TOWARDS UNDERSTANDING THE EFFECTS OF STOCKING DENSITY ON FARMED SOUTH AFRICAN ABALONE, *HALIOTIS MIDAE*

A thesis submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

At

Rhodes University

By

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January 2014

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ABSTRACT

The profitability of abalone farms is heavily influenced by their production per unit of grow-out space. With farms having physically expanded to the maximum, and with increasing production costs, one of the most realistic ways for farms to increase their production is through optimizing stocking densities. The effect of stocking density on *Haliotis midae* performance is undocumented and optimal stocking densities for this species have not been determined. Experiments were conducted under farm conditions to investigate the effects of four different stocking densities (16 %, 20 %, 22 % and 24 % of available surface area) on growth, production and health of three different size classes of abalone (15-35 g, 45-65 g, and 70-90 g start weight). Each treatment was replicated four times and trials ran over a period of eight months with measurements being made at four month intervals. Abalone behaviour was observed during the trials in the experimental tanks. Weight gain per abalone decreased with an increase in density for all tested size classes (5.04 ± 0.18 to 2.38 ± 0.17; 5.35 ± 0.21 to 4.62 ± 0.29; 7.97 ± 0.37 to 6.53 ± 0.28 $g.abalone^{-1}.month^{-1}$ for the 15-35, 45-65 and 70-90 g classes respectively, with an increased density of 16 to 24 %). Individual weight gain of 15-35 g abalone was similar at stocking densities of 16 % and 20 % while weight gain of 45-65 g and 70-90 g abalone decreased when density was increased above 16 %. Biomass gain ($kg.basket^{-1}.month^{-1}$) was not affected by stocking density in the 15-35 g and 45-65 g size classes (1.29 ± 0.02 and 0.97 ± 0.02 $kg.basket^{-1}.month^{-1}$ respectively). However, the biomass gained by baskets stocked with 70-90 g abalone increased with stocking density (1.08 ± 0.02 to 1.33 ± 0.02 $kg.basket^{-1}.month^{-1}$) with an increased density of 16 to 24 %) and did not appear to plateau within the tested density range (16 to 24 %). Food conversion ratio did not differ significantly between densities across all size classes. Stocking density did not have a significant effect on abalone condition factor or health indices. The proportion of abalone above the level of the feeder plate increased with density (7.26 ± 1.33 to 16.44 ± 1.33 with an increased density of 16 to 24 %). As a proportion of abalone situated in the area of the basket, the same proportions were situated on the walls above the feeder plate and on the feeder plate itself irrespective of stocking density ($p > 0.05$). Higher proportions of animals had restricted access to feed at higher stocking densities ($p = 0.03$). The amount of formulated feed available on the feeder plate did not differ between stocking densities throughout the night ($p = 0.19$). Individual abalone spent more time above the feeder plate at higher stocking densities ($p < 0.05$). The percentage of time above the feeder plate, spent on the walls of the basket and on the feeding surface was not significantly different at densities of 20 %, 22 % and 24 % ($p > 0.05$) but abalone stocked at 16 % spent a greater percentage of time above the feeder plate on the feeding surface (83.99 ± 6.26 %) than on the basket walls (16.01 ± 6.26 %). Stocking density did not affect the positioning of abalone within a basket during the day or at night. Different size *H. midae* are affected differently by increases in stocking density in terms of growth performance. Findings from this research may be implemented into farm management strategies to best suit production goals, whether in terms of biomass production or individual weight gain. The fundamental mechanisms resulting in reduced growth at higher densities are not well understood, however results from behaviour observations suggest that competition for preferred attachment space and feed availability are contributing to decreased growth rates. With knowledge of abalone behaviour at different densities, innovative tank designs may be established in order to counter the reduction in growth at higher densities.

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ACKNOWLEDGEMENTS

This research was funded by Marifeed (Pty) Ltd, Aquafarm Development (Pty) Ltd, HIK Abalone Farm (Pty) Ltd, Roman Bay Sea Farm (Pty) Ltd, the National Research Foundation (NRF) and the Technology and Human Resource for Industry Program (THRIP). Without this financial backing the completion of this project would not have been possible. The relationship between industry partners and aquaculture student research is one which needs to be commended; I hope the support continues a long way into the future.

I would like to thank my supervisor Dr Cliff Jones for his support and patience during my MSc studies. Your input and assistance from start to finish cannot go unnoticed. From help with developing objectives and accurate experimental designs to chapter editing, it is all greatly appreciated.

Thank you to the staff, friends and colleagues from both Aquafarm Development (Pty) Ltd and HIK Abalone Farm (Pty) Ltd. You have all made the running of this project so simple and not only have you assisted in the completion of my MSc research, you have opened my eyes to the world of aquaculture and provide a stepping stone into the industry for me; I couldn't be more grateful to all of you for that. Special thanks must be given to Rowan Yearsley and Matthew Naylor who were always available when assistance was required, often throwing their own work aside in order to guide me and share their knowledge with regard to the on-farm portion of this research.

Thanks to my fellow students Nick Riddin, Morgan Brand and Devin Ayres for all of your help with weighing, measuring and counting. Never one complaint came from you guys and many hands certainly made tedious days more bearable.

I would like to thank all the friends I have made along the way. Hermanus has proven to be one of my favourite places in South Africa and it's not only the spectacular surroundings, but all of you that make it so beautiful. My final thanks go to my parents and close family. You have always gone out of your way to make sure I am able to achieve what I am capable of, but more importantly you have encouraged me to follow the paths I wish to follow and supported me every step of the way. I can never thank you enough for your love and support.

CHAPTER 1

General introduction

The South African abalone, *Haliotis midae* is a marine gastropod which occurs naturally in shallow coastal waters of the southern and western coastlines of the country (Newman 1965). Water temperatures, in their natural distribution range, vary below 12 ° C in Western Cape to warmer Eastern Cape waters which may reach 21° C (Greenwood and Taunton-Clark 1994). *Haliotis midae* naturally feeds on both micro and macro-algae (Erasmus *et al*. 1997). It is the largest of the abalone species which inhabit South African waters, making it the only commercial species found in the country (Hecht 1994). Commercial fishing for *H. midae* began in 1949 (Tarr 1995) and the fishery managed to remain stable for several years before signs of overexploitation began to appear and commercial quotas had to be introduced in 1970 (Steinberg 2005). Quotas were based on total allowable catch (TAC) which was continuously overestimated causing natural populations of *H. midae* to decline despite constant monitoring and adjusting of annual TAC (Steinberg 2005).

The first attempts to cultivate *H. midae* were made in 1981 when specimens were successfully spawned and juvenile abalone were reared in captivity (Genade *et al*. 1988), yet commercial aquaculture remains a relatively young sector in the South African aquaculture industry. Commercial farming under intensive conditions began in the 1990's (Sales and Britz 2001) and saw rapid expansion through input into research and development, and the realisation of high market demand for the South African abalone (Sales and Britz 2001). By 2002, almost forty percent of South Africa's abalone production was derived from farmed sources (Cook and Gordon 2010). In 2005, there were 13 commercial abalone farms operating in South Africa providing around 508 tonnes (ZAR 82 million) of abalone to the market annually (Botes *et al*. 2006). In the context of global production, by 2004, South Africa was listed as the largest producer of farmed abalone outside of Asia (FAO 2004).

Research into Haliotis midae

The rapid growth of the abalone farming industry in South Africa is due largely to thorough and continuous research aimed towards optimising culture conditions. Sales and Britz (2001) provide a review of research performed on farmed *H. midae* prior to 2001. During this time, research was aimed mainly towards cultivation strategies and included investigations of: spawning and seed production (Wood 1993, Tarr 1995); the effect of temperature on abalone growth (Hecht 1994); the effects of handling, transport and anaesthetics on abalone well being (Genade *et al*. 1988, Cook and Ruck 1991, White *et al*. 1996); the development of formulated diets (Britz *et al*. 1994, Fleming and Hone 1996, Cook 1998); and post larval feeding (Matthews and Cook 1995). Beyond 2001, research has delved into fine tuning production techniques and investigated methods of working towards achieving optimum production strategies. This research has been broad and encompassing, investigating subjects such as: refined diet formulations and nutrient digestibility (Sales and Britz 2002, Shipton *et al*. 2002, Sales and Britz 2003, Sales *et al*. 2003, Naidoo *et al*. 2006, Green *et al*. 2011); use of probiotics in formulated feeds (Macey and Coyne 2005); Ammonia toxicity and water quality effects on growth and health (Reddy-Lopata *et al*. 2006, Yearsley 2007, Naylor *et al*. 2011); parasite susceptibility and infestation (Simon *et al*. 2006, Macey and Coyne 2006); genetic research and gene isolation (Slabbert *et al*. 2008), understanding energy investment (Laas and Vosloo 2010, Riddin 2012) and integrated aquaculture possibilities (Robertson-Andersson *et al*. 2008, Bolton *et al*. 2009).

Despite the depth of research aimed towards improving production techniques, no documented research could be found on the effects of stocking density on farmed *H. midae* performance and no attempt has been made to establish optimum stocking densities for this species of abalone under farm conditions.

Stocking density and aquaculture

Stocking density describes the proportion of farmed aquatic animals within a defined water body (Ellis *et al*. 2001). In aquaculture, particularly on land-based farms where space is limited, stocking densities are considered important tools in achieving optimum production and economic yields (Christianssen *et al*. 1992, Wickert 2011, Villanueva *et al*. 2013). Research has shown that if stocking densities are increased above threshold levels, they will have negative effects on water quality parameters (Suresh and Lin 1992, Yearsley 2007), animal condition (Capinpin *et* al. 1999, North *et al*. 2006), growth and survival rates (Mgaya and Mercer 1995, Schram *et al*. 2006, Badillo *et al*. 2007) and yield and productivity (Holliday *et al.* 1991, Parsons and Dadswell 1992, Salas-Leiton *et al*. 2008). Therefore, the effects of stocking density limit the amount of animals that can be housed in a water body (El-Sayed 2006). Despite the problems associated with high stocking densities, economic analyses show that densities can significantly affect production costs and revenue of an aquaculture operation (Villanueva *et al*. 2013); higher stocking densities result in increased costs of production but, by producing larger quantities of organisms, can increase the value of the biomass produced (Forsberg 1996, El-Sayed 2006, Wassnig *et al*. 2009). The choice of stocking density has been described as a trade-off between maximum growth rates, optimal biomass gain and economic considerations which would indicate which densities will result in a net reduction in production costs (Mgaya and Mercer 1995). It is therefore important to develop optimal stocking densities for cultured species in order to achieve maximum productive output with minimum expenditure, thereby maximising profit margins (Rönnbäck 1999, Pomerleau and Engle 2003, El-Sayed 2006).

Most studies performed on the stocking densities of farmed finfish and molluscs have focussed on the effect of stocking densities on growth, reproduction and behaviour in order to try and establish appropriate densities for optimum culture (Holliday *et al*. 1991, Mgaya and Mercer 1995, Capinpin *et al*. 1999, Lui and Chen 1999, Huchette *et al*. 2003, Schram *et al*. 2006, Lloyd and Bates 2008, Wassnig *et al*. 2009, Wu *et al*. 2009). Flatfish such as halibut and sole species can be compared to abalone on a stocking density basis because they are also reliant on surface area as a measurement of density under aquaculture conditions. Björnsson (1994) estimated the optimum stocking density for two kilogram halibut (*Hippoglossus hippoglosus L.*) to be between 25 and 50 kg/m^2 after establishing that growth rates were only affected by stocking densities once a threshold was reached. Similarly, growth rates of the Dover sole (*Solea solea*) were reduced with an increase in stocking density from 0.5 – 12 kg/m^2, however a peak in productivity in terms of biomass gain was found at 7.4 kg/m^2 despite a drop in growth rates (Schram *et al*. 2006). The growth rates of juvenile European abalone (*Haliotis tuberculata*) decreased with increasing stocking density but the amount of biomass gained was greater at higher densities (Mgaya and Mercer 1995). Growth rates of *Haliotis corrugate* were also negatively affected with an increase in stocking density when housed in flow through systems (Badillo *et al*. 2007). This density dependant trend in growth rate has been established for several other molluscs and abalone species under farm conditions (Holliday *et al.* 1991, Allan and Maguire 1992, Parsons and Dadswell 1992, Lloyd and Bates 2008, Wu *et al*. 2009, Wassnig *et al*. 2009) and is expected to be similar for *H. midae*.

Although it is largely accepted that abalone growth rates decrease with an increase in stocking density, the underlying mechanisms causing this reduction in growth is not fully understood (Mgaya and Mercer 1995, Huchette *et al*. 2003, Wassnig *et al*. 2009). The majority of research aimed at understanding this trend has focused on abiotic aspects of production (Harris *et al*. 1998, Higham *et al*. 1998, Capinpin *et al*. 1999, Lui and Chen 1999). It is suggested that a decrease in growth rates at higher stocking densities may be as a result of competition for space and food which is based largely on abalone behavioural characteristics (Douros 1987, Mgaya and Mercer 1995, Huchette *et al*. 2003, Wassnig *et al*. 2009, Wu *et al*. 2009). There is no documented research in relation to stocking density and its effect on the behaviour of farmed *H. midae* but an understanding of this could be important in mitigating the effect of stocking density on growth rates, and possibly allow farms to increase stocking density through implementing innovative tank or basket design strategies. Huchette *et al*. (2003) investigated the effects of stocking density on the behaviour and growth of *Haliotis rubra* and found that the spatial distribution of abalone within a tank was closely related to the availability of preferred shelter space. This resulted in abalone stacking in preferred areas and this occurrence increased with an increase in stocking density. When tanks were situated in a completely shaded environment, the behaviour of abalone changed and their distribution within a tank was less crowded, reducing competition for preferred shelter space and allowing for easier access to formulated feed (Huchette *et al*. 2003).

The aim of this study was to investigate the effect of stocking density of the growth, health, production and behaviour of different size *H. midae* under farm conditions in order to develop a better understanding of optimal stocking densities for farmed South African abalone.

The research objectives were to:

1) quantify the effect of four different stocking densities on the growth and production of three size classes of abalone;
2) establish whether stocking density has an effect on abalone health under farm conditions;
3) identify behavioural characteristics of farmed *H. midae* which could be quantified; and
4) investigate the effect of stocking density on these behavioural characteristics.

CHAPTER 2

The effect of stocking density on health, growth and production

2.1 Introduction

The profitability of abalone farms is heavily influenced by their production per unit of grow-out space (Holliday *et al.* 1991, Capinpin *et al.* 1999). With farms having physically expanded to the maximum and with increasing costs of production (electricity, maintenance etc.) (Wurts 2000), one of the most realistic ways for an abalone farm to increase its production and therefore profitability is through optimizing stocking densities.

Research aimed towards understanding the effects of stocking density on the growth and production of the South African abalone, *Haliotis midae* has not been documented. Work done on other species of abalone suggests however that growth performance will decrease with an increase in stocking density (Mgaya and Mercer 1995, Capinpin *et al*. 1999, Lui and Chen 1999, Huchette *et al*. 2003, Lloyd and Bates 2008, Wassnig *et al*. 2009, Wu *et al*. 2009). Huchette *et al*. (2003) concluded that the growth of *Haliotis rubra* at high densities was reduced directly through competition for space and indirectly via the degradation of water quality. The growth of individual juvenile European abalone, *Haliotis tuberculata*, showed a decreasing trend when stocking density was increased (Mgaya and Mercer 1995). The same decreasing trend was observed when *Haliotis asinina* were exposed to increased stocking densities (Capinpin *et al*. 1999). The stocking densities used on South African abalone farms currently range from 16-18 % of the available surface area in a basket. Although optimal stocking densities for South African abalone have not yet been determined, preliminary, anecdotal on-farm research has shown that stocking densities may be increased until a size dependant threshold is reached at which growth rates and animal health may be reduced (Yearsley, *pers. comm*., Aquafarm Development (Pty) Ltd., March 2011). This threshold seems to be size dependant (Mgaya and Mercer 1995).

Growth rates of juvenile European abalone *H. tuberculata* decreased with increased stocking densities, although the total biomass gained per unit area illustrated an interesting trend in that the biomass gained increased with density to a point before decreasing slightly (Mgaya and Mercer 1995). The research suggested that the choice of on farm stocking density will be based on a trade off between maximum growth and optimal biomass gain (Mgaya and Mercer 1995), which will be based on a farm's production plan. Fallu (1991) suggests because abalone stocked at higher densities show reduced growth rates, farmers need to find a compromise between the costs of additional facilities and reduced growth rates at high densities. However, perhaps an optimal stocking density could be one at which maximum profits are attained at the expense of high growth rates? It is likely that although growth of *H. midae* may be reduced at higher densities, production or biomass gain per unit grow-out space may be higher than at lower densities where faster growth is achieved. This situation has been observed in other aquaculture studies (Neudecker 1981, Holliday *et al.* 1991, Wassnig *et al.* 2009). Despite this, if animal health is negatively affected, the risk of infection by parasites or disease will be increased and perhaps the market quality of the abalone will be compromised.

The degradation of water quality is often associated with increased stocking densities under aquaculture conditions (Suresh and Lin 1992, Wu 1995, Yearsley 2007). Although water is expensive to pump, sea water is not considered a major limiting resource for South African abalone farmers (Yearsley 2007). If the availability of sea water was to become limiting under current farm conditions, farmers could increase the pumping capacity of their infrastructure in order to increase production; the cost of suitable land required for extra tank space is likely to be a greater expense. With sea water not currently being limiting, increased stocking densities can be mitigated through increased flow rates and water should not remain a dependant variable in research or development which aims to optimise stocking densities for land-based abalone farms in South Africa.

This study aims to evaluate the effects of different stocking densities on the growth, health and production of abalone when water quality is not a confounding factor in order to develop a better understanding of optimal stocking densities for different size *H. midae*.

The objectives of this study were to:

1) compare the growth of three size classes of abalone stocked at four different stocking densities over eight months;
2) quantify the effect of stocking density on the production of different size abalone; and
3) establish whether stocking density has an effect on abalone health under farm conditions.

2.2 Materials and methods

Experimental system

Experiments were conducted at Aquafarm Development (Pty) Ltd, in Hermanus, South Africa (34º26'04.35"S; 19º13'12.51"E; Figure 2.1). Abalone were housed in rectangular farm tanks (length, width and depth of 3.90, 0.85 and 0.65 m respectively) which were part of a flow-through sea water system (Figure 2.2). Sea water was pumped from the ocean, directly into a header tank and was filtered through a micro-screen drum filter (85 µm) before being fed by gravity into the tanks. Water entered each tank from one end and was drained from the opposite end through an up-stand pipe. Each tank contained seven oyster mesh baskets (Yearsley *et al*. 2009). Each basket contained a farm designed rack made up of seven vertically positioned plastic plates, and an asbestos feeder plate which was positioned horizontally above the vertical plates and approximately 10 cm below the water surface (Yearsley *et al*. 2009). The total surface area available to the abalone was calculated as 3.101 m^2, and was based on the surface area of the rack, the bottom of the basket and the underside of the feeder plate. This figure was used throughout the study to calculate stocking densities. Water temperatures and photoperiod were not controlled and fluctuated with the environmental conditions. Airlines were installed in each tank using 20 mm polyvinylchloride (PVC) piping that ran the length of the tank and that were raised slightly from the tank floor. Blowers were used to deliver air through these pipes and into the water (Naylor *et al.* 2011). Each tank was drained and cleaned once every two weeks. Prior to cleaning, abalone were moved, in their baskets, to already cleaned tanks as part of standard farm practise.

Figure 2.1: Map imagery indicating the location of Hermanus within the Western Cape, South Africa (inset image) and an aerial view of Aquafarm Development (Pty) Ltd (outlined area) (Source: “Aquafarm Development (Pty) Ltd.”, 34º26’05.34”S and 19º13’24.26”E. Google Earth. 20 January 2014. 28 August 2014.)

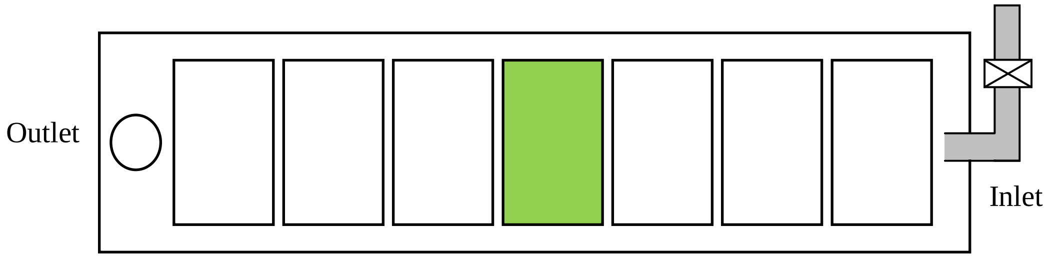


Figure 2.2: Schematic aerial view of the experimental tank layout containing seven oyster mesh baskets, a water inlet on one end and outlet up-stand pipe on the opposite end. Abalone in the centre basket in each tank were used to obtain individual abalone measurements.

Experimental animals and feed

Animals were spawned using farm broodstock and were obtained from the farm's production, therefore no acclimation was required. Spawning takes place regularly and larvae are kept in pre-on growing tanks for approximately six months before entering the farms grow-out system. The month in which abalone enter the grow-out system is recorded as their batch number. Abalone from the same batch are assumed to be of a similar age and were used within each of three size classes. The average starting weights (mean ± std error) of individual abalone in size class A, size class B and size class C were 29.28 ± 0.25 g.abalone^{-1} (Batch: November 2009), 51.88 ± 0.40 g.abalone^{-1} (Batch: April 2009) and 75.07 ± 0.57 g.abalone^{-1} (Batch: July 2009) respectively. Each size class was considered an independent experiment and experiments were run at slightly different times of year due to the availability of abalone.

Abalone were fed formulated diets according to size class, based on farm procedure. Abalone in size class A were fed Abfeed® S34 (Marifeed (Pty) Ltd; 34.7 % protein, 2.4 % lipid, 57.3 % carbohydrate, 1.6 % fibre and 5.6 % ash), abalone in size class B and C were fed Abfeed® S34K (Marifeed (Pty) Ltd; 34.5 % protein, 3.3 % lipid, 53 % carbohydrate, 1 % fibre and 6 % ash). Feeding occurred once daily by distributing pellets from a feeding cup (30.66 ± 0.23

g.cup^{-1}; n = 30) onto the feeder plate. Abalone were fed to apparent satiation based on the amount of feed remaining on the plate the following day (Yearsley 2007).

Stocking density

The effects of four different stocking densities were tested on the three different size classes of abalone mentioned above. The tested stocking densities included 16 %, 20 %, 22 % and 24 % of the available surface area covered by abalone in a basket. These stocking densities represented the treatments for each experiment. The treatments were replicated four times, and a tank (including seven baskets) was used as a unit of replication. Tanks were individual farm units and were positioned randomly within the farms tank layout system.

Before stocking the baskets, length-weight and breadth-weight relationships were established for each size class of abalone by weighing (0.01 g) (electronic balance: Kern PLS 4200-2F, serial number: WIC1200486) and measuring (0.1 mm) a random sample of 200 animals from the batch of abalone to be used in the trials. These relationships were used to predict the average length and breadth of an abalone from a known average weight and therefore calculate the predicted area covered by an individual abalone. With this information, the number of abalone needed to achieve the required stocking density for each size class was calculated. The number required was multiplied by the average weight of animals from the basket and an experimental basket was stocked by weight (kg.basket^{-1}). Stocking density tables were established using the length-weight and breadth-weight relationships. Before stocking each basket, an average weight was obtained from a sample of 50 randomly selected abalone. This average weight was read off the stocking density tables which provided an estimate of the biomass (kg.basket^{-1}) required to stock a basket at the desired density.

Length-weight and breadth-weight relationships were recalculated for experimental animals after four months, and stocking density tables were adjusted to account for abalone growth during the trials. These tables were used to correct stocking densities within the baskets using the same procedure described previously.

Table 2.1: Mean (± standard error) starting weight of abalone stocked into each basket to achieve stocking densities of 16, 20, 22 and 24 % of the available surface area and the approximate number of abalone required to achieve these weights according to calculated stocking density tables.

	Stocking Density (% surface area covered)			
	16	**20**	**22**	**24**
Exp 1: 15 - 35 g Size Class				
Mean basket weight (kg.basket^{-1})	8.34 ± 0.03	10.39 ± 0.02	11.32 ± 0.04	12.43 ± 0.05
Approximate number of abalone	275	346	389	420
Exp 2: 45 - 65 g Size Class				
Mean basket weight (kg.basket^{-1})	9.33 ± 0.04	11.86 ± 0.05	12.36 ± 0.07	14.15 ± 0.07
Approximate number of abalone	177	217	242	262
Exp 3: 70 - 90 g Size Class				
Mean basket weight (kg.basket^{-1})	10.82 ± 0.03	13.58 ± 0.05	14.87 ± 0.05	16.16 ± 0.04
Approximate number of abalone	148	184	204	224

Data collection - Growth and biomass production

The trials were run for eight months, with a four month splitting interval, at which time growth data were collected. Trials for each size class started at different times according to when abalone were available. The start and end dates of each experiment were as follows (DD-MM-YYYY): 24-06-2011 to 06-03-2012 (size class A); 12-05-2011 to 16-01-2012 (size class B); 15-12-2011 to 20-08-2012 (size class C). Abalone were purged for 24 hours before handling. Prior to handling, abalone were anaesthetized by placing the basket into a tank containing seawater which was saturated with carbon dioxide. Once anaesthetised, abalone were removed from the basket and weighed and measured. The total weight of abalone in each basket was measured. A sample of 50 abalone were removed from the basket and weighed to obtain an average individual abalone weight for each basket. The number of mortalities in each basket after four months was recorded.

The basket positioned in the middle of each tank was labelled with a coloured tag (Figure 2.2). Fifty randomly selected abalone from the tagged basket were individually weighed (0.01 g) using the same electronic balance and measured (0.1 mm) with vernier callipers at the start, four months into and at the end of the trial which lasted eight months.

Abalone growth was calculated as mean individual weight gain ($g.abalone^{-1}.month^{-1}$) and mean biomass gain ($kg.basket^{-1}.month^{-1}$) for all treatments. Final weight was subtracted from initial weight to calculate the mean weight gain of individual abalone. Condition factor was calculated using Equation 1 (Britz 1996):

$$\text{Condition factor} = \text{weight (g)}/\text{length (mm)}^{2.99} \times 5575 \tag{1}$$

The number of cups (30.66 ± 0.23 $g.cup^{-1}$; $n = 30$) of formulated feed, which were administered to an individual basket over the eight month trial period was recorded daily. Feed conversion ratio (FCR) was calculated using Equation 2 (Britz *et al*. 1997):

$$\text{FCR} = \text{dry feed supplied (g)} / \text{wet weight gain (g)} \tag{2}$$

Data collection - Health Analysis

A qualitative health examination was carried out on abalone in the 45-65 g size class only. It was carried out by an independent, commercial veterinary laboratory (Amanzi Biosecurity (Pty) Ltd, Hermanus, South Africa). At the start of the experiment, thirty abalone were randomly selected from the batch that was used to stock experimental baskets; these animals served as reference to the health of the abalone before they were exposed to different stocking densities. After eight months, thirty abalone were randomly selected for analysis from each of the 16 % and 24 % density treatments; eight animals were selected from two tanks and seven animals from the other two tanks in each treatment. The selected abalone were examined for parasites and their shell condition, nutritional status, environmental stress status and gonad development status were evaluated. They also underwent histological examination of the kidney and digestive gland. Samples were fixed in Davidson's fixative and processed using standard methods for paraffin wax embedding (Austin and Austin 1989). Sections were stained using Harris' haematoxylin and eosin and were observed under light microscopy. Scores were used to describe different abalone health parameters (Table 2.2). Proportions were used to quantify the prevalence of parasites.

Table 2.2: Health parameters were given scores with the following explanations for each value (Amanzi Biosecurity Pty Ltd, Hermanus, South Africa).

	Score Value				
Parameter	**0**	**1**	**2**	**3**	**4**
Sabbelid prevalence	Absent	Less than 10 tubes on growth edge	More than 10, but tubes do not overlap	New tubes overlaid on older ones	More than two thirds of growth edge completely covered in tubes
Gonad development	Absent	Immature. No mature gametes	Predominantly immature with few mature gametes	Predominantly mature gametes but immature stages visible	Mature gametes only
Nutritional status	No changes	Mild shrinkage of digestive gland tubules	Marked shrinkage of digestive gland tubules	Advanced atrophy of tubules with metaplasia of epithelium	
Environmental Stress	No changes	Mild dilation of lumen in right kidney tubules	Moderate dilation of lumen in right kidney tubules	Marked dilation of lumen and degenerative changes in epithelium right kidney tubules	

Data collection - Water Quality

Temperature (°C), pH, dissolved oxygen (DO) levels (mg.L^{-1}) and total ammonia nitrogen (TAN.µg.L^{-1}) were measured in each of the experimental tanks once every two weeks. The pH was measured using a pH meter (YSI Model # 60 / 10 FT; Yellow Springs, OH, USA), DO was measured with an oxygen meter (YSI Model # 55 D, Yellow Springs, OH, USA) and temperature readings were recorded from both the pH and oxygen meters. Water samples were collected from experimental tanks in sterilized glass bottles. The samples were processed using the phenol hypochlorite method (Solórzano 1969) to determine the concentration of TAN in the water. Absorbance was read using a spectrophotometer (Prim Light, Secomam, Ales, France) at 360 nm. Total ammonia nitrogen measurements were calculated from a standard curve that was constructed from solutions containing known TAN concentrations.

Flow rates were standardised and calculated according to tank biomass, using flow rate charts, in order to avoid restrictive water quality. Flow rates were measured and set as litres of water entering each tank per second (L.s.$tank^{-1}$) (0.32, 0.39, 0.43 and 0.47 L.s.$tank^{-1}$ for tanks stocked with 15-35 g abalone; 0.40, 0.52, 0.56, 0.60 L.s.$tank^{-1}$ for tanks stocked with 45-65 g abalone; 0.38, 0.48, 0.53, 0.58 L.s.$tank^{-1}$ for tanks stocked with 70-90 g abalone; at 16 %, 20 %, 22 % and 24 % densities respectively). They were checked and corrected at least once per day.

Statistical analysis

Treatment means were compared after eight months using a one-way analysis of variance (ANOVA) (Fisher 1928) at a significance rate of $p < 0.05$. The ANOVA's assumptions of equal variance and the normal distribution of the residuals

were tested using a Levene's test (Levene 1960) and the Shapiro-Wilk test (Shapiro and Wilk 1965) respectively. Tukey's *post-hoc* (Tukey 1960) test was used to identify any significant differences between treatments. If the assumptions of an ANOVA were not met, the data were log transformed and if the log transformed data did not meet the assumptions then a non-parametric Kruskal Wallis ANOVA (Kruskal and Wallis 1952) was used to compare treatment means. Linear regression models were calculated for all growth data using the mean parameter of each tank as the unit of measure ($p < 0.05$). Analysis of pH was run on un-logged data; mean values were then logged again to obtain the presented pH values. All analyses were carried out using a software package (Statistica, version 10). The statistical files will be made available on request (Contact: c.jones@ru.ac.za).

2.3 Results

Growth and production

There were fewer than ten recorded mortalities across all treatments in each of the experiments after eight months. The effect of stocking density on abalone mortality was considered negligible and no statistical analyses were performed.

Experiment 1: 15-35 g size class

Mean individual weight gain per abalone decreased with an increase in density from 16 % to 22 % (5.04 ± 0.18 to 2.59 ± 0.08 g.abalone^{-1}.month^{-1}) for animals in the 15-35 g size class (ANOVA: $F_{(3,12)} = 103.36$, $p < 0.0001$; Regression analysis: $r^2 = 0.87$, $p = 0.0000001$; Figure 2.3 A). No significant differences were found in individual weight gain between 16 % and 20 % or 22 % and 24 % stocking densities; however these two groups were significantly different from each other (Figure 2.3 A). The mean biomass gain per basket of 15-35 g abalone was not significantly affected by an increase in stocking density after eight months (overall mean: 1.29 ± 0.02 kg.basket^{-1}.month^{-1}; ANOVA: $F_{(3,12)} = 4.23$, $p = 0.30$; Figure 2.3 B).

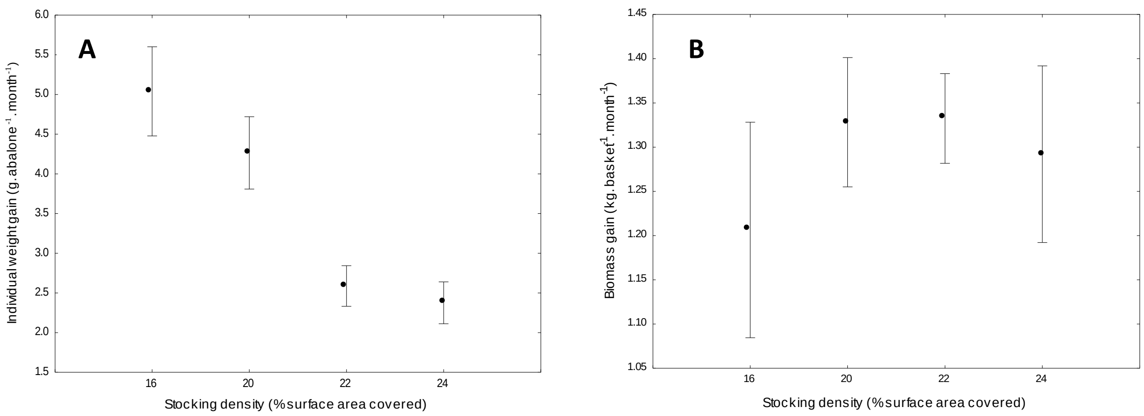


Figure 2.3: Mean (± 95 % confidence interval) (A) individual weight gain (g.abalone^{-1}.month^{-1}) (ANOVA: $F_{(3,12)} = 103.36$, $p < 0.0001$; Regression analysis: $y = -0.3605x + 10.9667$, $r^2 = 0.87$, $p = 0.0000001$) and (B) biomass gain (kg.basket^{-1}.month^{-1}) (ANOVA: $F_{(3,12)} = 4.23$, $p = 0.30$; Regression analysis: $y = 0.0122x + 1.0393$, $r^2 = 0.26$, $p = 0.04$) of 15-35 g abalone stocked at different densities for eight months.

Food conversion ratio values were not affected by stocking density (16 %: 1.05 ± 0.03, 20 %: 0.90 ± 0.08, 22 %: 0.97 ± 0.03, 24 %: 1.05 ± 0.04, overall mean: 0.99 ± 0.03; ANOVA: $F_{(3, 12)} = 2.31$, $p = 0.13$) after eight months. No significant differences were found in condition factor of 15-35 g abalone stocked at different densities over eight months (16 %: 1.12 ± 0.02, 20 %: 1.08 ± 0.01, 22 %: 1.09 ± 0.01, 24 %: 1.06 ± 0.02, overall mean: 1.09 ± 0.01; ANOVA: $F_{(3, 12)} = 2.65$, $p = 0.10$).

Experiment 2: 45-65 g size class

Mean individual weight gain per abalone decreased significantly with an increase in density from 16 % to 22 % for animals in the 45-65 g size class, however individual weight gain remained similar across 20 %, 22 % and 24 % stocking densities (ANOVA: $F_{(3,12)} = 3.59$, $p = 0.047$; Figure 2.4 A). There was a significant trend of decreased weight gain with increased stocking density (Regression analysis: $r^2=0.29$, $p=0.03$; Figure 2.4 A). The mean biomass gain of 45-65 g abalone was not significantly affected by an increase in stocking density after eight months (overall mean: 0.97 ± 0.02 kg.basket^{-1}.month^{-1}; ANOVA: $F_{(3,12)} = 1.92$, $p = 0.18$; Regression analysis: $r^2 = 0.23$, $p = 0.06$; Figure 2.4 B).

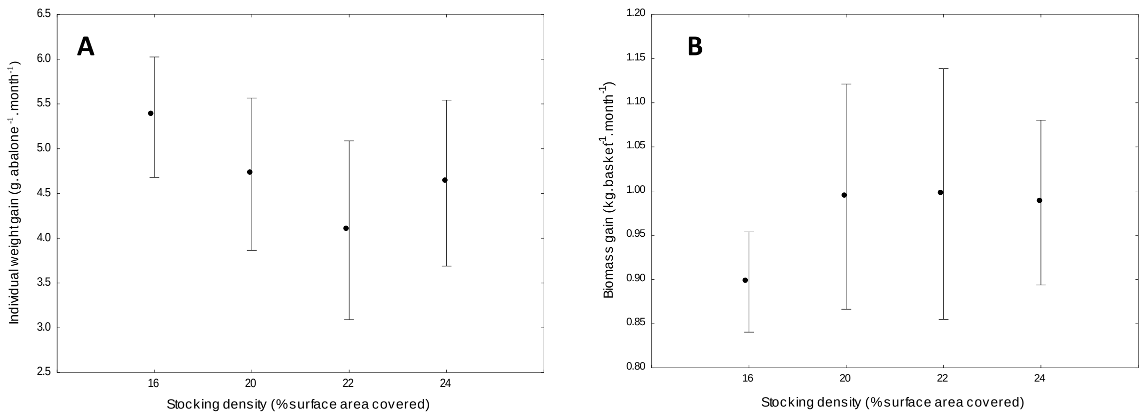


Figure 2.4: Mean (± 95 % confidence interval) (A) individual weight gain (g.abalone^{-1}.month^{-1}) (ANOVA: $F_{(3,12)} = 3.59$, $p = 0.048$; Regression analysis: $y = -0.1187x + 7.1268$, $r^2 = 0.29$, $p = 0.03$) and (B) biomass gain (kg.basket^{-1}.month^{-1}) (ANOVA: $F_{(3,12)} = 1.92$, $p = 0.18$; Regression analysis: $y = 0.0119x + 0.7253$, $r^2 = 0.23$, $p = 0.06$) of 45-65 g abalone stocked at different densities for eight months.

Food conversion ratio values were not affected by stocking density (16 %: 1.27 ± 0.06, 20 %: 1.17 ± 0.10, 22 %: 1.15 ± 0.05, 24 %: 1.12 ± 0.06, overall mean: 1.17 ± 0.03; ANOVA: $F_{(3,}$

$_{11)}$ = 1.45, p = 0.28) after eight months. No significant differences were found in condition factor of 45-65 g abalone stocked at different densities over eight months (16 %: 1.04 ± 0.02, 20 %: 1.02 ± 0.02, 22 %: 1.02 ± 0.02, 24 %: 1.04 ± 0.01, overall mean: 1.03 ± 0.03; ANOVA: $F_{(3, 12)} = 0.57$, $p = 0.64$).

Experiment 3: 70-90 g size class

In this size class, abalone stocked at 16 % density had significantly higher individual weight gain than those stocked at 20 %, 22 % and 24 % densities which all showed similar growth (ANOVA: $F_{(3,12)} = 7.94$, $p = 0.004$; Figure 2.5 A), with an overall significant decrease in weight gain with increasing density (Regression analysis: $r^2 = 0.51$, $p = 0.002$; Figure 2.5 A). Mean biomass gain was not significantly different between densities of 16 % and 20 %, 20 % and 22 % and 22 % and 24 % stocking densities; baskets stocked at 24 % however, had a higher mean biomass gain than those stocked at 16 % and 20 %, and those stocked at 22 % also had a significantly higher mean biomass gain than baskets stocked with a density of 16 % (ANOVA: $F_{(3,12)} = 14.21$, $p < 0.001$; Figure 2.5 B). The increase in biomass gain for the 70-90 g abalone did not appear to plateau in the tested stocking density range, and there was a significant linear increase in biomass gain with increasing stocking density (Regression analysis: $r^2 = 0.73$, $p = 0.00002$; Figure 2.5 B).

Food conversion ratio values were not affected by stocking density (16 %: 1.02 ± 0.06, 20 %: 0.95 ± 0.02, 22 %: 1.07 ± 0.05, 24 %: 0.96 ± 0.05, overall mean: 1.00 ± 0.02; ANOVA: $F_{(3, 12)}$=1.19, p = 0.35) after eight months. No significant differences were found in condition factor of 70-90 g abalone stocked at different densities over eight months (16 %: 1.10 ± 0.01, 20 %: 1.09 ± 0.01, 22 %: 1.10 ± 0.01, 24 %: 1.08 ± 0.01, overall mean: 1.09 ± 0.02; ANOVA: $F_{(3, 12)} = 0.65$, $p = 0.60$).

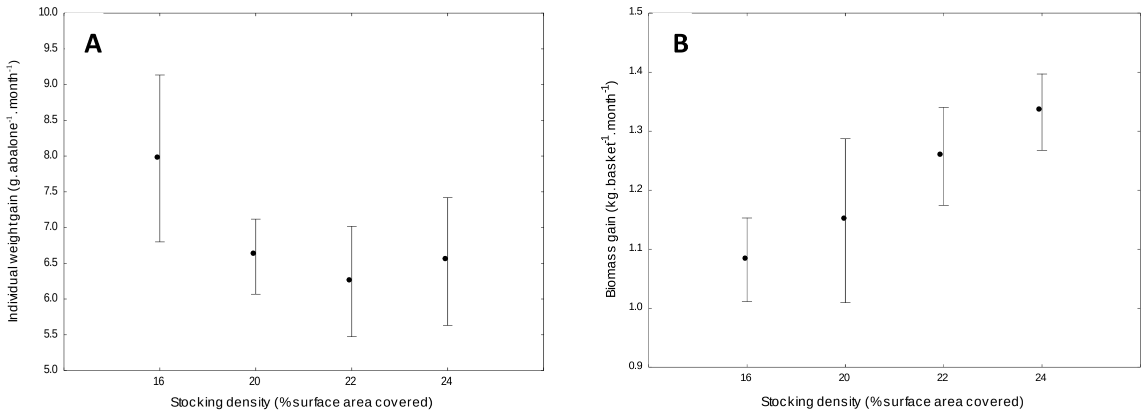


Figure 2.5: Mean (± 95 % confidence interval) (A) individual weight gain (g.abalone^{-1}.month^{-1}) (ANOVA: $F_{(3,12)} = 7.94$, $p = 0.004$; Regression analysis: $y = -0.1984x + 7.1268$, $r^2 = 0.51$, $p = 0.002$) and (B) biomass gain (kg.basket^{-1}.month^{-1}) (ANOVA: $F_{(3,12)} = 14.21$, $p < 0.001$; Regression analysis: $y = 0.0315x + 0.5586$, $r^2 = 0.73$, $p = 0.00002$) of 70-90 g abalone stocked at different densities for eight months.

Health analysis

An increase in stocking density from 16 % to 24 % did not affect abalone health. Abalone stocked at 16 % densities had more constant indications of new shell deposition, but their sabbelid scores appear to be higher than those stocked at 24 % densities (Table 2.3). Gonad development and environmental stress scores appear similar between treatments after eight months. Abalone stocked at densities of 24 % had more signs of early digestive gland atrophy. Average sabbelid, gonad development, nutritional status and environmental stress scores (Table 2.3) did however fall into the same score value brackets indicating similarity between 16 % and 24 % stocking density treatments (Table 2.2). Gonad development scores were higher at the end of the experiments than at the start, and environmental stress scores were substantially higher in sampled abalone after being exposed to experimental treatments for 8 months than they were at the start of experiments. It must be emphasised that these results are based on qualitative scores only.

Table 2.3: Mean scores and indications, provided for each of the examined health parameters, before experiments commenced (*n* = 30) and at the end of 16 % (*n* = 30) and 24 % (*n* = 30) stocking density treatments (Amanzi Biosecurity Pty Ltd, Hermanus, South Africa). Data were collected from animals in the 45-65 g size class only.

	Start	**16 % End**	**24 % End**
Sabellids	0.4	0.6	0.4
Shell condition	Good	Good	Acceptable
Gonad Development Score	2.2	3	3.3
Nutritional Status	0.5	0.4	0.9
Environmental Stress	0.1	1.5	1.3
Parasite Presence	Coccidia (3 %) Gut Protozoa (3 %)	Coccidia (13 %) Rickettsia (13 %)	Gut Protozoa (13 %) Boccardia (13 %)

*Mean values were received from Amanzi Biosecurity Pty Ltd Raw data was not available. Therefore no standard errors are presented.

Water Quality

Experiment 1: 15-35 g size class

No significant differences were found in temperature (ANOVA: $F_{(3, 204)}$= 0.011, p = 0.998), pH (ANOVA: $F_{(3, 204)}$= 0.145, p = 0.933), DO (ANOVA: $F_{(3, 204)}$= 1.332, p = 0.265) and TAN (Kruskal-Wallis ANOVA on ranks: $H_{(3, 208)}$ = 6.450, p = 0.092) at different stocking densities, throughout the eight month growth trial (Table 2.4).

Experiment 2: 45-65 g size class

No significant differences were found in temperature (ANOVA: $F_{(3, 220)}$= 0.023, p = 0.995), pH (ANOVA: $F_{(3, 220)}$= 0.353, p = 0.788), DO (ANOVA: $F_{(3, 220)}$= 1.937, p = 0.124) and TAN

(ANOVA: $F_{(3, 220)}$= 1.952, p = 0.122) at different stocking densities, throughout the eight month growth trial (Table 2.4).

Experiment 3: 70-90 g size class

No significant differences were found in temperature (ANOVA: $F_{(3, 204)}$= 0.032, p = 0.992), pH (ANOVA: $F_{(3, 204)}$= 0.028, p = 0.994), DO (ANOVA: $F_{(3, 204)}$= 0.892, p = 0.446) and TAN (ANOVA: $F_{(3, 204)}$= 1.950, p = 0.123) at different stocking densities, throughout the eight month growth trial (Table 2.4).

Table 2.4: Means (± standard error) of tested water quality parameters for different size class experiments over eight months. No significant differences were found in water quality parameters between stocking densities for any of the experiments. Statistical analyses are presented in text.

	Stocking Density (% surface area covered)				
	16	**20**	**22**	**24**	**Pooled mean**
Exp 1: 15 - 35 g size class					
Temperature (°C)	19.10 ± 0.25	19.16 ± 0.25	19.13 ± 0.24	19.13 ± 0.24	19.13 ± 0.12
pH	7.78 ± 0.04	7.76 ± 0.03	7.78 ± 0.04	7.75 ± 0.04	7.77 ± 0.02
Dissolved Oxygen (%)	90.48 ± 0.46	89.70 ± 0.45	90.60 ± 0.44	89.65 ± 0.39	90.12 ± 0.22
TAN ($\mu g.L^{-1}$)	21.75 ± 3.24	32.24 ± 4.92	33.61 ± 4.62	38.11 ± 6.18	31.42 ± 2.44
Exp 2: 45 - 65 g size class					
Temperature (°C)	18.46 ± 0.22	18.49 ± 0.22	18.41 ± 0.22	18.44 ± 0.22	18.45 ± 0.11
pH	7.80 ± 0.01	7.82 ± 1.00	7.80 ± 0.02	7.79 ± 0.01	7.80 ± 0.01
Dissolved Oxygen (%)	94.26 ± 0.05	93.48 ± 0.60	92.54 ± 0.57	92.68 ± 0.59	93.28 ± 0.29
TAN ($\mu g.L^{-1}$)	24.31 ± 1.45	25.65 ± 1.35	27.80 ± 1.57	29.11 ± 1.74	26.72 ± 0.77
Exp 3: 70 - 90 g size class					
Temperature (°C)	16.93 ± 0.35	17.06 ± 0.36	17.07 ± 0.36	17.05 ± 0.36	17.03 ± 0.18
pH	7.69 ± 0.04	7.69 ± 0.04	7.67 ± 0.04	7.68 ± 0.04	7.68 ± 0.02
Dissolved Oxygen (%)	91.23 ± 0.34	90.59 ± 0.36	91.50 ± 0.34	90.60 ± 0.34	90.87 ± 0.17
TAN ($\mu g.L^{-1}$)	23.54 ± 1.69	26.75 ± 1.10	28.28 ± 2.07	29.93 ± 2.01	27.13 ± 0.98

2.4 Discussion

The individual weight gain of abalone decreased with an increase in density from 16 % to 22 % for all size classes tested in this study. Similar results have been reported in other abalone studies with animals being housed in a variety of culture systems where growth rates decrease in response to increased stocking densities (Mgaya and Mercer 1995, Capinpin *et al*. 1999, Badillo *et al.* 2007). This density dependant growth has also been demonstrated in other shellfish species under culture conditions (Holliday *et al.* 1991, Allan and Maguire 1992, Parsons and Dadswell 1992). Most research regarding abalone stocking densities has worked with juvenile and smaller abalone which allow for results to be seen over a shorter experimental time frame. Results from this study suggest however that the growth of larger abalone may not be largely affected by increases in stocking densities. Animals in the middle and larger size class showed no significant difference in individual weight gain between 20 %, 22 % and 24 % stocking densities. Larger, and normally older, abalone and other molluscs are known to have slower growth rates than younger animals due to the need for energy investment in other metabolic activities such as reproduction (Barkai and Griffiths 1988, Beaumont and Fairbrother 1991, Farias *et al.* 2003). This might raise the question to whether these trials were too short to observe a difference in weight gain between densities. However, abalone were able to gain a significant amount of weight, almost doubling in mass over the eight month trial period, and significant differences in growth were seen between 16 % and 24 % densities across all size classes.

The choice of stocking densities to be used by abalone farms and other aquaculture facilities is not only based on individual animal growth but also other economic considerations which include biomass gain and animal condition (Maguire and Leedow 1983, Holliday *et al.* 1991, Björnsson 1994, Mgaya and Mercer 1995). In this study, maximum individual growth was obtained at the lowest stocking densities across all size classes, however higher stocking densities were able to achieve higher levels of production in terms of biomass gain ($kg.basket^{-1}.month^{-1}$) in some cases. Biomass gain was not affected by an increase in stocking densities for 15-35 and 45-65 gram animals. However, baskets containing animals from the largest size class (70-90 grams) that was tested showed an increase in biomass gain when stocking density was increased up to 24 %. As a result of there not being much difference in individual growth of larger animals at the tested stocking densities, and because there are a larger number of abalone in more densely stocked baskets growing at similar rates, a higher gain in biomass per basket at higher stocking densities could logically be expected. Although straight line regression models were fitted to the data in these results, the two smaller size classes of abalone show a plateau in biomass gain at which more biomass was not achieved with an increase in stocking density. This suggests that alternative regression models, such as logarithmic models, may better describe the trend. Similar peaks hav been reported in other aquaculture related stocking density experiments (Holliday *et al*. 1991, Mgaya and Mercer 1995, Salas-Leiton *et al*. 2008, Wassnig *et al*. 2009) and perhaps suggests that the growth rates of individual abalone at lower densities are sufficient enough to overcome the effect of increased numbers in higher stocked baskets and therefore the biomass gain will remain similar for animals in these size classes whether stocked at 16 % or 24 % over a period of eight months. The reason for this could be that young animals have higher relative growth rates than older and therefore larger animals (Mgaya and Mercer 1995, Abele *et al*. 2009). The biomass gained per unit area could also be expected to increase with an increase in the size of abalone. Björsson (1994) suggests, in a study on halibut, that the optimal stocking density should increase with the size of halibut if expressed in terms of weight per unit area. This is simply because flatfish become thicker as they grow larger which results in a layer (surface area) of large flatfish weighing more than that same size layer of smaller flatfish (Björsson 1994, Salas-Leiton *et al*. 2008). This theory should hold true in the case of *H. midae* as unpublished on-farm length-weight relationships demonstrate that longer animals gain more weight per unit length than shorter abalone ($L = 16.861\ W^{0.3343}$, where L represents shell length and W represents weight). Similar length-weight relationships are documented for *H. discus hannai* (FAO 1990).

It has been suggested that a decrease in growth performance with an increase in stocking density is as a result of density dependant intraspecific competition in the form of competition for space and food exploitation (Jarayabhand and Newkirk 1989, Mgaya and Mercer 1995). Mgaya and Mercer (1995) suggest that food limitation is the main factor affecting growth at higher densities because the movement of animals toward feed and feeding areas is restricted by stacking (Douros 1987) and their tendency to cluster together in preferred areas of the tank. Badillo *et al.* (2007) suggests that even when food availability was not a confounding factor, excess food resulted in poorer water quality parameters which could have accounted for poorer growth rates and stress. Ammonia levels and other water quality parameters did not differ between treatments in this study which might rule out the argument suggested by Badillo *et al.* (2007). However, deterioration of water quality in localised areas of the basket where abalone stack, for example, has not been ruled out and should be investigated in future research. It may be possible that changes in stocking densities result in a change in behaviour by the abalone when under culture conditions or perhaps the innate behaviour of these animals leads to them being unable to overcome changes in their environment and so they tend to stack and gather in preferred areas of a tank despite there being other surface area available to them (Mgaya and Mercer 1995, Huchette *et* *al*. 2003). The behavioural differences of animals in this study are investigated further in Chapter 3.

Poor water quality and animal health have previously been suggested as possible reasons for decreased growth performances at higher stocking densities under aquaculture conditions (Harris *et al*. 1998, Huchette *et al*. 2003, Björnsson and Ólafsdóttir 2006, Schram *et al*. 2006). Water quality parameters recorded during this study are similar to those from previous research performed on abalone farms in Hermanus (Yearsley 2007, Riddin 2012). In this study, no differences were found in water quality parameters between treatments and care was taken to ensure that flow rates were adequately set and maintained to prevent any degradation of water quality with an increase in tank biomass and density. Stocking densities in the range of 16 to 24 % did not negatively affect the health of *H. midae* over the eight month period. Although the condition factor of animals in the 45-65 gram size class decreased throughout the trial period, there was no significant difference in the condition factor of abalone between different densities for any of the tested size classes, providing evidence that an increase in stocking density does not affect the condition of farmed abalone. This result appears to be in coherence with previous research performed on fish and molluscs under aquaculture conditions (Mgaya and Mercer 1995, North *et al*. 2006). This all suggests that the decreased growth rates observed with an increase in stocking density from 16 % to 24 % in this study were an unlikely result of poor water quality or degradation of animal health.

Farm implications

The relationship described between stocking density and individual growth, biomass gain and abalone condition will be useful in making farm management decisions. One of the major limiting factors to the production and success of land based aquaculture facilities is physical space and the efficient use of infrastructure can be critical to profitability (Parsons and Dadswell 1992, Wassnig *et al*. 2009). It is essential that tank space is used wisely to obtain maximum economic yields and the optimising of stocking densities can be seen as an important tool in achieving financial goals in terms of farm grow out strategies (Schram *et al*. 2006). Results from this study suggest that farms could increase stocking densities up to 24 % without affecting the condition of abalone across all tested size classes. An increase in density will negatively affect the growth rates of animals in all tested size classes, however the overall production in terms of biomass gain per unit area will likely remain similar in 45-65 g and 15-35 g size classes while the production of 70-90 g animals stocked at higher densities may be increased. When making management decisions based on these results, farms should take the value of the end product into account and the financial effects of increased growth rates versus increased biomass gain should be considered (Wassnig *et al*. 2009). If a farmer's strategy is to mass produce a lower market value abalone and aims to achieve maximum biomass gain at the expense of individual growth, larger animals could be, for example, housed at much higher densities than current management practises allow. On the other hand, larger animals tend to fetch a higher market price (Cook and Gordon 2010) and so maximum individual growth may be the driving force behind management decisions. In this scenario, it may still be plausible to increase stocking densities slightly within certain size classes (15-35 g) without having a significant impact on individual growth rates.

Conclusion

This study conformed to the results of previous stocking density studies in which the general conclusion reached is that individual growth rates decline as stocking density is increased. It has been established that the growth of different size *H. midae* is affected differently by increases in stocking density. Biomass gain per tank can be increased substantially with an increase in stocking density for abalone in the 70-90 gram size class, while smaller size classes are able to achieve similar rates of biomass production with increasing densities despite a decrease in individual growth. The health and condition of *H. midae* was not affected by increases in stocking density up to a rate of 24 % available surface area covered within the time frame of these experiments. The fundamental mechanisms which result in reduced growth at higher densities are largely unexplored in the literature. Behavioural studies focussed on the reasons for reduced growth with increasing stocking densities may prove valuable to solving this problem.

CHAPTER 3

The influence of stocking density on behaviour

3.1 Introduction

The effect of stocking density on abalone growth performance has been well documented (Mgaya and Mercer 1995, Capinpin *et al*. 1999, Huchette *et al*. 2003, Badillo *et al*. 2007, Wassnig *et al*. 2009) and results from Chapter 2 support previous findings which suggest that individual abalone growth is largely density dependant. The reasons for a decrease in growth performance at higher stocking densities are not fully understood and it is possible that density may affect abalone behaviour. The majority of research, towards understanding the reduction in growth seen at higher densities, has focussed on abiotic factors such as water quality parameters (Harris *et al*. 1998, Huchette *et al*. 2003), space (Capinpin *et al*. 1999), rate of water flow (Higham *et al*. 1998) and water depth (Lui and Chen 1999). With regard to stocking density and its effect on abalone behaviour, several studies have reported that higher stocking densities result in a higher occurrence of stacking behaviour (Douros 1987, Wassnig *et al*. 2009) and possibly greater competition for space and food (Mgaya and Mercer 1995, Huchette *et al*. 2003, Wu *et al*. 2009). No work has been published on the effect that stocking density has on the behaviour of farmed *H. midae*.

Previous research under aquaculture conditions has mainly attributed the slow growth rates of abalone at high stocking densities to competition for space (Mgaya and Mercer 1995, Huchette *et al*. 2003, Wassnig *et al*. 2009). Huchette *et al*. (2003) investigated the effect of stocking density on the behaviour of *Haliotis rubra* and concluded that abalone behaviour was affected by light, habitat type, space and density. Abalone were found to have preferred areas of the tank which became crowded when density was increased. Abalone which were forced to settle in unfavourable areas may have been subject to stressors which could have had an impact on growth performance (Huchette *et al*. 2003). Under natural circumstances, abalone have been known to occupy a specific home area and in some cases settle in precisely the same spot time after time (Dixon *et al.* 1998). When food availability decreases (Prince 1992) and as habitat space is reduced (Dixon *et al.* 1998), abalone tend to become more mobile. This may result in excess energy expenditure and stress which could be reflected in growth rates at higher stocking densities. Huchette *et al*. (2003) suggest that *H. rubra* demonstrate similar behaviour under farm conditions which further suggests that space limitations may be a reason for reduced growth at higher stocking densities. Competition for attachment space could result in shell damage and stress (Tarr 1995) which in turn would result in energy investments being directed to shell repair rather than growth (Hindrum *et al*. 1999).

Stacking behaviour in natural abalone populations is often demonstrated when space is limited (Douros 1987). Stacking behaviour is probably the most obvious indication of high density conditions and that stacking is perhaps a response which reduces the pressure of abalone constantly competing for primary attachment space (Wassnig *et al*. 2009). Stacking behaviour was also reported as a reason for reduced growth at high stocking densities in *Haliotis tuberculata* (Mgaya and Mercer 1995), *Haliotis fulgens* (Aviles and Sheppard 1996), *Haliotis asinina* (Capinpin *et al*. 1999), *Haliotis diversicolor* (Liu and Chen 1999), *H. rubra* (Huchette *et al*. 2003) and *Haliotis kamtschatkana* (Lloyd and Bates 2008).

Tank design should be adapted to suit the behaviour of abalone which could well be species specific (Huchette *et al*. 2003). With an understanding of the effect of stocking density on the behaviour of farmed *H. midae*, it may be possible to relate behavioural responses to decreased growth performances at higher stocking densities. This knowledge may allow for the mitigation of reduced growth at high stocking densities through innovative basket design and farm management strategies, and subsequently improve economic performance. It is therefore important to develop an understanding of abalone behaviour at different stocking densities.

The aim of this study was to develop a better understanding of the behaviour of *H. midae* stocked at different stocking densities in an attempt to establish what behavioural traits may be contributing to decreased growth performance at higher densities.

The objectives of this research were to:

1) identify possible behaviour characteristics which could be quantified;
2) to quantify behaviour observations; and
3) to determine if behaviour characteristics differed at different stocking densities.

3.2 Materials and methods

Experimental system, animals and feeding

Behaviour observations were performed on the same animals which were used in the growth experiments described in Chapter 2, and over the same period. In summary, experiments were conducted at Aquafarm Development (Pty) Ltd in flow through. Abalone were stocked into 16 farm tanks with seven oyster mesh baskets per tank at 16, 20, 22 and 24 percent densities (% surface area covered) so that each stocking density was represented in four different farm tanks (n = 4 treatment^{-1}). All the behavioural observations were carried out on the 45-65 g animals only, unless otherwise stated. Observations commenced after the first splitting period, which ensured that abalone had been subjected to their respective stocking densities for a minimum of four months. Animals were fed once daily and subject to the farm husbandry routines (Chapter 2).

Data Collection

Haliotis midae, like many other abalone species, are nocturnal feeders and are therefore most active at night when they move above the feeder plate to feed (Shepherd 1973, Barkai and Griffiths 1987, Knauer *et al*. 1995, Lloyd and Bates 2008). For this reason, behaviour observations were conducted at night only. Visual observations were made and behaviours were identified and described (Table 3.1). The frequency of these behaviours and the frequency that animals were seen in different areas of the basket were recorded (Figures 3.1 and 3.2; Table 3.1).

Table 3.1: Description of different basket positions and activities which were used in abalone counts during behaviour observation periods.

Position or Activity	**Description**
On the feeder plate	Abalone which had more than half of their body positioned on the surface of the asbestos feeder plate
On the walls above the feeder plate	Abalone which had more than half of their body positioned on the basket walls above the level of the asbestos feeder plate
Active	Abalone which were changing their location
Restricted	Abalone which could not move towards feed because their movement was being hampered by other individuals
On feed	Abalone on the feeder plate which were positioned with their mouth parts on commercial feed

A B

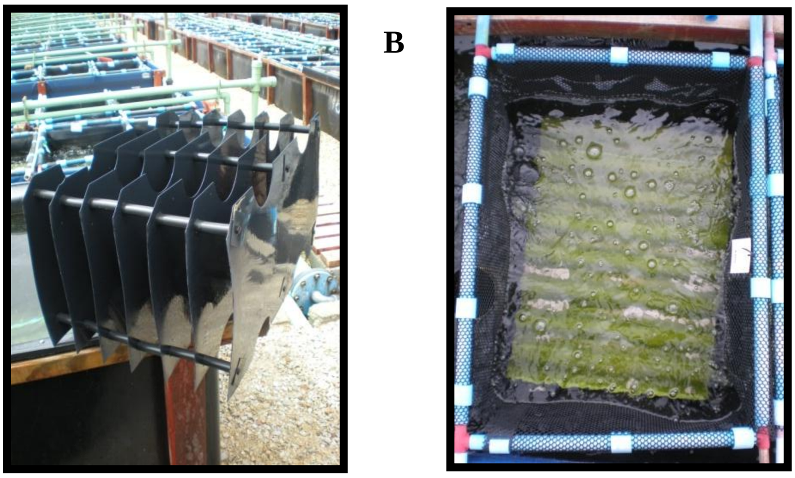


Figure 3.1: Each basket contained (A) a rack comprised of seven plastic sheets (0.6 x 0.35 m each) and (B) a corrugated asbestos feeder plate which rests above the rack. The rack provides extra available surface area in the basket while the feeder plate serves as shading during the day and a feeding platform at night.

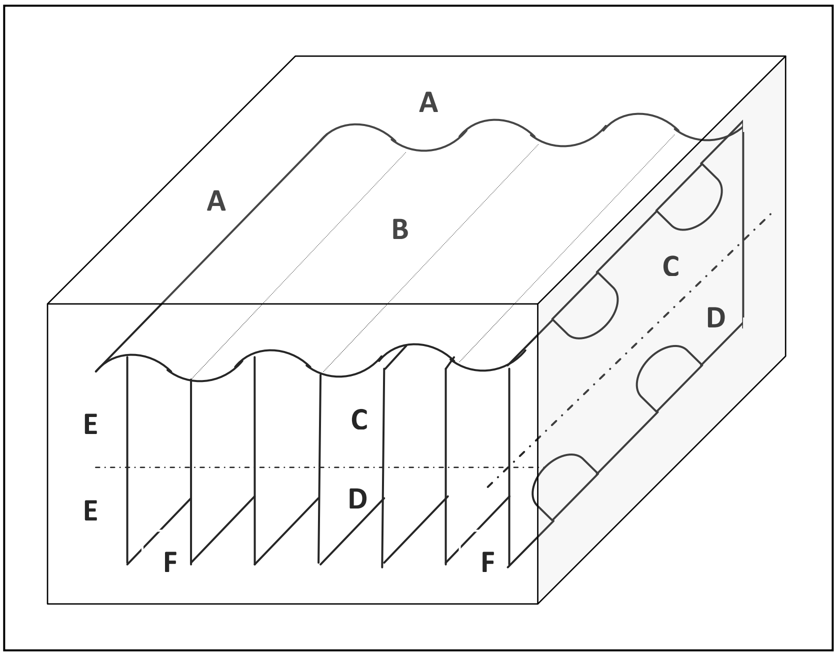


Figure 3.2: Illustration of the inside of a basket used to house abalone in the on farm and experimental systems. The basket holds a rack of vertical plastic plates and a corrugated asbestos feeder plate which rests on top of the rack. Labels indicate areas of the basket which are used descriptively throughout the behaviour study and are described as follows: A) Basket walls above the feeder plate, B) Feeder plate, C) Top half of the rack, D) Bottom half of the rack, E) Basket walls below the feeder plate, and F) Bottom of the basket.

Preliminary study

In order to establish the best time and time intervals in which to observe abalone behaviour during the night, a preliminary investigation of abalone activity took place. A single basket in each replicate tank from the growth study (Chapter 2) was marked and the area above the feeder plate was observed repeatedly every hour from 20:00 to 04:00 for three consecutive nights. These observations provide a snapshot of what was happening above the feeder plate in each basket once every hour. During each observation, tank aeration was briefly turned off to prevent distorted vision, as a result of bubble disturbance, and turned on again once frequency counts had been performed. A red light head lamp was used as a light source which allowed abalone to be viewed in the dark with minimal disturbance. The number of abalone situated on the walls of the basket above the feeder plate and the number of animals on the feeder plate itself were counted in each basket. The total number of animals in each basket was known from the time of stocking and from this a proportion of abalone above the feeder plate at different times and at different densities was established.

Distribution counts and activity observations

Behaviour observations were carried out on the same single basket of abalone in each experimental tank for each tested stocking density (16 %, 20 % 22 % and 24 %) during each observation period. Behaviour experiments and observations took place 10 days after splitting to allow adequate acclimation, the same baskets were marked and repeatedly observed 60 days and 120 days after splitting. Each observation period consisted of three consecutive nights of measurements. When it rained, observations were postponed until the following night because abalone are less active during periods of rainfall (Lloyd 2013).

On an observation night, each experimental basket was observed every hour between 21:00 and 02:00, when abalone were most active according to results of the preliminary study (Figure 3.3). The abalone were counted by an individual person in order to standardise any observer bias which may have occurred. It took approximately 15 minutes to view the 16 baskets which were being observed; baskets were viewed in random order and in a different sequence at each measurement interval. The same observation protocol that is described in the methods for the preliminary study was employed in this experiment.

Distribution areas and activities were described and used as references for frequency counts (Table 3.1) which were performed on abalone situated above the level of the feeder plate (Figure 3.2). This divided the studied area into two main parts, these being (a) the walls of the basket above the level of the feeder plate and (b) the feeder plate itself (Figure 3.2). The proportions of abalone, of those within each basket or of those situated above the feeder plate, that were found in each of the positions or which were involved in each of the activities described (Table 3.1), were calculated and used in further analyses.

Feed availability throughout the night

In order to investigate whether or not stocking density within a basket affected the length of time that feed was available to abalone on the feeder plate, equal amounts of commercial feed was administered to abalone stocked at each density. The amount of feed remaining on the feeder plate was recorded at hourly intervals throughout the night.

Two baskets, in the same position, in each experimental tank were used in this study. Measurements were recorded on two consecutive nights between 20:00 and 01:00, with eight replicate baskets being examined per density on each night. Two cups of the commercial feed (30.66 ± 0.23 g.cup^{-1}; n = 30) were placed into each basket at 18:00 on the night of observation. The amount of feed remaining on the feeder plate was estimated and recorded hourly, based on a score system (Table 3.2).

Table 3.2: Scores and their corresponding values used to estimate the amount of Abfeed® remaining on the feeder plate at any observed time as a percentage of the two cups (30.66 ± 0.23 g.cup^{-1}; n = 30) of feed on which was evenly distributed across the feeder plate at 18:00 on the night of observation.

Score	Amount of Abfeed (%)
5	100
4	75 - 99
3	50 - 74
2	25 - 49
1	0 - 24
0	0

Time spent above the feeder plate by individual abalone

Two baskets per experimental tank were randomly selected and five percent (six, eight, nine and ten individual abalone per basket stocked at 16 %, 20 %, 22 % and 24 % densities respectively) of the abalone within those baskets were marked by drying the shell and sticking numbered pieces of reflective tape to the animal using waterproof glue (BOSTIK© blits stik gel super glue, Bostik, Cape Town, South Africa). Abalone in the 70-90 g tanks were used in this study. After tagging, a 10 day acclimation period was allowed for tagged abalone to recover from any stress that may have occurred during the procedure before observation trials began.

With observations being performed actively and in real time, a maximum of four baskets could be observed each night with constant monitoring. For this reason, one basket per stocking density treatment was observed per night for a period of eight nights, by which time each basket containing tagged animals had been observed once. Using red light to enhance vision with minimal disturbance of the abalone, each basket was constantly observed for a period of 180 minutes between 22:00 and 01:00. During that period, the appearance of any tagged abalone above the level of the feeder plate was documented and the amount of time spent by that individual on the walls of the basket above the feeder plate and on the feeder plate itself was recorded. Every time the individual entered or left one of these areas the time was recorded and later the time period of a single visit could be calculated along with the total amount of time spent in each area above the feeder plate.

Position of animals in the basket during day and night times

The position of the 70-90 g abalone within the basket during the day and at night was observed in two randomly selected baskets within each replicate tank. Observations occurred 30 days after splitting. Day time observations were made between 12:00 and 14:00 during peak light intensity hours and night time observations were made between 22:00 and 00:00 within the same 24 hour period, different baskets of animals within the same tank were observed at day and at night.

The process of counting the number of animals in each defined area of the basket (Figure 3.2) required two people. Once the number of abalone on the feeder plate and on the walls above the feeder plate had been counted and recorded, the feeder plate was removed and the number of abalone on the underside of the plate was counted. The rack within the basket was quickly removed and placed into a basket lined with shade cloth which would catch any falling abalone. The number of abalone on the top half and on the bottom half of the rack was quickly counted by one person while the second observer counted the number of abalone on the walls of the basket below the feeder plate. The number of abalone situated on the bottom of the basket was calculated by subtracting all of the count values from the total number of abalone within the basket. Once disturbed, abalone tend to move relatively quickly towards the bottom of the basket and so this process had to be rapid and thorough in order to get accurate counts of abalone position before they change location due to disturbance and also to avoid recounting abalone which may have moved. This process was used both during the day and night time observations, red light was used during the abalone counts at night.

Statistical analysis

Abalone within the same basket were repeatedly observed and counted at hourly intervals over numerous nights in the distribution and activity counts as well as in the feed availability study. Thus, average values were calculated for each replicate at each observed time frame and the presence of a significant interaction between the main effects, stocking density and time, on the dependant variable, proportion of abalone above the feeder plate, was tested using a repeated measures analysis of variance (repeated measures ANOVA). Multifactor analysis of variance (multifactor ANOVA) was used to analyse the data from the time spent above the feeder plate investigation as these were individual tagged abalone observed for a single night and time period only. The Levene's test (Levene 1960) was used to test the equality of variance assumption of an ANOVA and the normal distribution of residuals was tested using a Shapiro-Wilk test (Shapiro and Wilk 1965). Tukey's *post-hoc* test was used to identify where significant differences occurred between treatments (Tukey 1960). An α-error level of 5 % was used for all analyses. Analyses were conducted using a computer software package (Statistica 10®). All data presented in text and tables are means ± standard error, while figures show means ± 95 % confidence interval, unless otherwise stated. The statistical files will be made available on request (Contact: c.jones@ru.ac.za).

3.3 Results

Preliminary study

Most animals were found above the feeder plate between 21:00 and 02:00 (repeated measures ANOVA: $F_{(8, 96)} = 38.98$, $p < 0.0001$; Figure 3.3). This period was assumed to be the time of maximum activity during the night and was used as the observation period for the rest of the behaviour experiments described in this chapter.

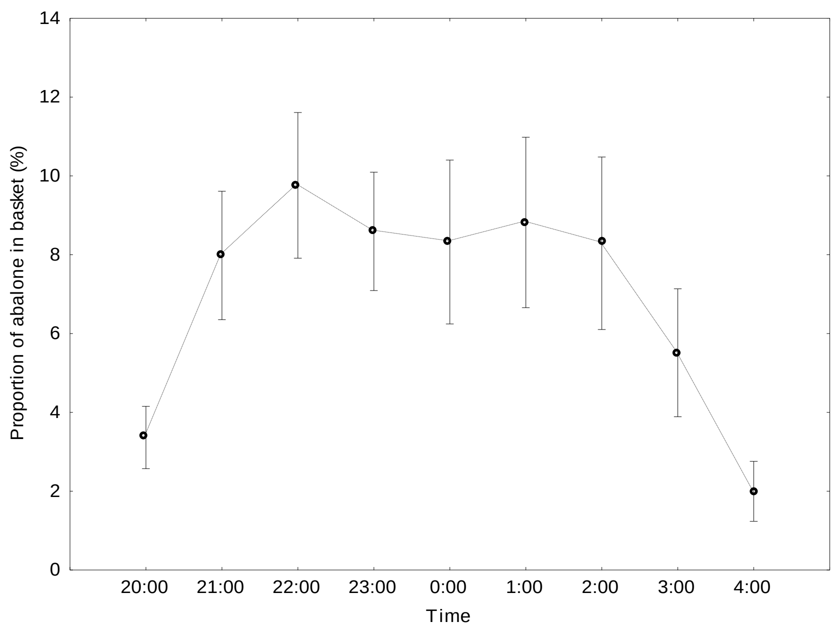


Figure 3.3: Proportion of abalone situated above the level of the feeder plate (% of the total number in the basket) (± 95 % confidence interval) (repeated measures ANOVA: $F_{(8, 96)} = 38.98$, $p < 0.0001$) at different times during the night, irrespective of stocking density.

Distribution counts and activity observations

The proportion of abalone situated above the feeder plate (on the walls above the feeder plate and on the feeder plate itself) was not influenced by an interaction between stocking density and time of night (repeated measures ANOVA: $F_{(15, 60)} = 1.26$, $p = 0.25$; Figure 3.4). This proportion changed significantly over time between 21:00 and 02:00, with the highest proportion of abalone above the feeder plate at 22:00 and 23:00 (repeated measures ANOVA: $F_{(5, 60)} = 33.88$, $p < 0.0001$; Figure 3.5 B). Furthermore, the proportion of animals above the feeder plate increased with an increase in stocking density from 16 % to 24 %. A higher proportion was found above the feeder plate at 24 % densities (mean: 16.44 ± 1.33 %) than at 16 % (mean: 7.26 ± 1.33 %) and 20 % (mean: 10.77 ± 1.33 %) densities, and baskets stocked at 22 % densities had a significantly higher proportion of abalone above the feeder plate (mean: 14.18 ± 1.33 %) than those stocked at 16 % density (repeated measures ANOVA: $F_{(3, 12)} = 9.05$, $p = 0.002$; Figure 3.5 A). As a proportion of abalone within the basket, similar trends to those described above are seen with abalone situated on the feeder plate itself and on the walls of the basket above the feeder plate. The proportion of abalone on the feeder plate and on the walls above the plate was not influenced by an interaction between stocking density and time of night (repeated measures ANOVA: $F_{(15, 60)} = 0.67$, $p = 0.81$, and repeated measures ANOVA: $F_{(15, 60)} = 1.54$, $p = 0.12$ respectively).

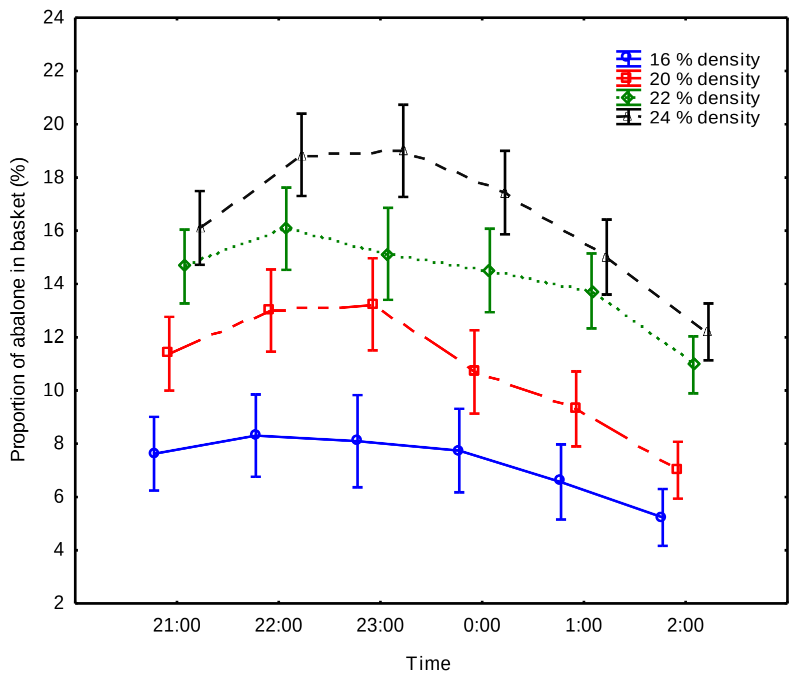


Figure 3.4: Proportion of abalone within a basket (± 95 % standard error) situated above the level of the feeder plate (on the walls and on the feeder plate itself), stocked at different densities over time (repeated measures ANOVA: $F_{(15, 60)} = 1.26$, $p = 0.25$).

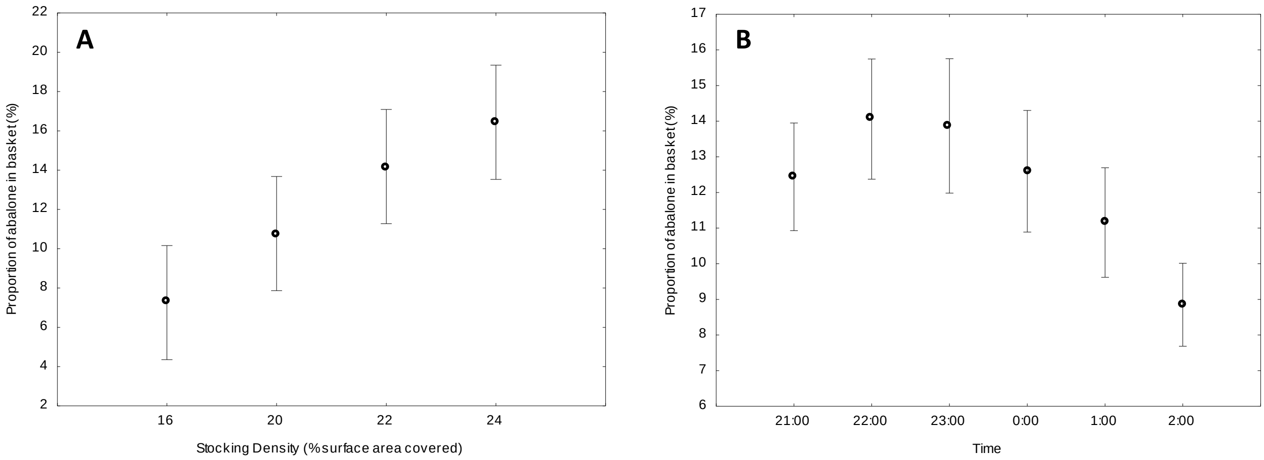


Figure 3.5: Proportion of abalone within a basket (± 95 % confidence interval) situated above the level of the feeder plate (on the walls and on the feeder plate itself) at (A) different stocking densities (repeated measures ANOVA: $F_{(3, 12)} = 9.05$, $p = 0.002$) and (B) at different times of night (repeated measures ANOVA: $F_{(5, 60)} = 33.88$, $p < 0.0001$).

The proportion of abalone situated on the feeder plate itself differed significantly over time between 21:00 and 02:00 (repeated measures ANOVA: $F_{(5, 60)} = 4.39$, $p = 0.002$) and a higher proportion of abalone were found on the feeder plate at the 24 % density (mean: 4.94 ± 0.52 %) than at the 16 % (mean: 1.77 ± 0.52 %) and 20 % (mean: 2.62 ± 0.52 %) densities (Figure 3.6). Baskets stocked at 22 % densities also had a significantly higher proportion of abalone on the feeder plate (mean: 4.54 ± 0.52 %) than those stocked at 16 % density (repeated measures ANOVA: $F_{(3, 12)} = 8.52$, $p = 0.003$; Figure 3.6).

The proportion of abalone in the basket which were situated on the walls above the feeder plate was significantly lower at 01:00 and 02:00 than at times between 21:00 and 00:00 (repeated measures ANOVA: $F_{(5, 60)} = 41.02$, $p < 0.0001$; Figure 3.7 B) and significantly more abalone were positioned on the walls above the feeder plate at a stocking density of 24 % (mean: 11.50 ± 1.1 %) than those at 16 %, 20 % and 22 % densities (means: 5.49 ± 1.11; 8.16 ± 1.11; 9.63 ± 1.11 % for abalone stocked at 16 %, 20 % and 22 % respectively; repeated measures ANOVA: $F_{(3, 12)} = 5.24$, $p = 0.02$; Figure 3.7 A).

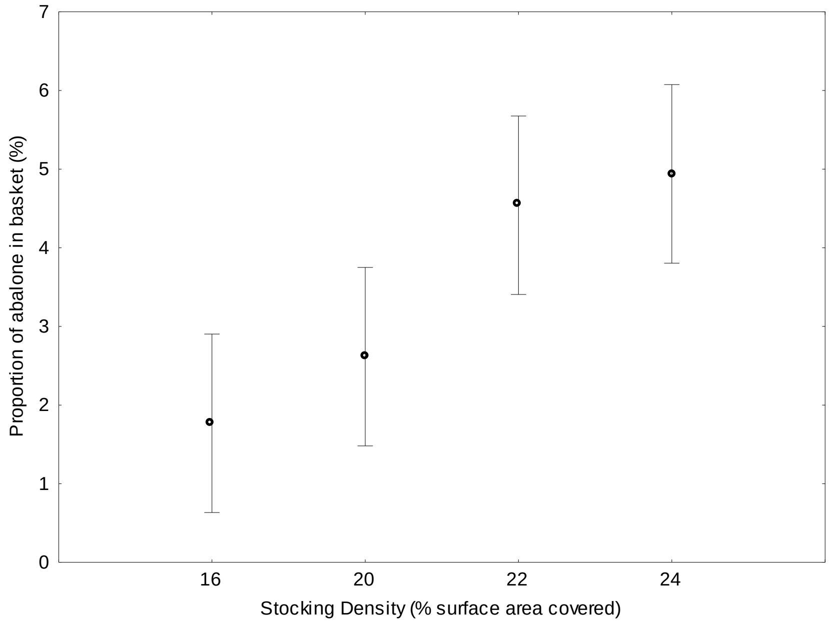
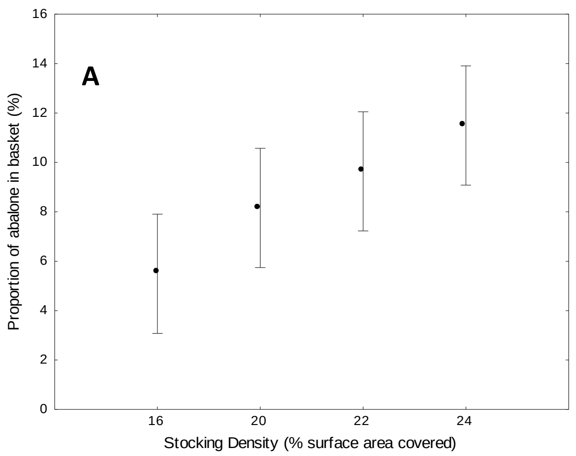


Figure 3.6: Proportion of abalone within a basket (± 95 % confidence interval) situated on the feeder plate itself at different stocking densities (repeated measures ANOVA: $F_{(3, 12)} = 8.52$, $p = 0.003$).



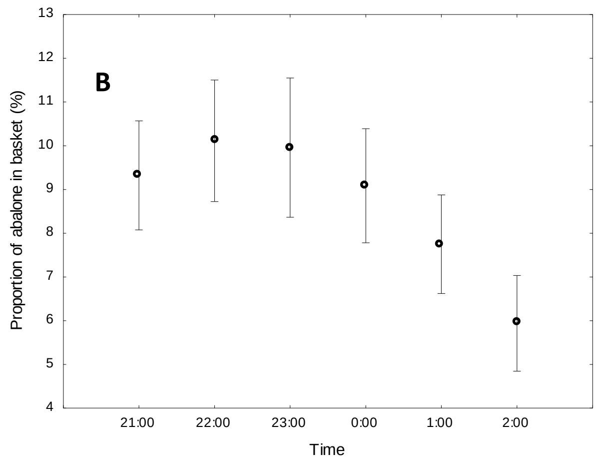


Figure 3.7: Proportion of abalone within a basket (± 95 % confidence interval) situated on the walls above the feeder plate at (A) different stocking densities (repeated measures ANOVA: $F_{(3, 12)} = 5.24$, $p = 0.02$) and (B) at different times of night (repeated measures ANOVA: $F_{(5, 60)} = 41.02$, $p < 0.0001$).

As a proportion of abalone which were situated above the feeder plate (i.e. number of abalone. number of abalone above feeder plate^{-1}), no interaction was found between density and time of night in the proportion of animals found on the feeder plate (repeated measures

ANOVA: $F_{(15, 60)} = 0.87$, $p = 0.60$), on the walls above the feeder plate (repeated measures ANOVA: $F_{(15, 60)} = 0.87$, $p = 0.60$) or in the proportion of animals whose access to the feeder plate was restricted (repeated measures ANOVA: $F_{(15, 60)} = 0.74$, $p = 0.73$). This proportion of abalone on the feeder plate (mean: 27.38 ± 3.24 %) was not affected by time of night (repeated measures ANOVA: $F_{(5, 60)} = 2.18$, $p = 0.07$) nor by stocking density (repeated measures ANOVA: $F_{(3, 12)} = 1.58$, $p = 0.25$). The same trend was observed with animals situated on the walls above the feeder plate (mean: 72.62 ± 3.24 %) where no significant differences were seen between stocking densities (repeated measures ANOVA: $F_{(3, 12)} = 1.58$, $p = 0.25$) nor at different times of night (repeated measures ANOVA: $F_{(5, 60)} = 2.18$, $p = 0.07$). The proportion of abalone above the plate whose access to feed was restricted was significantly higher at a stocking density of 24 % (mean: 6.41 ± 0.98 %) than at 16 % (mean: 1.51 ± 0.98 %) (repeated measures ANOVA: $F_{(3, 12)} = 4.30$, $p = 0.03$; Figure 3.8).

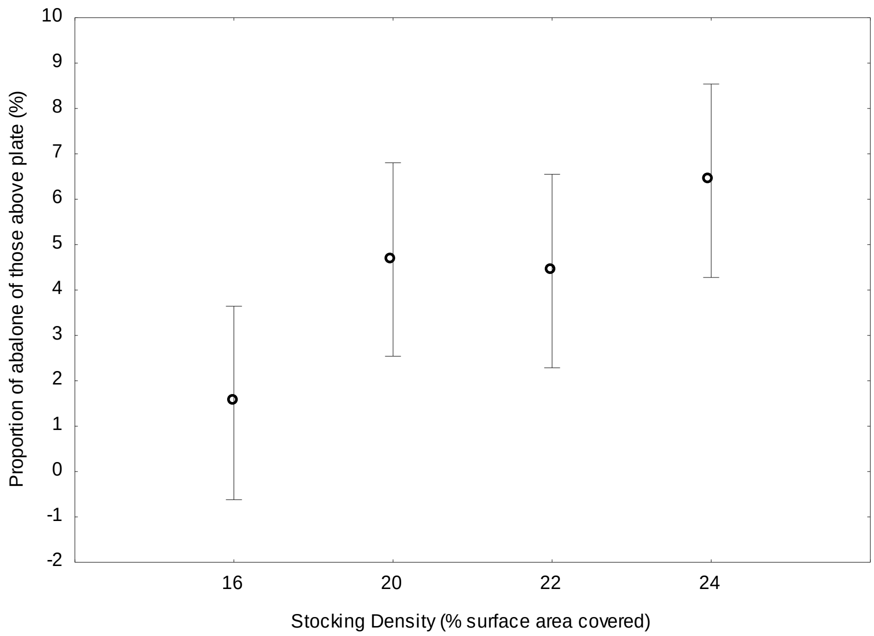


Figure 3.8: Proportion of abalone above the level of the feeder plate (± 95 % confidence interval) with restricted access to feed at different stocking densities (repeated measures ANOVA: $F_{(3, 12)} = 4.30$, $p = 0.03$).

Feed availability throughout the night

No interaction was found between stocking density and time, for the amount of Abfeed® remaining on the feeder plate throughout the night (repeated measures ANOVA: $F_{(12, 48)} = 1.52$, $p = 0.15$). There was no significant difference in the amount of food present on the feeder plate between stocking densities over the period that was tested (repeated measures ANOVA: $F_{(3, 12)} = 1.88$, $p = 0.19$). The amount of formulated feed remaining on the feeder plate decreased significantly at hourly intervals from 21:00 to 00:00 (overall means: 4.60 ± 0.14 to 2.63 ± 0.16, see Table 3.2 for explanation of scores) but remained similar between 00:00 and 01:00 (mean: 2.48 ± 0.17) (repeated measures ANOVA: $F_{(4, 48)} = 127.54$, $p < 0.0001$).

Time spent above the feeder plate by individual abalone

The amount of time spent above the feeder plate by individual abalone over a period of 180 minutes was significantly higher at stocking densities of 22 % and 24 % than the time spent above the feeder plate at 16 % densities (ANOVA: $F_{(3, 72)} = 6.30$, $p = 0.001$; Figure 3.9). Individual abalone stocked at 22 % and 24 % densities spent a similar amount of time above the feeder plate (Figure 3.9).

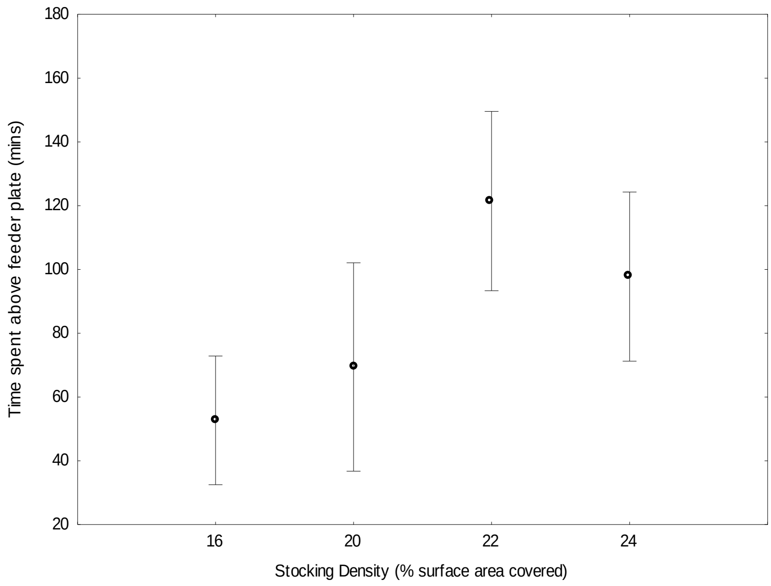


Figure 3.9: Mean amount of time spent above the feeder plate (± 95 % confidence interval) by individually tagged abalone at different stocking densities (one-way ANOVA: $F_{(3, 72)} = 6.30$, p = 0.001).

No significant interaction was found between stocking density and the amount of time spent, over a 180 minute period, in different areas above the level of the feeder plate (multifactor ANOVA: $F_{(3, 144)} = 1.85$, p = 0.14; Figure 3.10 A). The amount of time spent on the feeder plate within a 180 minute period did not differ between densities (one-way ANOVA: $F_{(3, 72)} = 0.80$, p = 0.50) while the amount of time spent on the walls above the feeder plate was higher 24 % densities than at 16 % densities (one-way ANOVA: $F_{(3, 72)} = 4.94$, p = 0.004). Abalone stocked at 16 % densities spent 83.99 ± 6.26 % of their time on the feeder plate which was significantly higher than the percentage of time which was spent on the walls of the basket (mean: 16.01 ± 6.26 %), whereas no significant difference was found in the percentage time spent on the walls in the top part of the basket and on the feeder plate for abalone stocked at 20 %, 22 % and 24 % densities (multifactor ANOVA: $F_{(3, 144)} = 16.10$, p < 0.0001; Figure 3.10 B).

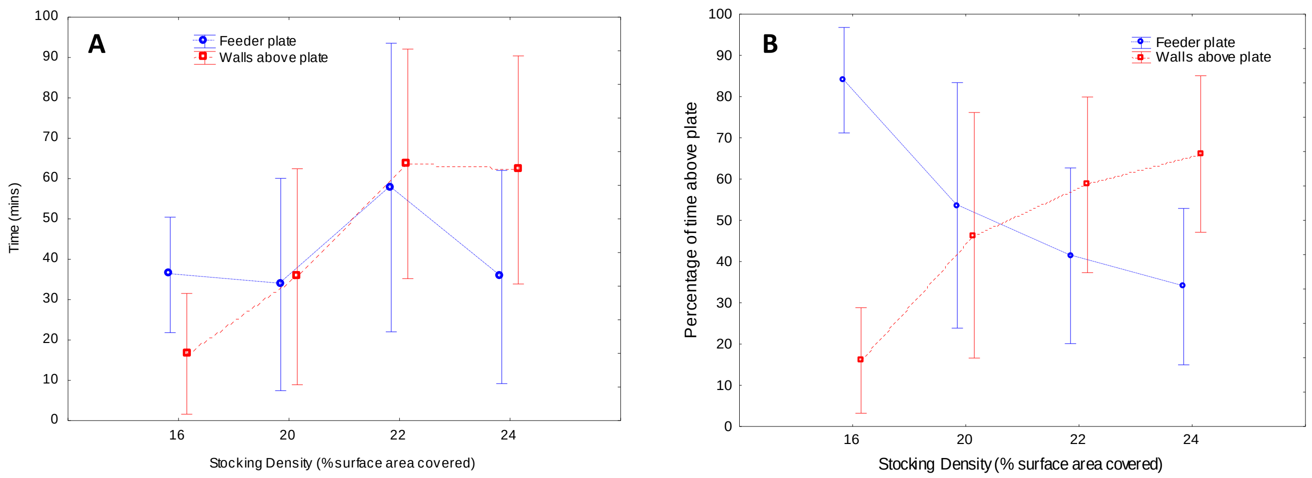


Figure 3.10: The (A) mean time (± 95 % confidence interval) spent on the feeder plate and on the walls above the feeder plate (minutes) (multifactor ANOVA: $F_{(3, 144)} = 1.85$, $p = 0.14$) and (B) the mean percentage of time above the feeder plate (± 95 % confidence interval) which was spent situated on the walls of the basket and on the feeder plate itself (multifactor ANOVA: $F_{(3, 144)} = 16.10$, $p < 0.0001$) by individually tagged abalone at different stocking densities.

The average amount of time spent in an individual visit to the feeder plate or the walls above the feeder plate by tagged abalone did not differ between stocking densities and was similar whether the visit was to the walls above the feeder plate or to the feeder plate itself (overall mean: 50.67 ± 4.70 min.visit^{-1}; ANOVA: $F_{(3, 94)} = 0.24$, $p = 0.87$).

Position of animals in the basket during day and night times

The proportion of abalone distributed in different areas of the basket, during the day and at night, showed a similar trend for all stocking densities. An increased proportion of abalone were seen above the feeder plate (i.e. on the walls above the feeder plate and on the feeder plate itself) at night compared with day time observations (Figure 3.11). The opposite was observed in the lower parts of the basket (i.e. on the bottom half of the rack and on the bottom of the basket), with a higher proportion of abalone being found in these areas during the day time rather than at night (Figure 3.11). The proportion of abalone situated on the top half of the rack and on the basket walls below the feeder plate appeared to be similar during the day and at night (Figure 3.11).

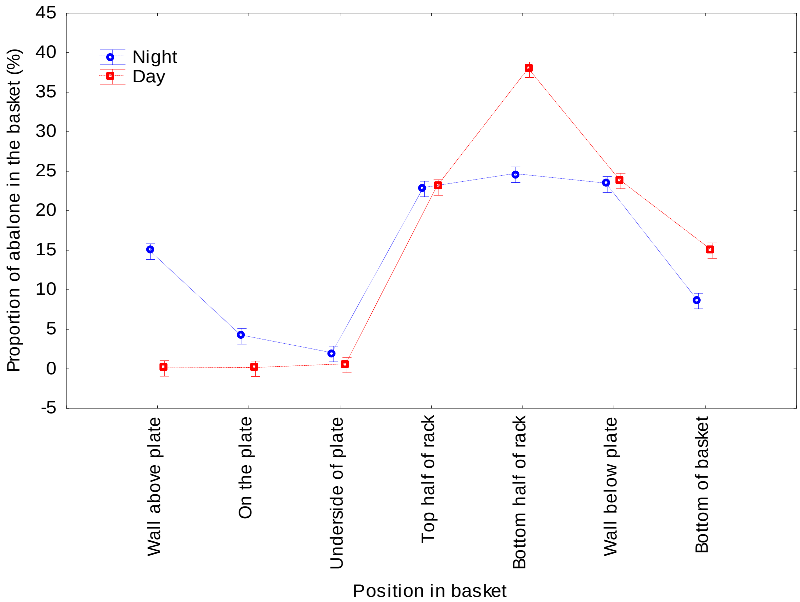


Figure 3.11: The proportion of abalone within a basket (± standard errors) which were situated in different areas of the basket during the day and at night when stocking densities were combined. No analyses were done to create this graph; it is included to illustrate an observed trend.

3.4 Discussion

A higher proportion of abalone within a basket were situated above the feeder plate at 24 % stocking densities than at 16 % densities. Competition for space has been reported as a reason for slow growth rates at higher stocking densities (Mgaya and Mercer 1995, Wassnig *et al*. 2009) and could be resulting in this observation. Abalone are light sensitive and have been shown to have preferred shelter areas within aquaculture tanks (Huchette *et al*. 2003). With abalone being sensitive to light and space availability, the onset of nightfall increases the surface area in which abalone will comfortably situate themselves (Huchette *et al*. 2003). It could therefore be argued that higher proportions of abalone at the higher densities are moving above the feeder plate at night due to spatial restrictions experienced on the rack below the feeder plate during the day as a result of their preference for shade. Other than spatial considerations, previous research has suggested that aggregations of abalone in certain areas of a basket can result in localised degradation of water quality parameters (Harris *et al*. 1998, Macey and Coyne 2005). This may result in physiological stress of abalone at higher densities which are tightly grouped in preferred shelter areas (Gaty and Wilson 1986, Huchette *et al*. 2003) and could be a possible reason for a larger proportion of animals moving out of these areas during the night. As a proportion of abalone which were situated above the feeder plate, no differences were observed in the number of abalone found on the walls of the basket or on the feeder plate itself at different stocking densities. This perhaps suggests that the reasons for moving above the feeder plate remain the same whether abalone are stocked at 16 % or 24 % densities. Previous studies have attributed the possible lack of access to feed as a contributor to poor growth rates at higher stocking densities (Mgaya and Mercer 1995, Huchette *et al*. 2003, Wassnig *et al*. 2009). If this was the case we might expect a higher proportion of abalone above the feeder plate to be situated on the feeder plate itself in an attempt to access feed at higher stocking densities and so would lead one to believe that the higher proportions of abalone above the feeder plate as a percentage of those within the basket is due to reasons other than the drive to access feed. It has however been noted that post larval abalone can depend largely on diatoms as a source of nutrition (Hahn 1989 Matthews and Cook 1995) and on-farm research is beginning to suggest that adult *H. midae* may also be feeding on diatoms and bacterial cultures which grow on light exposed surfaces within the baskets (Robinson, *pers. comm*., Department of Ichthyology and Fisheries Science, Rhodes University, 13 September 2013). Abalone have been found to spend large amounts of time above the feeder plate in areas where formulated feed is not available and was noted that *H. midae* were demonstrating grazing activities in these areas close to the water surface (Lloyd 2013). If this is the case, it may explain why although there are larger proportions of abalone above the feeder plate at higher stocking densities, as a proportion of abalone within the basket, the proportion of abalone above the feeder plate which are situated on the walls of the basket or on the surface of the feeder plate does not change with an increase in density. It also means that although abalone are not finding their way onto the feeder plate where the commercial feed is positioned, they may still be moving above the feeder plate in order to feed (i.e. on commercial feed and/or diatoms) and perhaps limited access to food remains a driver behind there being higher proportions of animals in the basket situating themselves above the feeder plate at higher densities. Further studies should focus on determining the dependence of farmed South African abalone on the natural biofilm that grows on the surfaces in the baskets and the contribution that this makes to their total nutritional requirements.

Stacking behaviour has been suggested as an indication of high stocking density conditions and is perhaps a result of abalone competition for primary attachment space (Wassnig *et al*. 2009). The occurrence of stacking behaviour has been shown to increase at higher stocking densities for several species of abalone under farm conditions (Mgaya and Mercer 1995, Aviles and Sheppard 1996, Capinpin *et al*. 1999, Liu and Chen 1999, Huchette *et al*. 2003, Lloyd and Bates 2008). In this study, the term restricted was used to describe abalone which were observed to have had their movement towards feed hampered. Abalone movement was restricted in several ways which included stacking, being wedged into a space by tightly grouped abalone or by having their access to the feeder plate limited by other individuals blocking access areas onto the plate. The proportion of abalone which had restricted access to formulated feed was found to be higher at stocking densities of 24 % than at 16 %. This is consistent with research mentioned above that has demonstrated that stacking behaviour increased with increased stocking density (Mgaya and Mercer 1995, Aviles and Sheppard 1996, Capinpin *et al*. 1999, Liu and Chen 1999, Huchette *et al*. 2003, Lloyd and Bates 2008). Restricted abalone movement and the competitive behaviours sometimes demonstrated by abalone are likely to prevent natural feeding behaviour of animals (Huchette *et al*. 2003) and possibly result in feed becoming a limiting factor even when excess formulated feed is supplied (Mgaya and Mercer 1995). These restrictions could therefore contribute to poorer growth performances often associated with higher stocking densities (Mgaya and Mercer 1995, Capinpin *et al*. 1999, Wassnig *et al*. 2009).

With the proportion of abalone above the feeder plate which are situated on the feeder plate not differing between 16 % and 24 % densities, as previously discussed, it could be expected that the amount of feed situated on the feeder plate throughout the night would vary between stocking density treatments because there are a greater number of individual abalone situated on the feeding surface at higher densities. This research found that this was not the case and that when equal amounts of formulated feed were given to baskets of abalone, there was no significant difference in the quantity of feed available on the feeder plate between 20:00 and 01:00 at different stocking densities. This suggests that individual abalone at higher stocking densities are either not eating as much formulated feed as those stocked at lower densities (Mgaya and Mercer 1995) or that abalone are moving onto the feeder plate at higher stocking densities for reasons other than to access formulated feed such as access to preferred surface area (Huchette *et al*. 2003, Wassnig *et al*. 2009). Even though feed availability remained the same across different densities, the score value for the amount of feed remaining on the feeder plate at 01:00 equated to approximately 25 % of the initially administered feed. It is possible that changes in feed availability could have decreased beyond the period of these observations.

Research on the Senegalese sole (*Solea senegalensis*) (Salas-Leiton *et al*. 2008) showed that feeding rates increased with an increase in stocking density but soles became more active in their search for food due to the need to overcome crowding, thereby increasing their energy expenditure on metabolic activities. Intraspecific interactions between Dover sole (*Solea solea*) have been suggested to hamper feed intake and reduce accessibility to formulated feed through movement of feed out of the feeding zone and by blocking access to feed (Schram *et al*. 2006). It has also been noted that these interactions increased with an increase in stocking density (Schram *et al*. 2006). It is possible for these behaviours to be related to abalone feeding behaviour as they too are surface feeders where stocking density is determined by the availability of surface area. Individual abalone would have to put more effort into finding feed at higher stocking densities due to crowding and the restrictions previously described. Mgaya and Mercer (1995) suggest that feeding rates of *H. tuberculata* may be suppressed at higher densities and that feed availability could become a limiting factor to growth performance even when supplied in excess. Other research has also suggested that abalone are able to forage more efficiently when the feeding surface is less crowded (Marsden and Williams 1996, Huchette *et al*. 2003). During behaviour observations in this study, it was noted that abalone would sometimes push formulated feed along the grooves of the feeder plate involuntarily with their movement across the feeding surface. With fewer abalone on the feeding surface at lower densities, it could be more common for pellets to be pushed off the edge of the feeder plate before being blocked or obstructed by other animals. This may contribute to feed availability remaining equal across densities throughout the night.

As previously discussed, larger numbers of abalone may be moving onto the feeder plate at higher stocking densities in search of preferred shelter space rather than in order to access formulated feed (Huchette *et al*. 2003, Wassnig *et al*. 2009). This would hamper the feeding rates of abalone which are on the feeder plate in order to feed by creating restrictions. Previous research has shown that individual abalone do not actively feed on formulated feed every night (Knauer *et al*. 1995) and it has been suggested that adult *H. midae* will only access the feeding surface once every three to five days in order to access commercial feed (Lloyd 2013). Further research into the numbers of abalone on the feeder plate each night in relation to these ideas and investigations of how often individual abalone are situated on the feeder plate at higher densities could suggest whether the animals are on the feeding surface in order to access feed or space. If individual animals are on the feeder plate at higher stocking densities for space and are not feeding, the similar feed availability indicators between stocking densities could be explained.

Individual abalone stocked at densities of 24 % spent more time above the feeder plate than those stocked at 16 % densities. There was no significant difference in the amount of time spent on the feeder plate within a period 180 minutes between stocking densities. Individual abalone spent significantly more time on the walls of the basket, above the level of the feeder plate, when stocked at 24 % than when stocked at 16 % density. Similar research found that the same amount of time was spent in a feed square by *H. midae* stocked at high and low densities and it was concluded that a decline in individual growth could not be attributed to the difference in time that abalone spent in proximity to feed (Lloyd 2013). Other research has previously suggested that growth of abalone could not be directly attributed by food consumption or access to feed (Mgaya and Mercer 1995, Capinpin *et al*. 1999, Lloyd and Bates 2008). The positioning of abalone above the feeder plate for longer periods of time suggests once again that individual abalone are moving above the feeder plate at night in order to access preferred space rather than to access formulated feed at higher stocking densities, which is consistent with previous suggestions (Mgaya and Mercer 1995, Capinpin *et al*. 1999, Huchette *et* al. 2003, Wassnig *et al*. 2009). Furthermore, as a percentage of time spent above the level of the feeder plate, individually tagged abalone stocked at a 16 % density spent over eighty percent of their time on the feeder plate itself while abalone stocked at higher densities spent similar amounts of their time above the feeder plate on both the feeding surface and the basket walls. This strongly suggests that abalone at 16 % densities are more likely to be moving above the feeder plate in order to access formulated feed than those stocked at higher densities.

Although the behaviour of different abalone species varies, *H. midae* (Knauer *et al*. 1995, Lloyd 2013), like most other abalone (Shepherd 1973, Mgaya and Mercer 1995, Huchette *et al*. 2003, Lloyd and Bates 2008), are nocturnal foragers and so are most active during the night. The behaviour of abalone is also affected by light, space and density (Huchette *et al*. 2003) and so it could be expected that positioning of abalone within the basket would change during the day and night. In this study, larger proportions of abalone were positioned on the bottom of the basket and on the bottom half of the rack during the day time while more abalone were situated above the level of the feeder plate during the night. These findings correspond with previous research which has established that abalone will congregate in preferred areas of the basket during the day, which are limited to areas of low light intensity and adequate shelter (Huchette *et al*. 2003) before emerging in the evening to forage and return to areas of shelter again at dawn (Shepherd and Turner 1985, Jarayabhand and Paphavasit 1996). Stocking density did not have any effect on the proportion of abalone which were positioned in different parts of a basket during the day or at night. Previous studies have shown abalone to congregate in high numbers in certain areas of the basket, which can be considered preferred shelter areas (Maguire *et al*. 1996, Huchette *et al*. 2003). This explains the proportion of animals in each of these areas remaining similar regardless of stocking density.

Conclusion

The most commonly suggested reasons for decreased abalone growth performance at higher stocking densities, in terms of behaviour, include competition for space and food (Mgaya and Mercer 1995, Huchette *et al*. 2003, Wu *et al*. 2009) which often result in stacking behaviours (Douros 1987, Wassnig *et al*. 2009) and reduced feed availability (Prince 1992, Wu *et al*. 2009). Results from this study largely support these ideas. Higher proportions of abalone situated above the feeder plate, increased occurrence of restrictions and longer periods of time spent above the feeder plate at night by individual abalone at higher stocking densities could be considered as indications of competition for space and preferred shelter areas within baskets (supported by Huchette *et al*. 2003, Wassnig *et al*. 2009). Feed availability is likely to have been reduced through restrictions and larger numbers of abalone on the feeder plate at higher densities, despite similar amounts of formulated feed being present for the majority of the night across stocking densities. Results from this study demonstrate that abalone positioning within a basket changes between day and night but is not affected by stocking density.

This research has provided insight towards understanding the effects of stocking density on farmed *H. midae* behaviour which will prove valuable to establishing ideas as to what might be causing a reduction in growth at higher densities. These ideas can be taken into consideration and put to use through the innovation of tank designs that may be able to counter a reduction in growth by manipulating abalone behaviour (Huchette *et al*. 2003). Observations of foraging behaviour and stacking behaviour should be used as tools in developing basket designs which will allow for maximum potential use of space and water by abalone and ensure sufficient access to formulated feed. Through the reduction of restrictive behaviours both during the day and night, farms could ensure that feed consumption and growth are maximised at higher stocking densities.

Future research is needed before technologies can be developed to mitigate the negative effect of stocking density on abalone growth. This research should further investigate the effects of increased stocking density on the use of preferred shelter spaces by *H. midae* and whether dense aggregations of abalone in certain areas of the basket are resulting in a localised degradation of water quality (Macey and Coyne 2005) or in any physical shell damage (Tarr 1995), both of which may be causing unnecessary stresses to abalone and diverting energy expenditure away from growth targets. The feeding habits of farmed *H. midae* should be investigated to establish an accurate idea of how often abalone are moving above the feeder plate and to quantify their dependence on feed sources other than formulated feed. It is also essential to develop an understanding of abalone behaviour below the feeder plate during the day. Results from suggested research topics, along with findings from this study, will allow for an adequate understanding of *H. midae* behaviour, and the effect of stocking density on abalone behaviour, under farmed conditions. Different basket designs that account for the outcomes of this and future research should be developed and the effect these technologies have on countering the reduction in growth of abalone at higher stocking densities should be tested.

CHAPTER 4

Concluding discussion

The research documented in this thesis was successful in its aims of (1) evaluating the effects of stocking density on the growth, health and production of different sized *Haliotis midae* under farm conditions and (2) providing insight towards a better understanding of the effect of stocking density on the behaviour of *H. midae*.

Abalone growth and biomass production

Different size *H. midae* react differently to changes in stocking density under farmed conditions. Individual growth of larger abalone (45-65 g and 70-90 g size classes) decreased when stocking density was increased from 16 % to 20 % but showed no differences in individual growth when stocked at densities of 20 %, 22 % and 24 % of the surface area in the basket. Although abalone in the 15-35 g size class were able to achieve similar growth rates at 16 % and 20 % densities, regression analyses suggest a decreasing trend in growth with an increase in stocking density. These growth responses to increased stocking density are supported by previous research which suggests that abalone and other shellfish demonstrate density dependant growth patterns (Mgaya and Mercer 1995, Capinpin *et al*. 1999, Badillo *et al*. 2007). The growth of individual abalone differed for abalone in the different size classes; this difference was explained by older animals demonstrating proportionally slower growth rates due to investment of energy into other metabolic activities (Barkai and Griffiths 1988, Beaumont and Fairbrother 1991, Farias *et al.* 2003). Behaviour observations (Chapter 3) revealed that increased numbers of abalone were being restricted in their access to formulated feed and larger proportions of abalone were moving above the feeder plate during the night, not necessarily to access feed, at higher stocking densities. Animals moving above the feeder plate for reasons other than to access feed suggest crowding of preferred space (Huchette *et al*. 2003). Previous research on abalone behaviour has reported that competition for space and food availability could contribute to decreased growth rates at higher stocking densities (Mgaya and Mercer 1995, Huchette *et al*. 2003, Wu *et al*. 2009).

Despite individual growth rates decreasing at higher stocking density, results from this study showed no differences in biomass gain at different stocking densities in 15-35 g and 45-65 g abalone. Abalone in the 70-90 g size class showed an increase in biomass gain as density was increased from 16 % to 24 %. If individual growth rates were the same across densities, biomass gain would increase with density due to the larger number of individuals housed in a basket. The growth rates of abalone at lower densities are high enough to overcome the effect of increased numbers when density is increased for abalone in 15-35 g and 45-65 g size classes. The reason for this not being the case in the largest tested size class (70-90 g abalone) was again related to the relative growth rates of older animals (Mgaya and Mercer 1995, Abele *et al*. 2009). With reference to halibut (Björsson 1994) it is suggested that larger abalone will result in greater biomass per unit surface area due to increasing body depth with size.

Effect of density on behaviour

The results from this research have corresponded with other behavioural studies performed on abalone at different stocking densities. Notable observations and findings attributed to an increase in stocking density include: an increased occurrence of abalone which had their movement towards formulated feed restricted; higher proportions of abalone situating themselves above the level of the feeder plate during the night and larger amounts of time spent by individual abalone above the feeder plate between 22:00 and 01:00. Several potential reasons for these findings have been discussed (Chapter 2); however, these observations have largely been attributed to the limited access of preferred shelter areas which may be resulting in intraspecific competition for space (Huchette *et al*. 2003). Stocking density did not have a significant effect on the proportion of abalone above the feeder plate which were situated on the walls or on the feeding surface; neither the average duration of a visit to the feeder plate by individual abalone, the quantity of formulated feed available on the feeder plate between 20:00 and 01:00, nor the position of abalone within a basket during the day or at night was affected by stocking density.

The behavioural differences between stocking densities are attributed largely to availability of space and feed in this research which provides a platform to suggest that changes in basket design and farming techniques may allow for these observations to be countered. It has previously been suggested that shelter design should follow a species specific approach and be aimed at suiting the behaviour of the farmed abalone (Huchette *et al*. 2003). It may be plausible to alter basket design in an attempt to manipulate or account for behavioural responses to increasing stocking density, and in that way reduce unnecessary stress and intraspecific competition associated with high densities. By reducing stress and competition, through understanding abalone behaviour, higher abalone growth rates might be achieved at higher stocking densities.

Identifying optimum stocking densities

The choice of optimum stocking densities is based on a number of considerations which, other than economic criteria (Maguire and Leedow 1983, Spencer *et al*. 1985), include survival, individual growth (Holliday *et al*. 1991, Capinpin *et al*. 1999, Park *et al*. 2008) and biomass gain (Neudecker 1981, Fallu 1991, Wassnig *et al*. 2009). If we consider that abalone mortality is not heavily influenced by stocking density (Mgaya and Mercer 1995, Wassnig *et al*. 2009), the major biological criteria which need to be focused on in working towards achieving optimal stocking densities are individual growth and biomass gain, provided that the health and condition of abalone is not negatively affected.

An increase in stocking density does not have an effect on the health or condition of different size abalone when stocked at densities up to 24 % of the available surface area covered by abalone. Maximum individual growth rates were achieved at densities of up to 20 % for 15-35 g abalone, while maximum individual growth was achieved at 16 % densities for 45-65 g and 70-90 g abalone. No differences were found in biomass gain across tested stocking densities for 15-35 g or 45-65 g abalone which suggests that although individual abalone growth would be effected, animals in these size classes may be stocked at densities of up to 24 % without having an effect on yield in terms of biomass gain ($kg.basket^{-1}.month^{-1}$). Furthermore, 70-90 g abalone are able to increasingly gain more biomass when stocking density is increased, from 16 % to 24 % despite a decrease in individual growth from 16 % to 20 %, 22 % and 24 % densities.

Farm production strategies are likely to vary according to the market that they occupy and the end product which they are aiming to produce. If farming strategies are based on abalone growth, a farm needs to decide whether it will produce large quantities of smaller abalone or achieve maximum growth and therefore a higher price for larger individual abalone (Cook and Gordon 2010). Results from this study suggest if individual growth is less important than achieving large amounts of biomass, baskets may be stocked at up to 24 % stocking densities for 15-35 g, 45-65 g and 70-90 g abalone, thereby allowing higher numbers of abalone to be held in a given unit of space, and even increasing the amount of biomass produced in baskets containing 70-90 g animals. If individual growth and the production of larger individual abalone is important to a farm grow-out strategy, abalone should be housed at lower stocking densities in order to achieve maximum individual growth rates in shorter periods of time.

Research and management implications

The research presented in this thesis has provided valuable information about the effect of stocking density on the growth, health and production of different sized farmed *H. midae*. It has also investigated the effects that increased stocking densities have on abalone behaviour under farm conditions. No previous work has been documented with regard to these matters for *H. midae* and so this study will act as a foundation for further research aimed towards understanding on-farm stocking densities and their influence on growth, production and behaviour.

By understanding farm stocking densities and their effect on different size *H. midae*, farmers have gained a useful tool which can be applied to management strategies. This research has provided evidence that 15-35 g abalone may be housed at slightly higher stocking densities (than the 16 % stocking density which is currently used by the South African abalone farming industry) without having a significantly detrimental effect on growth rates and also that 70-90 g abalone may be held at much higher stocking densities (up to 24 % available surface area covered) in order to increase biomass production per unit of grow out space. These findings in themselves are useful to farmers and will benefit production and commercial success if implemented into grow out strategies. Along with implementing the results from this work in management strategies, farms will be able to use this research as a template for future stocking density studies.

Behaviour observations and findings will serve as a platform for future work and provides initial insight to the behavioural responses of *H. midae* to increasing stocking densities. The confirmation of research done on other abalone species, suggesting that behaviour responses to increased stocking densities are largely a result of competition for preferred attachment space (Mgaya and Mercer 1995, Huchette *et al*. 2003), will be of importance to future *H. midae* farming research and practise. Possible reasons for a reduction in growth at high densities, described in this study, include stressors due to intraspecific competition for space and reduced feed availability as a result of crowding and restrictive behaviour. Understanding and identifying any reasons for a reduction in growth rate is of vital importance if farms intend to optimise production. Information described in Chapter 3 provides a foundation which allows for further research into optimising basket design and farming conditions with the aim of improving growth at higher densities (Huchette *et al*. 2003). Fleming and Hone (1996) describe an ideal system as one which promotes the even distribution of abalone while allowing easy access to feed. This idea is strongly supported by research from this study. For farms to optimise production at increased densities, it is important to allow for adaptations to grow out conditions.

Conclusions

Increasing stocking densities have a detrimental effect on *H. midae* growth under current farm conditions but did not have an effect on the health and condition of abalone. Smaller abalone (15-35 g size class) are able to withstand slightly higher stocking densities than animals in larger size classes before individual abalone growth is affected. The biomass gain per basket increased with density for large abalone (70-90 g size class) and remained similar across densities for 15-35 g and 45-65 g animals. Behaviour studies showed that competition for space and/or reduced availability of formulated feed are likely causes of reduced growth at higher stocking densities. These results conformed to previous research on other species of abalone under farm conditions.

The results of this research will act as a useful tool to the adaptation of farm management strategies toward achieving optimum production. Although the growth related results of this study correspond with findings of work done on other abalone species, future research should aim to further understand the behaviour of farmed abalone at different stocking densities so that innovative basket designs and grow out strategies may be developed. These innovations should aim to counter the reduced growth rates of individual abalone seen at higher stocking densities. Aspects of future research should aim to develop an understanding of: (1) the physical stressors related to higher stocking densities, such as localised water quality parameters and excessive shell damage due to crowding; (2) the feeding habits of *H. midae*, with regard to how often individuals are finding their way onto the feeding surface and how reliant abalone are on sources of food other than formulated diets; and (3) abalone behaviour below the feeder plate during the day and at night at different stocking densities. Results from these suggested research topics along with findings from this study will provide the knowledge necessary to begin developing and implementing different basket designs. Growth rates of different size abalone stocked at different densities within these baskets should be tested and compared to current technologies in order to establish if it is possible to counter the decreased growth rates associated with higher stocking densities through innovative management practices.

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