Effects of illumination, shading and temperature on the settlement, survival and growth of juvenile abalone (*Haliotis midae*)

By

Liezl Müller

May 2003

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

at the University of Cape Town

Supervisors: Prof. P. A. Cook & Prof. C. L. Griffiths

This thesis is dedicated to my family and husband for their continuous support and encouragement throughout my studies. Thanks.

Table of Contents

Acknowledgements

The Research and Development Organization funded this project. Their support is gratefully acknowledged.

Sincere appreciation goes to my supervisors: Prof. Peter Cook for his continuous support, assistance and input in this study, it would not have been possible without him. Also Prof. Charles Griffiths for all his help, constructive criticism and advice on the finer points of scientific writing in this thesis. His patience with an "Afrikaans" student struggling with English grammar is greatly appreciated.

Special thanks to: Mr. Andrè du Plessis for providing me with the opportunity to use his facilities and abalone larvae for my project. Gavin Johnston for all his help, support and encouragement as well as for checking on my experiments when I was unable to do so. All the workers of Aquafarm for helping me clean tanks and plates and for all the other assistance they gave throughout the experiments.

Thanks to: Raymond Ferriera from I & J in Gansbaai for his help in teaching me how to identify the different diatom genera. Neville Eden for his help and instructions on the use of the inverted light microscope at UCT. Meg Ledeboer and all the staff from the Zoology Department at UCT who directly or indirectly contributed to the success of this study. Alun Darwood for spending so much time on "proof reading" this thesis for me, I really appreciate all the effort he put in. Dr Anesh Govender for helping me when I was most desperate, with the statistical aspects of my thesis, I know it wasn't always easy and he still helped me under difficult circumstances and I really appreciate all his efforts.

Thanks to my family for their support and encouragement. Finally thanks to my husband, Andrew for his understanding and support through the late nights in the write-up of this thesis.

Abstract

In aquaculture, a thorough knowledge of the specific environmental requirements of a species is needed in order to maximize growth and survival rates. Several studies have helped develop the technology necessary for the intensive commercial culture of *Haliotis midae*. However, a considerable amount of research is still required to develop the most appropriate and optimal method for the cultivation of this species. In particular there remains a lack of information on the feeding habits of the early life stages and as a result the survival rates of spat are still low and variable. This prompted the design of three experiments to determine the best illuminance and water temperature conditions to maximize the settlement, growth and survival of *H. midae* post-larvae. These experiments also investigated what effect different illumination and water temperatures would have on the growth of diatom colonies as substrate for the abalone post-larvae during and after settlement.

Different illumination levels were achieved by using different colours and numbers of layers of shade-cloth. Elevated water temperature was achieved by supplying heated seawater to parts of one of the experiments. Difference in water temperature between the other two experiments was achieved by conducting the experiment in both summer and winter. Diatom-scrapes were taken from settlement plates every ± 14 days in each experiment. These scrape samples were analyzed and showed no significant difference in the diatom colonies identified under different illumination or water temperature conditions. These results were, however, inconclusive, because the ratios of the different diatom genera were not determined.

After 69 days all experiments were terminated and the post-larvae were counted and measured to determine survival rates and growth. The data were analyzed using one-way ANOVA. Illumination, shading and water temperature had a significant effect on the survival and growth of juvenile *H. midae*. In the winter experiment the use of green shade-cloth resulted in a significant increase (p value < 0.00000, F = 19.87 and *Fcrit.* = 2.61) in post-larval growth (average shell length 3.42 - 3.59 mm) as well as a significant

increase (p value < 0.00000, F = 14.08 and *Fcrit.* = 2.68) in the survival rate (average 0.0043 %). However, in the summer experiment the red shade-cloth covered tanks supplied with ambient seawater (17.35 ± 0.15 °C) resulted in a significant increased (p value < 0.00000, F = 10.78 and *Fcrit.* = 2.61) larval growth (average shell length 4.13 - 4.45 mm). The survival rate (average 0.0043 %), however showed no significant increase (p value < 0.10550, F = 2.09 and *Fcrit.* = 2.69) for *H. midae* post-larvae in summer. In the heated seawater experiment red shade-cloth covered tanks supplied with heated water (19.32 ± 0.96 °C) showed a significantly increased (p value < 0.00000, F = 695.16 and *Fcrit.* = 2.61) larval growth (average shell length 3.86 - 4.06 mm) as well as increased (p value < 0.00000, F = 53.61 and *Fcrit.* = 2.69) survival rate (average 0.0035 %).

It was concluded that red shade-cloth together with elevated water temperature produced the optimum growth of *H. midae*. The costs involved in providing heated seawater will, however, determine whether this would be feasible in commercial farming. If not, the use of green shade-cloth in winter and red shade-cloth in summer also provides increased growth and survival for *H. midae*. This would be less expensive than heating the seawater of an entire abalone farm.

Haliotis midae

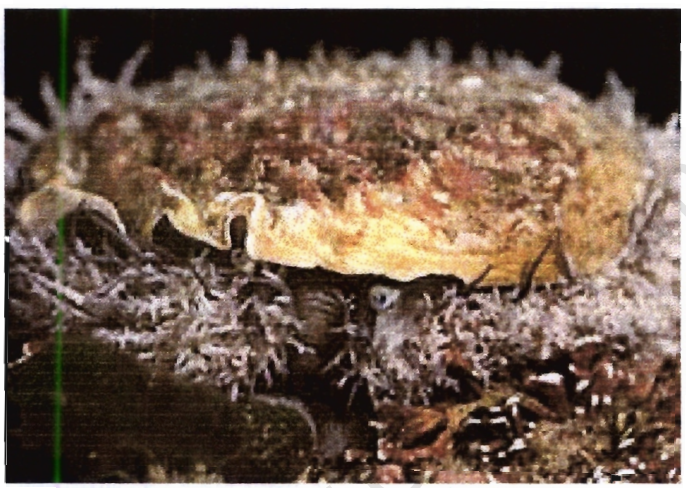


Photo courtesy of Rob Tarr

Chapter 1

General Introduction

Worldwide abalone culture and fisheries

Abalone are considered one of the most commercially important groups of gastropods around the world. The majority of the world's abalone harvest is exported to South East Asia. World production peaked at 28 000 tons per annum in 1968 (Rudd, 1994). However, world supply declined (*Fig. 1.1*) to 14 000 tons per annum by 1991, due to over-exploitation (Rudd, 1994). In recent decades, the exploitation of abalone has risen above the level at which they can maintain stocks by natural reproduction and many fisheries relying on wild abalone stocks have collapsed (Fallu, 1991). For example, due to over-exploitation of abalone in California, USA, white abalone was extinct (Fallu, 1991), but fortunately a farm in California has now produced about 100 000 *H. sorenseni* for genetic analysis and eventual use in restoration projects. The demand for abalone is, however, always increasing and has led to the development of abalone farming.

There are approximately 90 species of abalone worldwide, only 15 of which are commercially harvested. Countries such as Japan, Australia, New Zealand, South Africa, Korea, Taiwan and China had large abalone fisheries, but no longer have this luxury. The depletion of the ocean's abalone resources is a problem in all of these countries, with

reductions running from 50 - 95 % in the commercial catch over the past 25 years (Fishtech, 2003).

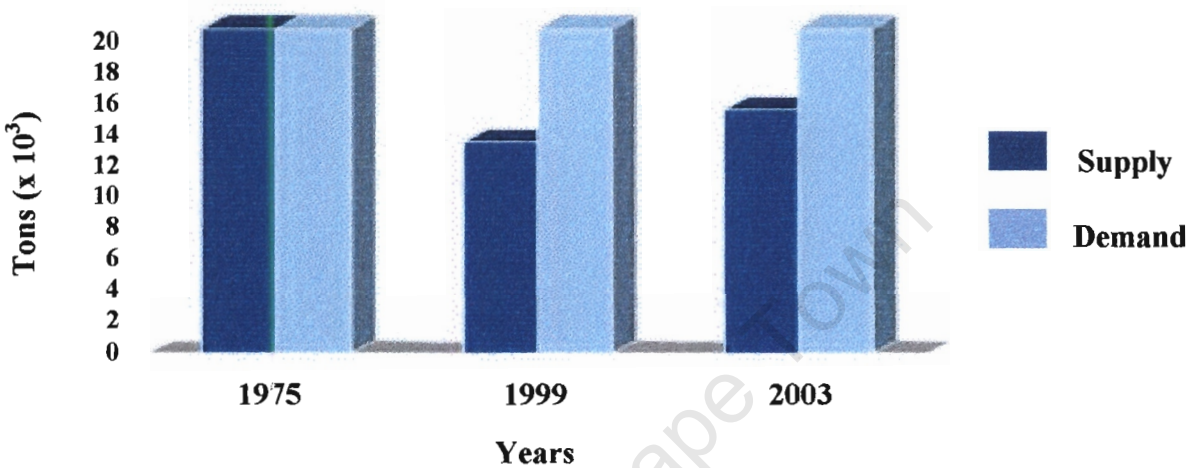


Figure 1.1: World abalone supply and demand from 1975 - 2003.

(From Fishtech, 2003. http://www. Fishtech.com/farming.html.)

Due to this drastic reduction of natural abalone populations, abalone culture projects for reseeding have been initiated as part of the farming technology development in an attempt to re-establish wild stocks (Hahn, 1989; Kawamura *et al.,* 1998). Under artificial conditions abalone have been reared, from egg to adulthood, with great success (Seki, 1980; Ebert *et al.*, 1984; Uki *et al.,* 1984; Hahn, 1989). Following the successes of such programs, the emphasis has definitely shifted towards the intensive culture of abalone in onshore facilities.

South African abalone culture and fisheries

Avery (1974) suggests that Strandlopers (coastal bushmen) were the first to harvest abalone *Haliotis midae* for food, during prehistoric times. However, the first official records of commercial harvesting of South African abalone date from the early 1950's (Payne *et al.*, 1989). It is during this period that the large-scale global exploitation of wild abalone began (Rudd, 1994).

Since the 1990's, land-based farming of *H. midae* has developed to commercial scale production (Pitcher *et al.*, 2000). Today there are farms situated from the south to the west coast of South Africa, from Port Nolloth on the west coast down to Kleinmond, Hermanus, Gansbay and through to Port Elizabeth. According to Marine and Coastal Management (2002) there were 11 farms situated in this region in 2002, though this number is always increasing. The estimated total production from these is about 600 tons per annum, but most farms have not yet achieved full production status (Cook, 2002). Two newly-established abalone farms have only started marketing their product in 2001. Production on these two farms, one at Hougham Park and the other at Marsh Strand, are expected to grow to 80 tons per annum, worth R 22.4 million (Britz *et al.*, 2002). The farming of abalone is a growing business in South Africa and in 2002 there were seven farms exporting cultured abalone to the Far East (Marine and Coastal Management, 2002).

H. midae is the largest of six South African abalone species and the only one to be commercially exploited (Muller, 1986). The other South African species are small and therefore commercially unattractive. *H. midae* is present (*Fig. 1.2*) from St. Helena on the South West coast right through to Port St. Johns on the East coast (Branch *et al.*, 1994; Zoology, 2002[a]).

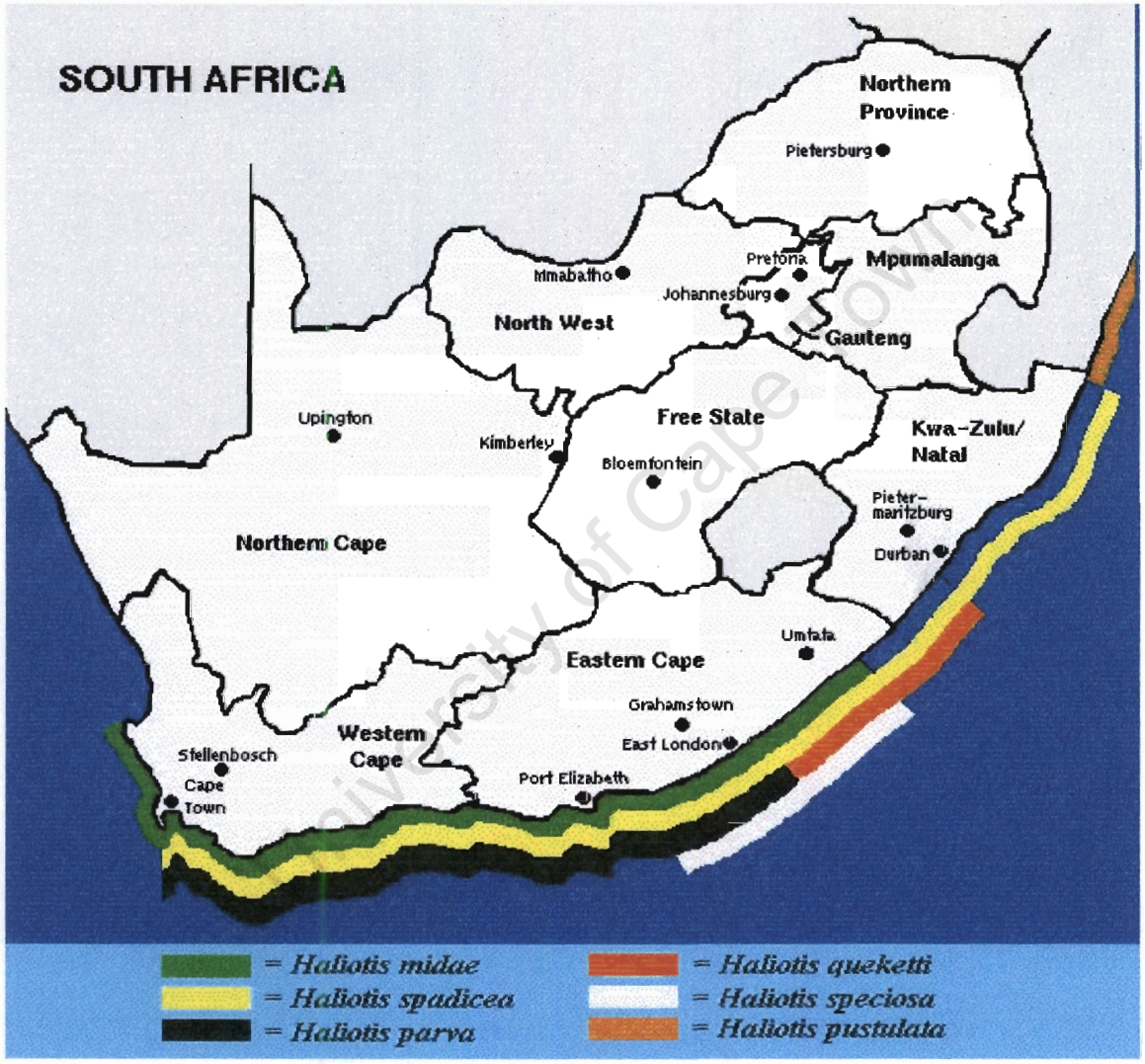


Figure 1.2: South African abalone species distribution map.

(From: Zoology Department, UCT. 2002[b].

http://web.uct.ac.za/depts/zoology/abnet/safrica.html)

According to Cook and Britz, (1990) abalone culture can be broken down into the following components:

- Conditioning of the sexually mature male and female abalone.
- Induction of spawning to produce sperm and eggs.
- Fertilization and hatching.
- Rearing and management of the pelagic larval stages.
- Settlement and first feeding of the spat.
- Management of juvenile abalone throughout the microalgae feeding stage.
- Weaning onto macrophytes.
- Grow out.
- Marketing.

Problems associated with abalone culture

In the past there were several reasons why farmers were reluctant to become involved in abalone farming. Scientists such as Newman (1986) and Payne *et al.* (1989) suggested that the growth rate (*Fig. 1.3*) in natural populations was extremely slow, taking up to seven years for the abalone to reach maturity. Furthermore, the legal harvesting size of 114.30 mm, meant that the animals had to be between eight and nine years old to attain legal marketable size.

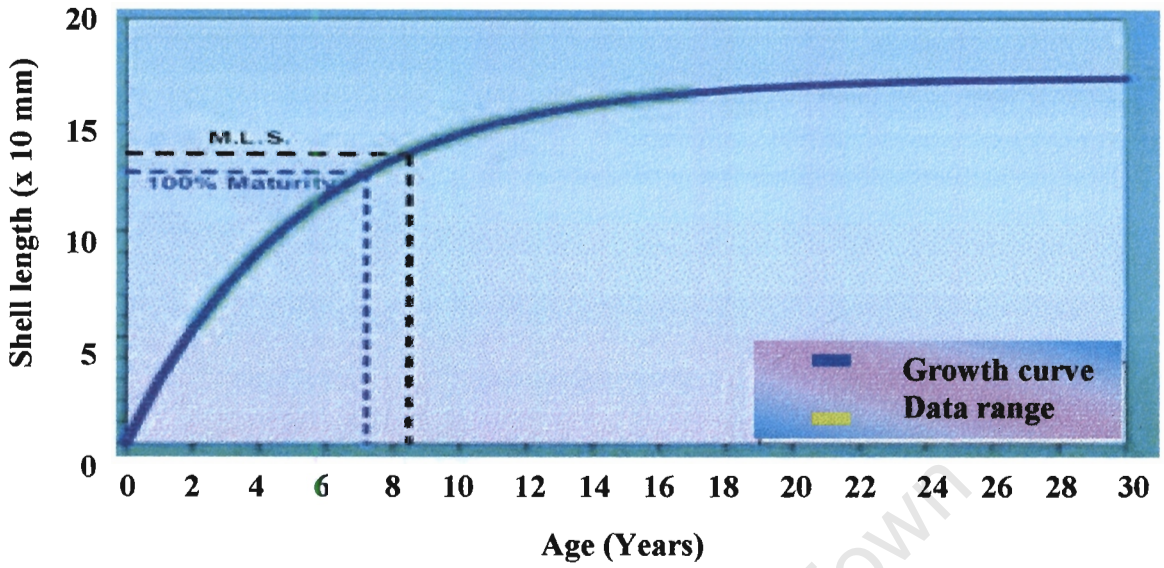


Figure 1.3: The average growth curve *of Haliotis midae*.

(From Tarr R, 2002. htpp://www.environment.gov.za/mcm/inshore/abstory.htm)

Another problem arose because the natural abalone population on the west coast of South Africa mainly feeds on kelp, *Ecklonia maxima*. To supply sufficient quantities of fresh kelp for a full-scale production unit has been a major problem for successful farming operations (Cook and Britz, 1990). However, Genade *et al.* (1988) proved that it was possible to spawn abalone successfully in captivity and together with work done by Seki (1980); Ebert *et al.* (1984); Uki *et al.* (1984) and Hahn (1989) they proved that abalone farming is feasible. However a considerable amount of research still has to be done to develop the appropriate and optimal technology for intensive culture of abalone. Hahn (1989) and Fallu (1991) have reviewed studies on abalone aquaculture. Research on *H. midae* has included various aspects of taxonomy (Muller, 1986; Hahn, 1989), physiology

(Barkai *et al.*, 1986, 1987; Dixon, 1992), ecology and distribution (Stephensen, 1944; Newman, 1969), growth (Newman, 1968), and reproduction (Newman, 1967; Genade *et al.*, 1988). These studies have provided important information towards the technological foundation for the commercial culture of *H. midae*, though more research needs to be done to improve the efficiency of farming operations in future.

Some of the previously mentioned problems that abalone farmers face have been solved to some extent. The food (i.e. kelp) needed during grow-out stages of abalone production can be cultured in certain areas and added at regular intervals or otherwise prepared diets can be used (Cook and Britz, 1990). Abalone survival should be high, once the appropriate grow-out structures are in place with adequate water quality, water exchange rates and food supply (Cook and Britz, 1990).

Another major concern for abalone farmers is obtaining high growth rates. By providing either elevated temperature water and/or increasing the food conversion efficiency, higher growth rates can be achieved. The best way to ensure the adequate water temperature is to situate the abalone farm in a region where the ambient water temperatures are at the upper end of the natural temperature range. By using artificial foods, higher food conversion efficiencies can also be achieved (Cook and Britz, 1990).

The most critical step in abalone culture is the induction of settlement. To maintain a larval survival rate of greater than 95 % from fertilization to veligers competent to settle, is fairly easy to achieve. The survival rate, however, usually drops to approximately 10

% during the transition from planktonic veliger to benthic juvenile (Hahn, 1989). Some researchers think this low survival is unimportant, since a single female can spawn a million eggs. These researchers suggest that as long as annual productions are small, low survival rates are not a problem. However, because of the ever-increasing demand for abalone the low survival rate poses a problem for commercial farmers. Therefore, any research that can maintain consistently higher survival in abalone settlement, will have a great impact on abalone production on farms. For example, in Japan there are some laboratories which produce over two million juveniles per annum. Higher survival rates will allow greater efficiency, which in turn equals lower cost per animal (Hahn,1989).

Furthermore, studies have shown that settlement and survival rates can be increased by using the pre-grazed plate method, where abalone settlement is induced by diatoms and mucus trails from juvenile and/or adult abalone. This means that plates that were previously used for settlement are re-used. Another way to increase the settlement and survival rates of juvenile abalone is to use red crustose algae or γ-aminobutyric acid (GABA) as a stimulant to induce settlement (Seki *et al.*, 1981). When GABA is added at 10^{-6} Molar concentrations to settlement containers, the settlement success rate is claimed to be in excess of 90% (Hooker *et al.*, 1985).

Abalone metamorphosis

Understanding the habitat requirements of larvae and post-larvae from commercially important abalone species are necessary both for the development of aquacultural methods and the optimal management of natural stocks (Shepherd *et al.*, 1985). A

critical, but very poorly understood aspect of most marine invertebrate life cycles, which may be crucial to their survival, is the newly settled larvaes' required habitat and their ability to discriminate between substrata (Shepherd *et al.,* 1985). There are certain easily-observed larval structures which indicate when larval development is complete and the larvae are ready or "competent" to settle (Seki *et al.,* 1981). Larvae that are competent have not lost their ability to swim or crawl, and have not changed shape yet. After the first epipodal tentacle forms, the larvae are capable of crawling on the substratum and after the first snout protrusions form, settlement is initiated (Seki *et al.,* 1981). By repeatedly alternating swimming upwards and sinking to the bottom, as they are carried in water currents, the competent veligers effectively "sample" the bottom, until suitable substrata are found. After the veligers find a possible suitable site, they alternatively swim and crawl on the substratum, a kind of "searching behavior" (Seki *et al.,* 1981). Initially most of the competent veligers time is spent swimming, but as time goes by, they spend more and more time crawling on the substratum (Morse *et al.,* 1979).

Irreversible metamorphoses, which would mean the loss of the veligers swimming cilia, is prevented by this "searching behavior" and ensures a highly specific induction of settlement. This prevents the larvae from undergoing metamorphoses into benthic juveniles (*Fig. 1.4*) in an inappropriate, for example nonalgal, microhabitat. It would be common for larvae to make mistakes by settling in an inappropriate microhabitat, if settlement were stimulated by diffusible inducers from algae in the agitated water (Morse *et al.,* 1979), but insoluble organic materials (diatoms) on the substratum are believed to be the settlement inducers (Crisp, 1974; Akashige *et al.,* 1981). If the appropriate

settlement substratum is not found, the larvae can delay settlement and metamorphosis (Hahn, 1989).

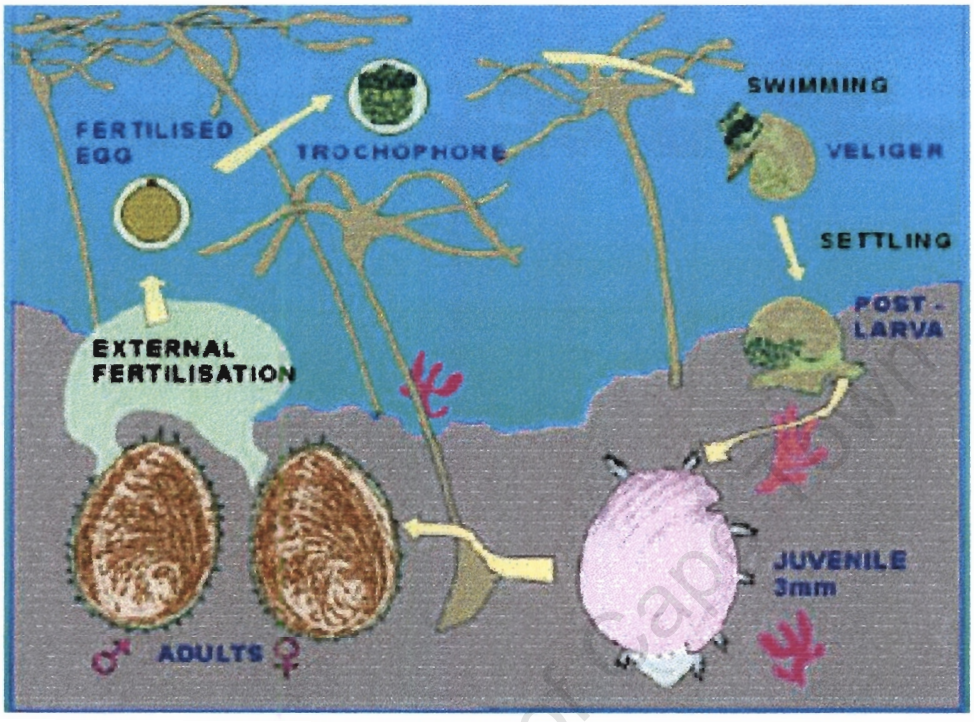


Figure 1.4: The life cycle of the South African abalone, *Haliotis midae*.

(From Tarr R, 2002. htpp://www.environment.gov.za/mcm/inshore/abstory.html)

The appropriate diatoms would have to be present, in order for the adequate substratum to induce settlement of abalone larvae and to serve as a food source after metamorphosis has taken place. Only then will the benthic juveniles have the capability to ingest diatoms. Corallines are also considered an important cue for settlement in the natural environment of abalone.

Abalone food supply and water temperature

Two of the main factors that effect the settlement and survival of abalone are food supply and water temperature. There is a lack of information on the feeding habits of the early life stages of abalone (Kawamura, 1996) and the ability to control the initial food in hatchery seed production, also remains one of the most critical problems (Hahn, 1989; Seki, 1997). In abalone hatcheries the survival rates of spat are still low and variable, especially in the first few months (Searcy-Bernal *et al.*, 1992). Fig.1.5 illustrates just how much the survival rate drops within the first few weeks after settlement.

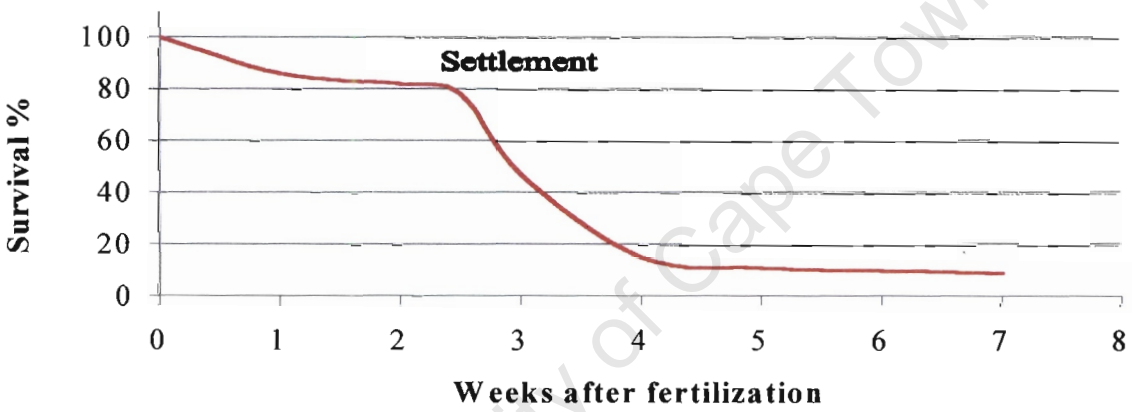


Figure 1.5: Average mortality of spat of *Haliotis* spp. (From Fallu, 1991.)

Benthic diatoms have traditionally been used as an initial food source for post-larvae in abalone hatcheries (Seki, 1980; Ebert *et al.*, 1984; Hahn, 1989). Most hatcheries used diatom films that developed naturally on the settlement substrate (e.g. plastic plates), or pure strains with unknown dietary value. In Japan, the pregrazing method is used in most hatcheries (Seki, 1980; Ebert *et al.*, 1984; Hahn, 1989). This method relies on the grazing action of juvenile or adult abalone leaving behind the strongly adhesive, solitary diatoms suitable for settlement (Suzuki *et al.*, 1987; Kawamura *et al.*, 1992). As

mentioned earlier, this means that settlement plates that have been used for adults or juveniles previously, are re-used to settle new larvae on, without first cleaning the diatom film off the plates. This "pregrazed plate method" is used to ensure consistently high settlement (Seki, 1980; Slattery, 1992), and also provides a good supply of food for post-larvae after settlement (Takami *et al.*, 1997).

Water temperature plays an important role in the growth of both juvenile abalone and diatoms. Excess temperatures are lethal for diatoms (Roberts *et al.*, 2000). In the genus *Haliotis*, many species have different specific temperature preferences. This has important implications for any potential abalone culture operations, such as abalone hatcheries, as well as experimental projects on abalone.

Seawater temperatures also play an important role in the early life stages of abalone. The success of gamete fertilization in abalone is dependent on seawater temperature (Ebert *et al.*, 1983). Hatch-out timing varies between *Haliotis* species, as well as with seawater temperature. High temperatures terminate larval development, while low temperatures slow it down. The metamorphosis rate is also directly related to seawater temperature, where warmer water will induce accelerated metamorphosis (Cuthbertson, 1978; Hahn, 1989).

Diatom biology

To understand the feeding of abalone post-larvae, it is necessary to understand what they feed on, which is mainly diatoms. Diatoms are plant-like organisms and one of the

largest algal groups known. Diatoms need light for photosynthesis, which in turn raises the degree of oxygen saturation and pH. The opposite is also true, since when diatoms respire in darkness, they cause a drop in oxygen and pH levels (Roberts *et al.*, 2000). Light intensity plays an important role in diatom growth. With increasing light intensity, diatom growth increases, up to saturation. Excess light levels reduce diatom growth (Roberts *et al.*, 2000). Plant pigments absorb particular wavelengths, and diatom pigments mostly absorb wavelengths in the 400 - 500 nm range (Roberts *et al.*, 2000).

Diatoms are found throughout all seasons and in all types of aquatic habitats. Although they are unicellular, they appear in various kinds of colonies. A characteristic of diatoms is their siliceous cell, which incorporate the features that are mostly used for identification to family, genus, and species. These features are clearly detectable - namely the shape of the cell, the number and shape of chloroplasts, and the structure of the colony (Newell *et al.*,1963). Diatoms can unite to form long chains several centimeters long, although on their own they range in size from a few μ to about 1mm (Newell *et al.*, 1963).

Diatoms are divided into two main sub-classes, Centricae and Pennatae, the nature of the sculpturing on the frustule being the main difference between the two sub-classes. The sculpture patterns on the frustule in Centricae radiate out from a central, or from a lateral point, or points. In the Pennatae the sculpture lines on the frustule are more or less straight lines, almost feather-like (Newell *et al.*,1963).

Diatoms in abalone culture

There are two ways in which abalone hatcheries use diatoms, some use naturally occurring diatoms and others supplement settlement tanks with a slurry of diatoms retrieved from an algal culture (Ebert *et al.*, 1984). There are two basic groups in which abalone farmers classify colonizing diatom communities. The first community is made up mainly of long filamentous diatoms. When juvenile abalone >10 mm graze on this primary diatom community, it encourages the development of the secondary community, which are encrusting diatoms (Suzuki *et al.*, 1987).

Settlement tanks are covered by shade-cloth. This shading needs to be controlled carefully. Too much cover will result in a very thick diatom film, which will smother the juvenile abalone. On the other hand too little diatom growth, due to excess light, will result in the abalone juveniles starving. The light intensity can be measured with an illuminance meter. By manipulating the photoperiod and/or light intensity, it is possible to balance microalgae production against abalone grazing. This type of feeding carries on until the abalone can be weaned onto macroalgae, which can normally be done when they reach a size of approximately 8 mm (Cook and Britz, 1990).

To improve the growth and survival of post-larvae, the feeding and growth of young abalone in hatcheries needs to be understood. This is important to achieve consistent and optimal production and therefore decrease the proportion of slow-growing animals (Kawamura *et al.*, 1998). Intensive aquaculture strives to achieve maximum production, while reducing associated capital and operating costs. Some of the major capital and

operating costs on abalone farms are the provision of enough space to grow the abalone, and the appropriate water supply systems. These are necessary to ensure that there is sufficient water exchange to prevent oxygen depletion and to dilute and remove waste products, such as ammonia (Botes, 1996).

The main objectives of this study were:

1. To investigate whether the different illuminance and shading of tanks have an effect on the variety of diatom colonies present on settlement plates.
2. To test if different illuminance and shading of tanks will have an effect on the settlement, survival and growth of *Haliotis midae* larvae and post-larvae.
3. To see what effect the different illuminance and shading will have on the respective pH and degree of oxygen saturation, during settlement.
4. To test the effect seawater temperature will have on the settlement, survival and growth of *H. midae* larvae and post-larvae.
5. To investigate what effect the seawater temperature will have on the respective pH and degree of oxygen saturation, during settlement.

The results of these investigations are presented in the following chapters.

Chapter 2 presents the general materials and methods and explain how different colour shade-cloths were used to test the effect illuminance and shading would have on the settlement of *Haliotis midae*. Chapter 2 also gives a broad outline of the experiment and system design used in this study. Chapter 3 then looks at what effect water temperature,

as well as illuminance and shading, had on the diatom composition, during and after the settlement of *H. midae*. Chapter 4 consists of a more detailed experiment on the effect of illuminance and shading on the settlement, survival and growth of *H. midae* larvae and post-larvae. Chapter 5 examines the effect that seawater temperature, together with illuminance and shading had on the settlement, survival and growth of *H. midae* juveniles. In Chapters 4 and 5 the specific colours of shade-cloth, pH and oxygen levels for each experiment are also recorded and discussed. Finally Chapter 6 gives the conclusion of the entire study.

Chapter 2

General Materials and Methods

Origin of juvenile abalone

Three separate experiments on the settlement of *Haliotis midae*, the West-coast abalone, were conducted at the Aquafarm Development farm in Hermanus. The spawning procedure was similar for all three experiments, while the settlement procedure differed between experiments.

Water temperature was controlled at a constant 18°C in the brood stock room, and the abalone were spawned according to normal South African abalone farm practice, as outlined in Cook and Britz, (1990). The sperm and eggs were harvested from the brood stock tanks and mixed for about two minutes. Fertilization was generally limited to a 15-minute period, whereafter excess sperm were separated from the eggs, as outlined in Cook and Britz, (1990). The fertilized eggs (fused gametes) were then transferred into larval rearing tanks, where they divided repeatedly to form larvae (Fallu, 1991). The larvae were retained in the larval rearing tanks for approximately six days, depending on water-temperature. The larval rearing tanks were supplied with ambient seawater, but if the water temperature were warmer in the summer season then the larval period would be shorter than six days. Abalone larvae are tiny at first, with no shell (Fallu, 1991). During the larval rearing stage the larvae develop from trochophores to immediately pre-settlement veliger larvae (Cook and Britz, 1990; Fallu, 1991). The larvae rely on their

"egg" energy (yolk) during this metamorphic stage and therefore it is unnecessary to give the larvae additional feed. Larvae apparently obtain energy supplements from dissolved free amino acids (Cook and Britz, 1990).

System design and management

The readiness of larvae to settle can be recognized either by behavioral responses, or by the number of rows of teeth that have developed on the radula (Cook and Britz, 1990). For example, *H. iris* larvae which have three or more teeth on their radula have been found to settle, though larvae possessing eight teeth had a higher post settlement survival rate (Henry, 1995). When the larvae were ready for settlement, according to these characteristics, the larval rearing tanks were drained and the larvae collected. At this stage the settlement experiments started.

In all three settlement experiments, 12 rectangular plastic settlement tanks were used, each 1.14 m^3. Each of the tanks had 10 plastic settlement plates placed inside. The 10 plates were split into two groups of five each, which fitted into a settlement plate rack, made from PVC pipe. Therefore there were two settlement plate racks, with five plates each, in each settlement tank. Each of the tanks was covered with three layers of shade-cloth, which varied in colour between the different tanks and experiments *(Chapters 4 & 5)*. The tanks were supplied with seawater and with air through PVC-pipes. The larvae were collected from the larval rearing tanks, and their density estimated by counting the larvae in 10 of each, of 1 ml sub-samples randomly drawn from each larval container. The total number of larvae was calculated by multiplying the mean number counted per

ml by the volume of the container used. Equal numbers of larvae were then placed into each settlement tank. Settlement plates were conditioned by placing them in the settlement tanks three to four days prior to the transfer of the larvae into the tanks. This was done so the plates would have a coating of suitable microalgae, e.g. flagellates, pennate diatoms of 10 μm or less, for the larvae to settle and feed on (Cook and Britz, 1990).

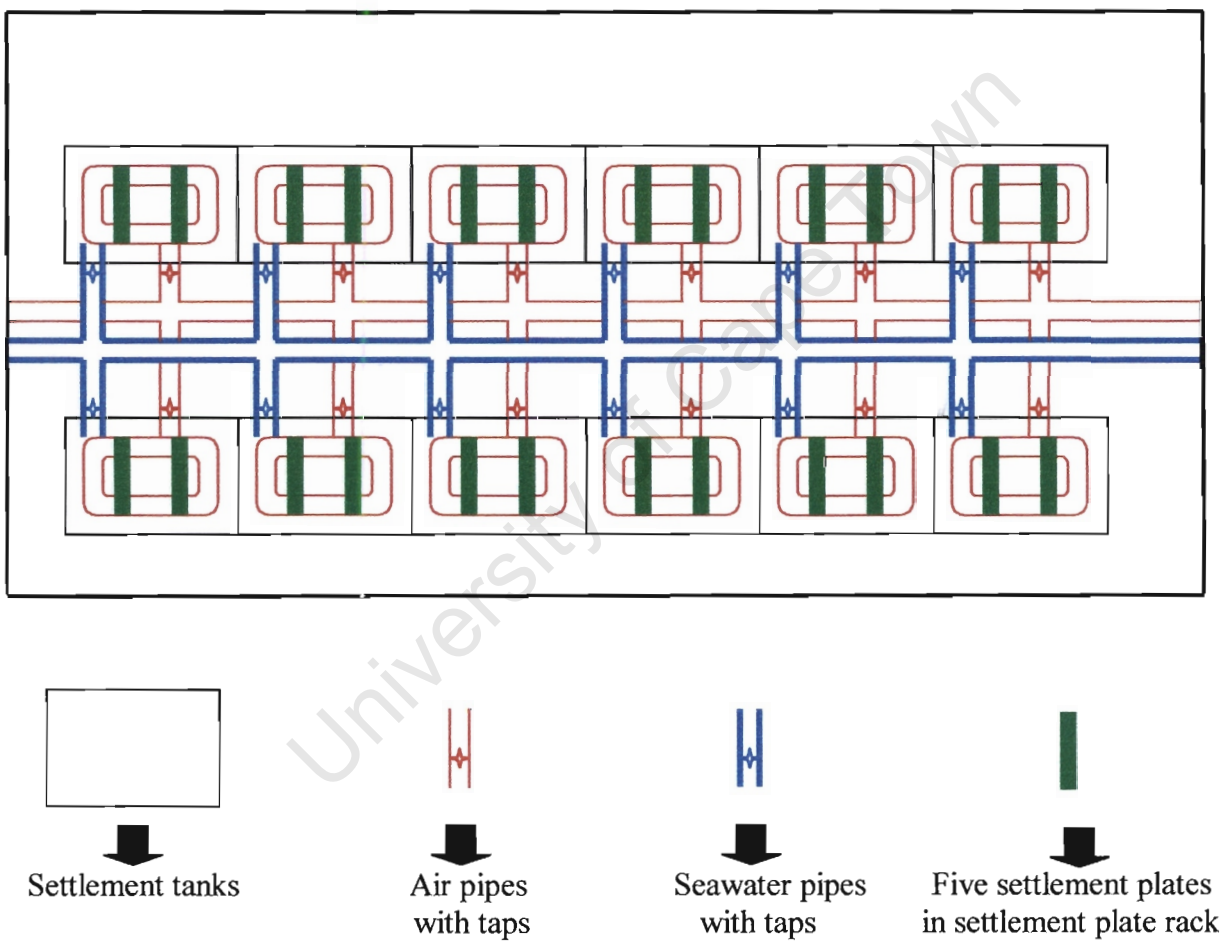


Figure 2.1: Arrangement of air pipes, water pipes and settlement plates in and/or between the tanks.

Each tank was supplied with ambient seawater, which could be drained through draining pipes at the bottom of each tank. For the first two to three days of each experiment, no water was circulated through the tanks. Caps were placed onto the draining pipes with silicone to prevent the water from leaking out, and the water was manually topped up twice daily. After this period, when the larvae had settled, the caps were removed and the seawater was allowed to flow through the tanks at a constant rate of 0.67 l/min per tank. The airflow was manually controlled and was also increased after the larvae had settled. All the pipes used were made from PVC.

Experimental procedures and methods

Throughout the experiments the degree of oxygen saturation and pH levels were recorded. These measurements were done with a digital pH and oxygen meter, the equipment were calibrated before each set of measurements was taken. The pH meter was calibrated using a pH 4 and pH 10 buffer solution respectively. The oxygen meter had an automatic calibration function and was calibrated by exposing the probe to saturated air and pressing the CAL button on the meter. The oxygen meter also had a function by which the water temperature could be measured with a range of 0 °C up to 50 °C. It was therefore used to record the seawater temperature for each experiment. Abalone require relatively high oxygen concentration levels, therefore these levels must be monitored closely to ensure that they remain high (Fallu, 1991). Seawater is alkaline by nature but waste products turn the water more acidic. The measuring of the pH can give an indication of how much degradation has occurred (Fallu, 1991).

Approximately every 14 days, scrape samples were taken off the settlement plates in each tank. Two scrapes were done for each tank, resulting in 24 samples after each scraping procedure. The scrapes were taken off the plates with cotton ear-buds, a clean ear-bud being used for each tank, to prevent contamination. An area of approximately 3 cm on each plate was scraped with the ear-bud to obtain each sample. Diatom scrapes from randomly chosen plates in each tank were placed into a solution of 3 ml seawater and 3 ml 2 % Glyceraldehyde. The 2 % Glyceraldehyde functions as a fixative to preserve the diatoms for later analysis. Thereafter the samples were stored at room temperature until they could be analyzed to determine which diatoms were present on the settlement plates.

The scrape samples were analyzed at the University of Cape Town. In the analyzing process, each of the samples was transferred onto a microscope slide with a pipette and covered with a cover slip. The samples were then examined under an inverted light microscope at x 40, x 60 and x 80 magnification. The analyses were done only to identify which diatom genera were present in the samples and the ratio of the different species were not determined. The various diatoms present in each sample were also photographed. The analysis was repeated for every batch of scrape samples, collected every 14 days, for the duration of each experiment.

A light meter fitted with a lumidisc was used to record the illuminance (lx) for the single, double and triple layers of shade-cloth, in order to determine the light intensity inside each settlement tank. By manipulating the light intensity diatom growth could either be

sped up or slowed down, increasing or decreasing the feed of the juvenile abalone (Cook and Britz, 1990).

Counting and measuring of juvenile abalone

After 69 days the juvenile abalone on each of the settlement plates were counted, to determine the average survival rate. The juvenile abalone were then washed off the settlement plates with anaesthetic, the most commonly recommended anaesthetic being benzocaine (ethyl para-aminobenzoate) (Fallu, 1991). Juvenile abalone were collected from three randomly chosen settlement plates in each tank and 100 of these were randomly chosen for measurements. This was done to determine the average juvenile growth from each tank and measurements were taken with a digital calliper (vernier) in millimeters down to two decimal places. All the juveniles from each tank were subsequently dispersed to other settlement tanks on the farm for production purposes.

Data Analysis

The data were analyzed by using the computer programs Microsoft Excel and Statistica 6.0. Means and standard deviations for pH, degree of oxygen saturation and respective seawater temperatures, for each colour shade-cloth were calculated in Microsoft Excel. Statistica 6.0 was used for the more advanced statistical functions such as one-way ANOVA, to test for differences in the shell length and survival of the juveniles for each colour shade-cloth. The normality of each set of data were tested before ANOVA analyses were conducted, all data were found to be normally distributed.

Chapter 3

Effects of illumination, shading and temperature on the growth of diatoms as substrate for *Haliotis midae* larvae and post-larvae, during and after settlement.

Introduction

It is important to understand the habitat requirements of larvae and post-larvae from commercially important abalone species in order to develop aquacultural methods and manage natural stocks (Shepherd *et al.,* 1985). Insoluble organic materials on the substratum surface are believed to be the inducers of settlement in abalone larvae (Crisp, 1974; Akashige *et al.,* 1981). Larvae can delay settlement and metamorphosis if the appropriate substratum is not found (Hahn, 1989) (*Chapter 1*).

The appropriate substratum would contain the preferred diatoms to induce settlement of abalone larvae and serve as foodsource after metamorphosis (*Chapter 1*). The principal food of post-larval abalone are benthic diatoms (Seki, 1980; Ebert *et al.,* 1984; Hahn, 1989; Kawamura *et al.*, 1998). An important factor determining diatoms dietary value seems to be the digestion efficiency of their strains. Diatom morphology, attachment strength, frustule strength and the post-larval size and age can influence the digestion efficiency of diatom strains (Kawamura *et al.*, 1997). With growth the ability of post-

larval abalone to ingest large diatoms increases (Kawamura *et al.*, 1998, Roberts *et al.*, 1999). The isolation, culture and use of digestible strains in hatcheries could make a positive impact on abalone culture (Kawamura *et al.*, 1997). A critical factor controlling the nutritional value of diatoms seems to be the proportion of cells broken down during post-larval grazing, however, this has only been shown for *Haliotis discus hannai* (Kawamura *et al.*, 1997). Due to differences in the ability of post-larvae to ingest and digest diatoms, their growth and survival will differ among diatom diets (Kawamura, 1996; Kawamura *et al.*, 1998, Roberts *et al.*, 1997, 1999), growth form (Seki, 1980; Kawamura *et al.*, 1992) and density (Kawamura, 1996).

A natural biofilm takes between one to three weeks to develop and is generally used as settlement cue in abalone nurseries depending on the site, season and temperature (Hone *et al.*, 1997). There is considerable variability in algal growth of a natural biofilm on settlement plates depending on the filtration, shading, nutrients and water exchange. Biofilms are considered a strong cue for settlement of invertebrate larvae (Pawlik, 1992; Keough *et al.*, 1995), however, the responses of individual species to such films are highly variable (Todd *et al.*, 1994; Keough *et al.*, 1995, 1996). In the natural environment, larvae of several abalone species preferentially settle on non-geniculate coralline red algae (Saito, 1981; Morse *et al.*, 1984; Shepherd *et al.*, 1985; McShane *et al.*, 1988).

Diatoms are plant-like organisms from one of the largest algal groups. Light plays a very important role in the development of the biofilm. Micro-algae utilises light as an energy

source for photosynthesis and as an information source about their local environment (Bowler, 2003). Diatom photosynthesis (Roberts, 2000) in turn causes a raised degree of oxygen saturation and pH levels (*Chapter 1*). Interception of the informational components of light - both in terms of its intensity and spectral composition, are critical for adjustments in photosynthetic reactions. For example, plants and algae growing at low light intensities increase their light-harvesting capabilities by synthesizing photosynthetic pigments (Bowler, 2003. Therefore different colours of light may affect photosythesis and the subsequent growth of plants and algae.

Pigments are by definition, any substances that absorb light, and their colours are determined by the wavelengths of light they do not absorb. Diatom pigments capture light energy and are located inside organelles called chloroplasts and play an important role in photosynthesis. The pattern of wavelengths that pigments absorb are called their absorption spectrum (ATJ. 2002). The photosynthetic pigments utilised by diatoms are chlorophyll *a* and *c*; α-, β-, ε-carotenes, fucoxanthin and several other xanthophylls (ATJ. 2002). Green chlorophylls absorb light from the blue-violet and red regions of the visible spectrum and reflect green light. The yellow, orange and red carotenoids only absorb light from the blue-violet region of the spectrum but are mostly masked by the more dominant chlorophyll. In clorophyll *a*, the wavelength absorption range is 420 - 660 nm, while in chlorophyll *c* it is 445 - 625 nm, when disolved in organic solvents. When a different solvent is used, the values might be slightly different (Aquatic-Plants, 2002). Diatoms absorb light (wavelengths) within the visible range of the ligth-spectrum (Overmann, 2001). The approximate visible range for the naked eye is between 390 -

800 nm (Aquatic-Plants, 2002). Diatom pigments absorb wavelengths mostly in the 400 - 500 nm range (Roberts, 2000).

Light intensity also plays an important role in diatom growth. With increasing light intensity, diatom growth increases, up to saturation. Rapid diatom growth takes place between 30 - 50 $\mu E/m^2/sec$ and for different species of diatoms the saturation intensities vary between approximately 20 - 200 $\mu E/m^2/sec$. Bright sunlight is about 1 500 $\mu E/m^2/sec$ and such excess light reduces diatom growth (Roberts, 2000). Therefore settlement tanks are covered by shade-cloth and need to be controlled, to maintain the right balance between diatom growth and juveniles grazing pressure. Too much or too little cover will be undesirable (*Chapter 1*). By manipulating the photoperiod and/or light intensity in farming situations, it is possible to find the equilibrium between microalgae production and abalone grazing (Cook and Britz, 1990).

Water temperature plays an important role in both juvenile abalone and diatom growth. Excess temperatures are lethal for diatoms and water temperatures exceeding 25 °C harm many. Successful growth of diatoms occurs between 15 - 22 °C (Roberts, 2000).

Diatoms are found throughout all seasons and in all types of aquatic habitats. Although they are unicellular, they appear in various kinds of colonies. A characteristic of diatoms is their siliceous cell, surrounding features used for identification like the shape of the cell, the number and shape of chloroplasts, and the structure of the colony

(Newell *et al.*, 1963). Though diatoms show great diversity in size and form, they are immediately recognizable by their cases, or frustules, of silica, which are often elaborately ornamented, as well as by their brownish or yellowish chloroplasts. There is a central mass of cytoplasm, containing the nucleus, joined to the wall of the cell by cytoplasmic strands (Newell *et al.*,1963). A very unique feature to diatoms is that the frustule is in two parts, or valves, which fits tightly into one another, like a petri-dish and are connected by girdle bands (Newell *et al.*, 1963; Asada *et al.*, 2002). Diatoms range in size from a few μ to about 1 mm though many of them unite to form long chains, often several centimeters long (Newell *et al.*, 1963). For example, the sizes from a few of the more commonly found diatom species in abalone culture are *Nitzschia closterium* spp. which are ± 40 μ long, *Bacillaria paradoxa* spp. ± 50 μ long, *Chaetoceros* spp. between 15 - 75 μ long (Newell *et al.*, 1963), *Navicula cancelatta* spp. ± 40 μ long, *Licmophora opehoroides* spp. between 30 - 72 μ long, *Grammatophora* spp. between 19 - 75 μ long, *Rhabdonema* spp. ± 18 μ long, *Cocconeis sublitoralis* spp. between 35 - 37 μ long and *Surirella scopuli* spp. ± 24 μ long (Giffen, 1970).

In Japan, the pregrazing method (*Chapter 1*) is used (Seki, 1980; Ebert *et al.*, 1984; Hahn, 1989). This "pregrazed plate method" presumably ensure consistently high settlement (Seki, 1980; Slattery, 1992), and provides feed for post-larvae after settlement (Takami *et al.*, 1997). Most local hatcheries use diatom films that develop naturally on the settlement substrate (e.g. plastic plates). Another way hatcheries use diatoms are to supplement settlement tanks with a slurry of diatoms retrieved from an algal culture (Ebert *et al.*, 1984). Some of the typically used diatom genera for these supplements are

Navicula spp., *Cocconeis* spp., *Amphora* spp., *Nitzschia* spp., and unicellular green algae, *Tetraselmis* spp. (Ebert *et al.*, 1984). Benthic diatoms like *Cocconeis* spp. and *Nitzschia* spp. are widely used in laboratories to induce settlement in abalone larvae (Ebert *et al.*, 1984). The highest rates of settlement are induced by diatoms such as *Navicula* spp., and *Nitzschia* spp., which then also serve as feed for the newly settled larvae (Hahn, 1989).

Abalone farmers classify colonizing diatoms in two basic groups. The first community is made up mainly of *Navicula* spp. and *Nitzschia* spp. When juvenile abalone >10 mm graze on the primary diatom community, it encourages the development of the secondary community (encrusting diatoms), such as *Cocconeis* spp. With age, the structure and composition of a microbial film changes (Suzuki *et al.*, 1987). Juvenile abalone of ~ 15 mm will graze on the primary community for approximately two weeks at a seawater temperature of 20 °C, after which the plates change to a greenish-yellow colour, indicating a film of encrusting diatoms (Hahn, 1989). The encrusting diatoms (secondary community) are an excellent foodsource for newly settled juveniles, since their developing mouthparts can easily handle them. Newly metamorphosed larvae (~ 220 µm) have not yet developed mouthparts or a mouth opening capable of feeding on diatoms larger than 10 µm. It seems that bacteria are their main foodsource during this period (Cook and Britz, 1990).

According to Newell *et al.* (1963), two main sub-classes of diatoms are recognized namely, Centricae and Pennatae (*Chapter 1*). Many of the pennate diatoms, those which can move independently, have a narrow slit running along one or both valves, called the

raphe. Diatom species are widely distributed and there are few that are typical of particular water-masses.

In the sub-class Centricae some of the more common centric diatoms are *Melosira, Paralia, Coscinodiscus, Lepticylindrus, Rhizosolenia and Chaetoceros.* More of the commoner members of the sub-class Pennatae are *Licmophora, Navicula, Pleurosigma, Bacillaria and Nitzschia* (Newell *et al.*, 1963).

The aim of this chapter is to investigate whether illuminance and shading of settlement tanks had an effect on the variety of diatom colonies present during the settlement experiments done in this study. Also what effect water temperature had on the diatom composition, during and after the settlement of *H. midae*.

Materials and methods

Three separate experiments were conducted at Aquafarm Development in Hermanus. The experiments were done on the settlement of *Haliotis midae*, the West-coast abalone. In each experiment 12 settlement tanks were used. Two of the experiments were done using four shade-cloth colours, namely black, blue, green and red (*Chapter 4*), thus resulting in three settlement tanks, covered with the same shade-cloth colour. One of the two experiments was conducted during the summer (November through to the beginning of January), while the other was done in winter (July through to the beginning of September). Both of these experiments were supplied with ambient seawater (*Chapter 4*). In the third experiment (done in April through to the beginning of June) only two

shade-cloth colours were used, namely black and red on tanks that were either supplied with ambient or heated seawater (*Chapter 5*). Therefore there were six tanks covered with each of the two shade-cloth colours, of which three received ambient seawater, and the remaining three heated seawater. The shade-cloth colour used for each tank was randomly selected. In all three experiments each tank was covered by three layers of the respective shade-cloth, which were manually varied in order to maintain the satisfactory growth of diatoms in each tank. Each shade-cloth layer had a shade factor of 88 % and up to 96.7 % U. V. blockage. Black shade-cloth was used as control for each of the three experiments, because the black shade-cloth absorbs all the wavelengths of light.

The basic methods as outlined in Chapter 2 were followed in each experiment. A light meter fitted with a lumidisc and ISO at 100, was used to record the illuminance (lx) for the single, double and triple layers of shade-cloth used in each experiment. The measurements were actually taken in EV and were converted by aid of a convertion table supplied with the Sekonic L308B light meter, to lux values. These measurements were taken once a week at 12h00 am in the center of each tank, for the duration of each experiment.

Diatom scrape samples were taken off the settlement plates in each tank (*Chapter 2*). The samples were stored at room temperature and analyzed at the University of Cape Town with an inverted light microscope (*Chapter 2*). The diatoms identified in each sample were also photographed. The analyses were repeated for every batch of scrape samples, collected every 14 days, for each experiment.

After 69 days in each experiment the average shell length and survival rate of the juvenile abalone in each tank were determined (*Chapters 4 & 5*). The data were analyzed as explained in Chapter 2. To make the results more accessible, each set of data will be presented under each of the three experiments, namely, experiment 1, 2 and 3. Photos taken of the diatom genera will also be presented.

Results

Experiment 1: Summer settlement experiment

The results (*Fig. 3.1*) shows that during the summer experiment, the light intensity was high and that there was a considerable drop in illuminance(lx) between the number of shade-cloth layers used. Less shade-cloth layers resulted in higher illuminance values except in the case of the red shade-cloth. The double layer of red shade-cloth shows an increased illuminance value in comparison to the single layer of red shade-cloth. The reason for the increased illuminance value must be due to inaccurate measurements taken in this experiment and was not repeated in any of the other experiments. The standard deviation of the illuminance for certain shade-cloth colours and number of layers used are large, though for others there are little or no variance. For example, black and green single layer shade-cloth had a mean illuminance of 14 000 ± 0.00 lx, where blue and red single layer shade-cloth had respective illuminance means of 9370 ± 8019.40 lx and 11733 ± 3925.98 lx.

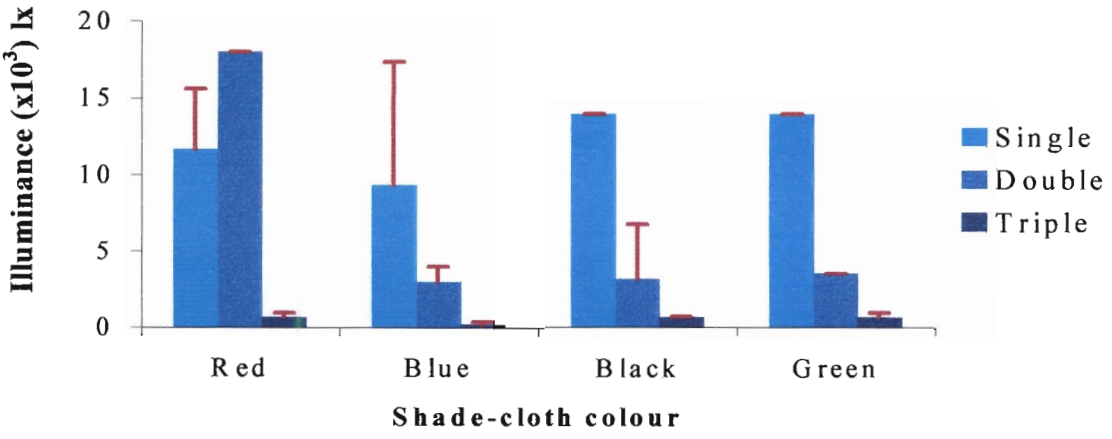


Figure 3.1: Mean illuminance (lx) for single, double and triple layers of four shade-cloth colours in a summer experiment with *Haliotis midae* post-larvae. Error Bars represent Standard Deviation. *n* = 3 replicates.

According to Table 3.1 the diatom genera that were present in all tanks, regardless of shade-cloth colour during a summer settlement experiment, were *Bacillaria* spp., *Grammatophora* spp. *and Navicula* spp. Other diatom genera like *Gyrosigma* spp., *Licmophora* spp. and *Striatella* spp. were found in most of the tanks, though not in all (*Table 3.1*). *Chaetoceros* spp., *Cylindrotheca* spp. and *Rhabdonema* spp. were identified in very few of the tanks in the summer experiment.

All of the diatom genera mentioned above were recorded at some time during the experiment regardless of shade-cloth colour. *Chaetoceros* spp. and *Cylindrotheca* spp., were only found in tanks covered by certain colours of shade-cloth. *Chaetoceros* spp. were only recorded in scrapes taken from tanks covered with blue shade-cloth and only on day 28 of the summer experiment. *Cylindrotheca* spp. on the other hand were present in all tanks except for those covered by red shade-cloth.

Diatoms genera	Red	Black	Blue	Green
Bacillaria spp.	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4
Chaetoceros spp.	****	****	2	****
Cylindrotheca spp.	****	1,3	1	1
Grammatophora spp.	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4
Gyrosigma spp.	1,3,4	1,3,4	1,3,4	1
Licmophora spp.	2,3,4	2,3,4	2,3,4	1,2,3,4
Navicula spp.	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4
Nitzschia spp.	1	1	1	1
Rhabdonema spp.	3	3	2,3	2,3
Striatella spp.	2,3	2,3	2,3,4	2,3,4

Present in scrapes:

1 = 14 days

2 = 28 days

3 = 42 days

4 = 56 days

* = Not present

Table 3.1: Diatom genera identified in diatom-scrapes taken every ± 14 days during a summer experiment with *Haliotis midae* post-larvae using four shade-cloth colours. *n* = 3 replicates.

Experiment 2: Winter settlement experiment

Figure 3.2, shows that the illuminance was much higher when less layers of shade-cloth were used on the tanks. For the winter experiment, only the double layer of red shade-cloth had no variance (1800 ± 0.00 lx) between the illuminance (lx) measurements taken, though the other three shade-cloth colours and levels of shading (layers of shade-cloth) all had fairly big standard deviations.

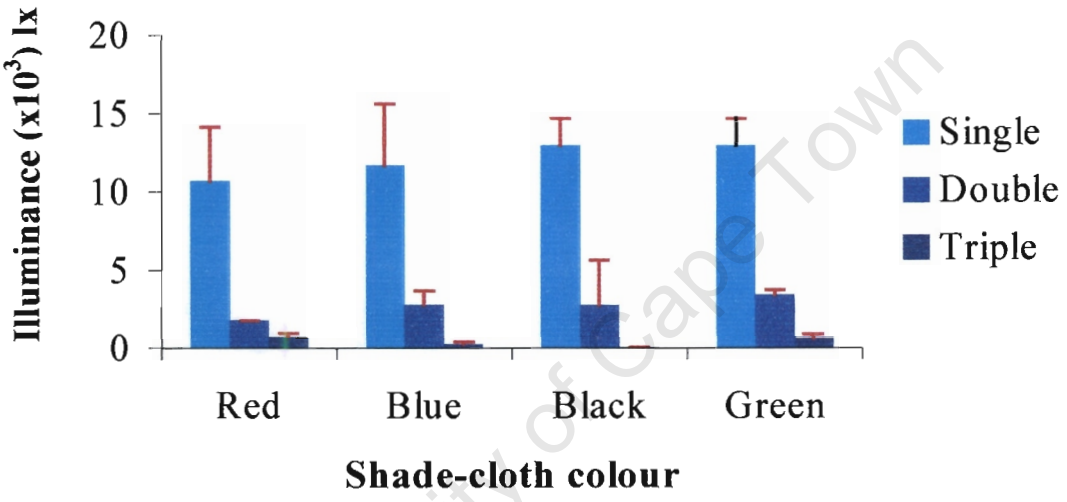


Figure 3.2: Mean illuminance (lx) measured for single, double and triple layers for four shade-cloth colours used in a winter experiment with *Haliotis midae* post-larvae. Error bars represent Standard Deviation. *n* = 3 replicates.

Table 3.2 shows that the diatom genera present in all tanks regardless of shade-cloth colour, for the winter settlement experiment were *Grammatophora* spp., *Navicula* spp., and *Rhabdonema* spp. Other diatom genera like *Bacillaria* spp., *Licmophora* spp., *Nitzschia* spp. and *Striatella* spp. were found in most tanks, though not in all (*Table 3.2*). *Gyrosigma* spp. were only identified in scrape samples taken from tanks covered by red and black shade-cloth and only on the 14th day of the winter experiment.

Diatom genera	**Red**	**Black**	**Blue**	**Green**
Bacillaria spp.	1,2,3,4	1,2,3	1,2,3,4	1,2,3,4
Grammatophora spp.	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4
Gyrosigma spp.	1	1	****	****
Licmophora spp.	1,2,	1,2,3,4	1,2,3,4	1,2,3,4
Navicula spp.	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4
Nitzschia spp.	1	1,2,3	1	1,2
Rhabdonema spp.	1,2,3	1,2,3,4	2,3	1,2
Striatella spp.	1,3,4	3,4	4	2,3,4

Present in scrapes:

1 = 14 days

2 = 28 days

3 = 42 days

4 = 56 days

* = Not present

Table 3.2: Diatom genera identified in diatom-scrapes taken every ± 14 days during a winter experiment with *Haliotis midae* post-larvae using four shade-cloth colours. *n* = 3 replicates.

Experiment 3: Settlement experiment with heated and ambient seawater

In Figure 3.3, the illuminance was much higher when less layers of shade-cloth covered the tanks. The results show that there was no significant difference in the light intensity between the two shade-cloth colours used in the settlement experiment.

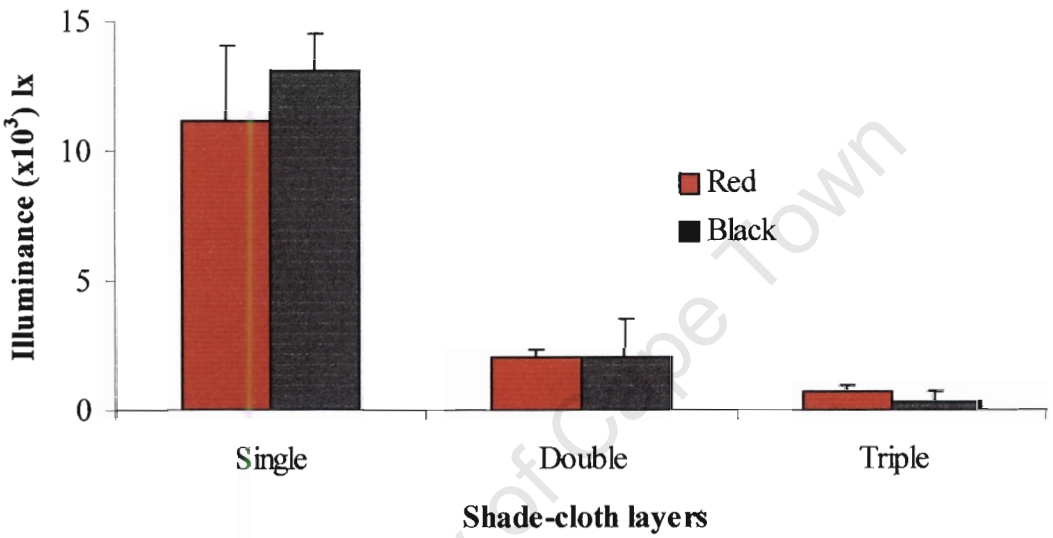


Figure 3.3: Mean illuminance (lx) measured for single, double and triple layers of red and black shade-cloth used for a experiment with *Haliotis midae* post-larvae. Error bars represent Standard Deviation. *n* = 6 replicates.

Table 3.3 indicate that the diatom genera present in all the tanks, regardless of water temperature or shade-cloth colour, were *Navicula* spp. Other diatom genera like *Nitzschia* spp., *Licmophora* spp. and *Rhabdonema* spp. were found in most but not in all (*Table 3.3*). Though *Bacillaria* spp., *Striatella* spp. and *Cocconeis* spp. were only recorded in some scrapes taken from tanks supplied with heated seawater, *Surirella* spp.

were found in both heated and ambient tanks for both shade-cloth colours, but only on day 56 of the experiment. *Gyrosigma* spp. were only present in scrapes taken on day 14 from the red covered tanks, supplied with ambient seawater (*Table 3.3*).

	Heated		Ambient	
Diatom genera	**Black**	**Red**	**Black**	**Red**
Bacillaria spp.	1,2,3	1,2,3,4	****	****
Cocconeis spp.	2	3	****	****
Cylindrotheca spp.	1,3	1	1,3	1
Grammatophora spp.	2,4	1,2,3,4	1,2,3,4	1,2,3,4
Gyrosigma spp.	****	****	****	1
Licmophora spp.	2,3,4	3,4	2,3,4	2,3,4
Navicula spp.	1,2,3,4	1,2,3,4	1,2,3,4	1,2,3,4
Nitzschia spp.	1,2,3	1,2,4	1,2	1,2
Rhabdonema spp.	2,4	1,2,3,4	****	2,3
Striatella spp.	2,3,4	4	****	****
Surirella spp.	4	4	4	4

Present in scrapes:

1 = 14 days

2 = 28 days

3 = 42 days

4 = 56 days

* = Not present

Table 3.3: Diatom genera identified in diatom-scrapes taken every ± 14 days during an abalone experiment with *Haliotis midae* post-larvae using two shade-cloth colours on tanks supplied with either heated or ambient seawater. *n* = 3 replicates.

Photos: Diatom genera of all three experiments

Photos (*Figs 3.4 - 3.13*) were taken of most diatom genera identified under the inverted microscope. Photos were taken of the following diatom genera: *Bacillaria* spp.; *Striatella* spp.; *Licmophora* spp.; *Gyrosigma* spp.; *Surirella* spp.; *Grammatophora* spp. and *Navicula* spp.

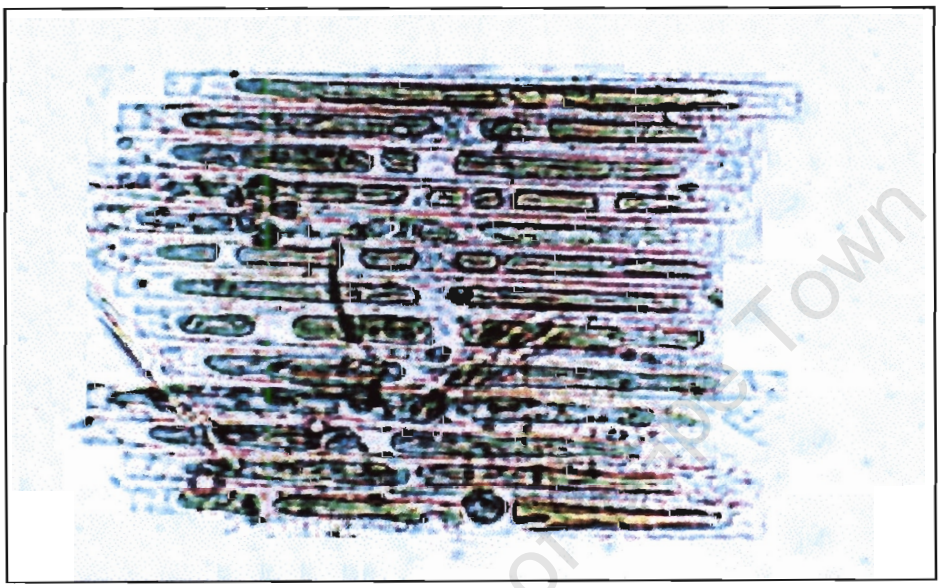


Figure 3.4: *Bacillaria* spp. (x 80 magnification, inverted light microscope)

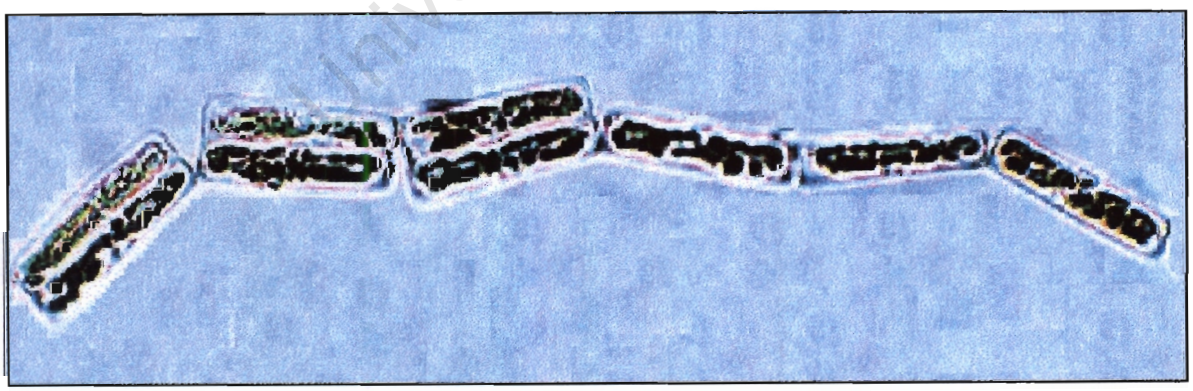


Figure 3.5: *Grammatophora* spp. (x 80 magnification, inverted light microscope)

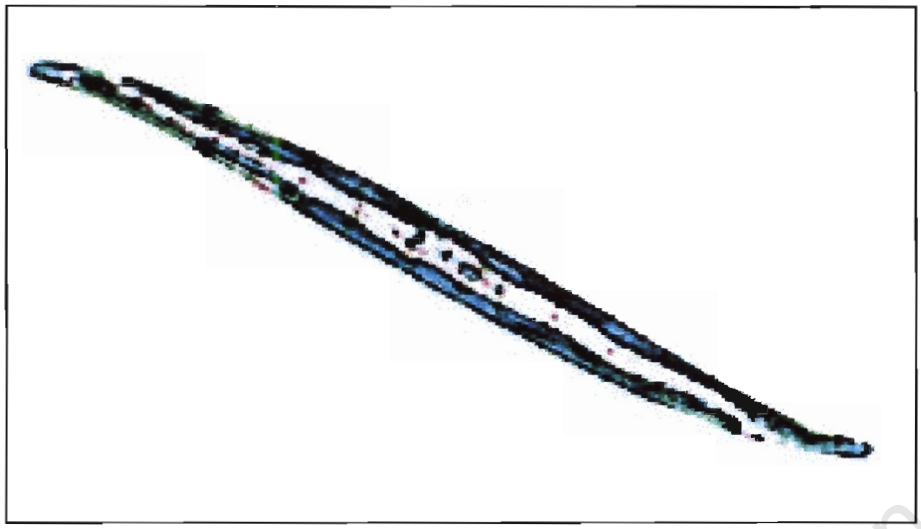
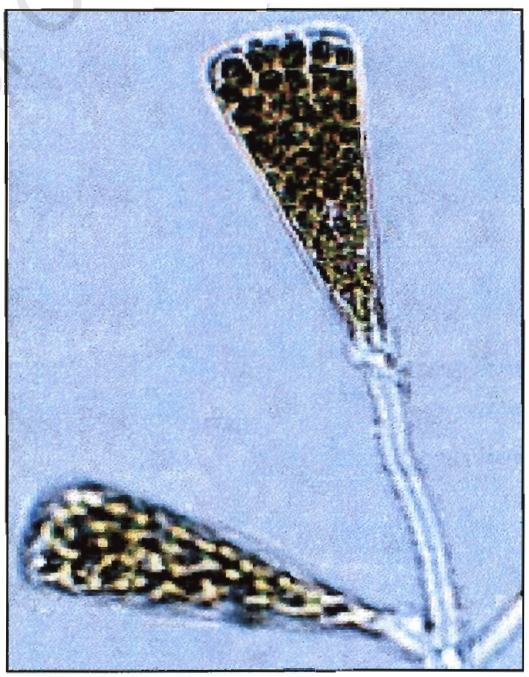


Figure 3.6: ***Gyrosigma*** **spp. (x 80 magnification, inverted light microscope)**



Figures 3.7 & 3.8: ***Licmophora*** **spp. (x 80 magnification, inverted light microscope)**

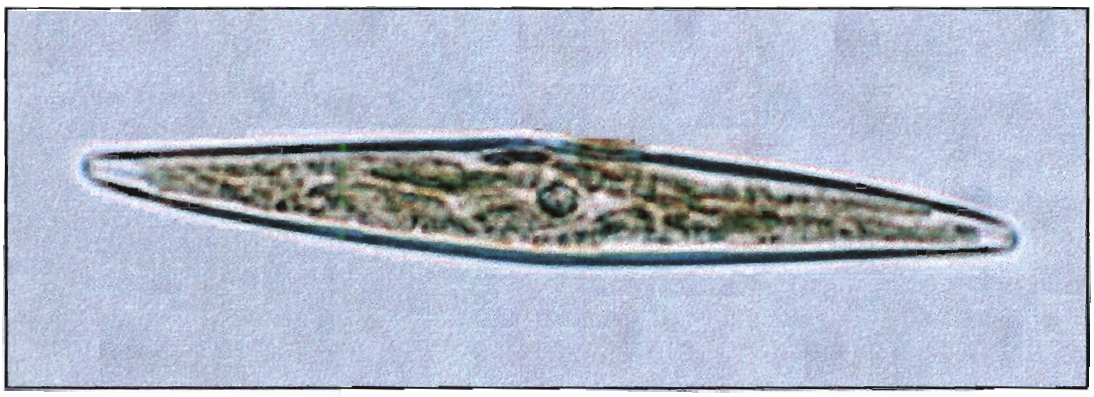


Figure 3.9: ***Navicula*** **spp. (x 80 magnification, inverted light microscope)**

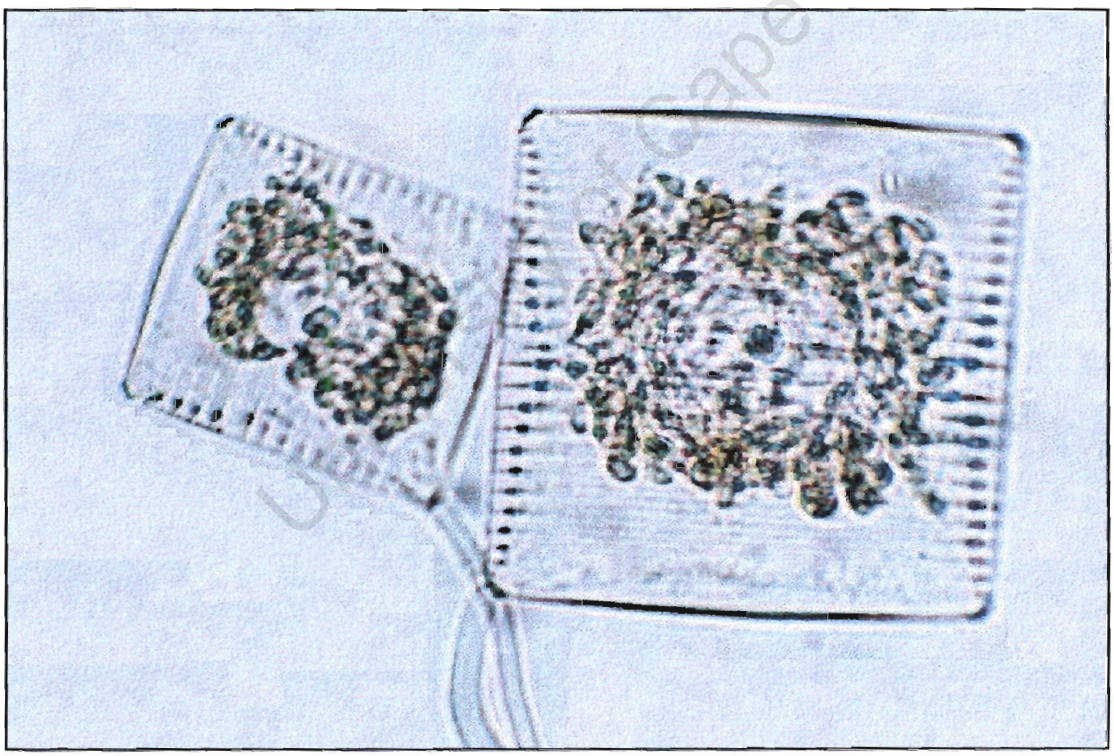
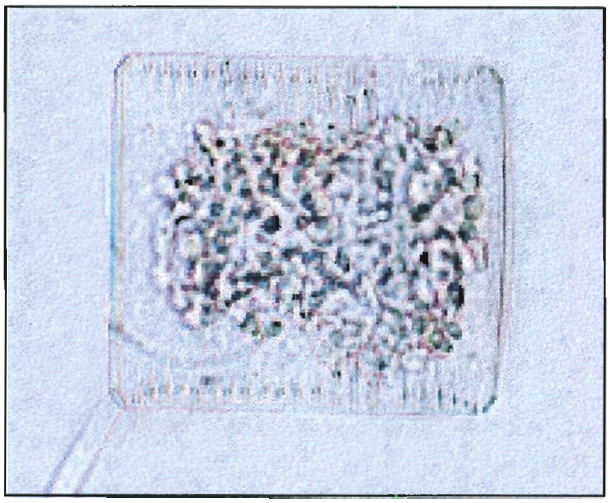
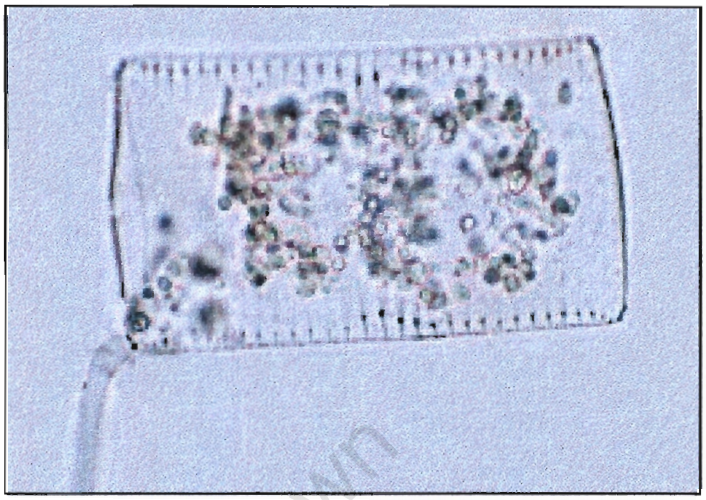


Figure 3.10: ***Striatella*** **spp. (x 80 magnification, inverted light microscope)**





Figures 3.11 & 3.12: ***Striatella*** **spp. (x 80 magnification, inverted light microscope)**

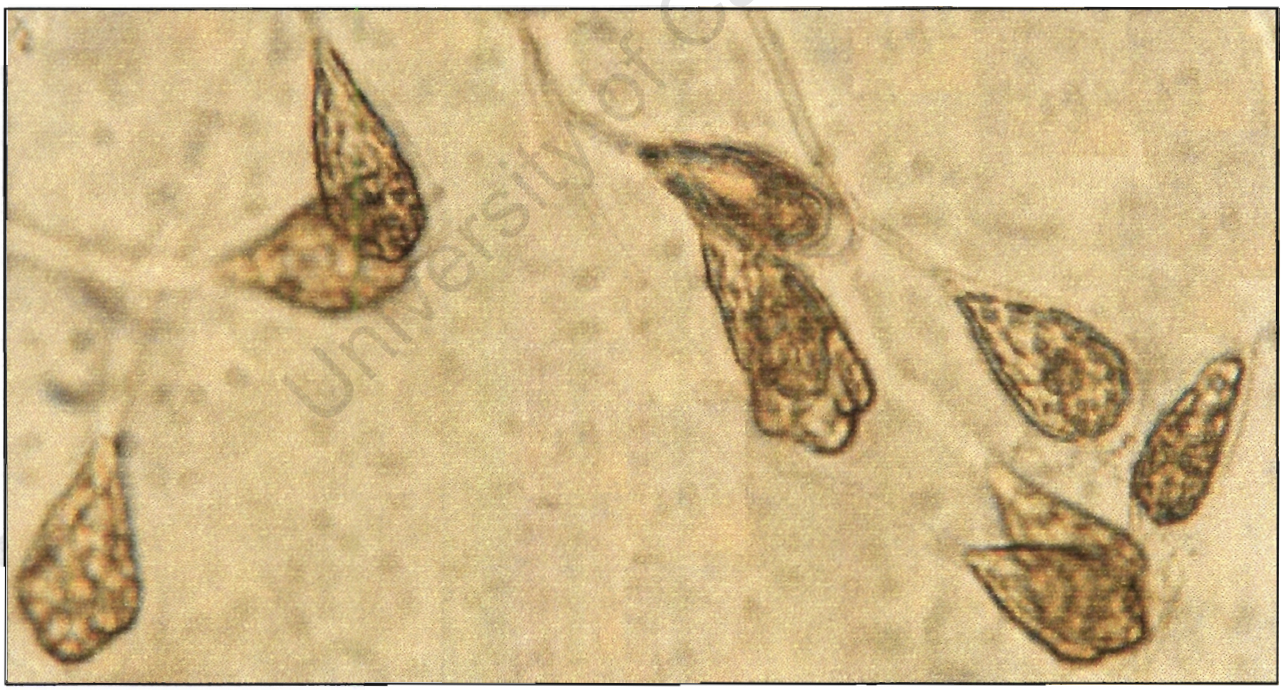


Figure 3.13: ***Surirella*** **spp. (x 80 magnification, inverted light microscope)**

Discussion

In general, the red/blue and green colour of the shade-cloths used in all the experiments implied that other colours of light were absorbed, so that the light passing through them were enrinched in red/blue and green wavelenghts respectively. The black shade-cloth absorbed all wavelengths and was therefor used as control in all three experiments. The shade-cloth colours absorbed the following respective wavelengths (energy levels), according to their visible colour as outlined in Aquatic-Plants, 2002:

Black……..all wavelengths

Blue……460 - 480 nm

Green……..490 - 530 nm

Red……650 - 800 nm

Therefore the black, blue and green shade-cloth's absorption spectrum were approximately within the wavelengths mostly absorbed by diatoms, which are between 400 - 500 nm. The red shade-cloth's absorption spectrum, however, was longer than the wavelengths mostly absorbed by diatoms. This could explain why in Figure 3.1 the red shade-cloth showed a significantly higher illuminance (lx) value, when two layers of shade-cloth (double) covered the settlement tank, in comparison to the other three shade-cloth colours, although this was most likely due to inaccurate measurement. In all three settlement experiments the light intensity (illuminance) increased as the number of layers of shade-cloth covering the tanks, decreased (*Figs 3.1; 3.2 & 3.3*). There was no significant difference found within the mean illuminance between the different experiments (*Figs 3.1; 3.2 & 3.3*). The result of zero variance in illuminance (lx) for

certain layers of red, black and green shade-cloth were unexpected (*Figs 3.1 & 3.2*), but the readings were all checked and it seemed that they were identical by chance.

By shading the feeder plates with shade-cloth, it seemed that the growth of micro-algae was slowed down. The diatom film on the settlement plates provided feed for growing post-larvae. Manipulating the light intensity by shading the tanks with shade-cloth, together with passive seeding (new cells brought in with incoming seawater), was how the diatom film was maintained (Fisheries Research and Development Cooperation, 2002). Therefore care was taken in the amount of shading used during the settlement procedures, because too little cover would have resulted in smothered spat and too much cover would have lead to starving spat, due to lack of diatoms (Fallu, 1991). The level of shading differed between tanks, depending on the thickness of the diatom growth present in each tank and had to be dealt with manually, day by day and tank by tank, for the best results. For example, if the diatom film got too thick, shading was increased to slow diatom growth down and visa versa.

In both summer and winter settlement experiment the diatom genera *Bacillaria* spp., *Grammatophora* spp. (*Fig. 3.5*) and *Navicula* spp. were present in all tanks and for all shade-cloth colours. The different illuminance had no significant effect on these three diatom genera. There was no significant effect shown between the different shade-cloth colours on the presence of *Licmophora* spp., *Nitzschia* spp., *Rhabdonema* spp. and *Striatella* spp. (*Figs 3.10, 3.11 & 3.12*). However, these diatom genera were not found throughout, but only at some stages of both experiments. *Gyrosigma* spp. (*Fig. 3.6*) were identified in all tanks in the summer experiment, though in the winter one they were only

present in tanks covered by either red or black shade-cloth and only in the scrapes taken on day 14. *Chaetoceros* spp. and *Cylindrotheca* spp. were found in tanks from the summer experiment. However, *Chaetoceros* spp. was only identified in scrapes taken on day 28 from blue shade-cloth covered tanks. *Cylindrotheca* spp. on the other hand were recorded in scrapes taken on day 14 from tanks covered by all shade-cloth colours used, except for red. They were also present on day 42 in tanks covered by black shade-cloth. Therefore it seemed that the different illuminance had an effect on *Gyrosigma* spp., *Chaetoceros* spp. and *Cylindrotheca* spp., whether it was significant could, however, not be determined.

In the temperature controlled experiment (*Exp. 3*), the different illuminance and water temperature had no significant effect on the presence of *Grammatophora* spp., *Licmophora* spp., *Nitzschia* spp. or *Navicula* spp. A difference was displayed between red and black shade-cloth, regardless of water temperature for the diatom genus, *Cylindrotheca* spp. This diatom genus was found in scrapes taken on day 14 and 42 for the black shade-cloth, where as for the red shade-cloth it was only recorded on day 14 of the experiment. *Bacillaria* spp., *Cocconeis* spp. and *Striatella* spp. were only in scrapes taken from tanks supplied with heated water. Whereas *Gyrosigma* spp. was only identified in tanks supplied with ambient water and covered by red shade-cloth on day 14. Therefore water temperature seemed to have an effect on these diatom genera. *Rhabdonema* spp. was recorded in tanks covered by either shade-cloth colour and heated water supply. However, only ambient tanks covered by red shade-cloth proved to contain *Rhabdonema* spp. It seemed that illuminance, together with water temperature, had an

effect on *Rhabdonema* spp. *Surirella* spp. (*Fig. 3.13*) was only visible in scrapes taken on day 56 of the temperature controlled experiment, for all shade-cloth colours and water temperatures.

In conclusion there seemed to be no significance between the different diatom colonies found under different illuminance or water temperatures. This will have to be investigated in more detail and the actual ratios of the different diatom genera need to be determined in order to fully understand whether illuminance, together with water temperature, could indeed have an impact on the diatom colonies on the feeder plates used in hatcheries. If so it would enable farmers to have more control over which diatom genera are grown on the settlement plates, ultimately growing the desired diatom species by manipulating illuminance and water temperature. This could mean that the costs of different shade-cloth colours for certain times of the year, together with controlled water temperatures, might be worth while, if it leads to better production rates.

Chapter 4

Effects of illumination and shading on the settlement, survival and growth of *Haliotis midae* larvae and post-larvae.

Introduction

In abalone development, the end of the larval phase and the beginning of the settlement phase, occurs when the larvae cease to swim and settle on the bottom. At this time the abalone are termed spat and start to look like tiny replicas of the adult. Generally, no feeding is required during settlement, therefore no special arrangements need to be made during this stage of development (Fallu, 1991).

Three major transitions can be recognized during post-larval feeding and growth. Firstly, there is the transition from lecithotrophy to particle feeding. Lecithotrophic abalone larvae do not have a functional mouth or digestive tract. However, active feeding begins within a day of the velum being shed, when the mouth forms (Crofts, 1937; Seki *et al.*, 1981; Norman-Boudreau *et al.*, 1986; Ohashi, 1993; Kawamura *et al.*, 1995[a]). In abalone nutrition, there seems to be an overlap between lecithotrophy and early ingestion. The second transition is visible at around 600 - 800 µm shell length. Below this size, post-larvae grow at similar rates regardless of diatom strain, provided they receive an adequate supply of biofilm material. Larger post-larvae, however, experience increased growth on certain diets, such as highly digestible diatoms. Final transition takes place from a biofilm-dominated diet to a macroalgae-

dominated diet. Data from both, natural habitats and hatcheries suggest that this transition happens when the post-larvae are 5 -10 mm in shell length (Kawamura *et al.*, 1998). In aquaculture, abalone are encouraged to settle on special plates, such as plastic plates, with cultures of micro-algae that will ultimately supply the spat with food (Fallu, 1991). Generally both physical and chemical characteristics of substrata play a role in settlement (Genade *et al.*, 1988). By understanding the feeding and growth of young abalone in hatcheries, the growth and survival rates of post-larvae can be improved (Kawamura *et al.*, 1998), which in turn leads to higher production rates.

Micro-algae live and grow on the feeder plates even after settlement, despite the grazing of the spat. The ideal situation would be where the growth of micro-algae exactly balances the amount grazed by the spat, while still having a steady supply of food for the young abalone. In other words, the system must be in equilibrium (*Chapter 3*). However, this balance might shift in favour of too much algal growth or too little. If the micro-algae grows more rapidly than the spat can graze them, the layer of micro-algae will become too deep, resulting in the spat being smothered. This is likely to happen when the spat are still very small, with a shell length of 1 mm or less. On the other hand, if the spat over-graze they will have no micro-algae left for food or, alternatively, they will remove the species of algae they prefer, and other, or less desirable, forms may take over. A successful abalone farmer needs to influence the equilibrium to keep the conditions satisfactory for the growth of spat and there are several ways in which this can be done.

The food consumption of the spat increases with growth. Fine tuning of the equilibrium of spat consumption towards micro-algae growth, has to be undertaken and continued for the duration of the spat stage. There are several techniques which the farmer can use to control this balance. Micro-algae, like other plants, need and utilise light for growth (*Chapter 3*). By shading the feeder plates with shade-cloth, the growth of micro-algae can be slowed down. Newly settled spat eat the least, due to their small size, so at this stage the micro-algal growth needs to be slowed down the most. Experimentation will determine the exact amount and time of shading needed, but it may be adviseable to cover the feeder plates totally for the first week. After that, shading can be progressively decreased (Fallu, 1991).

The main aims for the two experiments explained in this chapter were to examine the effect different illuminance and shading of tanks, by using various shade-cloth colours, would have on the settlement, survival and growth of *Haliotis midae* post-larvae. As well as the effect on the respective pH, degree of oxygen saturation and ambient water temperature, during settlement in both summer and winter experiments. Another aim of these experiments (*discussed in Chapter 3*) was to determine what effect the different illuminance and shading had on the variety of diatom genera found on the feeder plates.

Material and Methods

Two fairly similar settlement experiments were conducted at the Aquafarm Development farm in Hermanus. One of the main differences concentrated on was that the one settlement experiment was done during summer, while the other was

performed during winter. This was the main factor to be investigated in this study. The other factors such as differences in day length, intensity of solar insolation and wavelengths reaching the surface, to name only a few, were considered insignificant as far as the results were concerned. These differences might have an impact on the results from these experiments, but were left for a different study altogether. Both experiments were supplied with ambient seawater. Therefore the main difference investigated for this study was the seawater temperature, due to the different seasons in which the experiments were performed.

The basic methods outlined in Chapter 2 were followed in each of the two experiments. In both summer and winter settlement experiments 65 000 larvae were transferred into each tank. To ensure that approximately the same amount of larvae were transferred into each tank, the larvae containers were stirred before each batch was measured out with a 1L measuring jug. This was done to ensure that the larvae were evenly dispersed throughout the container and to prevent the majority of them sinking to the bottom.

In both experiments four different colours of shade-cloth were used; namely black (control), blue, green and red. Three layers of the same color shade-cloth were used to cover each settlement tank (*Chapter 3*). During the first week all three layers of shade-cloth covered the tanks to prevent the micro-algae from growing too fast and smothering the spat.

One or two of the shade-cloth layers were manually removed according to how thick the micro-algae film was. Some tanks were more exposed than others, because the diatom film needed more light to grow, to provide an adequate food source for the spat. The tanks were checked daily in order to determine which tanks needed less layers and which needed all three layers of shade-cloth. This was done to ensure that the equilibrium was maintained between micro-algae growth and the grazing of juveniles on the micro-algae of the feeder plates. Water quality measurements were taken as outlined in Chapter 2.

The abalone juveniles were removed from each settlement tank after 69 days to determine the average shell length and survival rate (*Chapter 2*). The data were analyzed as explained in Chapter 2. To make it more accessible, the results of the two experiments were divided into firstly the summer experiment's results and secondly the winter experiment's results.

Results

Summer experiment

Figure 4.1 shows the pH values, which were mostly 7.38 - 8.06, just below abalone's optimum pH range, which is 8.0 - 8.3 (Spotte, 1979). There was no significant difference in the average pH values measured between the four shade-cloth colours used in the summer experiment.

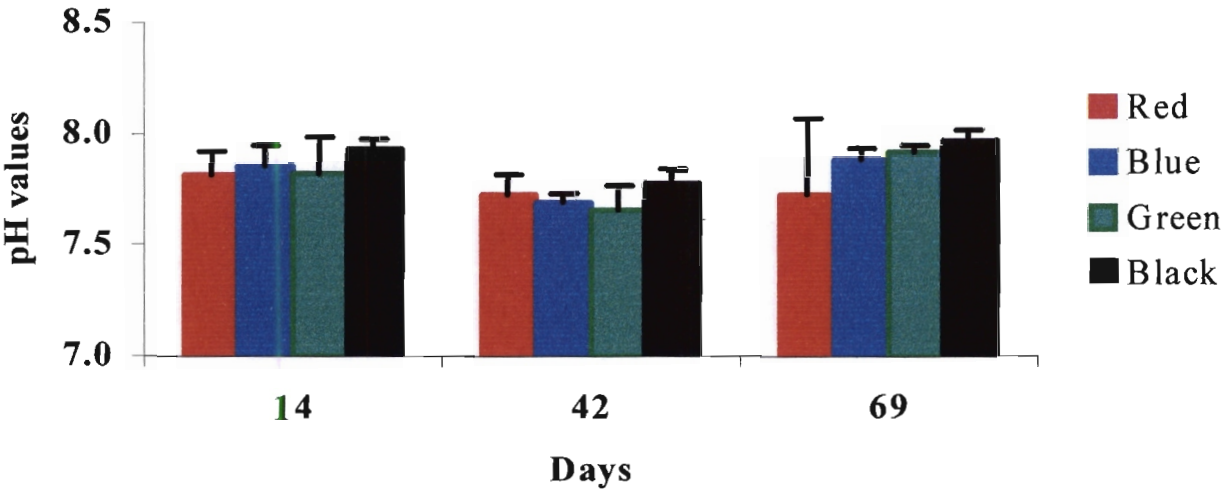


Figure 4.1: Mean pH values measured every ± 27 days during a summer experiment with *Haliotis midae* post-larvae using four shade-cloth colours. Error Bars represent Standard Deviation. *n* = 3 replicates.

Table 4.1 indicates that the summer settlement experiment's ambient seawater temperature was relatively high throughout the experiment. However, 30 days after the start of the experiment, there was a drop from 16.78 ± 0.20 °C (20 days), to 13.53 ± 0.17 °C (30 days) in ambient water temperature. This was due to a change in the weather (summer rain), but did not last very long and the water temperature increased again and remained high for the remainder of the experiment. Except for the sudden drop in water temperature at 30 days, the water temperature seemed to gradually rise from 16.95 ± 0.13 °C (10 days), to 18.58 ± 0.31 °C (69 days) as the experiment progressed.

Day	Black (Mean ± SD)	Blue (Mean ± SD)	Green (Mean ± SD)	Red (Mean ± SD)
10	16.87 ± 0.06	16.97 ± 0.12	17.00 ± 0.17	16.97 ± 0.15
20	16.70 ± 0.10	16.93 ± 0.12	16.60 ± 0.44	16.87 ± 0.15
30	13.47 ± 0.06	13.40 ± 0.10	13.83 ± 0.40	13.40 ± 0.10
40	18.03 ± 0.15	18.10 ± 0.10	18.07 ± 0.12	18.27 ± 0.15
50	18.50 ± 0.10	18.57 ± 0.06	18.77 ± 0.29	18.47 ± 0.12
60	18.80 ± 0.26	18.40 ± 0.26	18.80 ± 0.17	18.67 ± 0.25
69	18.37 ± 0.64	18.37 ± 0.15	18.80 ± 0.30	18.77 ± 0.15

Table 4.1: Mean seawater temperatures (°C) measured every ± 10 days during summer experiment with *Haliotis midae* post-larvae using four shade-cloth colours. *n* = 3 replicates.

Table 4.2 shows that during the summer settlement experiment, the O_2 saturation % values were all high (above 90 %), therefore the degree of O_2 saturation available to the juveniles was sufficient for their growth and survival. It was important to monitor the O_2 levels during the experiment to make sure that juveniles were not lost due to lack of oxygen. There was no significant difference between values found in the average percentages of O_2 saturation between the different shade-cloth colours used in the summer experiment.

Day	Black (Mean ± SD)	Blue (Mean ± SD)	Green (Mean ± SD)	Red (Mean ± SD)
12	97.23 ± 0.78	97.53 ± 0.61	96.90 ± 0.36	97.50 ± 0.70
24	96.83 ± 0.49	96.83 ± 0.76	96.40 ± 0.10	96.43 ± 0.91
36	99.43 ± 0.55	100.00 ± 0.50	99.27 ± 0.65	99.60 ± 1.57
48	97.80 ± 0.87	98.77 ± 1.16	97.23 ± 0.47	99.33 ± 3.35
60	96.57 ± 0.35	97.20 ± 0.95	95.93 ± 0.76	98.93 ± 3.78
69	100.90 ± 1.08	100.50 ± 1.95	101.80 ± 0.87	100.07 ± 2.10

Table 4.2: Mean O_2 saturation values (%) measured every ± 12 days during summer experiment with *Haliotis midae* post-larvae using four shade-cloth colours. $n = 3$ replicates.

The shell length (Fig. 4.2) of 300 juveniles measured from the three tanks covered by red shade-cloth (4.13 - 4.45 mm) were significantly longer in comparison with the other three shade-cloth colours, blue (3.60 - 3.99 mm), black (3.72 - 4.02 mm) and green (3.54 - 3.84 mm). This was an indication that shade-cloth colour, possibly due to the specific light spectrum present, could play an important role in the shell length of juvenile *H. midae*. Figure 4.3 shows that the survival rate of the juveniles during the summer experiment were higher for the tanks covered by blue shade-cloth, in comparison to the other three colours, though not significantly so (p value < 0.10550, $F = 2.09$ and *Fcrit.* = 2.69). During the summer experiment, the number of measurements (*Fig. 4.2*) taken of the shell length is only 200 and 20 for the number of

settlement plates used for the blue shade-cloth (*Fig. 4.3)*, due to the loss of juveniles from one of the settlement tanks. This was taken into consideration when analyzing the results and worked into the standard errors respectively, (16.84 & 0.10 mm) for the blue shade-cloth's survival and shell length average. Therefore, the loss of those juveniles did not compromise the results of the summer experiment in any way.

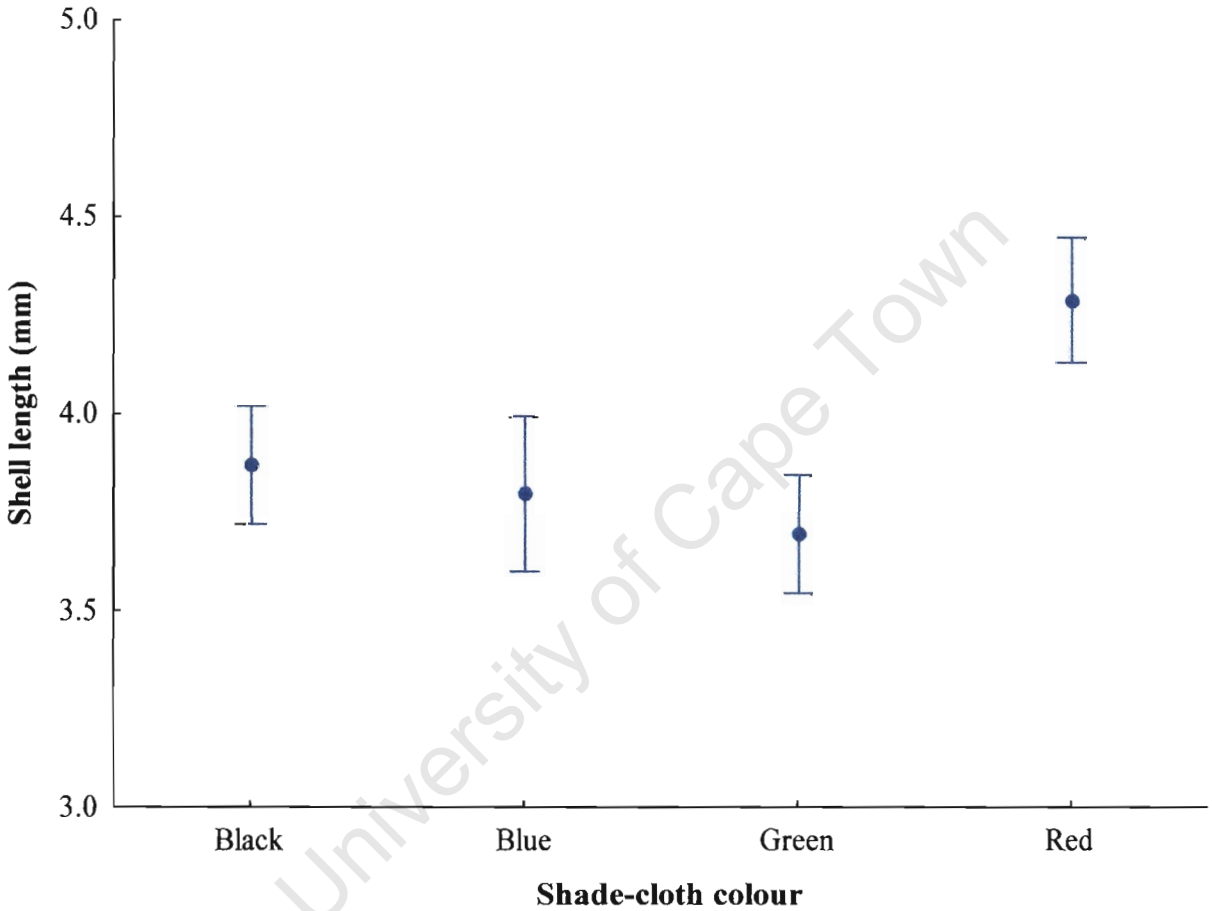


Figure 4.2: Mean juvenile shell length (mm) after 69 days, for four shade-cloth colours used during a summer experiment with *Haliotis midae* post-larvae. Current effect: F (3, 1096) = 10.78, *Fcrit.* = 2.61 and $p < 0.00000$. Vertical bars denote 0.95 confidence intervals. N(Black) = 300, N(Blue) = 200, N(Green) = 300 and N(Red) = 300.

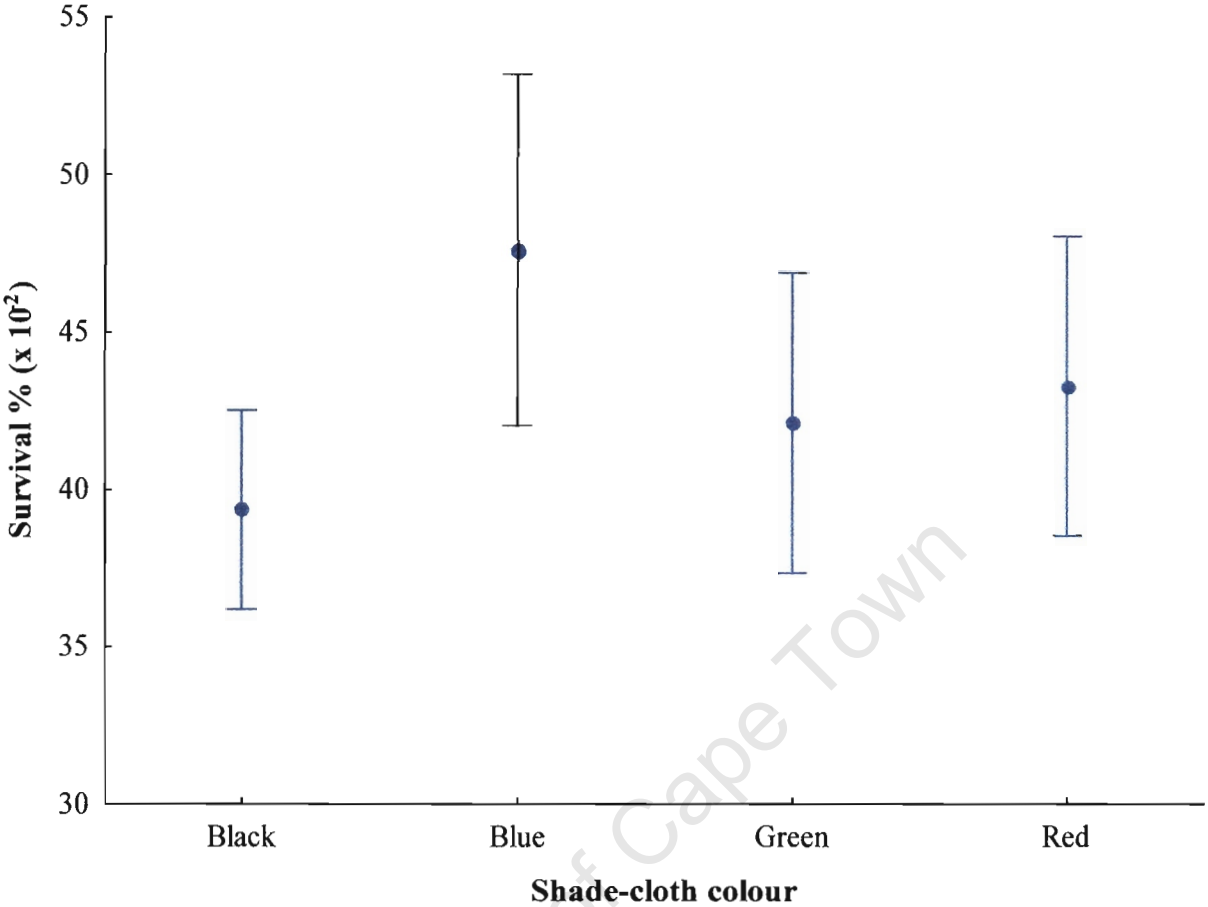


Figure 4.3: Mean juvenile survival (% of animals that survived from initial 65 000 larvae/tank) after 69 days in a summer experiment with *Haliotis midae* post-larvae for four shade-cloth colours. Current effect: $F(3, 106) = 2.09$, *Fcrit.* = 2.69 and $p < 0.10550$ (insignificant). Vertical bars denote 0.95 confidence intervals. N(Black) = 30, N(Blue) = 20, N(Green) = 30 and N(Red) = 30.

Winter experiment

Figure 4.4 shows that the pH values were higher during winter than in summer (*Fig 4.1*). The average pH values (8.12 - 8.25) were within the optimal pH range for abalone, (8.0 - 8.3) according to Spotte (1979). No significant differences in average pH values were noted between the four different shade-cloth colours used, in the winter experiment.

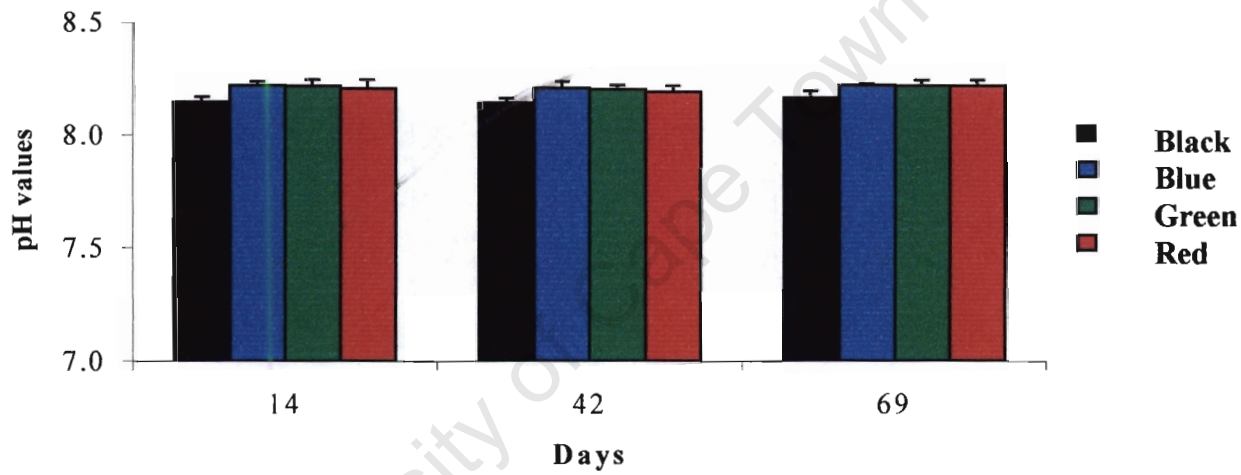


Figure 4.4: Mean pH values measured every ± 27 days during a winter experiment with *Haliotis midae* post-larvae using four shade-cloth colours. Error Bars represent Standard Deviation. *n* = 3 replicates.

Table 4.3 indicates as expected, that the ambient seawater temperatures were lower during the winter settlement experiment than in the summer one (*Table 4.1*). The ambient seawater temperatures fluctuated ~ 3 °C in winter and ~ 6 °C in the summer

experiment, due to the drop in water temperature 30 days into the summer experiment.

Day	Black (Mean ± SD)	Blue (Mean ± SD)	Green (Mean ± SD)	Red (Mean ± SD)
10	14.57 ± 0.06	14.60 ± 0.10	14.63 ± 0.06	14.57 ± 0.15
20	14.17 ± 0.06	14.17 ± 0.15	14.13 ± 0.29	14.60 ± 0.10
30	15.33 ± 0.06	15.30 ± 0.00	15.27 ± 0.12	15.33 ± 0.06
40	17.90 ± 0.17	17.83 ± 0.06	17.70 ± 0.17	17.87 ± 0.06
50	16.27 ± 0.06	16.43 ± 0.06	16.40 ± 0.20	16.20 ± 0.10
60	17.53 ± 0.15	17.00 ± 0.00	17.03 ± 0.15	17.00 ± 0.00
69	15.63 ± 0.15	15.80 ± 0.26	15.80 ± 0.26	16.00 ± 0.17

Table 4.3: Mean seawater temperatures (°C) measured every ± 10 days during a winter experiment with *Haliotis midae* post-larvae for four shade-cloth colours. n = 3 replicates.

Table 4.4 shows that for the greater part of the winter experiment (Days 36 - 69) the O_2 saturation (%) was significantly lower (86.80 ± 1.62 %) respective of shade-cloth colour, than that of the summer experiment (98.96 ± 1.31 %) displayed in Table 4.2. However, the O_2 saturation (%) levels were still above 80 % and therefore high enough too sufficiently sustain the juveniles growth and survival.

Day	Black (Mean ± SD)	Blue (Mean ± SD)	Green (Mean ± SD)	Red (Mean ± SD)
12	95.90 ± 0.95	95.27 ± 1.97	96.37 ± 1.22	96.60 ± 0.56
24	97.57 ± 1.29	97.60 ± 1.40	97.73 ± 2.14	97.43 ± 0.80
36	83.40 ± 2.80	82.83 ± 1.91	83.40 ± 1.21	83.03 ± 1.01
48	82.40 ± 0.56	82.67 ± 1.78	82.90 ± 1.51	83.27 ± 1.29
60	89.8 ± 2.79	83.13 ± 8.37	85.30 ± 0.87	84.53 ± 0.90
69	95.33 ±0.23	95.97 ± 0.29	95.83 ± 0.06	94.97 ± 0.38

Table 4.4: Mean percentage of O_2 saturation measured every ± 12 days during a winter experiment with *Haliotis midae* post-larvae using four shade-cloth colours. n = 3 replicates.

The 300 juveniles measured (*Fig. 4.5*) from the three tanks covered by red shade-cloth were significantly smaller (2.89 - 3.04 mm in shell length), than those from any of the other three shade-cloth colours, blue, black or green (3.17 - 3.59 mm). The juveniles in settlement tanks covered by green shade-cloth had the longest average shell length (3.42 - 3.59 mm), though not significantly longer than the black- or blue-shaded tanks (3.17 - 3.56 mm). They were, however, significantly longer than the juveniles from the red-shaded tanks (*Fig. 4.5*). This was an indication that shade-cloth colour, due to the specific light spectrum present, plays an important role in the growth of juvenile *H. midae*. Fig 4.6 shows that the survival rates of the juveniles during the winter experiment, were highest for the tanks covered by green and red shade-cloth (averaging respectively, 0.43 & 0.42 %) in comparison to the other two

colours, black and blue (averaging respectively, 0.25 & 0.32 %). The survival rates (*Fig. 4.6*) for red and green is significantly higher (p value < 0.00000, F = 14.08 and *Fcrit.* = 2.68). This was an indication that not only shell length was influenced by the light spectra of the different shade-cloth colours, but also the survival rate.

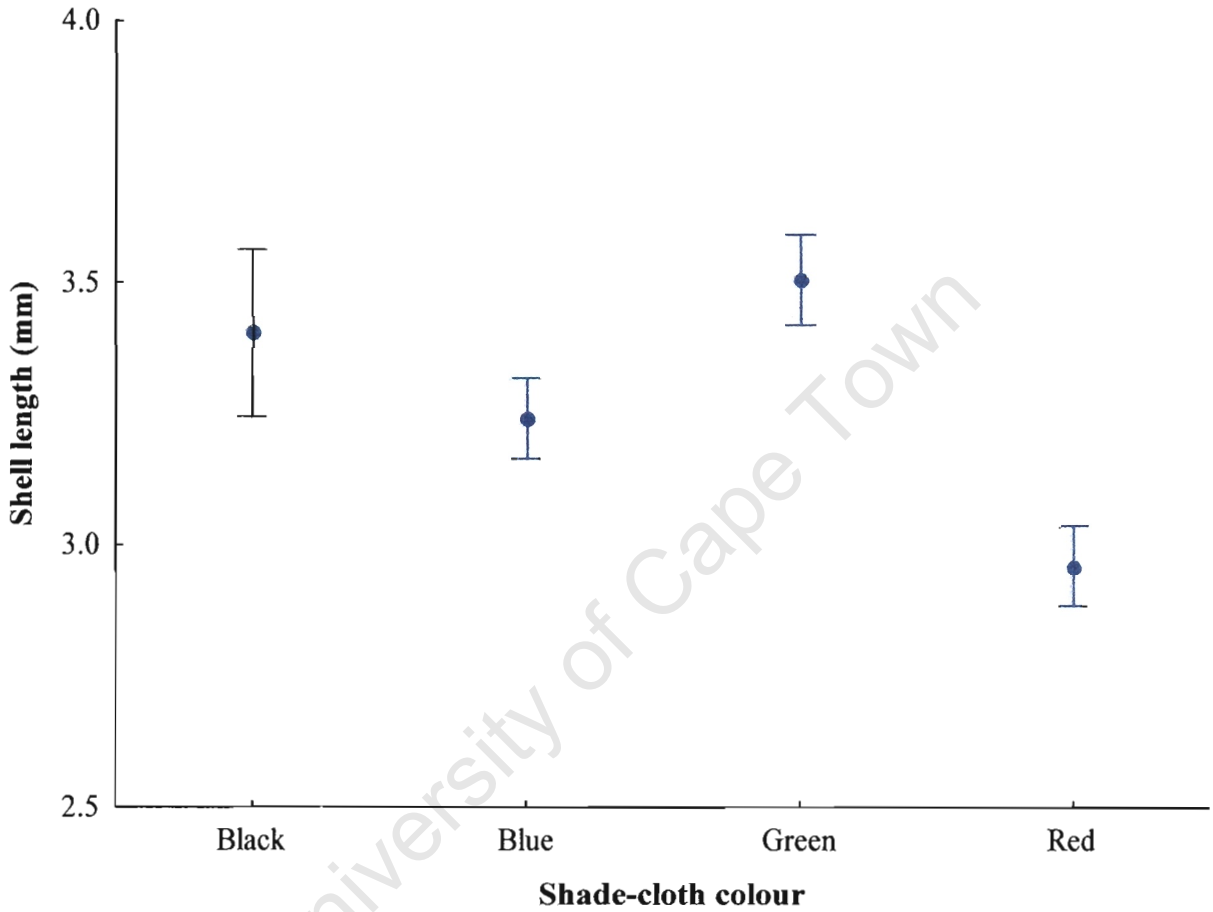


Figure 4.5: Mean juvenile shell length (mm) after 69 days in a winter experiment with *Haliotis midae* post-larvae using four shade-cloth colours. Current effect: F (3, 1196) = 19.87, *Fcrit.* = 2.61 and p < 0.00000. Vertical bars denote 0.95 confidence intervals. N(Black) = 300, N(Blue) = 300, N(Green) = 300 and N(Red) = 300.

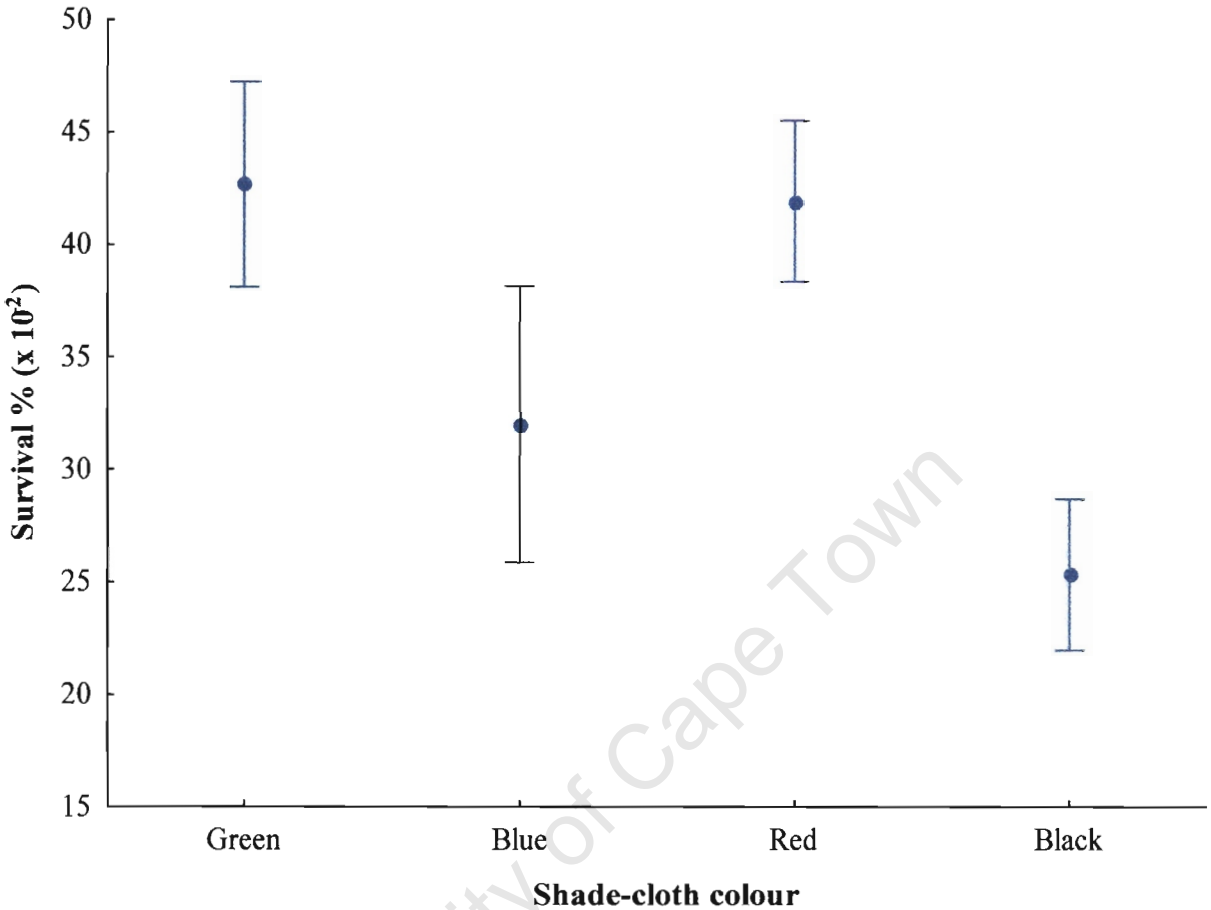


Figure 4.6: Mean juvenile survival (% of animals that survived from initial 65 000 larvae/tank) after 69 days, in a winter experiment with *Haliotis midae* post-larvae using four shade-cloth colours. Current effect: F (3, 116) = 14.08, *Fcrit.* = 2.68 and $p < 0.00000$. Vertical bars denote 0.95 confidence intervals. N(Black) = 30, N(Blue) = 30, N(Green) = 30 and N(Red) = 30.

Discussion

Seawater is alkaline by nature; waste products of degradation processes turn the water more acid. The measuring of the pH values gives an indication of how much degradation has occurred (Fallu, 1991). The optimum pH range for abalone is 8.0 - 8.3, though mature abalone can survive short exposures to an elevated pH of up to 9.3 (Spotte, 1979). The average pH values measured during the summer settlement experiment were significantly lower than those from the winter experiment (*Figs 4.1 & 4.4*). The reason for the increased pH during the winter could be due to lower water temperatures, slowing down the degradation processes, leaving the water more alkaline than in the summer. However, the pH values were suprisingly low in the summer experiment and because this was not repeated in the heated experiment (*Chapter 5*), this could also be ascribed to a malfunctioning pH meter or inaccurate calibration during the summer experiment. It is, however, important to monitor the pH levels in settlement procedures, to try and keep the pH values within the optimal pH range required by abalone.

In general, abalone can withstand any low temperatures that are likely to occur on an abalone farm. High temperatures seem to be more of a problem; excessive temperatures directly stress abalone. Abalone seem to grow faster as temperature increases until their optimum temperature are reached, above which growth will slow down. Temperatures of 2 - 3 °C above their optimum are likely to be fatal (Fallu, 1991).

The ambient water temperature was higher (average of 17.31 ± 1.74 °C) in the summer settlement experiment than in the winter one (average of 15.90 ± 1.23 °C), as expected due to the temperature difference between the two seasons (*Tables 4.1 & 4.3*). However, there was no significant difference in the average water temperature measurements between the four different shade-cloth colours (*Tables 4.1 & 4.3*). Water temperature, however, seemed to play a role where settlement and survival of *Haliotis midae* were concerned, because the survival (%) rates were over-all higher in the summer experiment (*Fig. 4.3*), in comparison to the winter one (*Fig. 4.6*).

According to Fallu (1991), abalone require the availability of very high concentrations of oxygen in their environment. In both the winter and summer settlement experiments, the O_2 saturation percentages were high (*Tables 4.2 & 4.4*). During the summer experiment the degree of O_2 saturation were above 90 % (*Table 4.2*), and for the winter experiment they remained above 80 % (*Table 4.4*), therefore the amount of O_2 available to the juveniles was sufficient for their growth and survival. It is important to monitor the O_2 levels during settlement procedures to ensure that juveniles have the required O_2 available and are not lost due to lack of oxygen.

Hahn (1989) and Fallu (1991) have reviewed studies on abalone aquaculture. Feeding requirements have been shown to change as abalone grow, as seen in feeding experiments of post-larvae on species of benthic diatoms (Kawamura *et al.*, 1992, 1995[a]; 1995[b]; Matthews *et al.*, 1995; Kawamura, 1996). Post-larvae grow more rapidly two to three weeks after settlement, when they become more responsive to the "digestibility" of the diatom strains (Kawamura *et al.*, 1998).

In the summer settlement experiment, the shell length varied between 3.54 - 4.45 mm (*Fig. 4.2*), whereas in comparison the winter experiment's shell length varied between 2.89 - 3.59 mm (*Fig. 4.5)*). According to these values it seemed that the shell had grown more in length during the same 69-day time period, in the summer than in the winter. Therefore it would be safe to suggest that an elevated water temperature (average of 17.31 ± 1.74) had a positive effect on the shell length of *H. midae* post-larvae during the settlement experiment.

In the summer experiment, the shell length of juveniles from tanks covered with red shade-cloth were significantly longer than those of juveniles from tanks covered with other three shade-cloth colours (*Fig. 4.2*). In the winter experiment, however, the shell length of juveniles from tanks covered by red shade-cloth were significantly shorter than those of juveniles from tanks covered with other three shade-cloth colours (*Fig. 4.5*). In the winter experiment the tanks covered by green shade-cloth, produced juveniles with the longest average shell length measured, and these were significantly longer than the averages from the red and blue shade-cloth covered tanks.

Therefore, it seemed that red shade-cloth produced better growth during the summer, but it was not advisable for use during the winter. This would mean abalone farms would need to stock more than one colour shade-cloth, one to be used in summer and one for winter, which increases the cost to a farm and is therefore not ideal.

A critical step in abalone culture is the induction of settlement. To maintain a larval survival rate of greater than 95 %, from fertilization to veligers competent to settle, is fairly easy to achieve (Hahn, 1989). Settlement, however, is very unpredictable and larval settlement and post-larval survival rates can be very low, only one to two percent of the larva might make the transition from planktonic veliger to benthic juvenile (Hahn, 1989; Fisheries Research and Development Cooperation, 2002). Because of the ever-increasing demand for abalone, this low survival rate poses a problem for commercial farmers. Therefore, any research that can obtain and maintain consistently higher survival in abalone settlement will have a great impact on the abalone production on farms. Higher survival rates will allow greater efficiency, which in turn equals lower cost per animal (Hahn, 1989).

In the winter settlement experiment, the survival percentages of the abalone juveniles from tanks covered by red and green shade-cloth, were significantly higher than that of the ones from the blue and black shade-cloth covered tanks (p value < 0.00000, F = 14.08 and *Fcrit.* = 2.68). No significance was found in the survival percentages of juveniles from the summer experiment between the different shade-cloth colours used to cover the tanks (p value < 0.10550, F = 2.09 and *Fcrit.* = 2.69). However, when comparing the actual number of animals counted for each shade-cloth colour in both the summer and winter experiment, it was found that the juveniles counted in both experiments were approximately similar in number for the green and red shade-cloth (*Figs 4.3 & 4.6*). However, the black and blue shade-cloth covered tanks numbers of juveniles counted in both experiments, varied considerably (*Figs 4.3 & 4.6*). The

black and blue covered tanks in the summer experiment produced more animals at the end of the 69-day period of the experiment than the winter experiment.

Therefore it appeared as if the red and green shade-cloth, when used as shading for juvenile abalone in settlement procedures would have approximately similar survival rates for each colour, in either winter or summer settlement procedures. While black and blue shade-cloth seemed to produce higher survival rates in summer compared to winter. These results were, however, restricted to using approximately the same initial amount of larvae in each tank for each season (winter & summer) and ambient seawater. Therefore where survival was concerned this suggested that either, red or green shade-cloth were better and constant all year round. In conclusion it seemed that red shade-cloth could make an impact on commercial farming, but the costs will determine if it is feasible to switch to a different colour shade-cloth. More studies will have to be initiated to give conclusive evidence in this matter. It was evident that illumination and shading had an effect on the settlement, survival and shell length of juvenile abalone, *H. midae*. Water temperature was also considered a key player in the findings of these two experiments. Therefore the main aims of these two experiments were achieved, though not conclusively. The subject of the illuminance and shading of settlement tanks, using shade-cloth, or more precisely, shade-cloth colour and its effect on abalone post-larvae, need to be investigated in more detail. The effect of water temperature on the abalone larvae and juveniles need to be studied further as well.

Chapter 5

Effects of illumination, shading and temperature on the settlement, survival and growth of *Haliotis midae* larvae and post-larvae.

Introduction

Species in the genus *Haliotis* have different temperature preferences. This is highlighted by the fact that abalone exist in different latitudes and oceans (Lindberg, 1992). Even sub-populations of the same species occupy different temperature regimes (Newman, 1969). For example larger individuals of *H. diversicolor* have higher optimal temperature ranges than smaller abalone (Chen, 1984). An important consequence of elevated seawater temperatures is the associated increase in the degree of oxygen consumption (Sagara *et al.*, 1971). This has important implications for any potential abalone culture operations.

Traditional studies on abalone have emphasized the critical importance of seawater temperatures in regulating and influencing reproductive cycles and spawning (Thorson, 1950). The importance of temperature in haliotid reproduction appears to vary among the various species. For example, in *H. cracherodii* maximal gonadal growth occurs during the summer months. Gonad size and initiation of gametogenises, however, showed no apparent relation to changes in seasonal water temperature. Gametogenises for *H. cracherodii* is initiated independently of water temperature. Completion of

gametogenises only results in spawning when a specific temperature threshold is reached (Webber *et al.*, 1969).

H. midae is the largest of six South African abalone species (Branch *et al.*, 1994) and the only South African species to be commercially exploited (Muller, 1986) (*see Chapter 1*). For *H. midae* reproductive changes relate closely with seawater temperature changes. The relatively low temperatures on the West coast of South Africa do not rise sufficiently to promote the intense spawning which occurs at Stony Point, on the Southeast coast, where annual temperature fluctuations are large (Newman, 1967).

Seawater temperatures also play an important role in the early life stages of abalone. Fertilization success of abalone gametes is dependent on temperature (Ebert *et al.*, 1983). The timing of hatch-out varies between *Haliotis* species and with temperature. For haliotid egg and larval stages, the species-specific optimal temperature varies by 1 - 2 °C (Leighton, 1974). High temperatures terminate larval development, while low temperatures slow it down. A sub-optimal temperature would increase the probability of mortality by predation, due to a longer planktonic larval phase (Underwood, 1979). This, however, poses no threat in culture, due to the lack of such predators. Rate of metamorphosis is also directly related to seawater temperature, where warmer water induces accelerated metamorphosis (Cuthbertson, 1978). In *H. discus hannai*, larval metamorphosis takes place at 20 °C (Seki, 1980).

In this chapter a temperature-controlled experiment was initiated at Aquafarm Development in Hermanus. All the other environmental aspects, such as the pH, degree of oxygen saturation (*Chapter 2*) and diatoms present with settlement (*discussed in Chapter 3*), were important factors that were monitored with this settlement experiment. The temperature was controlled by the use of a heated watertank to supply heated seawater to specific settlement tanks. This was done to determine what effect elevated seawater temperature would have on the settlement, growth and survival of *H. midae* juveniles.

Material and Methods

A settlement experiment was conducted, at Aquafarm Development in Hermanus, using the same 12 tanks described in Chapter 2. Six of the 12 tanks received heated seawater and the other six ambient seawater. The heated seawater was supplied from a heating tank, equiped with a 1KW glass and a 3 KW titanium heating element (*Fig 5.1*). Ambient seawater was pumped directly into the heating tank where it was heated to approximately 4 °C above ambient. The temperature level was controlled, so that the heated seawater remained approximately 4 °C above ambient.

Only two colours shade-cloth were used in this experiment, namely black (control) and red. Thus, three of each six tanks for both the ambient and heated part of the settlement experiment were covered with either, red or black shade-cloth. The shade-cloth colour for each specific tank was randomly chosen. As in Chapter 4, three layers of shade-cloth

were used for each tank, in order to manually control the diatom growth in the tanks and on the plates.

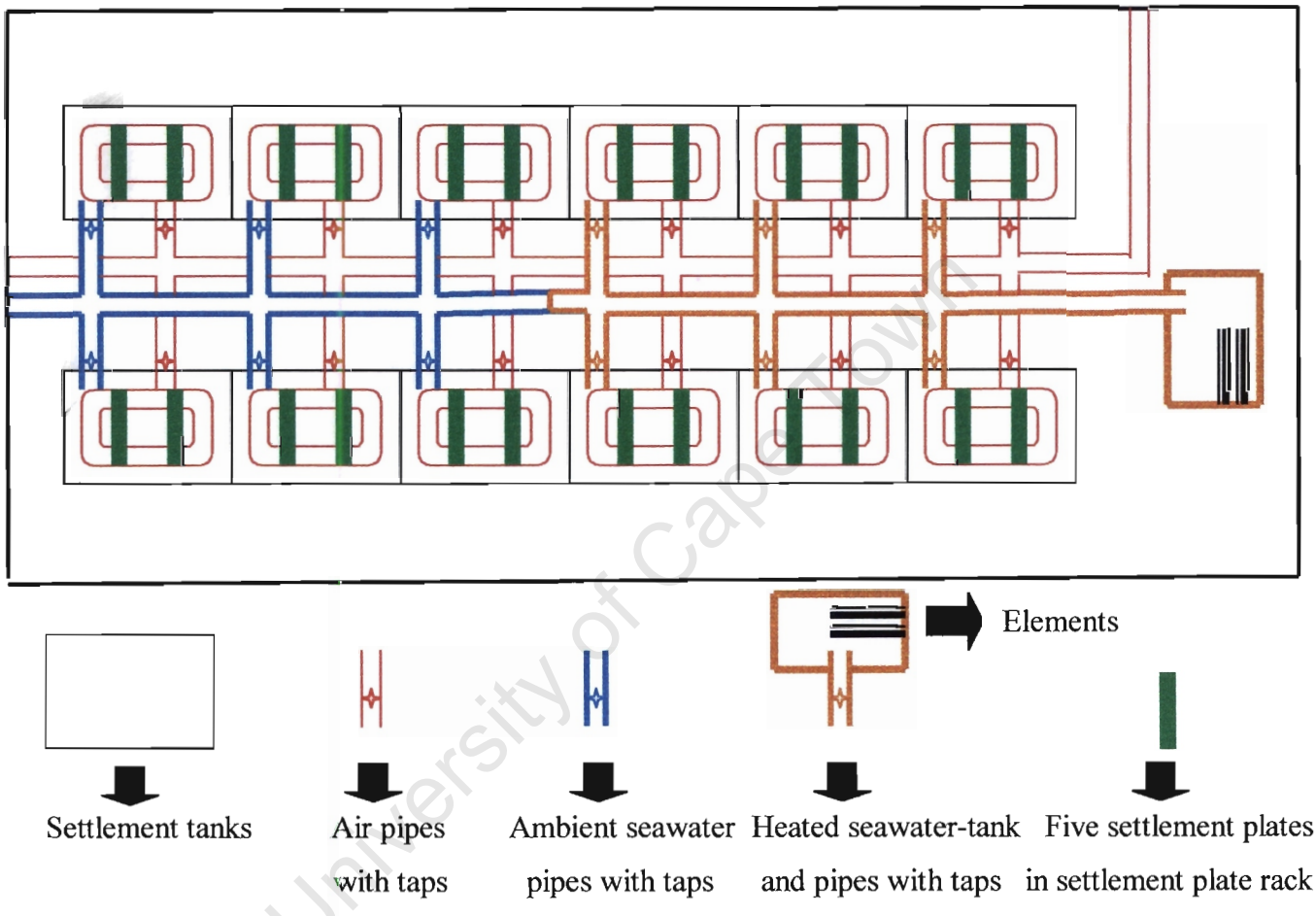


Figure 5.1: Arrangement of air pipes, water pipes (either supplying heated or ambient seawater) and settlement plates in and/or between the tanks.

Basic methods as outlined in Chapter 2 were followed in this experiment. Equal amounts of 90 000 larvae were transferred into each tank. The method used to ensure that each

tank had equal amounts of larvae is outlined in Chapter 4. The abalone juveniles were removed from the settlement tanks after 69 days and the average shell length and survival rates determined (*Chapter 2*). The data from this settlement experiment were analyzed as outlined in Chapter 2.

Results

Table 5.1, shows that the pH values (8.11 - 8.31), were within abalone's optimum pH range, which is 8.0 - 8.3 (Spotte, 1979). There was no significant difference in pH between the two shade-cloth colours or the different water temperatures used in this settlement experiment.

	Heated		**Ambient**	
Day	**Red (Mean ± SD)**	**Black (Mean ± SD)**	**Red (Mean ± SD)**	**Black (Mean ± SD)**
14	8.16 ± 0.03	8.15 ± 0.04	8.18 ± 0.01	8.18 ± 0.01
42	8.14 ± 0.01	8.17 ± 0.01	8.17 ± 0.01	8.18 ± 0.01
69	8.20 ± 0.02	8.24 ± 0.07	8.19 ± 0.00	8.21 ± 0.03

Table 5.1: Mean pH values measured every ± 27 days, starting 14 days into a experiment with *Haliotis midae* post-larvae using red and black shade-cloth on tanks supplied with either heated or ambient seawater. $n = 3$ replicates.

Figure 5.2 shows that the water temperature of tanks supplied with heated seawater were significantly higher than in tanks supplied with ambient seawater. The heated tanks had a mean temperature of 19.32 ± 0.96 °C, while the temperature in the ambient tanks was 14.73 ± 0.84 °C. Shade-cloth colour had no significant effect on the water temperature in either the heated or the ambient part of the experiment.

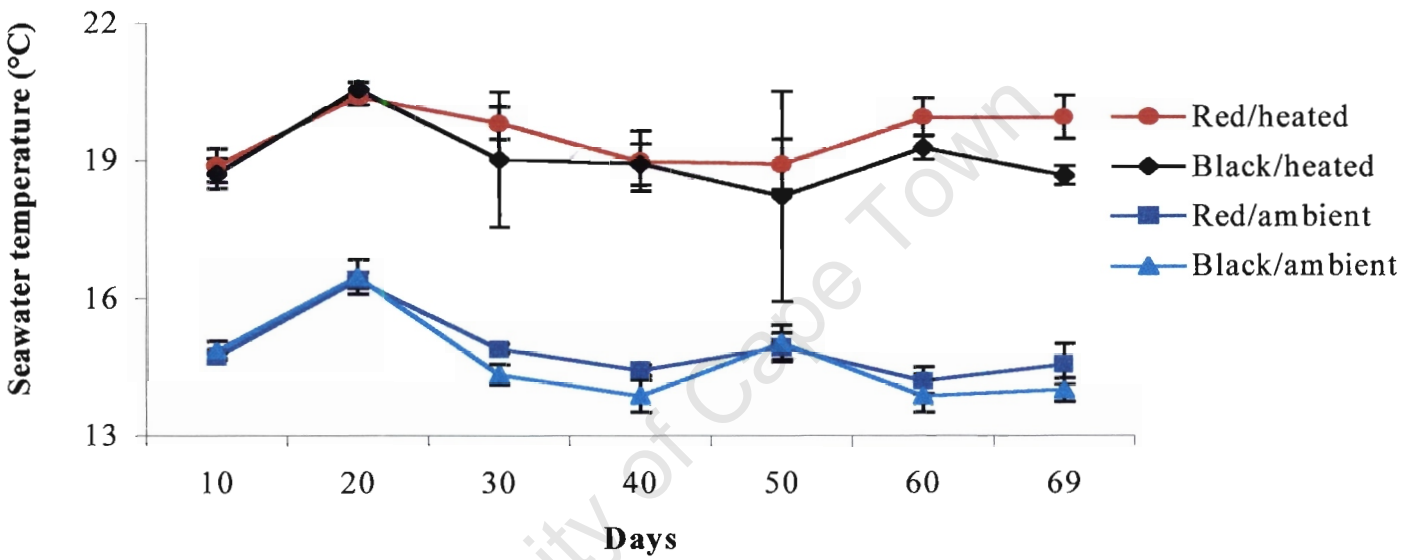


Figure 5.2: Mean seawater temperatures (°C) measured every ± 10 days during a experiment with *Haliotis midae* post-larvae using red and black shade-cloth on tanks supplied with either heated or ambient seawater. Error Bars represent Standard deviation. *n* = 3 replicates.

Fig. 5.3 shows that in all replicates, the percentages O_2 saturation were high (above 92 %). No significant differences in the degree of O_2 saturation were displayed between the two shade-cloth colours used, or between the heated and ambient part of the experiment.

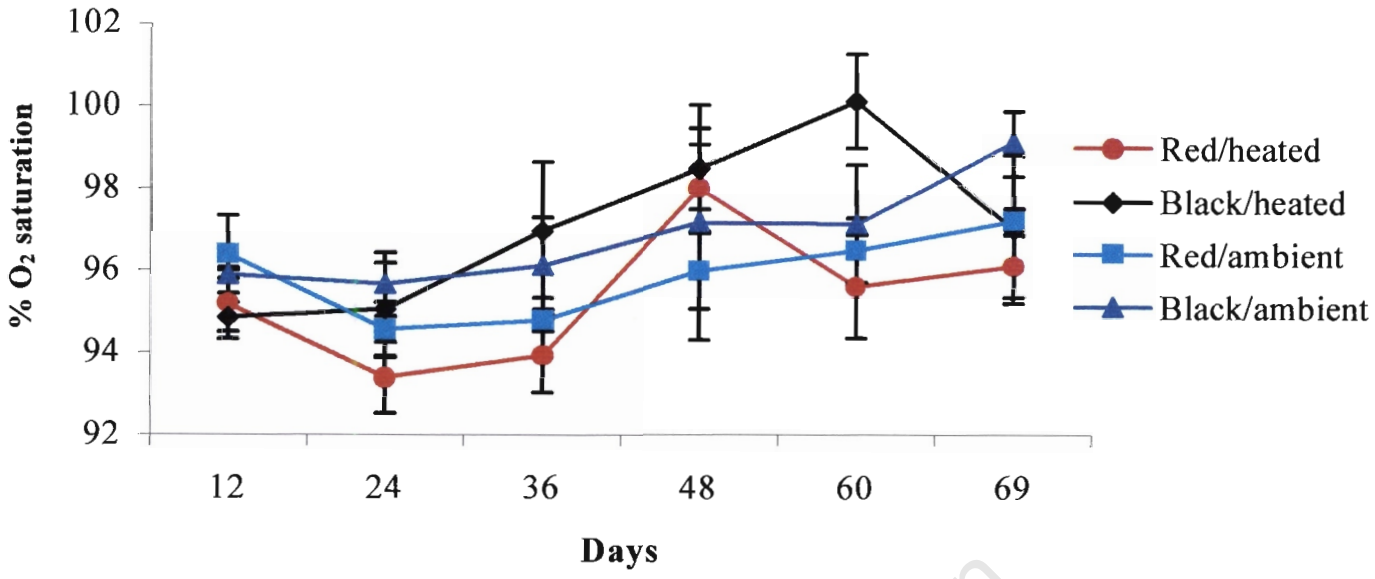


Figure 5.3: Mean O_2 saturation (%) measured every ± 12 days in a experiment with *Haliotis midae* post-larvae using red and black shade-cloth on tanks supplied with either heated or ambient seawater. Error Bars represent Standard Deviation. *n* = 3 replicates.

After 69 days the shell lengths of juveniles measured from the tanks receiving heated seawater were significantly longer in comparison to those receiving ambient water (*Fig. 5.4*). Furthermore the shell length of the juveniles measured from tanks covered by red shade-cloth were significantly longer than those from the black shade-cloth covered tanks, receiving heated water (*Fig 5.4*).

Figure 5.5 shows that the red shade-cloth covered tanks from the heated part of the experiment produced a significantly higher survival rate (%), in comparison to those of the other replicates in the experiment (black/heated; red/ambient or black/ambient).

There was also a significant difference in the survival rates between the black shade-cloth covered tanks from the heated and ambient part of the experiment (*Fig. 5.5*). Only 200 measurements were taken (*Fig. 5.4*) and 20 plates were counted (*Fig. 5.5*) for tanks supplied with heated water and covered by black shade-cloth, this was due to the loss of one of these specific replicates during the course of the experiment.

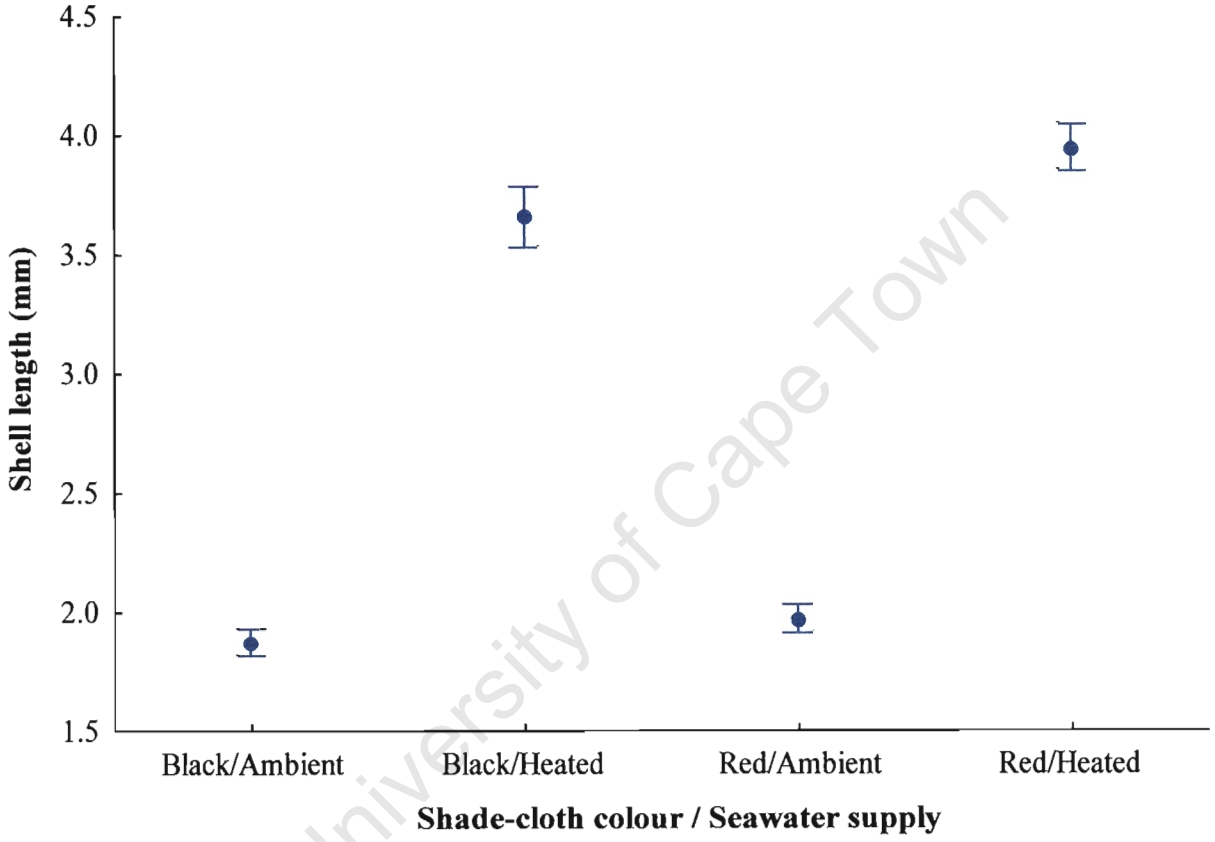


Figure 5.4: Mean juvenile shell length (mm) in a experiment with *Haliotis midae* post-larvae using red and black shade-cloth on tanks supplied with either heated or ambient seawater. Current effect: F (3, 1096) = 695.16, *Fcrit.* = 2.61 and $p < 0.00000$. Vertical bars denote 0.95 confidence intervals. N(Black/heated) = 200, N(Black/ambient) = 300, N(Red/heated) = 300 and N(Red/ambient) = 300.

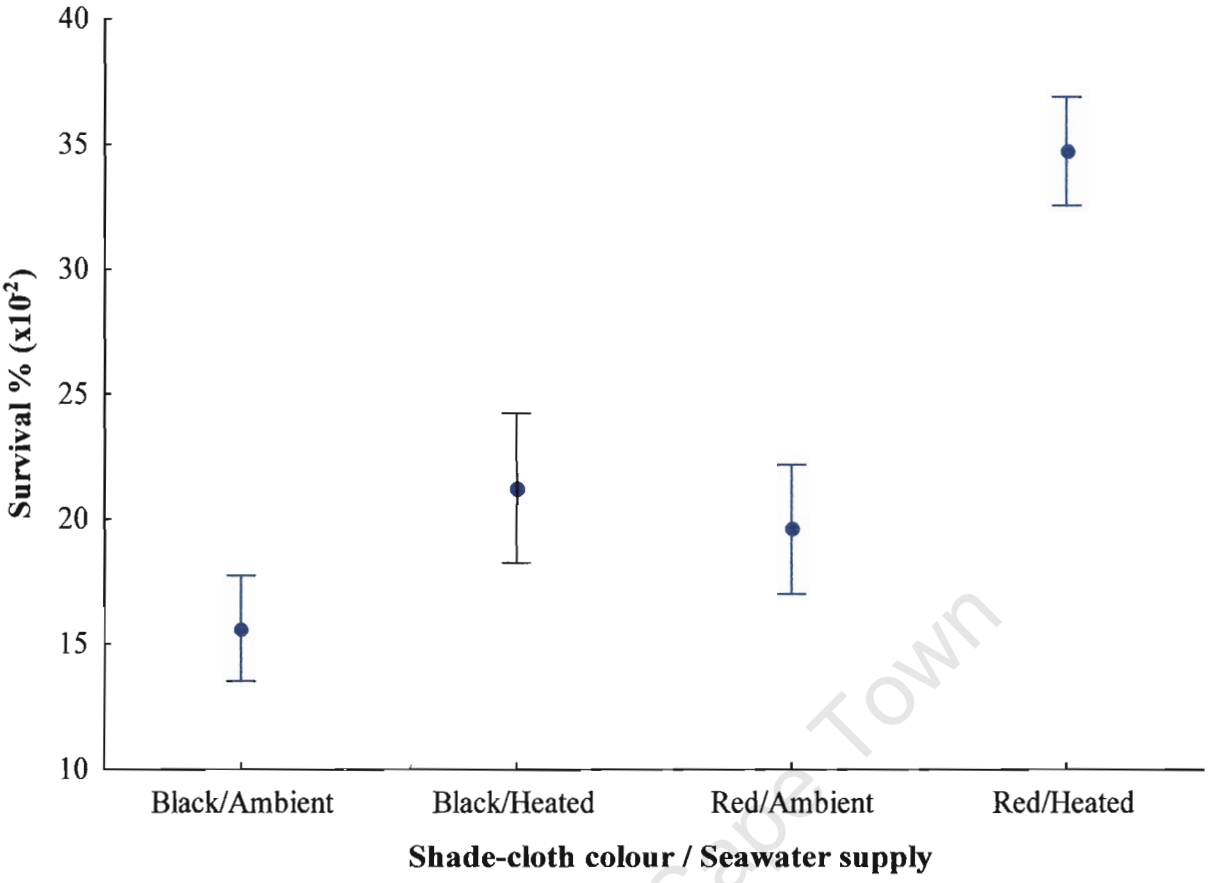


Figure 5.5: Mean juvenile survival (% of animals that survived from initial 90 000 larvae/tank) in a experiment with *Haliotis midae* post-larvae using red and black shade-cloth on tanks supplied with either heated or ambient seawater. Current effect: F (3, 106) = 53.61, *Fcrit.* = 2.69 $p < 0.00000$. Vertical bars denote 0.95 confidence intervals. N(Black/heated) = 20, N(Black/ambient) = 30, N(Red/heated) = 30 and N(Red/ambient) = 30.

Discussion

Seawater is alkaline by nature (*Chapter 4*). The average pH values (8.11 - 8.31) measured during the settlement experiment were within the optimum pH range (*Chapter 4*) for abalone (*Table 5.1*). It is important to monitor the pH levels in settlement procedures, to try and keep the pH values within the optimal pH range required by abalone.

Depending on their place of origin, different abalone species have different optimum temperatures. The red abalone (*Haliotis rufescens*) from the cold Californian waters performs best at around 15 °C. Other abalone prefer warmer water, i.e. the small abalone (*H. diversicolor*) does well at 21 °C. Determining the ideal temperature for a given species can be achieved by examining the temperature regime of the area in which it naturally occurs (Fallu, 1991). Fluctuation in temperature is not beneficial, especially for young abalone. Therefore, if abalone are to be kept in small containers, it is advisable to have them indoors (Fallu, 1991).

The water temperature (*Fig. 5.2*) in the ambient part of the settlement experiment was lower (average of 14.73 ± 0.84 °C) in comparison to the heated part (average of 19.32 ± 0.96 °C) as expected. However, there was no significant difference in the average water temperatures between the two shade-cloth colours used (*Fig 5.2*). Therefor shade-cloth colour seemed to play no significant role towards increasing or decreasing the temperature of the water.

According to Fallu (1991), abalone requires very high concentrations of oxygen in their environment. In the settlement experiment with black and red shade-cloth and tanks supplied with either heated or ambient seawater, the O_2 saturation percentages (*Fig. 5.3*) were high, above 92 %. Therefore the degree of O_2 available to the juveniles were sufficient for their growth and survival. There were, however, no significant differences displayed between the two shade-cloth colours used, or between the two water temperature ranges used. It is important to monitor the O_2 levels during settlement procedures to ensure that juveniles have the required O_2 available and are not lost due to lack of oxygen.

In this experiment the shell length of juveniles measured from tanks supplied by heated water, varied between 3.54 - 4.06 mm (*Fig. 5.4*), in comparison to those supplied with ambient water which varied between 1.82 - 2.04 mm (*Fig. 5.4*). These values suggest that the growth was higher in the heated, rather than the ambient tanks. Therefore it appears that the elevated water temperature of ~ 4°C, above ambient had a positive effect on the growth of *H. midae* post-larvae.

In the experiment the shell length of juveniles from tanks receiving heated water and covered with red shade-cloth, were significantly longer than those from tanks covered with black shade-cloth (*Fig. 5.4*). In the ambient part of the experiment, however, there was no significant difference between the two shade-cloth colours (*Fig. 5.4*). Therefore, where growth of juvenile *H. midae* was concerned, it seemed that red shade-cloth, together with heated seawater, provided the best growth performance.

In the heated part of the settlement experiment, the survival rates of the abalone juveniles from tanks covered with red shade-cloth, were significantly higher than those from the black shade-cloth covered heated tanks. Furthermore the survival rates (*Fig 5.5*) of the juveniles from the red shade-cloth covered heated tanks were significantly higher than either the red or black shade-cloth covered tanks from the ambient part of the experiment (p value < 0.00000, F = 53.61 and *Fcrit.* = 2.69). Therefore where survival rates were concerned, red covered tanks from the heated seawater tanks produced more animals (higher survival rate) at the end of the 69-day period of the experiment than the other tanks (*Fig. 5.5*).

In conclusion it seemed that red shade-cloth together with tanks supplied by heated seawater (~ 4°C, above ambient) could make an impact on commercial farming, but the costs will determine whether it is feasible to switch to a different colour shade-cloth and tanks supplied with heated seawater. More studies will have to be done to give conclusive evidence regarding this subject.

It was evident that illumination and shading had an effect on the settlement, survival and growth of juvenile *H. midae*. Water temperature was also definitely considered a key player in the findings of this experiment. Therefore the main aims of this settlement experiment were achieved, though not conclusively. The subject of the illuminance and shading of settlement tanks, using shade-cloth, or more precisely, shade-cloth colour and its effect on abalone post-larvae, need to be investigated in more detail. Furthermore the effect of water temperature on the abalone larvae and juveniles requires further study.

Chapter 6

Conclusion

Illumination and shading

Illuminance and shading seemed to have little effect on the diatom colonies growing on the settlement plates during and after the settlement of *Haliotis midae* post-larvae (*Chapter 3*). Further study on the ratios of the different diatom genera should be done to determine if the above conclusion is indeed correct.

The pH and degree of O_2 saturation were not affected significantly by the illumination and shading of the settlement tanks during and after the settlement of *H. midae* post-larvae (*Chapters 4 & 5*). It is however important to monitor these levels during settlement procedures to ensure that pH levels are within abalone's optimum pH range of 8.0 - 8.3 (Spotte, 1979) and that O_2 levels are sufficient for larval and post-larval growth and survival. The illumination and shading had no significant effect on the water temperature measured every 10 days of each settlement experiment with *H. midae* post-larvae (*Chapter 4*).

The growth of the *H. midae* juveniles were, however influenced by the illumination and shading of the settlement tanks (*Chapters 4 & 5*). In the summer settlement experiment the shell length of juveniles from tanks covered by red shade-cloth were significantly

longer than those from the other tanks (*Fig. 4.2*). However, in the winter experiment the opposite was true, the juveniles from tanks covered by red shade-cloth were smaller than those from the other tanks (*Fig. 4.5*). The green shade-cloth covered tanks produced the biggest juveniles in the winter experiment. In conclusion it seemed that when red shade-cloth was used in the summer experiment it had a significant influence on the growth of *H. midae* juveniles (*Fig. 4.2*). In winter, however, green shade-cloth seemed to result in maximum juvenile growth (*Fig. 4.5*). The costs of such procedures will determine if it is feasible to be used in commercial farming. The apparent value of red shade-cloth in summer but not winter could be explained by the sun angles. Low sun angles create the red colours of sunset and sunrise because light passes through a greater length of atmosphere before reaching the earth or it's clouds, enriching the red wavelengths at the expense of other colours of light. Therefore, in summer the proportion of red wavelengths may be reduced relative to winter by the higher sun angles.

There was no significant difference between the survival rates of juveniles from different shade-cloth covered tanks in the summer experiment (*Fig. 4.3*). In the winter experiment, however, the illumination and shading of the tanks made a significant difference in the juvenile survival rate. The red and green covered tanks had a significantly higher survival rate than those of the other two shade-cloth colours in the winter experiment (*Fig. 4.6*). Therefore green shade-cloth seem to be the colour of choice for the winter where settlement, survival and growth are concerned, but as mentioned earlier the costs associated with this will have to be determined. It would have been interesting to compare the juvenile survival rate achieved in these experiments with

the actual survival rate achieved by the abalone farm, but because such information is confidential it wasn't possible to do so. According to Knittex (Cape Town) the current cost of red or green shade-cloth is R 37.50 (vat excl.) per meter (2003). Abalone farms will therefore need to determine if the improved growth and survival of abalone under the suggested shading equals or exceeds the cost of buying these shade-cloth colours. This needs to be investigated to determine if this is a viable option, before deciding to undertake this change.

Water temperature

Water temperature (± 14 - 20 °C) had no significant effect on the different diatom colonies growing on the settlement plates during and after the settlement of *Haliotis midae* post-larvae (*Chapter 3*). More detailed study needs to be initiated to ultimately prove or disprove this observation. Water temperature seemed to have an effect on the mean pH values during and after settlement of *H. midae* post-larvae (*Chapters 4 & 5*). In the summer (17.31 ± 1 74 °C) settlement experiment (*Fig. 4.1*) the mean pH values were significantly lower than those measured during the winter (15.90 ± 1.23 °C) experiment (*Fig. 4.4*). This could be due to the higher temperature in summer that sped up degradation processes and therefore left the naturally alkaline seawater, more acid (Fallu, 1991). However in the elevated temperature experiment with *H. midae* post-larvae, there was no significant difference between the pH levels of the tanks receiving either ambient (14.73 ± 0.84 °C) or heated (19.32 ± 0.96 °C) seawater. Therefor the lower pH levels in the summer experiment could also be due to inaccurate calibration of the pH meter. Water temperature also had no significant effect on the degree of O_2 saturation measured

in the summer (*Table 4.2*), winter (*Table 4.4*) or elevated temperature (*Fig. 5.3*) settlement experiments (*Chapters 4 & 5*).

The growth of juvenile *H. midae* was significantly better during the summer experiment (*Fig. 4.2*) than in the winter experiment (*Fig. 4.5*). In the elevated temperature experiment the juveniles were significantly bigger from the tanks that received heated seawater in comparison with those from tanks supplied with ambient water (*Fig. 5.4*).

Water temperature had a significant effect on the survival rate of *H. midae* post-larvae during and after settlement. Survival rates were higher in summer (*Fig. 4.3*) than in winter (*Fig. 4.6*). In the elevated temperature experiment, the juvenile survival rate was significantly higher in the tanks supplied with heated seawater in comparison to those from tanks receiving ambient water (*Fig. 5.5*). Therefore it seemed that elevated seawater of between 17 - 20 °C led to increased growth and survival of *H. midae* post-larvae during and after settlement.

To conclude, experiments showed that red shade-cloth together with elevated seawater temperature could make a big impact on the settlement, growth and survival of *H. midae* post-larvae in hatcheries. Whether this is cost-effective will have to be determined with further study. Regarding water temperature, other possibilities for commercial abalone farm management to concider is to include spawning schedules to work with summer seawater temperatures as opposed to elevating the seawater temperature. Future research into the settlement, growth and survival with different illumination, shading and water

temperature needs to be conducted in order to establish where the optimal illuminance and water temperature exists for *H. midae* post-larvae and juveniles. In order to study this further and resolve some of the inconclusive patterns found in this study, quantification of the actual light spectrum under various shade-cloth colours would be recommended as a starting point.

Chapter 7

References

Aquatic-Plants. 2002. Color of light and color of plant leaves. Aquatic-Plants. http://fins.actwin.com/aquatic-plants/month.200209/msg00387.html. 15 December.

Asada R., Okuno M., Tazaki K. 2002. Structural anisotropy of biogenic silica in pennate diatoms under Fourier transform polarized infrared microspectroscopy. Department of Earth Sciences, Faculty of Science, Graduate School of Natural Science and Technology, Kanazawa University. Japan. http://wwwsoc.nii.ac.jp/jampeg/jmps97-5.htm. 15 December.

ATJ's Marine Aquarium Site. 2002. Photosynthesis. ATJ's Marine Aquarium Site. http://www.petsforum.com/personal/trevor-jones/photosynthesis.html. 17 November.

Avery G. 1974. Open station shell midden sites and associated features from Pearly Beach area, South-Western Cape South Africa. *Archaeol. Bull.* 29: 104 - 114.

Fisheries Research and Development Cooperation. 2002. Background. http://www.bluestoneit.com/abalone/background.html. 15 December.

Bardach J. E., Ryther J. H., McLarney W. O. 1972. Aquaculture. Wiley-Interscience. New York.

Barkai R., Griffiths C. L. 1986. Consumption, absorption efficiency, respiration and excretion in South African abalone *Haliotis midae*. *S. Afr. J. Mar. Sci.* 5: 523 - 529.

Barkai R. 1987. An energy budget for the South African abalone *Haliotis midae*. M.Sc. Thesis. University of Cape Town.

Botes L. 1996. Influence of biomass and water flow rate on water quality in abalone (*Haliotis midae*) aquaculture. B.Sc. Hons. Thesis. University of Stellenbosch.

Bowler C. 2003. Light signal transduction. Activity report 1996/1997. Stazione Zooligica Anton Dohrn. http://www.szn.it/actrep/aqua_205.htm. 7 January.

Branch G. M., Griffiths C. L., Branch M. L., Beckley L. E. 1994. Two Oceans: A Guide to Marine Life of South Africa. David Philip Publishers. South Africa.

Britz P. J., Sauer W. H. H., Mather D., Oellerman L., Cowley P. D., Ter Morshuizen L., Bacela N. 2002. Baseline study of the utilisation of living marine resources in the Eastern Cape Province. Department of Ichthyology and Fisheries Science. Rhodes University. http:// www.envirofishafrica.co.za/pdf/ecbaseline.pdf. 15 December.

Chen H. C. 1984. Recent innovations in cultivation of edible molluscs in Taiwan, with special reference to the small abalone *Haliotis diversicolor* and the hard clam *Meretrix lusoria*. *Aquaculture* 39: 11 - 27.

Cook P., Britz P. 1990. Perlemoen farming in South Africa. Proceedings of a workshop convened and published by the Mariculture Association of South Africa.

Cook P. 2002. Abalone farms in South Africa. In: A brief update of the South African abalone (*Haliotis midae*) resource. Tarr R. Zoology Department, University of Cape Town. http://web.uct.ac.za/depts/zoolgy/abnet/newslet1/saupdate.html. 20 November.

Crofts D. R. 1937. The development of *Haliotis tuberculata*, with special reference to organogenesis during torsion. *Philos. Trans. Roy. Soc. Lond. B.* 288: 219 - 268.

Cuthbertson A. 1978. The Abalone Culture Handbook. Hobart.

Dixon M. G. 1992. The effect of temperature and photoperiod on the digestive physiology of the South African abalone *Haliotis midae*. M.Sc. Thesis. Rhodes University.

Ebert E. E., Hamilton R. M. 1983. Ova fertility relative to temperature and to the time of gamete mixing in the red abalone, *Haliotis rufescens*. *Calif. Fish. Game* 69: 115 - 120.

Ebert E. E., Houk J. L. 1984. Elements and innovations in the cultivation of red abalone *Haliotis rufescens*. *Aquaculture* 39: 375 - 392.

Fallu R. 1991. Abalone Farming. Fishing News Books. Oxford.

Fishtech. 2003. World abalone farming. San Francisco and San Rafael California. Fishtech. http://www.fishtech.com/farming.html. 4 January.

Genade A. B., Kirst A. L., Smit C. J. 1988. Observations of the spawning, development and rearing of the South African abalone *Haliotis midae* Linn. *S. Afr. J. Mar. Sci.* 6: 3 - 12.

Giffen M. H. 1970. New and interesting marine and littoral diatoms from Sea Point, near Cape Town, South Africa. *Bot. Mar.* 13: 87 - 99.

Hahn K. O. 1989. CRC Handbook of Abalone and other Marine Gastropods. CRC Press. Florida.

Henry N. R. 1995. Studies related to the artificial spawning and culture of abalone, *Haliotis midae*. M.Sc. Thesis. University of Cape Town.

Hone P., Madigan S., Fleming A. 1997. Abalone Hatchery Manual for Australia. South Australian Research and Development Institute. Adelaide.

Hooker N., Morse D. E. 1985. Abalone: the emerging development of commercial cultivation in the United States. In: Crustacean and Mollusc Aquaculture in the United States. Huner J. V., Brown E. E. (eds.). AVI Publishers. Westport. pp 365 - 413.

Kawamura T., Kikuchi S. 1992. Effects of benthic diatoms on settlement and metamorphosis of abalone larvae (in Japanese with English abstract). *Suisanzoshoku* 40: 403 - 409.

Kawamura T., Takami H. 1995[a]. Analysis of feeding and growth rate of newly metamorphosed abalone *Haliotis discus hannai* fed on four species of benthic diatom. *Fish. Sci.* 61: 357 - 358.

Kawamura T., Saido T., Takami H., Yamashita Y. 1995[b]. Dietery value of benthic diatoms for the growth of post-larval abalone *Haliotis discus hannai*. *J. Exp. Mar. Biol. Ecol.* 194: 189 - 199.

Kawamura T. 1996. The role of benthic diatoms in the early life stages of the Japanese abalone (*Haliotis discus hannai*). In: Watanabe Y., Yamashita Y., Oozeki Y. (eds.). Survival Strategies in Early Life Stages of Marine Resources. A. A. Balkema. Rotterdam. pp. 355 - 367.

Kawamura T., Roberts R. D., Nicholson C. M. 1997. Factors affecting the food value of diatom strains for post-larval abalone *Haliotis iris*. *Aquaculture* 160: 81 - 88.

Kawamura T., Roberts R. D., Takami H. 1998. A review of the feeding and growth of postlarval abalone. *J. Shellfish. Res.* 17: 615 - 625.

Keough M. J., Raimondi P. T. 1995. Responses of settling invertebrate larvae to bioorganic films: effects of different types of films. *J. Exp. Mar. Biol. Ecol.* 185: 235 - 253.

Keough M. J., Raimondi P. T. 1996. Responses of settling invertebrate larvae to bioorganic films: effects of large-scale variation in films. *J. Exp. Mar. Biol. Ecol.* 207: 59 - 78.

Leighton D. L. 1974. The influence of temperature on larval and juvenile growth in three species of Southern California abalones. *Fish. Bull.* 72: 1137 - 1145.

Lindberg D. R. 1992. Evolution, distribution and systematics of the *Haliotidae*. In: Abalone of the World. Shepherd S. A., Tegner M. J., Guzma'n del Pro'o (eds.). Fishing News Books. Oxford. pp 3 - 18.

Marine and Coastal Management. 2002. Abalone: a fishery in crisis. Marine and Coastal Management. http://sacoast.uwc.ac.za/education/resources/abalone.htm. 15 November.

Matthews I., Cook P. A. 1995. Nutrition of newly settled larvae of the South African abalone *Haliotis midae*. *Mar. Freshw. Res.* 46: 545 - 548.

McShane P. E., Smith M. G. 1988. Measuring abundance of juvenile abalone *Haliotis rubra* Leach (Gastropoda: Haliotidae); Comparison of a novel method with two other methods. *Aust. J. Mar. Freshw. Res.* 39: 331 - 336.

Morse D. E., Hooker N., Jensen L., Duncan H. 1979. Induction of larval abalone settling and metamorphosis by gamma-aminobutyric acid and its congeners from crustose red algae. II Applications to cultivation, seed production and bioassays; principal causes of mortality and interference. *J. World Maricul. Soc.* 10: 81 -91.

Morse A. N. C., Morse D. E. 1984. Recruitment and metamorphosis of *Haliotis* larvae induced by molecules uniquely available at the surfaces of crustose red algae. *J. Exp. Mar. Biol. Ecol.* 75: 191 - 215.

Muller S. 1986. Taxonomy of the genus *Haliotis* in South Africa. *Trans. Roy. Soc. S. Afr.* 46: 69 - 77.

Newell G. E., Newell R. C. 1963. Marine Plankton a Practical Guide. Hutchinson & Co. London.

Newman G. G. 1967. Reproduction of the South African abalone, *Haliotis midae. Trans. Roy. Soc. S. Afr.* 46: 69 - 77.

Newman G. G. 1968. Groei van die Suid-Afrikaanse perlemoen *Haliotis midae. Invest. Rep. Div. Sea Fish. S. Afr.* 67: 1 - 24.

Newman G. G. 1969. Verspreiding van perlemoen *Haliotis midae* en die uitwerking van temperatuur op produktiwiteit. *Invest. Rep. Div. Sea Fish. S. Afr.* 74: 1 - 7.

Norman-Boudreau K., Burns D., Cooke C. A., Austin A. 1986. A simple technique for detection of feeding in newly metamorphosed abalone. *Aquaculture* 51: 313 - 317.

Ohashi S. 1993. Yolk absorption of plantigrade juvenile abalone *Nordotis discus discus* (in Japanese with English abstract). *Bull. Nagasaki Pref. Inst. Fish.* 19: 23 - 25.

Overmann J. 2001. Diversity and ecology of phototropic sulfur bacteria. *Microbiology Today.* 28:116 - 118.

Payne A. I. L., Crawford R. J. M. 1989. Oceans of Life off Southern Africa. Vlaeberg Publishers. Cape Town.

Pawlik J. 1992. Chemical ecology of the settlement of benthic marine invertebrates. *Oceanogr. Mar. Biol. Annu. Rev.* 30: 273 - 335.

Pitcher G., Franco J., Whyte K., Viljoen C. 2000. Abalone (*Haliotis midae*) farming and paralytic shellfish poisoning on the coast of South Africa. Abstract - 4th International Abalone Symposium. Cape Town.

Roberts R., Kawamura T., Hideki T. 2000. Diatoms for abalone culture: a workshop for abalone farmers. Cawthron Report No. 547. 4th International Abalone Symposium. Cawthron. Cape Town.

Roberts R. D., Nicholson C. M. 1997. Variable response from abalone larvae (*Haliotis iris, H. virginea*) to a range of settlement cues. *Moll. Res.* 18: 131 - 142.

Roberts R. D., Kawamura T., Nicholson C. M. 1999. Growth and survival of post-larval abalone (*Haliotis iris*) in relation to development and diatom diet. *J. Shellfish. Res.* 18: 243 - 250.

Rudd M. 1994. Abstract: A review of the international abalone trade patterns and pricing. Second International Symposium on Abalone Biology, Fishery and Culture. Hobart.

Sagara J., Araki K. 1971. Oxygen consumption of abalone in early development stage and juvenile. *Bull. Tokai Reg. Fish. Lab.* 65: 11 - 16.

Saito K. 1981. The appearance and growth of 0 - 1 year ezo abalone. *Bull. Jpn. Soc. Sci. Fish.* 47: 1393 - 1400.

Searcy-Bernal R., Salas-Garza A. E., Flores-Aguilar R. A., Hinojosa-Rivera P. R. 1992. Simultaneous comparison of methods for settlement and metamorphosis induction in the red abalone (*Haliotis rufescens*). *Aquaculture* 105: 241 - 250.

Seki T., Kan-no H. 1977. Synchronised control of early life in the abalone, *Haliotis discus hannai* Ino, Haliotidae, Gastropoda. *Bull. Tohoku Reg. Fish. Res. Lab.* : 38 - 143.

Seki T. 1980. An advanced biological engineering system for abalone seed production. In: International Symposium on Coastal Pacific Marine Life. Western Washington University. Bellingham. pp. 45 - 54.

Seki T., Kan-no H. 1981. Observations on the settlement and metamorphosis of the veliger of the Japanese abalone, *Haliotis discus hannai* Ino, Haliotidae, Gastropoda (in Japanese with English abstract). *Bull. Tohoku Reg. Fish. Res. Lab.* 42: 31 - 39.

Seki T. 1997. Biological studies on the seed production of the northern Japanese abalone, *Haliotis discus hannai* Ino (in Japanese with English abstract). *Bull. Tohoku Natl. Fish. Res. Inst.* 59: 1 -71.

Shepherd S. A., Turner J. A. 1985. Studies on southern Australian abalone (genus *Haliotis*): VI. Habitat preference, abundance and predators of juveniles. *J. Exp. Mar. Biol. Ecol.* 93: 285 - 298.

Slattery M. 1992. Larval settlement and juvenile survival in the red abalone (*Haliotis rufescens*): an examination of the inductive cues and substrate selection. *Aquaculture* 102: 143 - 153.

Spotte S. H. 1979. Fish and invertebrate culture: Water management in closed systems. John Wiley and Sons. New York.

Stephensen T. A. 1944. The constitution of the intertidal fauna and flora of South Africa. *Ann. Natal Mus.* 10: 261 - 358.

Suzuki H., Ioriya T., Seki T., Aruga Y. 1987. Changes of algal community on the plastic plates used for rearing the abalone *Haliotis discus hannai*. *Nippon Suisan Gakkaishi* 53: 2163 - 2167.

Takami H., Kawamura T., Yamashita Y. 1997. Survival and growth rates of post-larval abalone *Haliotis discus hannai* fed conspecific trail mucus and/or benthic diatom *Cocconeis scuttllum* var. parva. *Aquaculture* 152: 129 - 138.

Tarr R. 2002. The abalone story. Department of Environmental Affairs and Tourism. Marine and Coastal Management. Cape Town. http://www.environment.gov.za/mcm/inshore/abstory.htm. 10 October.

Thorson G. 1950. Reproductive and larval ecology of marine bottom invertebrates. *Biol. Rev.* 25: 1 - 45.

Todd C. D., Keough M. J. 1994. Larval settlement in hard substratum epifaunal assemblages a manipulative field study of the effects of substratum filming and presence of incumbents. *J. Exp. Mar. Biol. Ecol.* 181: 159 - 187.

Uki N., Kikuchi S. 1984. Regulation of maturation and spawning of an abalone, *Haliotis* (Gastropoda), by external environmental factors. *Aquaculture* 39: 247 - 261.

Underwood A. J. 1979. The ecology of intertidal Gastropods. *Adv. Mar. Biol.* 16: 111 - 210.

Webber H. H., Giese A. C. 1969. Reproductive cycle and gametogenesis in the black abalone *Haliotis cracherodii* (Gastropoda: Prosobranchiata). *Mar. Biol.* 4: 152 - 159.

Zoology (a). 2002. *Haliotis midae.* Zoology Department, University of Cape Town. http://web.uct.ac.za/depts/zoology/abnet/midae.html. 15 December.

Zoology (b). 2002. South African abalone. Zoology Department, University of Cape Town. http://web.uct.ac.za/depts/zoology/abnet/safrica.html. 15 December.