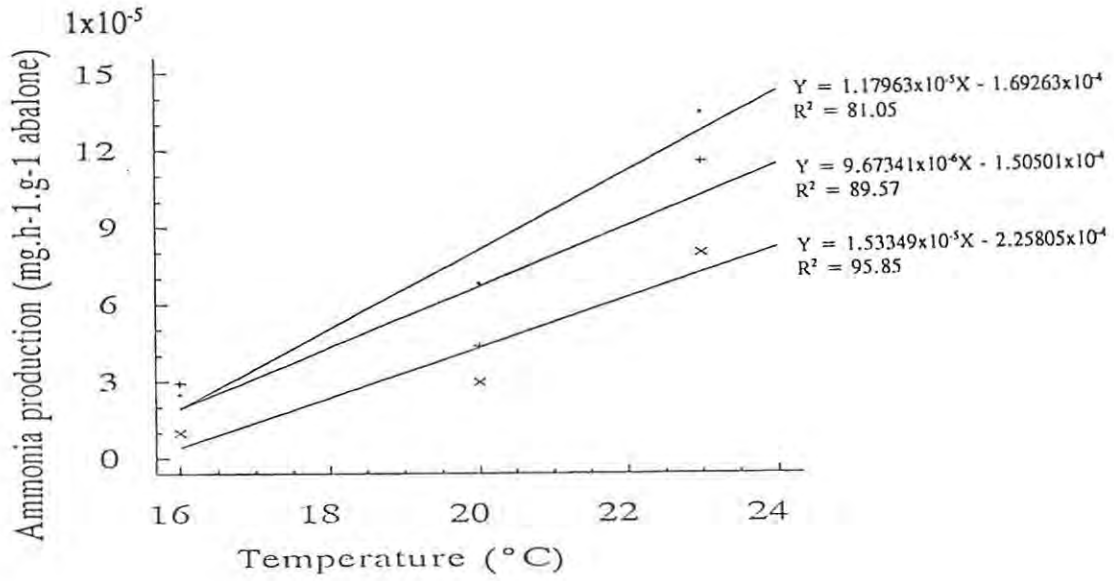


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Figure 8. Plot of regressions of ammonia production in milligrams per hour per gram abalone for fed abalone for each size class against temperature.

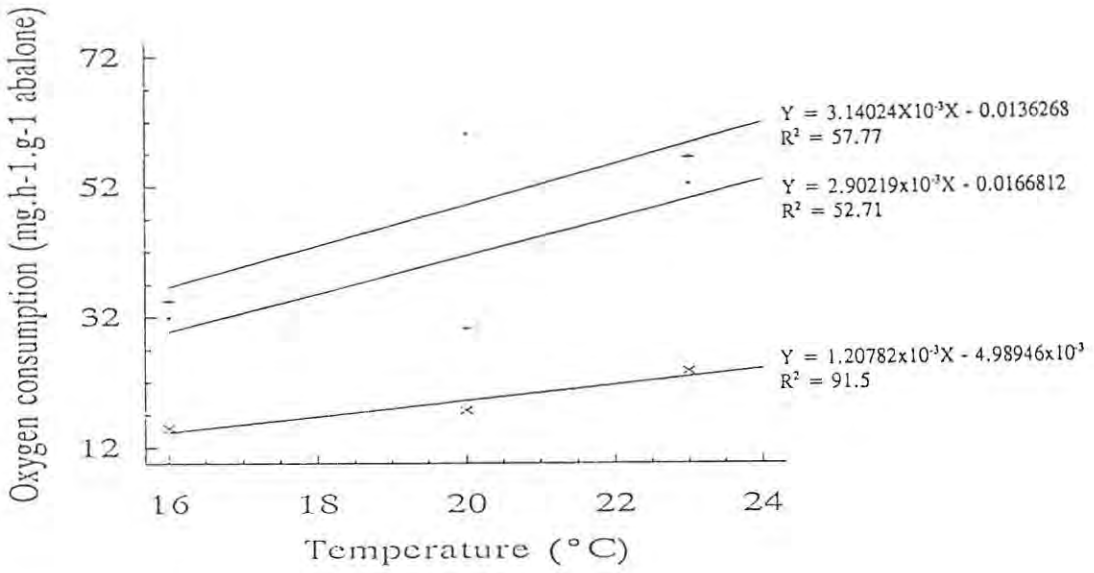

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Figure 9. Plot of regressions of oxygen consumption in milligrams per hour per gram of abalone (unfed) for each size class against temperature.

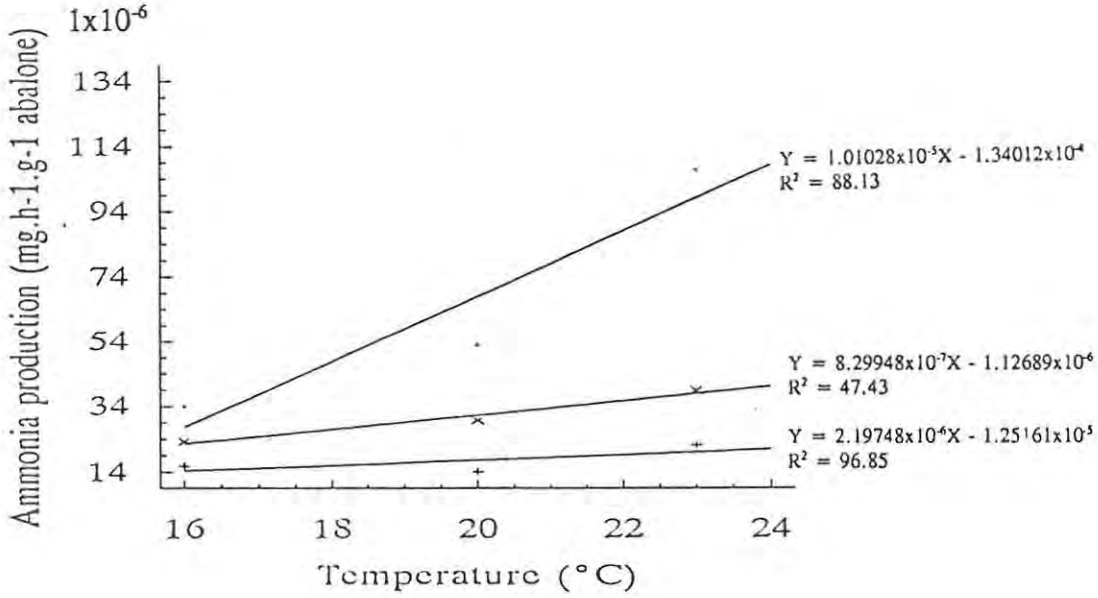

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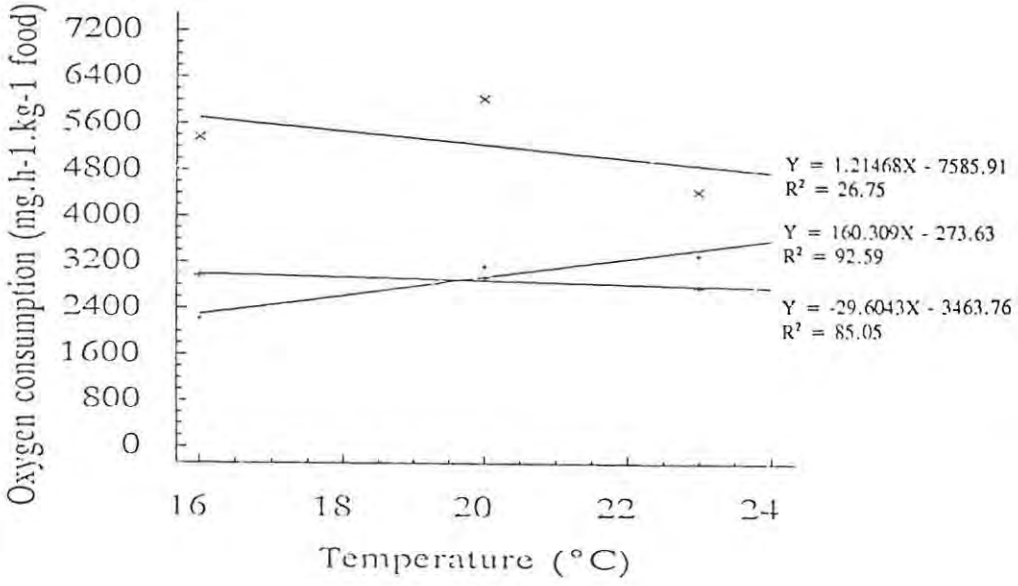
Figure 10. Plot of regressions of ammonia production in milligrams per hour per gram abalone (unfed) for each size class against temperature.



. 15mm + 30mm x 50mm

Figure 11. Plot of regressions of oxygen consumption in milligrams per hour per kilogram of food consumed for each size class against temperature.

Table 1. Oxygen consumption and ammonia production in milligrams per hour per gram of abalone for fed and unfed animals as functions of size class and temperature.

Oxygen consumption ($mg.h^{-1}.g^{-1}$ abalone)

		Size class (mm)		
Temperature		10-20mm	20-40mm	40-60mm
16	fed	0.034±.01	0.035±0.007	0.018±0.002
16	unfed	0.032±0.008	0.034±0.006	0.015±0.004
20	fed	0.074±0.007*	0.038±0.01	0.036±0.007*
20	unfed	0.060±0.01*	0.030±0.006	0.017±0.004*
23	fed	0.074±0.017*	0.046±0.01	0.029±.007
23	unfed	0.052±0.009*	0.056±0.009	0.023±0.003

Ammonia production ($mg.h^{-1}.g^{-1}$ abalone)

		Size class (mm)		
Temperature		10-20mm	20-40mm	40-60mm
16	fed	25.10^{-6}±4	29.10^{-6}±5	9.10^{-6}±4
16	unfed	34.10^{-6}±3	15.10^{-6}±12	23.10^{-6}±5
20	fed	67.10^{-6}±29	43.10^{-6}±19*	29.10^{-6}±12
20	unfed	53.10^{-6}±28	14.10^{-6}±1.6*	29.10^{-6}±11
23	fed	134.10^{-6}±24*	115.10^{-6}±25*	79.10^{-6}±17*
23	unfed	106.10^{-6}±25*	22.10^{-6}±3*	38.10^{-6}±14*

* denotes a statistically significant difference between fed and unfed animals ($P > 0.05$).

Table 2. Mean food consumption levels expressed as a percentage of body weight.

Temperature	Size class (mm) 10-20mm (% b.wt.)	20-40mm (% b.wt.)	40-60mm (% b.wt.)
16	1.537	1.177	0.336
20	2.394	1.307	0.614
23	2.239	1.692	0.676

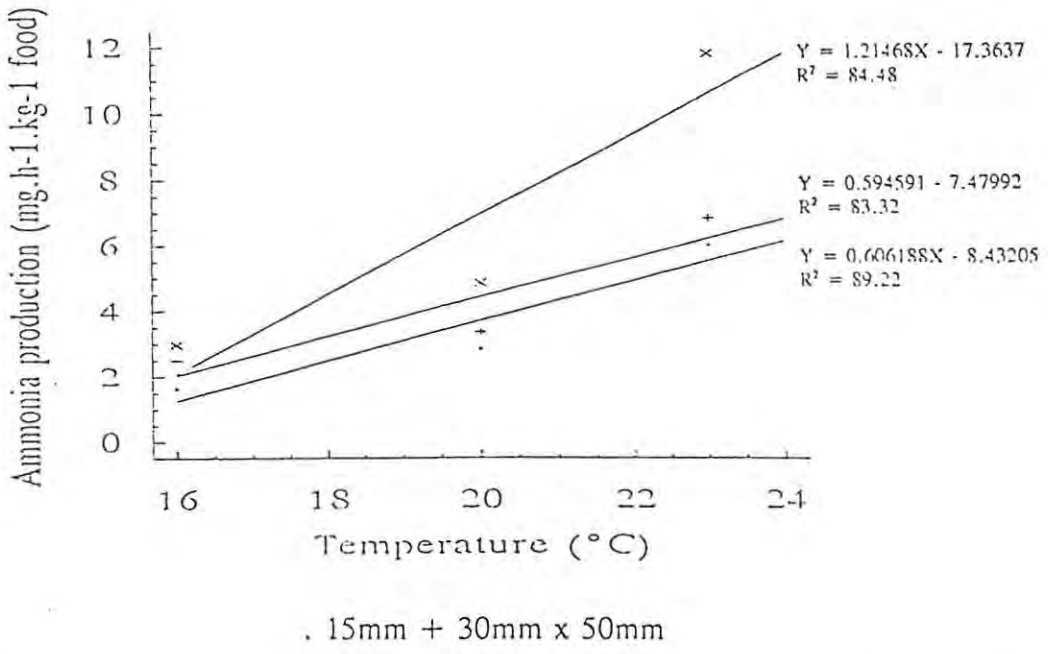


Figure 12. Plot of regressions of ammonia production in milligrams per hour per kilogram food consumed for each size class against temperature.

Table 3. Mean values of oxygen consumption ($mg.h^{-1}$) per kg of food consumed.

Size class	Temperature		
(mm)	16°C	20°C	23°C
10-20	2212±747	3115±296	3308±774
20-40	2968±618	2921±812	2754±639
40-60	5359±715	6014±1223	4420±1095

Table 4. Mean values of ammonia-nitrogen production ($mg.h^{-1}$) per kg of food consumed.

Size class	Temperature		
(mm)	16°C	20°C	23°C
10-20	1.63±0.31	2.84±1.24	5.99±1.07
20-40	2.49±0.42	3.33±1.46	6.8±1.51
40-60	2.97±1.31	4.83±2.03	11.77±2.56

DISCUSSION:

Despite an observed increase in nocturnal feeding and foraging activity amongst the experimental animals there were no significant peaks in oxygen consumption and ammonia production per gram of abalone over a 24 hour period, a trend also observed by Peck et al. (1985) on *H. tuberculata* and Barkai and Griffiths (1986) on *H. midae*. Uki and Kikutchi (1982) however discovered that *H. discus hannai* has a circadian rhythm of oxygen consumption, which corresponded to its activity rhythm, with the rate increasing from dusk to midnight and decreasing from midnight to midday. One can only speculate as to why the studies of Peck et al. (1985), Barkai and Griffiths (1986) and the present one yielded a fairly constant 24h oxygen consumption rate. This trend is unlikely to be due to an experimental artifact since the three studies obtained the same trend. Although the abalone were inactive during the light hours, oxygen consumption may not have shown a corresponding decrease, due to the increased energy requirements for digestion, absorption, assimilation and synthesis of excretory products, following feeding (Jobling 1994).

It is well known that smaller poikilotherms have higher metabolic rates per unit mass than larger ones. This has been ascribed to differences in surface area per unit mass, which affects energy requirements for control of heat loss, and various other physiological processes such as oxygen uptake in the lungs and food uptake in the intestine. It is also a fact that the metabolic rate of a poikilotherm increases in response to an increase in temperature (Schmidt-Nielson 1979). The present results demonstrated that the metabolic rate (reflected by oxygen consumption and ammonia production) of *H. midae* conformed to the poikilotherm pattern, increasing in a predictable fashion with increasing temperature and decreasing abalone size.

The present results are also consistent with the trends found by other authors working on *Haliotis* species (Tamura, 1939, Uki and Kikutchi, 1975, Hahn 1989, Peck et al. 1987, Jobling 1994) who found that the consumption of oxygen increased as the abalone size decreased and the temperature increased. Peck (1989) working on *H. tuberculata* found that ingestion, egestion, absorption and metabolism plus activity costs all had maximum values at the highest temperature tested which was 26.7°C. Thus temperature acts as a controlling environmental factor by exerting an influence on the metabolic rate of *H. midae.*

There was an observed increase in feed consumption with temperature which is probably due to an increased energetic requirement as a result of the faster metabolic rate of abalone at higher temperatures. Sano and Maniwa (1962) and Uki (1981) working on *H. discus hannai*, showed that the duration of feeding, the feeding rate, and hence the quantity of feed consumed increased with increase in temperature. The quantity of feed consumed expressed as a percentage of body weight also increased with decreasing abalone size. The food consumption levels in this study approximate those values determined by Mangold (pers. comm) in his study on food consumption in *H. midae* (Appendix 1: Table 9).

The present study revealed that fed animals displayed a higher metabolic rate (reflected by higher oxygen consumption and ammonia production) than unfed animals. This difference can be ascribed to the increased energetic cost associated with digestion, absorption, assimilation and excretion. This has important implications for the management of abalone stocks as the levels of ammonia produced decrease considerably in unfed abalone. This means that, in the event of a pump failure, the abalone can be starved, which would decrease the levels of ammonia produced thereby reducing the water flow rate required to maintain

suitable water quality conditions.

The absolute values for oxygen consumption and ammonia production in the present study correspond well with rates measured by other authors working on abalone (Uki and Kikutchi 1979, Barkai and Griffiths 1987, Peck et al. 1987, Segawa 1991) (Appendix 1: Table 10) though differences were evident which can be ascribed to differences in experimental conditions (i.e. temperature, food type, and quantity of feed consumed during each trial). This means that the absolute values calculated are representative for *H. midae* and can therefore be applied in a practical situation. Oxygen consumption and ammonia production in this study have been expressed per gram body weight and per kilogram food consumed and the resulting trends are derived below.

Oxygen consumption expressed per kilogram food consumed increased with abalone size but did not increase with temperature. However, ammonia production per kg food consumed increased in proportion to both temperature and body size. These results imply that the metabolic costs of digestion, assimilation and excretion for a unit of feed do not increase with temperature, but that larger animals expend more energy in processing a unit of feed. The increase in ammonia production per kg feed suggests that the efficiency of feed assimilation decreases with increasing temperature and abalone size. This is consistant with the suggestion of Barkai and Griffiths (1987) that the proportion of energy consumed and made available for growth declines with animal size. They reasoned that absorption efficiency remains constant but that the respiration rates (and at higher temperatures the excretion rates) increase more rapidly in relation to body mass than does consumption suggesting that larger animals expend relatively more energy to process their food. The present results therefore do not

support Haskell's contention that metabolic rates (oxygen consumption and ammonia production) are constant irrespective of animal size for a given quantity of food fed. However, oxygen consumption does remain relatively constant for a given quantity of feed consumed at different temperatures which is consistent with Haskell's findings.

Oxygen consumption and ammonia production of abalone expressed per gram body weight display linear relationships with the independent variables of temperature and size class for both fed and unfed animals (Appendix 1). From a water quality management point of view, it appears that ammonia production and oxygen consumption are more reliably predicted as a function of abalone biomass as opposed to feed consumed. The linear relationships of oxygen and ammonia, expressed per gram abalone body weight, with temperature and abalone size have been modelled using multiple regression analysis. From these relationships it is possible to predict the ammonia production and oxygen consumption rates for a given mass of animals of a given size class stocked in a raceway over a range of temperatures. Optimal flow rates for abalone rearing tanks can then be calculated using predetermined safe levels of oxygen and ammonia for abalone in order to enable efficient management of abalone in the rearing tanks.

Chapter 4 The effects of acute exposure to sublethal ammonia concentrations on growth, and the determination of the LC50 (96h) of ammonia in *H. midae*.

INTRODUCTION:

Haliotids are ammonotelic gastropods which primarily excrete their waste nitrogen as ammonia. Ammonia is the principle catabolic product which affects water quality in rearing ponds and water reuse systems (Burrows and Combs 1968). Determination of the effects of acute and chronic exposure of cultured fish species to various ammonia concentrations has therefore been the object of numerous studies (Redner and Stickney 1979, Thurston et. al 1983, Thurston and Russo 1983, Thurston et al. 1986). There are two forms of ammonia, an unionised form, NH_3 which is toxic and the ionised form, NH_4^+ which is non-toxic, which changes according to the physical and chemical conditions in the environment (Thurston et al. 1981), being mainly affected by temperature (Emerson et al. 1975), pH (Emerson et al. 1975), and the salinity (Westers and Pratt 1977, Bower and Bidwell 1978, and Wajsbrot et al. 1993).

Ammonia toxicity has been studied with reference to its effects on blood ammonia levels (Fromm and Gillette 1968), growth (Soderberg 1983, Robinette 1976, Alderson 1979), food conversion and survival (Tóth et al. 1982), and the associated gill and tissue damage caused by excessively high concentrations in fish (Wajsbrot et al. 1993). No work has been done on the acute and chronic toxic effects of unionised ammonia, on *H. midae*. Acute exposure to elevated but sublethal levels of ammonia may lead to sublethal histopathological changes, and suppression of growth (Burrows 1964, Robinette 1976). Suppression of growth reduces

productivity, overall yield and profitability. It is of prime importance therefore for the farm manager to know what levels of ammonia might be considered to be safe so that ammonia concentrations can be maintained at suitable levels. It was therefore important to determine what the lethal concentration of unionised ammonia is for acute exposure and what influence acute exposure to sublethal levels of ammonia would have on the growth and survival of *H. midae*.

METHODS AND MATERIALS:

Lethal Ammonia concentrations

Experimental animals

A total of 210 animals of three size classes (10-20mm, 20-40mm, and 40-60mm shell length) were used (Fig. 15) which were cultured abalone obtained from Sea Plant Products (Pty. Ltd.). The abalone were transported in polystyrene cooler-boxes in plastic bags filled with seawater and inflated with pure oxygen. All animals were fed on a formulated pellet diet developed by Britz et al. (1994) which they had been weaned onto from diatoms. Animals were acclimated for two weeks prior to the trial.

Experimental systems

The animals were kept in 15l glass aquaria (Fig. 13) in a recirculating system (Fig. 14) which remained static during each experimental run. These tanks were gravity fed from a 1500l asbestos header tank equipped with a heating element and a chiller unit which maintained the temperature at 18 ± 1°C. Used water was returned to four sunken biological

filters (total vol. ±4000l) filled with crushed shell and stones which were connected in series (Fig. 14). The system was topped up once a month with ±300l fresh seawater obtained from the Port Alfred estuary.

Experimental protocol

Five different unionised ammonia concentrations ranging from 0.2mg/l to 2.0mg/l were used (Table 5). Ammonium Chloride was used as a source of ammonia and was mixed up in 20l plastic buckets and then added to each of the tanks. Three tanks or replicates stocked with 10 animals each were used per concentration. A static lethal toxicity test was used, and there was no flow through the tanks during the experiment. The test solution was replaced every 24 hours. The tanks were all kept at the same temperature (±16°C). Since this experiment was a determination of the toxicity levels for acute exposure to ammonia, the experiment was run over the standard 96 hour (4 day) period.

Animals were weighed and measured individually at the beginning of the experiment, when mortalities occurred and at the termination of the experiment. The oxygen levels were determined after each mortality count. Mortalities were recorded after the first 15, 30, and 60 minutes and then 2, 4, 8, 14, 24 hours after that. Daily mortality observations, and temperature and pH were recorded from the second day until the termination of the experiment. Ammonia levels were quantified, using the indophenol method (APHA 1971). Levels of unionised ammonia were calculated from tables of pH and temperature (Rogers and Klemetson 1985). The median lethal concentration was calculated from a probability scale plot of the data where it was read off from the x-axis at the 50% mortality mark.

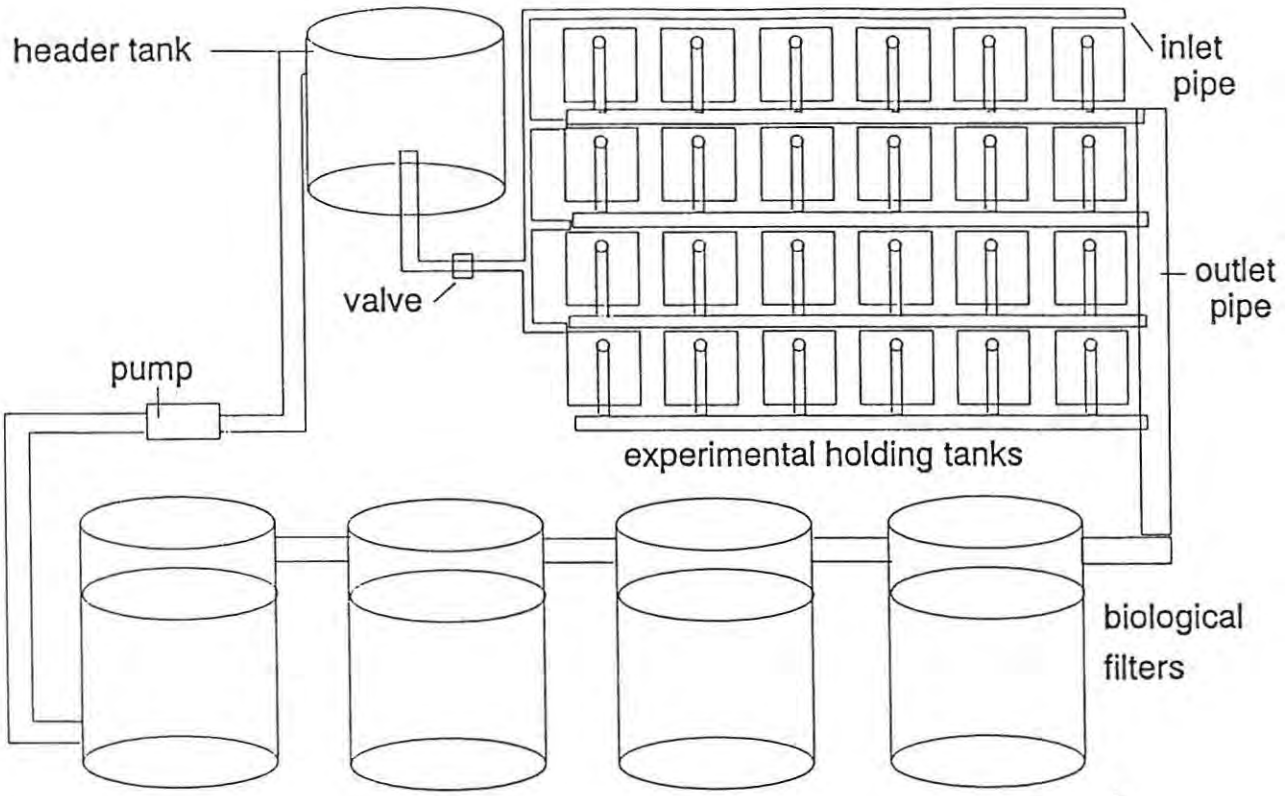


Figure 13. Diagram to show the recirculating seawater system used for the ammonia toxicity experiments.

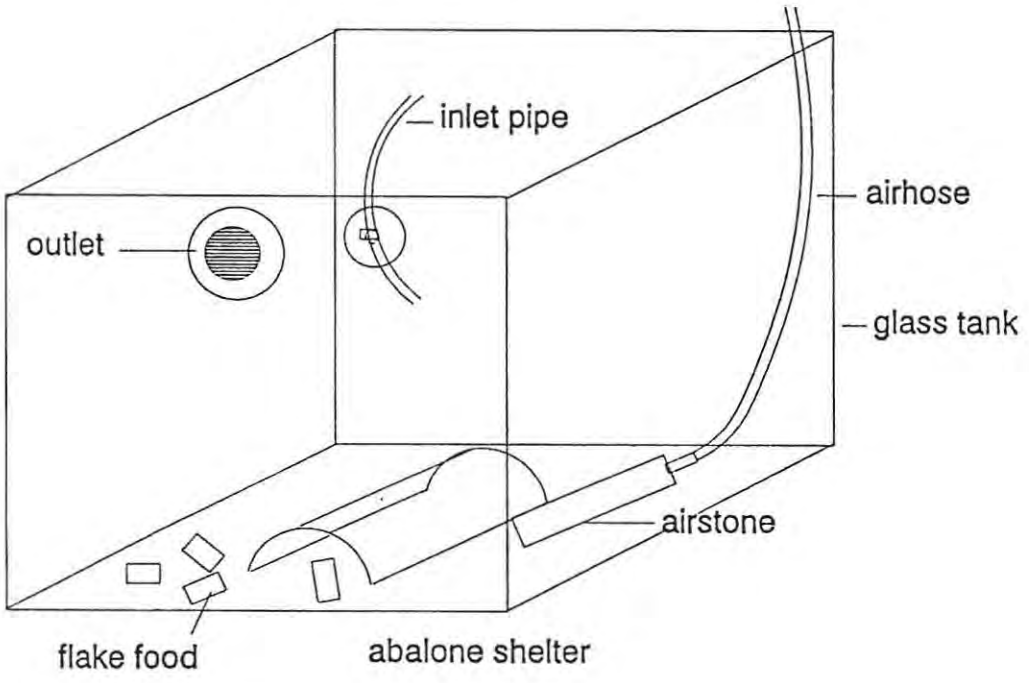


Figure 14. Diagram to show one of the 15l glass tanks used in the toxicity experiments.

Figure 15. Photographs to show the largest and smallest size classes of experimental animals used in each experiment (Photograph by Jentz Knauer).

Table 5. Concentrations of ammonia-nitrogen and proportions of ammonium chloride (mg/l) for ammonia concentrations used in LC50 test (T° ca. 16°C, pH 8.14±0.06, 35 ppt).

Ammonia-N	Total NH_3-N	NH_4Cl (mass)
0.2 mg/l	0.604 mg/l	1.43 mg
0.36 mg/l	1.087 mg/l	2.58 mg
0.64 mg/l	1.933 mg/l	4.6 mg
1.12 mg/l	3.383 mg/l	8.01 mg
2.0 mg/l	6.042 mg/l	14.37 mg

Sublethal Growth Trial

Experimental animals

A total of 630 abalone of a mean length of 18±3.3mm were used for this experiment. A total of thirty abalone were used per tank. The abalone were weighed and measured before the experiment. All tanks were kept at a temperature of ca. 16°C and the animals were fed on the formulated diet by Britz et al. (1994).

Experimental protocol

This trial was designed to simulate a system failure which would result in an overnight cessation of water flow of up to 12h. The abalone were exposed to five estimated sublethal concentrations between 0.064 mg/l and 0.88 mg/l (Table 6) in glass tanks (15l) for a period of 12 hours. Three replicates were used for each concentration and control. The water in each of the tanks was static and was not replaced until after the first 12 hours had passed. The abalone were then kept for a period of three months during which they were weighed and measured monthly to determine their respective growth rates. Since these animals were all of the same size class $\pm$18mm they were fed 2.67% (16°C) of their body weight during this period using a feeding table by Britz and Mangold (pers. comm.).

Statistical analysis

An analysis of covariance was used to compare the slopes of the growth curves to test for significant differences between slopes. A plot was also made of the Condition factor of the animals against time (Fig. 19). The condition factor was determined using an equation derived by Britz (pers. comm) for *H. midae*.

$$CF = (\text{mass}/\text{length}^{2.99}) \times 5575$$

Table 6. Concentrations of ammonium-nitrogen used in sublethal test.

Ammonia-nitrogen	Total NH_3	NH_4Cl
Control		
0.88 $mg.l^{-1}$	2.658 $mg.l^{-1}$	6.29 mg
0.64 $mg.l^{-1}$	1.933 $mg.l^{-1}$	4.6 mg
0.36 $mg.l^{-1}$	1.087 $mg.l^{-1}$	2.58 mg
0.2 $mg.l^{-1}$	0.604 $mg.l^{-1}$	1.437 mg
0.115 $mg.l^{-1}$	0.347 $mg.l^{-1}$	0.82 mg
0.064 $mg.l^{-1}$	0.193 $mg.l^{-1}$	0.46 mg

RESULTS:

Lethal Concentration of Ammonia

A plot of accumulated percentage mortality against resistance time (Fig. 16) showed that the first observed mortalities occurred at the highest concentration (2.0 mg/l of ammonia) after 24 hours with 60% mortality. After a further 48 hours there was 100% mortality for this concentration. The only other mortalities were for the concentration of 1.12 mg/l which showed 10% mortality after 48h and 68% mortality after 96 hours. Analysis of variance showed no significant differences in mortality in replicate tanks for each concentration of ammonia.

The probability scale plot of the percentage mortality at 96 hours against the log of the ammonia concentration (Fig. 17) gave an estimation of 1.08mg/l as the threshold LC50 ammonia concentration at a temperature of 16°C and a pH of 8.14. The salinity was 34 ppt.

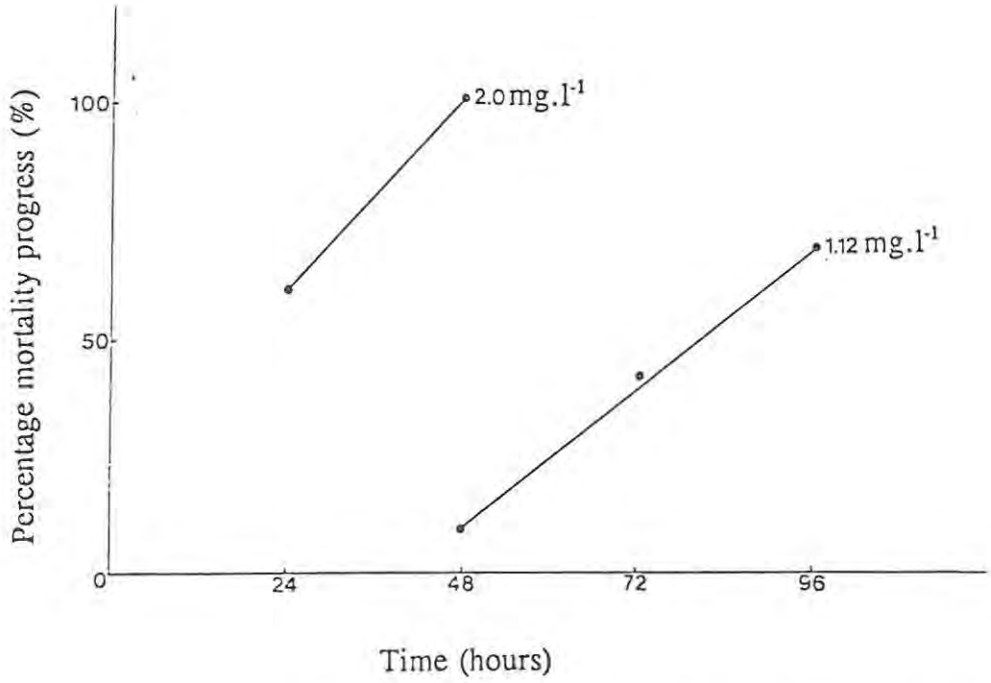


Figure 16. Percentage progress mortality plot as a function of time in hours.

Sublethal Effect of Ammonia to exposure (Growth Trial).

At the end of the three month period growth curves were plotted for the mass data (Fig. 18). Analysis of covariance showed no significant difference between the slopes of the curves for mass. Analysis of variance of length data also showed no significant difference in growth for

different treatments (Table 7). Condition factors (Fig. 19) decreased with time for all treatments and the control. There was no significant difference in condition between the control and the treatment tanks, however, the condition of all abalone in all treatments decreased over the experimental period. The percentage mortality was relatively high for all tanks (Table 8) but may be attributed to suffocation of abalone resulting from a cessation in the flow of seawater through the system because of accidental loss of seawater for $\pm$ 12 hours, two weeks into the experiment, from the header tanks. An ANOVA showed that there were no significant differences in mortality between treatments.

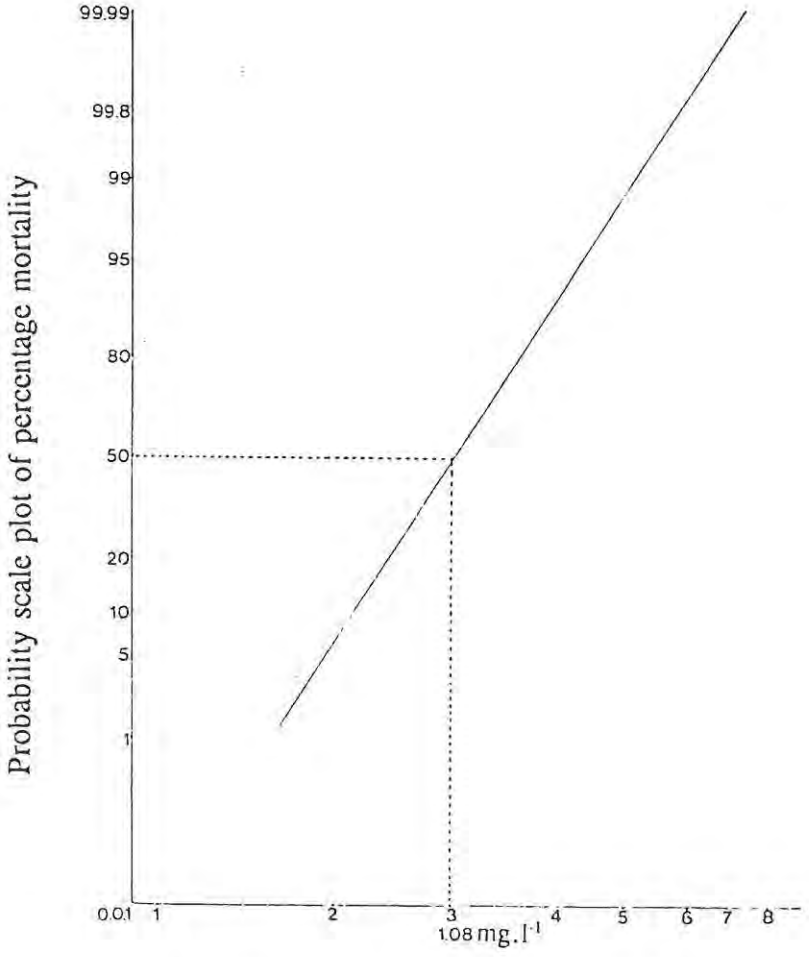


Figure 17. Probability scale plot of mortality as a function of the log of ammonia concentration.

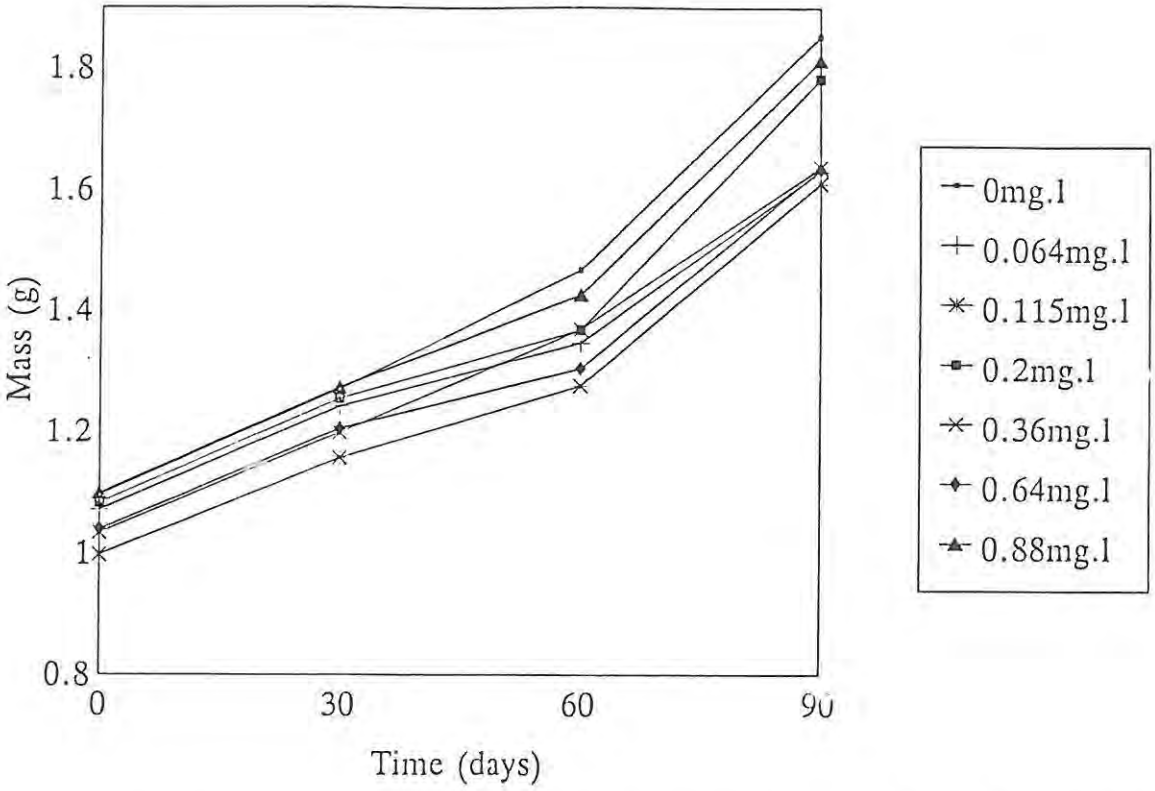


Figure 18. Plot to show the mass increase of *H. midae* as a function of the time in days.

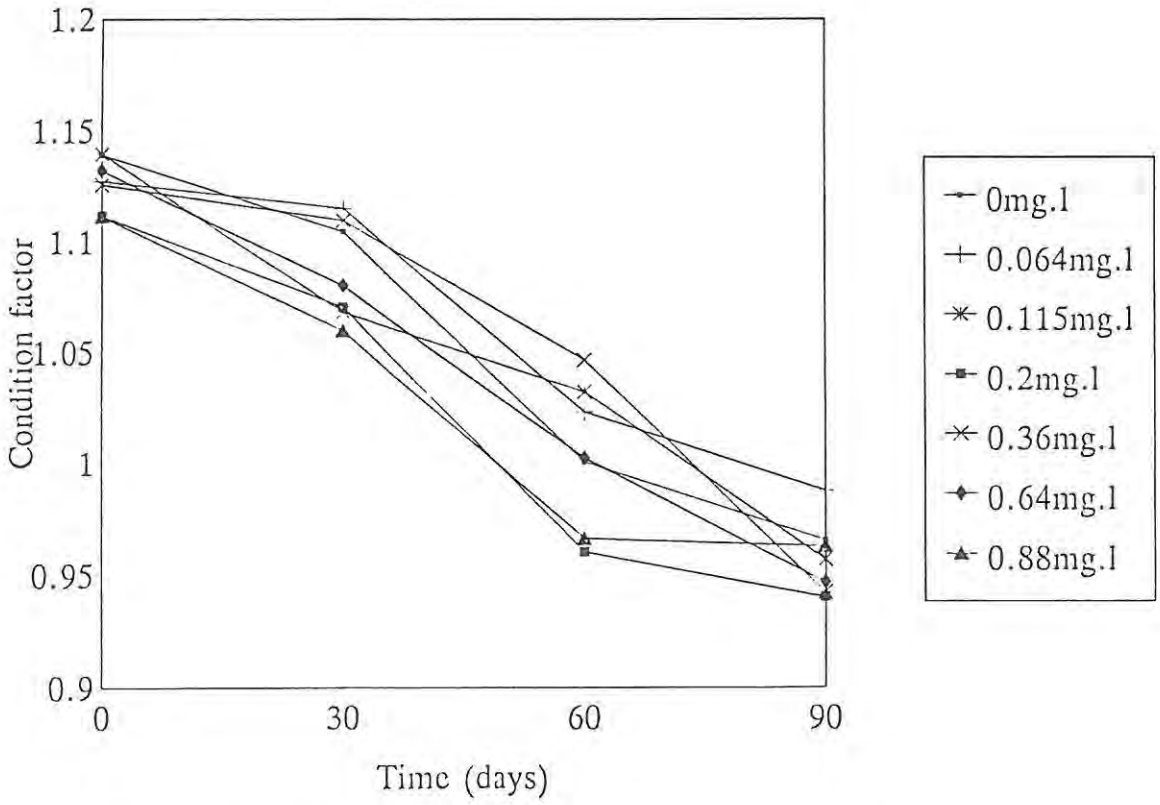


Figure 19. Condition factor of *H. midae* plotted as a function of time in days.

Table 7. Length data (mm) to show the growth of *H. midae* following acute exposure to sublethal ammonia levels ($mg.l^{-1}$).

NH_3		Period (days)		
Conc.	0	30	60	90
0.88	17.81±0.21	19.03±0.48	20.37±0.56	22.1±0.46
0.64	17.68±0.39	18.56±0.42	19.55±0.55	21.51±0.81
0.36	17.82±0.15	18.38±0.31	19.59±0.22	21.55±0.15
0.2	17.74±0.56	18.87±0.51	20.12±0.48	21.69±0.52
0.115	17.74±0.4	18.04±0.34	19.2±0.93	21.12±0.06
0.064	17.58±0.14	18.87±0.47	19.62±0.8	21.16±0.43
0	17.68±0.32	18.75±0.67	20.34±0.63	22.26±0.54

Table 8. Mean percentage mortality of abalone for each ammonia-nitrogen concentration.

Conc. NH_3	Percentage Mortality	Standard Deviation
0 mg/l	25.53%	11.33
0.064 mg/l	25.53%	4.15
0.115 mg/l	35.53%	17.28
0.2 mg/l	34.43%	5.66
0.36 mg/l	28.86%	1.57
0.64 mg/l	41.1%	11.33
0.88 mg/l	12.2%	3.14

DISCUSSION:

Lethal Concentration

The results from the present study suggest that the LC50 for unionised ammonia-nitrogen in *H. midae* is approximately 1.08mg/l at a temperature of $\pm$16°C and a pH 8.19$\pm$0.03. The literature shows that the LC50 of NH_3 for acute (96h) exposure in freshwater and marine fish is between 0.02 and 3.4 $mg.l^{-1}$ with most values falling between 0.16-1.74 $mg.l^{-1}$ (Thurston and Russo 1983, Rogers and Klemetson 1985, Hampson 1986, Hasan and Macintosh 1986, Thurston et al. 1986, Handy and Poxton 1993). Thus the (96h) LC50 of ammonia for *H. midae* lies within the expected limits shown for other poikilotherms. Handy and Poxton (1993) reviewed nitrogen pollution in mariculture. They found a paucity of information regarding ammonia toxicity in seawater. Although several papers have delt with ammonia excretion rates by abalone (Barkai and Griffiths 1987, Peck et al. 1987, Segawa 1991) no work has been done on ammonia toxicity in abalone.

The toxicity of ammonia and the relative concentrations of unionised ammonia have been found to be influenced by several factors including salinity, temperature and water pH (Alderson 1979, Alabaster and Lloyd 1980, Wajsbrot et al. 1991). The present LC50 trial was conducted at only one temperature, pH and salinity. Other authors have, however found that the toxicity of a given concentration of ammonia is relatively independant of pH (Wuhrmann and Woker 1948, Lloyd 1961, Hasan and Macintosh 1986), and the LC50 value of 1.08 mg/l^{-1} for *H. midae* should therefore apply to all pH values. The toxicity of ammonia has been found to increase with temperature in fish, thus, the tolerance of abalone to

ammonia may decrease with temperature. Thurston and Russo (1983) found a direct relationship between LC50 ($mg.l^{-1}$ of NH_3) and temperature over the range 10-19°C ($P < 0.002$). Further experiments should therefore be conducted over a range of temperatures and pH levels to determine the effects of these environmental factors on the toxicity of ammonia to *H. midae*.

Sublethal Growth Trial

The present results suggest that a period of acute exposure (12h) to sublethal levels of ammonia up to 0.88mg/l will have no affect on the subsequent growth of abalone. In practice chronic exposure to high concentrations of ammonia does not occur frequently, most often discrete events resulting in acute exposure of the animals to high NH_3 concentrations occur. Although mortality and a drop in condition was observed during the growth trial there were no significant differences between animals in the control and treatment tanks. The relatively high mortality is ascribed to anoxia which may have occurred when the flow of water was stopped for a day following problems with the water supply.

The present results suggest that an event which results in a sudden increase in ammonia such as a pump failure or blockage of a water inlet will have little or no effect on production provided that levels of ammonia remain below 0.88 mg/l. If the lethal concentration of ammonia is taken into account the results suggest that the acute effect threshold (threshold beyond which growth and survival are adversely affected) should lie between 0.88 $mg.l^{-1}$ and 1.08 $mg.l^{-1}$.

The lethal limit of ammonia determined in this study can be used to estimate a safe level of

ammonia using findings by Lloyd and Orr (1969) on non-toxic levels of ammonia in rainbow trout. They provided evidence that a non-toxic level of ammonia might exist for rainbow trout at 12% of the lethal threshold concentration. Similar results might be expected for *H. midae* though the relative level of non-toxicity might be higher than for rainbow trout since abalone have a slower metabolic rate. Using this method, a non-toxic level for acute exposure of 0.129 $mg.l^{-1}$ of unionised ammonia for *H. midae* is derived. This level appears high and is merely an estimate and should be viewed as such until it can be tested.

Prolonged or chronic exposure to sublethal levels of ammonia longer than 12 hours could have a negative effect on the growth of these animals. In practice, chronic exposure to high concentrations of ammonia does not occur frequently, most often discrete events resulting in acute exposure of the animals to high ammonia concentrations occur. Chronic ammonia poisoning can however be a major problem in some fish culture operations. Although no attempt was made to quantify the effect of chronic exposure to sublethal levels of ammonia in *H. midae*, a safe level can be estimated based on work on other organisms. Past research has shown that chronic-effect threshold concentrations resulting in suppressed growth and survival ranged from 0.27-0.91 $mg.l^{-1}$ (Thurston et al. 1986, Wajsbrot et al. 1993). The chronic effect threshold has still to be determined, however, based on past findings it can safely be assumed that the threshold level lies between 0.01 and 0.88 $mg.l^{-1}$. The acceptable safe level for chronic exposure to unionised ammonia for most marine organisms is between 0.01 and 0.04 $mg.l^{-1}$ (Huguenin and Colt 1989). Alabaster and Lloyd (1980) claim that adverse effects caused by prolonged exposure are absent only at ammonia concentrations lower than 0.025 $mg.l^{-1}$. Alderson (1979) working on sole and turbot has found evidence for a threshold level of ammonia below which little or no effect on growth was evident. This

Table 9. Established safe levels for unionised ammonia in freshwater and marine organisms.

	NH_3 conc.	Author
Marine organisms	0.01-0.04 mg/l	Huguenin and Colt (1989)
Freshwater fish	0.025 mg/l	Alabaster and Lloyd (1980)
Sole	0.066 mg/l	Alderson (1979)
Turbot	0.11 mg/l	Alderson (1979)

level was determined to be 0.066 mg/l for sole and 0.11 mg/l for turbot at 16°C, 34‰ salinity over a 42 day period. Handy and Poxton (1993) consider such ammonia levels as suspect since subtle metabolic disturbances probably occur before reduced growth becomes apparent. They therefore suggest caution with the recommendation of safe chronic ammonia levels. On the basis of the above values a conservative safe limit for chronic exposure of abalone would be 0.01-0.02 $mg.l^{-1}$ NH_3 (Table 9).

Chapter 5 Implications for management of water quality in intensive abalone farms.

In a rearing tank stocked with a high density of abalone, the rate of water flow is the most important factor moderating changes in water chemistry. A good flow of water replaces lost oxygen and removes polluting metabolites, such as ammonia, and accumulated wastes such as faeces and uneaten food. An important design criterion for a commercial abalone rearing unit is thus the rate of flushing. The present quantification of oxygen consumption and ammonia excretion rates for *H. midae* demonstrate the extent to which the metabolism of *H. midae* influences water chemistry. The model generated from this data makes it possible to calculate the oxygen consumption (Appendix 1: Tables 1 and 2) and ammonia production (Appendix 1: Tables 3 and 4) and hence the minimum flow rates required (expressed as litres per hour per kilogram of abalone) to maintain safe levels of oxygen (80 % saturation) (Appendix 1: Tables 5 and 6) and ammonia (0.02 $mg.l^{-1}$) (Appendix 1: Tables 7 and 8) for fed and unfed animals. The level of oxygen required for normal activity in abalone is above 4 $mg.l^{-1}$ O_2 (Jan 1980). Beyond a shell length of 50mm, some of the values calculated using the model are negative. These values have been replaced, in the tables, by the closest values at that temperature.

This study has shown that ammonia excretion is proportional to the quantity of feed consumed, hence the data of the ammonia excretion levels for unfed abalone will facilitate prediction of dissolved ammonia should a cessation of feeding be required to reduce ammonia production in the event of a pump failure or any other event leading to a reduction of water flow through abalone rearing tanks. A farm's water flow rate might thus also be managed by the quantity of feed fed, however, the results from this study for oxygen consumption and

ammonia production in milligrams per kilogram of food consumed were not as consistent as when they were expressed per gram abalone weight. Thus, their use as a management tool does not seem justified. The hypothesis, that rates of oxygen consumption and ammonia production are constant for the same quantity of feed consumed irrespective of the abalone size at each temperature, was also found not to be true. The results of the present study thus suggest that ammonia production and oxygen consumption are best predicted as functions of abalone weight.

Optimal flow rates can be expressed for given stocking densities of abalone for both fed and unfed animals, however, it is simpler to express flow rate per kg of abalone. Several authors have suggested optimal stocking densities for abalone that can be used as a baseline upon which to determine stocking densities and to predict optimal flow rates for those densities. Recommmended stocking densities for abalone range from 2500-5000 shells/m^2 (7.4-15 kg/m^2) for abalone less than 15mm, 731-1462 shells/m^2 (7-20 kg/m^2) for the culture of juvenile abalone of about 25mm, to 250 shells/m^2 (27.3 kg/m^2) for growout to commercial size (which is between 40-60mm) (Chen 1984a, Greenier and Takekawa 1992, Singhagraiwan 1992, Knauer 1994). Greenier and Takekawa (1992) stocked juvenile red abalone, *H. rufescens* (< 15mm) at three different densities, low (189 shells/m^2); 0.7 kg/m^2), medium (568 shells/m^2; 1.5 kg/m^2), and high (1420 shells/m^2; 4.5kg/m^2) at a flow rate of 1.5 ± 0.5 litres/minute or 60-120 litres/hour. The results of the present study show that at a temperature of 20°C, abalone of a shell length of 15-20mm stocked at a density of 1500 shells/m^2 would require a flow rate of 86.73 l/h for fed abalone and 73.32 l/h for unfed abalone based on their oxygen consumption rates. If determined from ammonia excretion rates, fed animals would require a flow rate of 43 l/h, while unfed animals would require a

flow rate of 26.54 l/h. There is therefore a close correlation between predicted flow rates from this study and the flow rates recommended by Greenier and Takekawa (1992) for the same stocking density of abalone. In addition oxygen consumption rates and ammonia excretion rates in this study compare favourably with those measured by other authors (Uki and Kikutchi 1975, Peck et al. 1987, Barkai and Griffiths 1987, Segawa 1991). This confirms the validity of the present results as a means of predicting water flow rates required in abalone rearing tanks for a given stocking density to maintain suitable water quality conditions. To this end, predicted water flow rates (l/h) for a 100 ton abalone stock, spread over the size range, have been presented for a range of temperatures (Appendix 1: Table 11).

Several events and factors can modify predicted relationships of ammonia production, stocking densities, and flow rates. The concentration of unionised ammonia increases ten-fold with a one point rise in pH. Ammonia threshold levels and excretion rates for this study have been determined at only one pH level. Although seawater is a relatively good buffer because of the high levels of $CaCO_3$, the pH may change in culture tanks for a number of reasons. Firstly a bacterial oxygen demand due to the large amounts of accumulated waste in the tanks at high stocking densities could lower the dissolved oxygen levels and increase the levels of CO_2 in the water causing a drop in the pH. The levels of unionised ammonia would decrease under such conditions resulting in an overestimation of flow rates required for the maintenance of suitable water quality conditions. This would increase pumping costs on an abalone farm. Accumulated levels of nitrates following denitrification could promote conditions for an algal bloom. Carbon dioxide would be removed by photosynthesis, causing a rise in pH and an increase in the levels of unionised ammonia. This scenario could result in an underestimation of the flow rates required to maintain suitable levels of ammonia and

the health of the abalone could be adversely affected.

The safe limit of unionised ammonia calculated for *H. midae* does not take into account that the animals tolerance may change with a change in pH or temperature, since the LC50 and sublethal growth trials were conducted at only one temperature and pH.

Tank volume is another important factor that might influence predicted estimates of relationships. Although the flow rate determines the rate at which ammonia is removed, tank volume might have an effect on the removal of ammonia. A tank with a high volume and low surface area may have poor mixing characteristics and a low exchange rate resulting in short circuiting of water flow, and the formation of dead volumes or spaces of poor mixing. This can lead to the build up of ammonia levels in spite of high flow rates that might be considered suitable for the maintenance of good water quality conditions. Aeration is however a good way of improving the conditions of mixing within a rearing tank so that high levels of ammonia become diluted. Aeration also works by removing gaseous ammonium thereby reducing the concentration of total ammonia and thus the levels of unionised ammonia.

The quantity of food fed can also affect the flow rates required by increasing the levels of ammonia produced. Leaching and the accumulation of uneaten food also raises the bacterial oxygen demand thereby lowering the pH. It is therefore vital that a strict feeding regime based on predicted food consumption levels is maintained in order to ensure that food wastage due to overfeeding is reduced and optimal flow rates for those feeding levels are maintained. The mass of food to be fed per size class in *H. midae* can be derived from a

practical feeding table by Britz and Mangold (pers. comm.) (Appendix 1: Table 9).

Other factors that might influence predicted values through synergistic effects are low dissolved oxygen levels and a high hydrogen sulfide concentration owing to decomposition of organic matter and respiration of organisms. Low dissolved oxygen concentrations increase the toxic effects of ammonia particularly in conjunction with higher CO_2 concentrations (Alabaster and Lloyd 1980, Handy and Poxton 1993). The toxic effects of ammonia are most evident at low oxygen concentrations because of hyperplasia at the gills. Abalone are very sensitive to H_2S concentrations as low as 0.05ppm which can retard growth (Chen, 1984b).

On commercial abalone farms the growout of abalone occurs in through-flow rearing tanks in which there is no water reuse. These tanks rely on aeration and a relatively high water exchange rate to replace oxygen and to remove ammonia and solid wastes. Other water treatment methods exist which rely on water reuse through recirculation, mechanical filtration, of solid wastes, and biological filtration, of ammonia. Solid waste may also be removed through siphon flushing systems (Braid 1987), sloping floors and centrifugal draining techniques. The control of water quality conditions within a tank using through-flow of water is much more practical as it doesn't require expensive construction and installation of treatment systems (filtration and sterilising equipment). In recirculating systems treatment systems are very often undersized as a result of underestimation of eventual metabolic waste loading levels (Rosenthal and Murray, 1986). This occurs most often during scaling-up where researchers fail to recognise the complexity of changes that occur in many critical variables other than the system size (Huguenin and Colt 1986). The use of through-flow systems allows for an immediate adjustment of the water flow rates through the system in the

eventuality of a sudden change in water quality under hyperintensive conditions. This makes farm management by water flow much more practical and cost effective, since a higher exchange rate means that less rearing space is required thus reducing capital outlay and operational costs.

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Appendix 1

Table 1. Oxygen consumption (mg.h-1.kg-1 abalone) for fed abalone per kilogram of abalone mass for different size classes and temperatures.

	class (mm)														
temp	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
14	41.57	37.15	32.73	28.31	23.89	19.47	15.05	10.63	6.21	1.79	1.79	1.79	1.79	1.79	1.79
15	45.18	40.76	36.34	31.92	27.50	23.08	18.66	14.24	9.82	5.40	0.98	0.98	0.98	0.98	0.98
16	48.78	44.36	39.94	35.52	31.10	26.68	22.26	17.84	13.42	9.00	4.58	0.16	0.16	0.16	0.16
17	52.38	47.96	43.54	39.12	34.70	30.28	25.86	21.44	17.02	12.60	8.18	3.76	3.76	3.76	3.76
18	55.98	51.56	47.14	42.72	38.30	33.88	29.46	25.04	20.62	16.20	11.78	7.36	2.94	2.94	2.94
19	59.58	55.16	50.74	46.32	41.90	37.48	33.06	28.64	24.22	19.80	15.38	10.96	6.54	2.12	2.12
20	63.18	58.76	54.34	49.92	45.50	41.08	36.66	32.24	27.82	23.40	18.98	14.56	10.14	5.72	1.30
21	66.78	62.36	57.94	53.52	49.10	44.68	40.26	35.84	31.42	27.00	22.58	18.16	13.74	9.32	4.90
22	70.38	65.96	61.54	57.12	52.70	48.28	43.86	39.44	35.02	30.60	26.18	21.76	17.34	12.92	8.50
23	73.98	69.56	65.14	60.72	56.30	51.88	47.46	43.04	38.62	34.20	29.78	25.36	20.94	16.52	12.10
24	77.58	73.16	68.74	64.32	59.90	55.48	51.06	46.64	42.22	37.80	33.38	28.96	24.54	20.12	15.70

Table 2. Oxygen consumption (mg.h-1.kg-1 abalone) for unfed abalone per kilogram of abalone mass for different size classes and temperatures.

	class (mm)														
temp	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
14	35.39	31.35	27.31	23.27	19.23	15.19	11.15	7.11	3.07	3.07	3.07	3.07	3.07	3.07	3.07
15	38.50	34.46	30.42	26.38	22.34	18.30	14.26	10.22	6.18	2.14	2.14	2.14	2.14	2.14	2.14
16	41.60	37.56	33.52	29.48	25.44	21.40	17.36	13.32	9.28	5.24	1.20	1.20	1.20	1.20	1.20
17	44.71	40.67	36.63	32.59	28.55	24.51	20.47	16.43	12.39	8.35	4.31	0.27	0.27	0.27	0.27
18	47.81	43.77	39.73	35.69	31.65	27.61	23.57	19.53	15.49	11.45	7.41	3.37	3.37	3.37	3.37
19	50.92	46.88	42.84	38.80	34.76	30.72	26.68	22.64	18.60	14.56	10.52	6.48	2.44	2.44	2.44
20	54.02	49.98	45.94	41.90	37.86	33.82	29.78	25.74	21.70	17.66	13.62	9.58	5.54	1.50	1.50
21	57.13	53.09	49.05	45.01	40.97	36.93	32.89	28.85	24.81	20.77	16.73	12.69	8.65	4.61	0.57
22	60.23	56.19	52.15	48.11	44.07	40.03	35.99	31.95	27.91	23.87	19.83	15.79	11.75	7.71	3.67
23	72.44	68.40	64.36	60.32	56.28	52.24	48.20	44.16	40.12	36.08	32.04	28.00	23.96	19.92	15.88
24	66.44	62.40	58.36	54.32	50.28	46.24	42.20	38.16	34.12	30.08	26.04	22.00	17.96	13.92	9.88

Table 3. Ammonia production (mg.h-1.kg-1 abalone) for fed abalone per kilogram of abalone mass for different size classes and temperatures.

temp	class (mm)														
	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
14	0.063	0.055	0.048	0.041	0.033	0.026	0.019	0.011	0.004	0.004	0.004	0.004	0.004	0.004	0.004
15	0.068	0.061	0.054	0.046	0.039	0.032	0.024	0.017	0.009	0.002	0.002	0.002	0.002	0.002	0.002
16	0.074	0.066	0.059	0.052	0.044	0.037	0.030	0.022	0.015	0.008	0.000	0.000	0.000	0.000	0.000
17	0.079	0.072	0.065	0.057	0.050	0.043	0.035	0.028	0.021	0.013	0.006	0.006	0.006	0.006	0.006
18	0.085	0.078	0.070	0.063	0.055	0.048	0.041	0.033	0.026	0.019	0.011	0.004	0.004	0.004	0.004
19	0.090	0.083	0.076	0.068	0.061	0.054	0.046	0.039	0.032	0.024	0.017	0.010	0.002	0.002	0.002
20	0.096	0.089	0.081	0.074	0.067	0.059	0.052	0.044	0.037	0.030	0.022	0.015	0.008	0.000	0.000
21	0.101	0.094	0.087	0.079	0.072	0.065	0.057	0.050	0.043	0.035	0.028	0.021	0.013	0.006	0.006
22	0.107	0.100	0.092	0.085	0.078	0.070	0.063	0.056	0.048	0.041	0.033	0.026	0.019	0.011	0.004
23	0.113	0.105	0.098	0.090	0.083	0.076	0.068	0.061	0.054	0.046	0.039	0.032	0.024	0.017	0.010
24	0.118	0.111	0.103	0.096	0.089	0.081	0.074	0.067	0.059	0.052	0.045	0.037	0.030	0.023	0.015

Table 4. Ammonia production (mg.h-1.kg-1 abalone) for unfed abalone per kilogram of abalone mass for different size classes and temperatures.

temp	class (mm)														
	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
14	0.039	0.034	0.029	0.025	0.020	0.015	0.011	0.006	0.001	0.001	0.001	0.001	0.001	0.001	0.001
15	0.042	0.038	0.033	0.028	0.023	0.019	0.014	0.009	0.005	0.005	0.005	0.005	0.005	0.005	0.005
16	0.046	0.041	0.036	0.032	0.027	0.022	0.017	0.013	0.008	0.003	0.003	0.003	0.003	0.003	0.003
17	0.049	0.045	0.040	0.035	0.030	0.026	0.021	0.016	0.012	0.007	0.002	0.002	0.002	0.002	0.002
18	0.053	0.048	0.043	0.039	0.034	0.029	0.024	0.020	0.015	0.010	0.006	0.001	0.001	0.001	0.001
19	0.056	0.051	0.047	0.042	0.037	0.033	0.028	0.023	0.018	0.014	0.009	0.004	0.004	0.004	0.004
20	0.060	0.055	0.050	0.045	0.041	0.036	0.031	0.027	0.022	0.017	0.012	0.008	0.003	0.003	0.003
21	0.063	0.058	0.054	0.049	0.044	0.039	0.035	0.030	0.025	0.021	0.016	0.011	0.006	0.002	0.002
22	0.066	0.062	0.057	0.052	0.048	0.043	0.038	0.033	0.029	0.024	0.019	0.015	0.010	0.005	0.001
23	0.070	0.065	0.060	0.056	0.051	0.046	0.042	0.037	0.032	0.028	0.023	0.018	0.013	0.009	0.004
24	0.073	0.069	0.064	0.059	0.054	0.050	0.045	0.040	0.036	0.031	0.026	0.021	0.017	0.012	0.007

Table 5. Flow rates (l.h-1.kg-1 abalone) required to maintain oxygen level for fed abalone at 80% saturation.

	class (mm)														
temp	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
14	5.90	5.27	4.64	4.02	3.39	2.76	2.13	1.51	0.88	0.25	0.25	0.25	0.25	0.25	0.25
15	6.56	5.92	5.28	4.63	3.99	3.35	2.71	2.06	1.42	0.78	0.14	0.14	0.14	0.14	0.14
16	7.17	6.52	5.87	5.22	4.57	3.92	3.27	2.62	1.97	1.32	0.67	0.02	0.02	0.02	0.02
17	7.88	7.22	6.55	5.89	5.22	4.55	3.89	3.22	2.56	1.89	1.23	0.56	0.56	0.56	0.56
18	8.53	7.85	7.18	6.51	5.83	5.16	4.49	3.81	3.14	2.46	1.79	1.12	0.44	0.44	0.44
19	9.30	8.61	7.92	7.23	6.54	5.85	5.16	4.47	3.78	3.09	2.40	1.71	1.02	0.33	0.33
20	10.10	9.41	8.70	8.00	7.30	6.58	5.87	5.16	4.45	3.75	3.04	2.33	1.62	0.91	0.20
21	10.84	10.10	9.40	8.68	7.97	7.25	6.53	5.81	5.10	4.38	3.66	2.94	2.23	1.51	0.79
22	11.57	10.84	10.10	9.39	8.66	7.94	7.21	6.48	5.76	5.03	4.30	3.57	2.85	2.12	1.39
23	12.49	11.75	11.00	10.25	9.51	8.76	8.01	7.27	6.52	5.77	5.03	4.28	3.53	2.79	2.04
24	13.20	12.52	11.77	11.00	10.25	9.50	8.74	7.98	7.23	6.47	5.70	4.95	4.20	3.44	2.68

Table 6. Flow rates (l.h-1.kg-1 abalone) required to maintain oxygen levelsof unfed animals at 80% saturation.

	class (mm)														
temp	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
14	5.02	4.45	3.87	3.3	2.73	2.15	1.58	1	0.43	0.43	0.43	0.43	0.43	0.43	0.43
15	5.59	5	4.42	3.83	3.24	2.65	2.07	1.48	0.89	0.31	0.31	0.31	0.31	0.31	0.31
16	6.11	5.52	4.92	4.33	3.74	3.14	2.55	1.95	1.36	0.77	0.17	0.17	0.17	0.17	0.17
17	6.73	6.12	5.51	4.9	4.29	3.69	3.08	2.47	1.86	1.4	0.64	0.03	0.03	0.03	0.03
18	7.28	6.67	6.05	5.44	4.82	4.2	3.59	2.97	2.36	1.74	1.12	0.51	0.51	0.51	0.51
19	7.95	7.32	6.69	6.06	5.43	4.79	4.16	3.53	2.9	2.27	1.64	1.01	0.38	0.38	0.38
20	8.65	8	7.36	6.71	6.06	5.41	4.77	4.12	3.47	2.83	2.18	1.53	0.88	0.24	0.24
21	9.27	8.61	7.96	7.3	6.65	5.99	5.33	4.68	4.02	3.37	12.71	2.05	1.4	0.74	0.09
22	9.9	9.24	8.57	7.91	7.24	6.58	5.91	5.25	4.59	3.92	3.26	2.59	1.93	1.26	0.6
23	12.2	11.55	10.87	10.1	9.5	8.82	8.14	7.45	6.77	6.09	5.41	4.73	4	3.36	2.68
24	11.37	10.68	9.99	9.3	8.6	7.81	7.22	6.53	5.84	5.15	4.45	3.76	3.07	2.38	1.69

Table 7. Flow rates (l.h-1.kg-1 abalone) required to maintain ammonia levels for fed abalone below 0.02 mg.l-1.

	class (mm)														
temp	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
14	3.13	2.76	2.4	2.03	1.66	1.29	0.93	0.56	0.19	0.19	0.19	0.19	0.19	0.19	0.19
15	3.41	3.04	2.67	2.31	1.94	1.57	1.2	0.84	0.47	0.1	0.1	0.1	0.1	0.1	0.1
16	3.69	3.32	2.95	2.58	2.22	1.85	1.48	1.11	0.75	0.38	0.01	0.01	0.01	0.01	0.01
17	3.96	3.59	3.23	2.86	2.49	2.12	1.76	1.39	1.02	0.65	0.29	0.29	0.29	0.29	0.29
18	4.24	3.87	3.5	3.14	2.77	2.4	2.03	1.67	1.3	0.93	0.56	0.2	0.2	0.2	0.2
19	4.51	4.15	3.78	3.41	3.04	2.68	2.31	1.94	1.57	1.21	0.84	0.47	0.1	0.1	0.1
20	4.79	4.42	4.06	3.69	3.32	2.95	2.59	2.22	1.85	1.48	1.12	0.75	0.38	0.01	0.01
21	5.07	4.7	4.33	3.97	3.6	3.23	2.86	2.5	2.13	1.76	1.39	1.03	0.66	0.29	0.29
22	5.34	4.98	4.61	4.24	3.87	3.51	3.14	2.77	2.4	2.01	1.67	1.03	0.93	0.57	0.2
23	5.62	5.25	4.89	4.52	4.15	3.78	3.42	3.05	2.68	2.31	1.95	1.58	1.21	0.84	0.48
24	5.9	5.53	5.16	4.8	4.43	4.06	3.69	3.33	2.96	2.59	2.22	1.86	1.49	1.12	0.75

Table 8. Flow rates (l.h-1.kg-1 abalone) required to maintain ammonia levels for unfed abalone below 0.02 mg.l-1.

	class (mm)														
temp	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
14	1.94	1.7	1.47	1.23	1	0.76	0.53	0.29	0.05	0.05	0.05	0.05	0.05	0.05	0.05
15	2.11	1.88	1.64	1.4	1.17	0.93	0.7	0.46	0.23	0.23	0.23	0.23	0.23	0.23	0.23
16	2.28	2.05	1.81	1.58	1.34	1.11	0.87	0.63	0.4	0.16	0.16	0.16	0.16	0.16	0.16
17	2.46	2.22	1.98	1.75	1.51	1.28	1.04	0.81	0.57	0.34	0.1	0.1	0.1	0.1	0.1
18	2.63	2.39	2.16	1.92	1.69	1.45	1.21	0.98	0.74	0.51	0.27	0.04	0.04	0.04	0.04
19	2.8	2.57	2.33	2.09	1.86	1.62	1.39	1.15	0.92	0.68	0.45	0.21	0.21	0.21	0.21
20	2.97	2.74	2.5	2.27	2.03	1.8	1.56	1.32	1.09	0.85	0.62	0.38	0.15	0.15	0.15
21	3.15	2.91	2.67	2.44	202	1.97	1.73	1.5	1.26	1.03	0.79	0.55	0.32	0.08	0.08
22	3.32	3.08	2.85	2.61	2038	2.14	1.9	1.67	1.43	1.2	0.96	0.73	0.49	0.26	0.02
23	3.49	3.25	3.02	2.78	2.55	2.31	2.08	1.84	1.61	1.37	1.13	0.9	0.66	0.43	0.19
24	3.66	3.43	3.19	2.96	2.72	2.48	2.25	2.01	1.78	1.54	1.31	1.07	0.83	0.6	0.36

Table 9. A practical feeding table for *H. midae* from Mangold (pers. comm.). (Figures in the columns are given as the recommended feed to be fed as a percent body weight per day).

Shell length (mm)	Temperature (°C)			
	14	16	18	20
10	4.38	5.54	7.68	5.8g
15	2.64	3.35	4.43	3.37
20	1.84	2.34	2.99	2.41
25	1.33	1.69	2.09	1.73
30	1.11	1.42	1.72	1.44
35	0.95	1.21	1.45	1.23
40	0.73	0.93	1.09	0.94
45	0.65	0.83	0.96	0.84
50	0.59	0.75	0.86	0.75
55	0.51	0.65	0.74	0.65
60	0.45	0.57	0.64	0.57
70	0.37	0.48	0.52	0.47
80	0.33	0.42	0.45	0.41

Table 10. Oxygen consumption and ammonia production rate values for abalone from various sources for comparison to present study.

Authors	Species	Rates**		T°
		*R (μl $O_2.h^{-1}.g^{-1}$)	U (μmole $NH_3.h^{-1}.g^{-1}$)	
Barkai and Griffiths (1987)	*H. midae*	135	0.68×10^{-5}	19°C
Segawa (1992)	*S. diversicolor aquatilis*	79.13	1.52×10^{-5}	24°C
Uki and Kikutchi (1975)	*H. discus hannai*	139		24°C
Peck et al. (1987)	*H. tuberculata*	82.43	1.35×10^{-5}	15°C
Present results	*H. midae*	45.6 (fed) 38.3 (unfed)	8.86×10^{-5} (fed) 5.45×10^{-5} (unfed)	24°C

* R - Oxygen consumption (μl $O_2.h^{-1}.g^{-1}$ abalone)

U - Ammonia production (μmole $NH_3.h^{-1}.g^{-1}$ abalone)

** All values are calculated for abalone of a shell length (30mm) and mass 4.96g.

Table 11. Predicted water flow rates (m^3/h) for a farm producing 95 tons of abalone a year over the size range. Assumptions are a projected monthly growth rate of 2 mm with a stocking rate of 125000 abalone/month and a 20% mortality over the growout period.

Temperature (°C)	14	15	16	17	18	19	20	21	22	23
Flow rate (m^3/h)	15	40	65	90	115	140	165	190	216	240